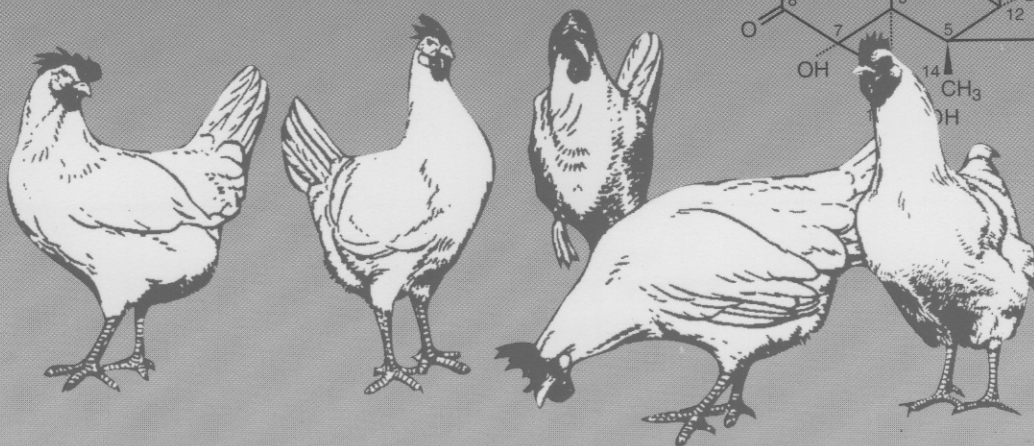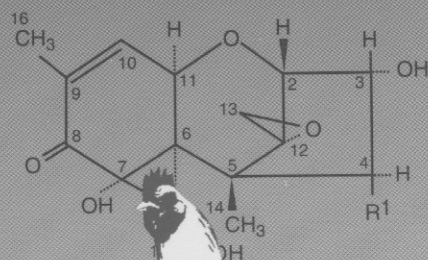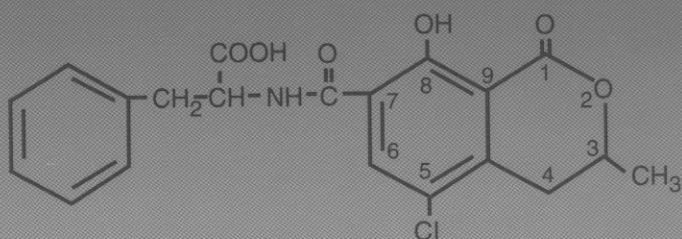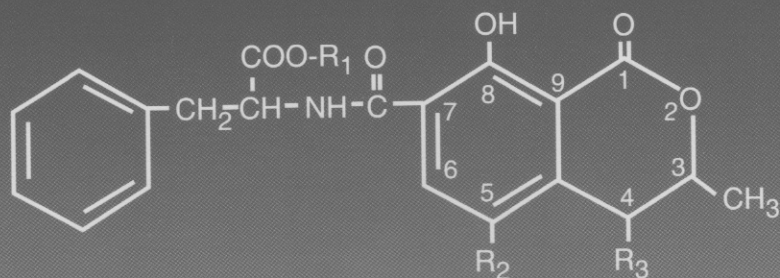# POULTRY METABOLIC DISORDERS AND MYCOTOXINS

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POULTRY METABOLIC DISORDERS AND MYCOTOXINS

By

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CHAPTER 1. SUDDEN DEATH SYNDROME

Other names:	ACUTE DEATH SYNDROME FLIP-OVERS
Species:	BROILER CHICKENS CONDITIONS REPORTED IN BROILER BREEDERS, TURKEY BREEDERS

1.   COMPENDIUM

Sudden Death Syndrome (SDS) is a condition afflicting fast growing broiler chickens, and especially males. Normal incidence is 1.5-2.5% of the flock and from 21-28 d will usually be the major cause of death. Afflicted birds appear healthy, are well fleshed and invariably have feed in their digestive tract. Death occurs within 1-2 minutes, the birds most frequently being found dead on their backs. There are few changes in gross pathology. The heart may contain blood clots, that are likely post-mortem in origin, and the ventricles are usually empty. Diagnosis is usually by exclusion of other diseases. Lungs are often edematous, although this usually occurs when birds spend time on their backs and fluid drains to the lung region by gravity. There are no specific changes in the tissue or blood profile that can be used for diagnosis. The condition is precipitated by fast growth rate, and so conversely it can be prevented by varying degrees of nutrient restriction. There are no clear relationships between any diet nutrients, ingredients and/or environmental factors that correlate with the onset or incidence of SDS. The condition seems to be more prevalent when ionophore anticoccidials are used or if the diet contains a readily available carbohydrate source such as glucose. SDS can be artificially induced by intubating with lactate, although the timing of onset can be modified by diet.

The condition can best be prevented or reduced in incidence by inducing a period of initial slow growth. This can be achieved by reduction in daylength, physical feed restriction and/or the use of low-nutrient dense diets. Economics dictate the degree of early growth suppression to be implemented. With 10-15% reduction in 20 d body weight, birds seem able to show complete growth compensation by 42-49 d.

2.   OCCURRENCE AND GENERAL SIGNS

Sudden death syndrome is a cause of mortality in apparently normal, fast-growing broiler chickens. Birds, which are most often males, are usually found dead on their backs with wings outstretched. The condition rarely occurs in other domesticated birds, although there have been reports of similar type deaths in adult meat breeders and periodically there is mention of individual

bird SDS mortality in flocks of growing Leghorn pullets. Unfortunately there are no specific signs, and so most often SDS is diagnosed by exclusion.

Gardiner *et al.* (1988a) attempted to correlate the incidence of SDS with bird age, body weight and season of the year. Studying records from 90,000 broilers collected over a five year period, these workers recorded a mean SDS mortality of 1.3-9.6%. Mortality peaked between 21-27 d of age and thereafter remained fairly constant at 0.08% per day to 39 d and then declined to 0.05% per day to 70 d. Based on these predictions, Gardiner *et al.* (1988a) conclude that within a flock of 100,000 male birds, 3,000 SDS mortalities can be expected up to 42 d, while for a mixed-sex flock, the mortality will be around 2,000 birds. The season of the year did not seem to influence these patterns of mortality. In Australia, Steele and Edgar (1982) conducted a comparable survey of a 64,000 bird flock. About 2.5% of birds died due to SDS, and this represented about 35% of total mortality. Between 7-28 d, SDS was the primary cause of death, and of these dead birds 75% were found to be male. Riddell and Springer (1985), in a survey of 51 broiler flocks, showed a mean SDS mortality of 1.95% with a range of 0.7-4.0%. In 39 of these flocks, SDS mortality peaked at 21 d of age, although in 12 other flocks, there was a gradual increase in occurrence throughout the 6 wk grow-out period.

Broilers that die of SDS show no specific abnormalities. Birds, usually male, appear healthy and are often above average flock body weight. Immediately prior to death they appear normal and are often seen feeding, drinking or walking. Birds extend their neck, squawk, and due to wing beating and leg extension, quickly work themselves onto their backs. Most poultry farmers describe the condition as a heart attack. Death occurs within minutes, although birds can be revived with limited long-term success if they are vigorously massaged during the early stages of the attack. Because subsequent post-mortem examination reveals few specific effects, Newberry *et al.* (1987) wondered if the condition was preceded by any behavioral changes, and so perhaps giving researchers a chance to study afflicted birds more closely. Through the use of video cameras, behavioral data was collected on a number of birds dying from SDS between 22-46 d of age. All affected birds exhibited a sudden attack prior to death, characterized by loss of balance, violent wing-flapping and strong muscular contractions. The apparent seizure usually lasted just under one minute, and as previously mentioned, most birds gave some type of squawk or high-pitched cry during the attack. Unfortunately, no single behaviour pattern or environmental change preceded these attacks. The majority of birds were eating and drinking within 2 h prior to the attack, and there was no apparent reduction in general activity during this preceding 2 h period.

Because SDS occurs more frequently in male birds, the role of female hormones has been questioned. Gardiner *et al.* (1988b) injected male broilers with estradiol and this had the expected effect on secondary sexual characteristics and a four-fold reduction in testes size. Both growth rate and the occurrence of SDS were unaffected.

3.   PATHOLOGY AND METABOLIC CHANGES

3.1.   *Gross Pathology*

Ononiwu *et al.* (1979a) extensively described the pathology of SDS birds and attempted to describe the events leading up to death. They found that birds are always well-fleshed, with edema and general pulmonary congestion. Feed is present along the entire digestive tract and the gall

bladder is usually empty. The liver and kidneys may be slightly enlarged and have patchy subcapsular hemorrhage. The heart may contain clotted blood, especially in the atria, although the ventricles are most often empty and the left ventricle may appear slightly hypertrophied. A microscopic examination of the heart muscle suggests some damage, because fibres are usually degenerated and infiltrated with heterophils. Ononiwu *et al.* (1979a) suggest that the pathological lesions seen in SDS are associated with some type of vascular disturbance. The process likely starts with circulatory lesions manifested by increased capillary permeability, followed by the congestion of many tissues. These workers concluded that death is caused by some type of damage to the heart which subsequently leads to lung edema, with the chicken then being unable to breathe. Squires and Summers (1993) cite edema as one of a number of factors linking SDS to ascites *(Chapter 3)*.

A general finding of SDS mortality is edematous lungs (Ononiwu *et al.,* 1979a). Initially this was thought to be a contributing factor, although it is now shown to be a normal occurrence of birds lying on their backs for some time. Since the lungs are in the dorsal region, then the apparent edema is more likely the gravitational settling of fluid into this region (Bowes and Julian, 1988). Certainly SDS birds found dead on their breasts usually do not show this sign and relative lung weight is often much less than seen with birds dying on their backs. Riddell and Orr (1980) collected 14-21 d old birds from 7 commercial flocks and confirmed that congested and edematous lungs were not always a consistent feature, and were rarely found in birds that were examined almost immediately after death. In a like fashion, care must be taken in interpreting the data on the intestinal weight following death, since the digesta will likely create positive osmotic gradients.

Bowes and Julian (1986) quantitated general organ development in broilers in an attempt to provide a comparative base-line during gross pathological examination. Broilers dying from SDS were examined 2-24 h after death. These workers concluded that relative weights of intestine, liver, lungs and heart were usually at the upper end of the range normally seen in healthy birds of the same age. Bowes and Julian (1986) conclude that SDS is not likely the cause of death if organ weights are much below normal, if the intestine is empty, and if the heart is not of normal size with contracted ventricles. In other studies, Bowes and Julian (1988) concluded that the apparent reduction in heart weight over time following SDS, is likely due to myocardial contraction which occurs until all ATP energy reserves are utilized. Such contraction physically empties the heart of blood and perhaps also of extracellular fluid.

There seems to be some discrepancy regarding the occurrence of enlarged and/or hemorrhagic livers. Bowes and Julian (1988) indicate such an enlarged liver, although Whitehead and Randall (1982) suggest that such hypertrophied livers may relate to fatty liver and kidney syndrome *(Chapter 6)* because they also observed pale fatty livers in their birds. This suggests that the fatty liver syndrome may predispose to SDS, although a common diagnosis with the former is an empty intestine since afflicted birds rarely eat as the condition progresses. On the other hand, with SDS, a full intestine is almost universal. It seems unlikely therefore that an enlarged liver, as part of the SDS condition, is in any way correlated to fatty liver and kidney syndrome.

Cassidy *et al.* (1975) carried out more detailed studies on the cardiac blood spots found in birds dying of SDS. These small clots were found in all chambers, usually in close proximity to the heart wall. A number of different types of clot can be found, although the most common contains a mixture of fibrin and erythrocytes, the latter giving the clots their typical dark red color. All clots were considered to be post-mortem in origin and formed by blood coagulation, leading Cassidy *et al.* (1975) to conclude that they were not causative agents.

3.2.   *Tissue analysis*

A number of studies have been aimed at correlating changes in tissue mineral, electrolyte and fat content as it relates to SDS. Rotter *et al.* (1985) observed a small but statistically significant reduction in the K content, and an increase in Na content of heart tissue of SDS birds. These same workers, together with Buckley *et al.* (1987) have also studied lipid composition of selected tissues, and produced some conflicting reports. While Rotter and co-workers observed a significant reduction in C20:4 fatty acid content of heart tissue, Buckley questioned the biological significance of the very small changes seen in their studies. Fatty acid and phospholipid profiles are of potential importance, due to their role in membrane function, and as precursors to essential prostaglandins. Buckley *et al.* (1987) indicated an elevated level of C20:4ω6, suggesting that a deficiency of this important prostaglandin precursor seems unlikely. In this same study, there was an indication of general desaturation of tissue lipids in SDS birds, a situation known to disrupt membrane enzyme activity. This scenario is seen in the hepatic lipids of infants dying from Sudden Infant Death Syndrome (Cairns *et al.,* 1983). In subsequent studies, Buckley and Gardiner (1990) sampled heart, liver, lung and kidney from 22-48 d old birds dying of SDS, and subsequently assayed these for 68 different elements. Unfortunately only relative mineral concentrations are reported, although a number of significant differences were observed. Most notably, SDS birds showed elevated levels of liver Ca and reduced Fe in lungs and kidneys. The heart, lungs, and especially the liver, also exhibited reduced levels of dry matter. These authors point out the valid problem of age-related changes occurring in tissue mineral levels following death, but conclude that SDS is related to change in tissue concentrations of some key elements. Obviously it is not known if these changes are causative, or the consequence of SDS. The elevated concentration of liver Ca is of interest in relation to the so-called SDS condition occurring in maturing broiler breeders, as discussed later *(Section 5)*. More recently Chung *et al.* (1993) showed SDS birds to have depressed Ca^{++} uptake and Ca^{++} and Mg^{++}-ATPase activity of cardiac sarcoplasmic reticular tissue, leading these authors to suggest cardiac membrane dysfunction is a factor in SDS.

3.3.   *Blood profile*

Because female broilers have a much lower incidence of SDS, and Leghorns of either sex rarely show the condition, Bowes *et al.* (1988) used these birds as controls, compared to male broilers in an attempt to correlate blood parameters. Blood samples were taken periodically from 9-42 d birds, housed under identical conditions and fed the same diet. Few differences were seen in the blood profiles of male and female broilers. Males consistently exhibited elevated blood glucose compared to females, although the actual levels found were not different to those seen in male Leghorns. Compared to the male Leghorn, the male broiler consistently exhibited elevated blood uric acid and lactate dehydrogenase. Bowes *et al.* (1989) conclude that higher levels of uric acid in broilers may have been due to the IB vaccination these birds alone received at day-of-age and this may have subsequently impaired kidney function to some degree. If this concept is valid, then it would be of interest to compare SDS susceptibility in vaccinated and non-vaccinated birds. Metabolically active skeletal and cardiac muscles are both significant contributors to lactate dehydrogenase, and this may relate to the higher levels seen in male broilers by Bowes *et al.* (1989). Conversely the higher level of this enzyme suggests higher concentrations of substrate,

and so perhaps elevated lactate levels. This concept is discussed more fully in the following *Section 4*.

Blood electrolyte levels have also come under close scrutiny, because SDS type symptoms can experimentally be induced by an overdose of key elements, such as K, administered directly into the heart. Riddell and Orr (1980) found higher levels of blood K in SDS birds compared to normal healthy controls. However the actual levels observed were not different to those assayed in blood taken from these same control birds, but stored for 60-90 minutes prior to assay. These data again pose the question of how to interpret biochemical profiles of SDS birds, because in most instances, birds have been dead for some time prior to blood or tissue sampling.

4.   **RELATED FACTORS**

A large number of factors have been studied with a view to isolating causative agents in SDS. Most studies have involved diet texture and composition. When reviewing these factors, it must be remembered that SDS is a condition exclusively seen in very fast growing birds, and so when SDS is claimed to be reduced, it is pertinent to consider growth rates achieved. Perhaps the easiest way to reduce SDS is to reduce growth rate, although obviously this is not usually economical.

4.1.   Diet texture and restriction programs

Because SDS occurs only in fast-growing birds that are assumed to be eating to near physical capacity, Bowes *et al.* (1988) investigated the effect of physical feed restriction. Male birds were fed conventional broiler diets either free-choice or restricted to 75% of normal feed intake. While 3% of the control birds died of SDS, no such mortality occurred in the feed-restricted birds. However, feed restriction did reduce the 39 d body weight of these birds (1760 vs 1270g) and in fact growth rate was tempered at all ages. These authors conclude that SDS can be prevented by growing birds at a slower rate, although as will be discussed later, *(Section 6)* the economics of such a management decision are not always so straightforward.

Along the same lines, Proudfoot and Hulan (1982) studied the effects of allowing birds access to the feeders for 8-24 h/d. Interestingly male broilers exhibited higher SDS when restricted to 8 h feeding per day, even though in this situation their body weight was less than that of birds with unlimited access to feed (2090 vs 2310 g at 49 d). Perhaps birds with only 8 h access to feed exhibited more frenzied activity around the feeder, because these birds are calculated to have consumed feed at 15 g/h vs 6 g/h for control birds, assuming that these control birds eat at a constant rate throughout the day. In this same study, Proudfoot and Hulan (1982) indicated a marked reduction in SDS when birds were fed mash (0.4 % SDS) rather than steam pelleted diets (1.6% SDS) even though birds from both treatments were of comparable body weight. This led the researchers to investigate further the effect of the pelleting process *per se*, and particularly the effect that such conditioning may have on selected dietary ingredients. Proudfoot *et al.* (1982) indicated a higher incidence of SDS in birds fed pellets (1.82%) or pellets reground to a mash consistency (1.85%) relative to birds fed untreated mash diets (0.64%). Unfortunately these results

are confounded with growth rate in that mash feeding resulted in a 220 g depression in body weight at 43 d of age.

In subsequent studies Proudfoot *et al.* (1984) assumed that there was a pelleting factor influencing SDS that was independent of body weight, and that these factors developed in the feed during the steam and pressure treatment employed during pelleting. In testing this theory, various diet ingredients by-passed the pelleting process, and so were added as "mash" ingredients to the other pelleted diet components. When all the protein-rich ingredients (soybean meal, fish meal and canola meal) by-passed pelleting, there was a significant reduction in SDS of male broilers to 49 d (3.6 vs 0.9%). While Proudfoot *et al.* (1984) conclude that toxic substances are produced by pelleting protein ingredients, and that these predispose to SDS, again it must be emphasized that reduction in SDS in this trial was associated with reduced growth rate (2160 vs 1950 g at 49 d).

4.2.   *Diet protein and amino acids*

Diet protein *per se* seems to have little effect on SDS. Julian and Leeson (1985) indicated some 4% incidence in all birds fed diets varying in protein from 17-31%. Mollison *et al.* (1984) used 19-24% CP in diets fed from 29-56 d and recorded a 50% reduction in SDS mortality with the lower protein levels. These authors attributed the effect to birds being smaller when fed the lower protein diet. Unfortunately these diets were also very different in ingredient composition, and so this is another confounding factor. For example the higher protein diets contained much more soybean meal, tallow, fish meal and less corn. In this same context, Blair *et al.* (1990) conducted studies to investigate field reports of higher SDS in birds fed wheat rather than corn. In controlled studies, wheat-based diets did produce more SDS, although as a proportion of total mortality there was no diet effect. In two other trials, either the incidence of SDS or its proportion of total mortality was reduced when meat meal was included in the diet. Blair *et al.* (1990) conclude that meat meal supplies some previously unidentified factor that provides protection against SDS, and this may relate to any wheat vs corn effects, since corn diets invariably contain more meat meal necessary to maintain protein and amino acid levels. Unfortunately there are also other confounding diet effects in the trials conducted by Blair *et al.* (1990). For example, meat-based diets contain no added phosphate source even though diet phosphate levels are calculated to be much higher in the soy-based control diet. Similarly the meat meal diet contains little added salt and less supplemental fat. These subtle changes in nutrient source and/or nutrient concentration could conceivably influence SDS. This type of example shows the dilemma facing nutritionists in attempting to study ingredient and nutrient factors, while at the same time using conventional rather than semi-purified diets.

There has been relatively little research aimed at investigating the role of diet amino acids. As with protein, graded levels of essential amino acids will likely influence growth rate, and therefore, will directly affect the occurrence of SDS. Independent of growth rate and the essential nature of known amino acids, taurine metabolism has received most attention. Taurine is considered to be a non-essential amino acid for poultry, being found in most animal tissues (*eg.* meat meal) but is almost non-existent in plant proteins (*eg.* soybean meal). Taurine is most concentrated in heart tissue and this source may represent 50% of the free taurine pool. In other species, myocardial metabolism can be altered by taurine depletion and in the classical situation with cats, deficiency can cause heart failure. While avian species are assumed to synthesize sufficient taurine, reduced cardiac taurine levels have been associated with heart tissue degeneration in turkeys. Furazolidone is known to cause heart muscle degeneration in turkeys and

this is accompanied by reduction in tissue taurine levels. Schaeffer *et al.* (1982) found that oral dosing of taurine to such furazolidone treated birds did restore heart taurine levels, although unfortunately cardiomyopathy still occurred. Following up on their early work investigating the role of meat-meal in SDS, Blair *et al.* (1991) indicated that taurine does not play a major role in its etiology. Taurine is known to affect calcium metabolism of the heart tissue (Huxtable, 1986) consequently Campbell and Classen (1989) made further studies on the role of this amino acid. Feeding up to 0.2% taurine to broilers resulted in a small reduction in SDS, although the differences were not significant. Further studies by Jacob *et al.* (1991) on the role of taurine in SDS involved the application of known taurine antagonists. Of the antagonists tested, β-alanine appeared the most effective, reducing taurine concentration in cardiac tissue from 29 to 3 mol/g wet weight. However even this dramatic change in tissue taurine level had no effect on SDS, adding to the conclusion that taurine plays little role in the etiology of SDS.

4.3.   Diet energy sources

Use of various sources of energy in the diet has also been suspected in affecting the occurrence of SDS. The replacement of carbohydrate by fat is suspected to be a factor, although in many instances the effects of the ingredients *per se* necessarily changes overall diet composition, and subsequent differential growth rate is a confounding factor. Rotter *et al.* (1987) showed that feeding graded levels of tallow had little effect on the incidence of SDS even though these diet changes promoted increased growth rate. In previous studies, Rotter *et al.* (1985) showed that the type of fat may be of significance, since birds fed hydrogenated coconut oil, (which is very saturated), had a very low incidence of SDS (0.6 vs 2.5%) compared to birds fed comparable levels of unsaturated sunflower oil. Unfortunately this reduced mortality up to 28 d of age was associated with reduced growth rate. Chung *et al.* (1993) also studied fat source as it affects SDS. Broilers fed sunflower oil showed less SDS than did birds fed tallow and this was associated with reduced phosphatidylcholine in cell membranes of the heart tissue. These authors conclude that fat source, and perhaps fat saturation, are implicated in the syndrome.

Looking at non-fat sources of energy, Julian and Leeson (1985) reported some interesting effects of using different carbohydrate sources in a diet. Mortality due to SDS was more than doubled to 6% SDS when glucose was the predominant energy source compared to 2.1% mortality when fat was a major contributor or 2.5% when corn starch was used. These results are of interest in relation to the later discussion on the role of lactate *(Section 4.7)*.

4.4.   Diet minerals and vitamins

Julian (1986) hypothesized that SDS may relate in some way to mineral availability, suggesting a potential hypomagnesemic tetany caused by nutrient interaction. For example, high levels of dietary fat, as commonly used in broiler diets, can lead to an increase in the formation of soaps that are complexes of minerals such as Mg, usually with saturated fatty acids. Similarly, Ca supplements added to combat potential skeletal abnormalities can compete with Mg for the sites of intestinal absorption. In testing this hypothesis, Julian (1986) carried out field studies with large commercial flocks in which supplements of 0.2% Ca, P or Mg were used. Unfortunately the diet had no effect on SDS, which averaged 1.5% for the 140,000 birds under study.

Vitamin nutrition has also come under close scrutiny. Hulan *et al.* (1980) investigating the role of various B-vitamins showed that SDS was highest when biotin, pyridoxine and thiamine were borderline while all other vitamins were at double the normal inclusion level. While these results were far from conclusive, they do pose the question of the adequacy of certain nutrients when others are fed in excess. More often it is simple deficiencies that are studied, whereas in reality, induced deficiencies may be of more concern. This scenario is obviously difficult to diagnose, since no apparent deficiencies are seen during analytical testing of diets.

Biotin has perhaps been singled out most frequently as a possible factor in SDS. Steele *et al.* (1982) showed no benefit from supplying up to 100 μg/d (via the drinking water) within a commercial flock of 60,000 birds to 49 d. A liver analysis confirmed an increased biotin uptake by the birds, although the authors conclude that there is little if any association between normal biotin status and SDS. Whitehead and Randall (1982) made similar conclusions, although suggested that biotin may be a factor if SDS is complicated by the incidence of fatty liver and kidney syndrome (FLKS). The SDS/FLKS syndrome seemed to be worse when biotin was marginal and the other B vitamins were in excess.

4.5.   Pharmaceuticals

Since heart function is obviously implicated in SDS, a number of related pharmaceutical products have been tested, though generally without success. Proudfoot and Hulan (1983) studied the role of aspirin, feeding birds up to 0.16% in the diet. Aspirin had no beneficial effect, and in two trials, the SDS mortality was in fact numerically increased. Reserpine has also been studied, because this anti-hypertensive drug has been used historically as a tranquillizer for large turkeys, and so Gardiner and Hunt (1984) used up to 3 ppm of this product in the diet but saw no change in the mortality patterns.

The role of anticoccidial drugs should perhaps have received more attention than has occurred to date. There is some evidence of higher SDS mortality when these products are used, and historically the occurrence of SDS does coincide with the introduction of the ionophore anticoccidials some 20 years ago. Julian and Leeson (1985) indicated that SDS was substantially higher when birds were fed diets containing ionophores. With two ionophores, namely monensin and maduramicin ammonium, the SDS mortality was 3.0 and 2.3% respectively, while with robenidene, a non-ionophore, SDS was only 0.5%. Riddell and Orr (1980) also indicated that among other things, birds surveyed from within 51 commercial flocks had a higher incidence of SDS when monensin was used in both the starter and grower period compared to birds fed monensin only in the starter period. In this situation the "grower" period was undefined, although it is assumed to coincide with the time of peak SDS mortality. Riddell and Classen (1992), however, showed no difference in SDS of broilers fed monensin or amprolium.

By definition, ionophore compounds increase the movement of metal ions across cell membranes. In practice, this means altered flux of Na^+ and K^+, where the predominant ion may change depending upon the ionophore used. In addition to the movement of monovalent ions, there is also a concern over the increased permeability of the membranes to H^+, consequently affecting acid-base balance. Perhaps just as important, and a major reason for acute toxicity, is the counter-current transport of Ca^{++} (Elsasser, 1984) which becomes critical in cardiac tissue. In dogs

at least, monensin toxicity leads to myocardial tissue degeneration, which can be explained on the basis of altered cation transport (Todd *et al.,* 1984).

The involvement of ionophores in SDS is far from clear, and it should be emphasized that pathological lesions typical of ionophore toxicity have not been reported in SDS diagnosed birds. On the other hand, ionophores can affect "moment to moment" flux of ions (Elsasser, 1984) and this is consistent with the sudden imbalance in the normal metabolic function as seen with SDS. If ionophores are involved in the occurrence or severity of SDS, any contribution will have to be balanced against their obvious effectiveness as anticoccidials.

4.6.   *Lighting programs*

Lighting programs can influence the growth and development of the broiler, and so there has been an interest in studying various regimes as they affect SDS. Ononiwu *et al.* (1979b) compared the SDS incidence of broilers grown under continuous light with those exposed to a step-down intermittent program of 3L:1D starting at 10 d, and ending up at 1L:3D during the final grow-out period. Photoperiod had no effect on SDS which showed the typical pattern related to age of 9, 19, 33, 30, 18 13 and 10% (of total mortality) in wk 1-7 respectively. Newberry *et al.* (1985) investigated the potential effect of high light intensity on SDS in birds grown to roaster weight. Intensity of 1-12 lux had no effect on SDS and different intensities recorded at different locations within the broiler house did not correlate with the location of dead birds. Extending this work, Newberry *et al.* (1986) showed SDS to remain constant at light intensities ranging from 0.5 to 100 lux.

A period of short day length during early growth has recently been shown to be beneficial in reducing both SDS and skeletal problems in birds. Classen (1991) describes a program in which broilers are subjected to only 6 h light from 3-14 d or various incremental programs of 6 h increasing to 23 h over the 3-35 d period. Early growth rate is reduced because birds have less time to eat, and this is obviously beneficial in reducing SDS. Using these types of programs, Classen (1991) indicates a 30-60% reduction in incidence of SDS. Birds show compensation of body weight after 21 d, and body weight is only slightly reduced at 42 d, with catch-up by 49-56 d. This type of program is one of the easiest ways to reduce SDS and can be modified for open-sided buildings as discussed later *(Section 6.1)*. More recently Riddell and Classen (1992) showed SDS to decline in 63 d old broilers from 5.9 to 3.4% using a step-down light program. Blair *et al*. (1993) recorded a comparable significant reduction in SDS using trials involving around 2500 birds.

4.7.   *Lactate metabolism*

There has been considerable discussion on the role of lactate imbalance affecting SDS. This idea stems from the fact that lactate dosing can bring about symptoms similar to SDS, and that comparable lactate-related conditions are seen in other species.

As previously discussed *(Section 3.3)*, Bowes *et al.* (1988) observed higher blood lactate dehydrogenase in male broilers compared to Leghorns. If this is in response to increased substrate, it indicates more lactate accumulation in male broilers. Most frequently, lactate

accumulates when there is inadequate oxygen to fuel the normal aerobic metabolism and $NADH_2$ is not oxidized. Production of lactate rather than pyruvate, due to anoxia, leads to acidosis. Weil and Abdelmonen (1970) developed a relationship between blood lactate levels and the probability of survival, indicating that one hundred percent mortality is likely when lactate levels reach 10 times normal. Weil and Abdelmonen (1970) suggest that survival is related to the irreversibility of the metabolic disturbance which occurs during acidosis, and that O_2 intake during recovery does not always lead to survival. This scenario is seen with SDS birds that are revived during an attack, where their ultimate survival is unpredictable. Lactate accumulation certainly causes problems in other species, most notably laminitis in horses and ruminants. Garner *et al.* (1977) working with horses, correlated onset of condition with a rise in plasma lactate, where those animals with the greatest change died of circulatory failure. This potential involvement of lactate in circulatory failure has led several researchers to pursue the involvement of lactate in SDS.

Summers *et al.* (1987) carried out the most extensive series of trials involving lactate dosing of broilers. When 5 ml of a 20% lactic acid solution was intubated into the crop, within minutes, birds showed signs characteristic of SDS. In subsequent studies, the results were not so clear cut with only 20% of the birds dying. However there was no mortality if the diet was buffered with carbonate or bicarbonate. By injecting 20% lactate into the wing vein, all birds died within several seconds, showing typical SDS signs. This led Summers *et al.* (1987) to suggest that SDS relates to the alteration of acid-base balance in some way. In other studies, these researchers repeated the lactate intubation for birds fed different energy yielding ingredients. When glucose was fed, birds showed SDS signs within 30 minutes, while for starch-fed birds, the onset was delayed for 90 minutes. SDS did not appear until 2 h after dosing when the birds were fed fat. Interestingly, birds fed glucose had the lowest initial levels of blood lactate, and so perhaps it is the change in level that is of importance, confirming the previous observation of Weil *et al.* (1970) discussed above.

In studying the effect of lactate in SDS, Jacob *et al.* (1990) fed broiler diets containing up to 7.5% calcium lactate and 45% glucose. Glucose was used because Summers *et al.* (1987) and Julian and Leeson (1985) had previously indicated higher SDS with such readily available carbohydrate. The diet had no significant effect on SDS, although there was a clear trend for increased incidence (1.9, 2.2 and 3.4%) as the calcium lactate increased from 0% through 2.5% and 5% respectively. Similarly, lactate feeding also resulted in a greater proportion of mortality attributable to SDS mortality (22, 35 and 48% respectively). Lactate feeding reduced growth rate, and so these results are even more interesting, because reduced growth itself is expected to dramatically reduce SDS. Rapid change in acid-base balance, caused by such factors as lactate accumulation, can obviously influence occurrence and timing of SDS.

5.   SDS IN BROILER BREEDERS AND TURKEY BREEDERS

SDS in meat-type breeding stock is described separately from the foregoing discussion on broilers because it is likely that the conditions are not the same. Hopkinson *et al.* (1983) described a syndrome that they termed SDS occurring in broiler breeders around the time of maturity. In these Australian flocks, SDS started at the time of 5% egg production with the highest mortality occurring when 20-30% production was achieved. Mortality averaged 0.5-8.0% each wk. Birds were thought to be quieter, and perhaps taking longer to clean-up their feed. Most birds died at feeding time exhibiting congestion of the comb, wattles and cloaca and with severe congestion of the lungs, liver, spleen, oviduct and ova. The heart was grossly enlarged and plasma K and P

levels were significantly reduced. The condition seemed worse on hot days, although Hopkinson *et al.* (1983) indicated that mortality was reduced when K_2CO_3 was added to the feed at 3.5 kg/t. However these workers concluded that hypokalemia was a secondary metabolic change, and not the causative agent. In other studies, Hopkinson *et al.* (1984) studied the role of diet K and P levels on the incidence of SDS in adolescent broiler breeders. Six percent of the birds died from so-called SDS when they were fed a diet low in P and K, with signs as previously described. Plasma K was depressed in affected birds (3.34 vs 4.38 meq/L) as was plasma P (2.69 vs 4.12 mg/dL). Hopkinson *et al.* (1984) conclude that because dietary K is normally alkalogenic, it is unclear why the control birds were close to a normal acid-base balance, while the test birds were alkalotic with a base excess of +7.5. Acidosis is more likely if the imbalance relates to potassium. Profuse watery droppings were a feature of affected birds, and this is also seen in field outbreaks. One wonders if this relates to the test diet containing a gross excess of calcium at 3.2% compared to feeding 1.5% calcium to control birds (up to 5% egg production).   Such an excess of calcium would place considerable stress on renal function, and possibly, a consequence is excess K excretion via the increased flow of urine. It would have been interesting to remove this confounding effect of diet calcium concentration. Hopkinson *et al.* (1984) rationalized the use of high calcium diets some 6 wk prior to maturity on the basis that this was normal industry practice.

In still more recent studies, Hopkinson (1991) hypothesized that the etiology of SDS in broiler breeders was related to mineral imbalance. Certainly most cases of SDS in Australian breeders occur when diets contain meat meal and this is notoriously variable in Ca and P concentration. Hopkinson (1991) attempted to simulate field conditions of SDS by developing a vegetable protein diet containing various mineral levels. No SDS occurred in birds fed any diet when there was a good mineral balance. However SDS was seen in birds fed all other diets. There was a 30% incidence for birds fed a vegetable protein diet with low total phosphorus (0.47 vs 0.73%) and low potassium (0.36 vs 0.55%). The low potassium diet was produced by substituting potassium-rich soybean meal with lupins.   Unfortunately these results are confounded with differences in diet protein (13.5 vs 16.2% control) and diet calcium (3.4 vs 2.9% control).

Swayne and Saif (1990) also reported a field case of a condition that they termed SDS in turkey breeders, at 31 wk of age. The condition was apparently predisposed by the physical relocation of birds from grower to breeder facilities some 2 d earlier. Necropsied hens had small spleens, congested lungs and enlarged livers. The most consistent lesion (not seen in broilers) was pulmonary hemorrhage, and edema, and congestion of the capillaries in most of the visceral organs.   Due to the inflammatory lung tissue, such SDS may be more closely related to hypertensive angiopathy as seen in younger turkeys. In fact Swayne and Saif (1990) compared these findings in turkey breeders to histopathological changes and clinical patterns seen in sudden death in horses which seems to relate to exercise induced atrial fibrillation or asphyxiation from breath-holding or bronchospasm related to subclinical respiratory disease (Gunson *et al.,* 1988).

6.   POTENTIAL TREATMENT AND PREVENTION

It is obvious that there is no one treatment or preventative system for the control of SDS in young broilers. The condition is undoubtedly related to fast growth rate, and as such, management techniques to reduce the early maximum genetic potential for growth offer the best preventative scenario.

6.1.   *Reduced growth rate*

One of the most successful techniques used to temper early growth rate, is a step-down lighting program as detailed in *Table 1.1.*

TABLE 1.1 Reduced daylength programs for broilers			
	Blackout housing		**Open-sided housing**
Age of bird (days)	**Option #1**	**Option #2**	
0 - 4	23 h	23 h	23 h
5 - 10	8 h	8 h	Natural daylength
11 - 14	10 h	8 h	Natural daylength
15 - 18	14 h	23 h	Natural daylength
19 - 23	18 h	23 h	18 h
23+	23 h	23 h	23 h

In all programs, broilers are subjected to reduced daylength during the 5 - 18 d period of growth. This effectively reduces feed intake and so tempers early growth rate. Birds will learn to eat in the dark period, but there may be problems of skin-tearing etc., when the stocking density is very tight, because birds clamber over each other to get to the feeders. However with more normal densities, the system works very well, reducing the incidence of SDS (and also skeletal problems) by up to 40% in male birds.

In extreme situations, feed restriction can also be practised, and this will virtually eliminate SDS.   Restriction programs are similar to those described for ascites *(Chapter 3).*   If restriction/reduced daylength is used, then it seems illogical to use high-nutrient dense diets. A more practical approach is to use diets with 5-7% reduction in nutrient density, and one may also question the need for growth promoting compounds in these specialized starter diets. Whatever system is used, tempering the early growth rate up to 18-20 d of age seems to reduce SDS, even though previously it was mentioned that peak SDS mortality normally occurs at 21-28 d. Depending upon the degree of growth restriction induced, birds will exhibit subsequent growth compensation by 42 d.   Slow early growth followed by catch-up growth, implies a faster growth rate during compensation, if normal weight for age is to be achieved. *A priori* one would, therefore, assume more SDS and other metabolic problems at this older age, if growth rate *per se* is a predisposing factor. Such late mortality would be more expensive since it involves older birds. To date, evidence suggests that this shift in mortality does not occur, and that tempering the early growth in some way protects against SDS and/or that SDS is age related in some way.

The economics of initial slow growth must be worked out by the individual producers. If catch-up growth is achieved, and normal weight-for-age is reached, then the program will likely be economical. On the other hand, if these programs delay the grow-out period, then the situation of slow growth for 97% of the flock for example vs SDS mortality for 3% of the flock, needs to be calculated for local economic conditions.

6.2.   Electrolyte balance

It is surprising that so few researchers have investigated the role of electrolyte balance. SDS-type signs can be induced in the laboratory by injecting electrolytes, such as potassium, into the heart, and the work with lactate loading, suggests an acid-base imbalance. Likewise the SDS condition reported in Australia with adult breeders seems to be affected by potassium level of the diet, and potassium salts given via the feed are recommended for prevention. The very sudden onset of signs and almost immediate death implies rapid change in some critical system. Acid-base/electrolyte balance seems the most likely candidate. Whether electrolyte imbalance is causative or symptomatic of other predisposing factors, is obviously unclear at this time. The "available potassium" status of diets warrants further investigation.

6.3.   Anticoccidials

There is circumstantial evidence that ionophore anticoccidials are in some way involved with SDS. Certainly the appearance of SDS some 15-20 years ago, coincided with the introduction of these products. Ionophores by definition affect ion balance, the most critical ones being Na and K. Again it would seem beneficial to study the available potassium status of diets as it relates to anticoccidial inclusion and the physical distribution of anticoccidial within pelleted diets. On the other hand, SDS occurs when non-ionophore anticoccidials are used, and so if they are involved in this condition, they are but one contributing factor.

6.4.   Lactate balance

An inherently high level of plasma lactate dehydrogenase in broilers, coupled with the dramatic effect of dosing birds with lactate, suggests that lactate (acid/base) imbalance could be a predisposing factor. Perhaps one of the most interesting research results is the differential response by the birds to lactate dosing dependent upon their dietary carbohydrate source. Lactate accumulation in other species can cause sudden death that is ultimately manifested as circulatory failure. The relationship between the available carbohydrate load, the metabolic rate and the oxygen supply warrant further study. From a practical viewpoint, readily available dietary carbohydrate sources would be suspect.

Chapter 1

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CHAPTER 2. ROUND HEART AND AORTIC RUPTURE IN TURKEYS

Other names:	ANGIOPATHY
	CARDIOMYOPATHY
	DISSECTING ANEURYSM
Species:	TURKEYS

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1.   COMPENDIUM

Round heart disease and aortic rupture are two unrelated conditions occurring in turkeys, and especially fast-growing toms. Round heart is most prevalent in 2-4 wk old toms where high mortality is associated with the enlargement and rounding of the apex of the ventricles. The condition is worse when stresses are involved and there may be a genetic component. Microscopic lesions consistently show myocardial congestion and degeneration, hemorrhage and epicardial fibrosis. Round heart is sometimes associated with ascites. Affected poults show reduced serum protein and deficiency of α-globulins. The condition seems to be worse when there is high salt/sodium intake, and round heart can be induced by feeding high levels of furazolidone. Aortic rupture most commonly affects older turkeys, and again males seem more susceptible. Affected birds appear normal immediately prior to death, the onset of which is very sudden. At necropsy, the musculature is often pale, presumably due to loss of blood, while the body cavity contains massive hemorrhage. The aorta is invariably split longitudinally, at close proximity to the heart. Aortic rupture can be induced by feeding a copper deficient diet. The role of copper is as a co-factor in monoamine oxidase enzyme, necessary for normal elastin production in the aorta. Birds dying from aortic rupture have reduced aorta elastin, and so the tensile strength of this vessel is affected. Feeding high levels of copper, however, does not totally resolve the problem. Lathyrism, as induced by feeding BAPN, also leads to aortic rupture, and again this toxin results in reduced aorta elastin anabolism. Aortic rupture occurs most frequently in cooler climates, although this may simply be a result of faster growth rate. The onset of aortic rupture can be significantly delayed by the use of anti-hypertensive/tranquilizer agents, such as reserpine, although these products are now not usually registered for use in turkey diets. Both conditions are induced by stress, and so the most effective preventative, is a stress-free growing environment.

2.   OCCURRENCE AND GENERAL SYMPTOMS

Round heart disease and aortic rupture are two unrelated conditions most commonly seen in turkeys, although there are reports of round heart in chickens. The etiology of both conditions is still unclear, although some metabolic derangements, possibly influenced by genetics, are likely the key factors involved.

2.1.   *Round heart disease*

Round heart disease is seen in all ages of turkeys, although the condition is most prevalent in poults 2-4 wk of age. High mortality is associated with the enlargement of the right ventricle which gives the heart a "rounded" appearance. The condition seems to be worse when stress situations occur, and there may be a genetic component. There is usually a greater incidence in males, and poults are often smaller than normal birds (Hunsaker, 1971). Round heart can be precipitated by a number of pharmacological compounds, the most notorious product being furazolidone.

2.2.   *Aortic rupture*

In older turkeys, aortic rupture causes sudden death in apparently healthy birds. As the name implies, there is a major rupture of the aorta associated with massive hemorrhage in the body cavity. The condition is associated with changes in elastin properties of the arterial wall, and these changes mimic those induced by certain nutrient deficiencies as subsequently detailed. Mortality can occur at any age, but it is most common in tom turkeys after 12 wk of age, and so causes major economic loss.

3.   <u>PATHOLOGY AND METABOLIC CHANGES</u>

3.1.   *Round heart disease*

Maywood and Fray (1962) were one of the first to describe round heart disease in turkey poults, although there are earlier references to a comparable condition in chicks. Affected birds show dilation of usually the right ventricle alone, although in some instances both ventricles may be enlarged (Rattner, 1976).   Hunsaker (1971) suggests that in some situations, dilation of the ventricles is followed by regression and complete recovery, or alternatively by heart failure. Microscopic lesions consistently show myocardial congestion and degeneration, hemorrhage and epicardial fibrosis (Czarnecki and Jankus, 1974), the latter being most prominent in the fat of the coronary groove (Sautter *et al.,* 1968). Sautter *et al.* (1968) indicate that round heart occurs in older turkeys and is often not associated with the thinning of the heart wall, and that the left ventricle, rather than the right, is most often affected.

Rattner (1976) and Sautter *et al.* (1968) indicate that round heart is often associated with an enlarged liver and sometimes ascites.   Rattner (1976) suggests the liver may contain intracytoplasmic globules that react positively against sera α-globulins. The sera of affected birds shows a marked reduction in the total protein and a characteristic deficiency of α-globulins. The presence of such liver globules is the basis of comparison to hereditary α_1-antitrypsin deficiency in man. Rattner (1976) did show that birds dying with round heart in the first 10 wk of life have a 50% reduction in trypsin inhibition capacity. Meirom *et al.* (1974) also showed that poults with round heart exhibited reduced serum protein while electrophoretic analysis revealed a characteristic α-globulin deficiency. Meirom *et al.* (1974) indicate that humans with inherited deficiency of serum α_1-

antitrypsin are predisposed to emphysema and that this is controlled by genes that produce variations of the normal antitrypsin molecule. Jankus *et al.* (1971) studied the heart rate and cardiac electrical activity of poults, indicating rate to peak at 8 d of age followed by subsequent decline. Using ECG analysis to diagnose round heart in poults, these workers showed different patterns in normal birds and those that were later diagnosed with or died from round heart.

3.2.   *Aortic rupture*

Aortic rupture most commonly occurs in fast-growing male turkeys after 12 wk of age. Affected birds appear normal immediately prior to death, the onset of which is very sudden. At necropsy, the musculature is often pale in colour, presumably due to loss of blood, while the body cavity contains numerous large blood clots. Close to the heart, the aorta is broken longitudinally, and in this region it will be thin-walled and often dilated. The major histological change in the aorta is reduction in elastin content as described more fully in *(Section. 4.2.1.)*.

4.   RELATED FACTORS

4.1.   *Round heart*

4.1.1. Dietary Salt

Morrison *et al.* (1975) give evidence of myocardial distension of poults fed high levels of salt, while Mohanty and West (1969) observed cardiac dilation in chicks receiving up to 1.5% salt in the drinking water. Dewar and Siller (1971) cite conflicting reports of the toxic effects of sodium for poults, and in their own work indicated that 0.57% Na caused extensive 0-18d mortality associated with marked cardiac enlargement and ascites being evident post-mortem. Morrison *et al.* (1975) also suggest that high levels of salt cause ascites and edema in poults. In a more extensive study, Leeson *et al.* (1976) failed to show any round heart in poults fed up to 5% dietary salt, or up to 1.5% dietary sodium. Field reports do not confirm any obvious relationship between dietary salt and incidence of round heart.

4.1.2. Dietary minerals

Jensen *et al.* (1974) indicate that in other species, a deficiency of selenium can cause an abnormal development of cardiac muscle. This concept was tested in studies involving Se deficiency induced by feeding high levels of silver. Feeding 900 ppm silver caused, among other things, growth depression and enlargement of the heart. A high percentage of birds exhibited a round shaped heart comparable to that seen in round heart. Feeding diets containing 10% distillers dried solubles or either copper or selenium reversed these effects.   While Jensen *et al.* (1974) originally proposed a role for selenium in abnormal heart development, these studies indicate copper metabolism to be a more likely candidate.

4.1.3. Furazolidone

Jankus *et al.* (1972) induced cardiac dilation, ascites and high mortality in poults fed 300 ppm furazolidone. While 700 ppm caused all poults to die, 500 ppm resulted in the most cases of cardiac dilation, and 100 ppm caused low incidence. These authors suggest these threshold levels for furazolidone to be comparable to that recorded for chicks. Czarnecki and Jankus (1974) subsequently showed that furazolidone resulted in a 2-3 times increase in the glycogen content of the heart, although they pointed out the important scenario that such change is not seen in birds exhibiting spontaneous round heart. By feeding 700 ppm furazolidone, Czarnecki *et al.* (1974) show almost 100% incidence of round heart with a significant increase in cardiac glycogen levels. These authors suggest round heart may be a manifestation of so-called glycogen storage disease, although with serum glucose levels being virtually unaffected there is little scope for diagnosis in live birds. Czarnecki *et al.* (1975) cite evidence for the 2-4 wk age period, which is when most round heart occurs, to be the time when there is the greatest general flux of enzyme systems, and that it is not too surprising that exogenous toxins may interfere with glycogen metabolism at this time. Czarnecki (1991) showed that administration of exogenous T4, while increasing glycogen in control birds, had no effect on glycogen reserves in furazolidone treated birds. Furazolidone is now known to be a potent inhibitor of monoamine oxidase, an enzyme linked to aortic rupture (*Section 4.2.1*). Powers *et al.* (1983) studied the role of monoamine oxidase inhibition in furazolidone-induced round heart in young poults. Furazolidone did reduce monoamine oxidase levels in only the heart, and not in liver or kidney, and many of these poults exhibited normal heart development. However, other, more potent inhibitors, depressed the heart monoamine oxidase levels to even lower levels, yet had no effect on the cardiomyopathy. From these results Powers *et al.* (1983) conclude that furazolidone-induced round-heart is not due to monoamine oxidase inhibition. O'Brien *et al*. (1993) showed that hypoproteinemia caused by furazolidone could be largely attributed to expansion of blood volume, and to a lesser extent, reduced feed intake.

4.1.4. Oxygen demand

Julian *et al*. (1992) recently showed spontaneous cardiomyopathy in turkeys subjected to artificially low oxygen pressure, as normally occurs at high altitude. Poults grown at the equivalent of 2050 m elevation with 16.3%O_2 in the environment, and fed a high nutrient dense diet, quickly developed a mainly right ventricular dilatory cardiomyopathy resembling round-heart disease. Interestingly, poults grown on a low nutrient dense diet exhibited a much lower incidence of heart problems.

4.2   Aortic rupture

4.2.1. Copper status

Although aortic rupture only occurs commonly in turkeys, the condition can be induced in chickens and other species by feeding copper deficient diets. Carlton and Henderson (1963) recorded over 50% mortality due to an aortic rupture in 4-6 wk old chicks fed copper deficient diets. Ruptures were most common in the aorta, although there were also lesions in the carotid and

abdominal arteries. In addition to aneurysm, there was disruption, fragmentation and loss of elastic fibres in the artery walls. Carlton and Henderson (1963) indicated that many of these copper-deficient chicks died suddenly, often while eating, while the hemorrhages were most often localized to the aorta. The elastin fibres of the arteries appeared to be decreased both in number and size. Starcher *et al.* (1964) studied the elastin development in normal and copper deficient birds. Aorta elastin levels in newly hatched chicks are about 5% by weight, although this increases to 12% by 17 d when birds receive adequate copper. With copper deficiency, elastin content increases more slowly and never equals that of control birds. Adding copper to 27 d deficient birds resulted in normal elastin by 43 d. In other studies, Starcher *et al.* (1964) indicate that once formed, elastin is a very inert material, and so copper affects synthesis rather than maintenance of elastin in the blood vessels.

It is likely that the biochemical lesion in the copper-deficient aorta relates to failure to synthesize desmosine, the cross-link precursor of elastin, since desmosine biosynthesis requires a copper-dependent monoamine oxidase enzyme (Graham, 1977). Starcher *et al.* (1964) also indicated that the lysine content of copper-deficient elastin was 3 times that seen in control birds, suggesting failure to incorporate lysine into the desmosine molecule. Graham (1977) also induced lesions in the connective tissue of the media of the aorta through inducing copper deficiency in poults. In field cases of naturally occurring aortic rupture, over 50% of the birds had <10 ppm Cu in the liver, compared to 15-30 ppm normally seen in birds of comparable age. Graham (1977) suggests that high levels of sulfate, molybdenum and ascorbic acid can reduce liver copper levels. Guenthner *et al.* (1978) found that 120 ppm Cu as the oxide or sulfate stimulated growth rate but reduced the incidence of aortic rupture. While these workers were unable to show any consistent effect of copper on aorta elastin content, they cite evidence of elastin content being more than double when diets contain 25 vs 0 ppm Cu. Guenthner *et al.* (1978) also showed that birds fed low rather than high protein diets had more elastin in their aorta, although such birds were obviously slower growing. A higher incidence of aortic rupture was also seen in turkeys fed 4-nitrophenylarsonic acid, although the problem could be resolved by feeding higher levels of copper (Guenthner *et al.,* 1978). This latter study suggests that products such as 4-nitro may complex with copper.

4.2.2. Lathyrism

Lathyrism, most commonly induced by administration of β-aminopropionitrile (BAPN) causes a number of pathological lesions including aortic rupture. Savage *et al.* (1966) compared the effects of feeding diets devoid of copper or diets containing BAPN. BAPN treated poults developed typical gross lesions of lathyrism, including leg weakness, subcutaneous hemorrhage and aortic rupture. Histopathology of copper-deficient birds was almost identical to that seen with BAPN, although no aortic rupture occurred. The aortas of birds from both treatments contained less elastin and more soluble collagen. All attempts at alleviating BAPN toxicity through diet copper, were without effect. Savage *et al.* (1966) show that BAPN can chelate with copper, but that the ligand is weak, and so the effect of BAPN is more likely a direct influence on crosslinking of desmosine and isodesmosine. While aortic rupture is a consequence of lathyrism in turkeys, Krista *et al.* (1964) suggest there to be many morphological and etiological differences between spontaneous and BAPN-induced aortic rupture.

4.2.3. Hormone balance

Because aortic rupture occurs most commonly in male turkeys, there has been speculation about the role of hormones. Certainly the incidence of aortic rupture can be markedly increased by the administration of diethylstilbestrol (DES) to male turkeys (Krista *et al.,* 1969), while Krista *et al.* (1964) question the role of testosterone since the highest incidence occurs in males approaching sexual maturity. However administration of exogenous testosterone reduced incidence of aortic rupture, while giving DES markedly increased the incidence. Interestingly, testosterone had no effect on blood pressure, while DES actually resulted in reduced blood pressure. The fact that males have higher blood pressure than do females (Krista *et al.,* 1964) is, therefore, likely coincidental.

4.2.4. Season

Harms *et al.* (1969) reviewed numerous factors that affected aortic rupture, citing among other things, DES, nutrient density and diet sodium. In reviewing over 20 experiments involving birds with a high incidence of aortic rupture due to DES administration, these workers show a clear relationship with time of year. There was an excellent correlation between incidence of DES-induced aortic rupture and environmental temperature and/or hours of natural daylight. In the colder winter months there was up to 60% mortality, while in the summer the incidence of aortic rupture was only around 13%. Harms *et al.* (1969) cite evidence for there being lower blood pressure in the summer, although work with testosterone and DES as cited previously (*Section 4.2.3*) shows little relationship with blood pressure. A more plausible effect is that of growth rate, since turkeys will likely have exhibited greater growth rate in the cooler winter period.

4.2.5. Tranquilizers/Hypotensive agents

A number of pharmacological agents have been used with some success in preventing or reducing the severity of aortic rupture. Reserpine has been studied and used most frequently, and there are a number of reports of beneficial effects. Waibel *et al.* (1962) showed reserpine at 1 ppm of the diet to significantly delay the onset of aortic rupture, although total mortality was little affected. Krista *et al.* (1963) tested a number of tranquilizers and hypotensive drugs. Four different drugs were found to delay the onset of aortic rupture, and these authors concluded that the efficacy of these agents was independent of their pharmacological classification. Speckmann and Ringer (1961) studied the hemodynamic pattern of turkeys following reserpine administration. All levels of reserpine used, even as low as 0.1 ppm, significantly lowered both systolic and diastolic blood pressure as well as reducing heart rate. Speckmann and Ringer (1961) speculate that the major influence is to reduce, or delay the natural increase over time, of systolic blood pressure, and that this relates to altered cardiac output. Simpson *et al.* (1968) investigated the potential of propranolol, a β-adrenergic blocker, to reduce severity of BAPN-induced aortic rupture. While 47% of control birds died, no mortality was seen with 0.1% propranolol treatment, while 0.01% inclusion level resulted in 40% mortality. The reduction in mortality was associated with reduced heart rate and also reduction in blood pressure as described previously. Also the tensile strength of the aorta was intermediate when turkeys were fed BAPN + propranolol vs BAPN alone.

5.   POTENTIAL TREATMENT AND PREVENTION

5.1.   Round heart disease

Round heart continues to be an industry problem, and there seem to be few options available for treatment. Certainly there seem to be more heart-related problems in poults fed furazolidone, and so this product should come under close scrutiny prior to inclusion in turkey starter diets. Although there are few scientific reports detailing the adverse effect of high salt/sodium levels in diet and drinking water, industry observations suggest more problems when salt intake is elevated. There is likely a genetic component, although it is obvious that this condition is seen in all commercial strains available at this time.

5.2.   Aortic rupture

Aortic rupture can easily be produced by inducing copper deficiency, either directly by feeding copper deficient diets, or by inclusion of copper antagonists or chelating agents. High levels of dietary copper will not prevent occurrence entirely, although care should be taken during formulation to ensure adequate levels (at least 10 ppm) of readily available copper. Since sulfates have been shown to reduce liver copper levels, then the usual industry practice of adding copper as the sulfate form, perhaps warrants careful consideration. Guenthner *et al.* (1978) correctly point out that repeated application of manure from turkeys fed high levels of dietary copper can result in serious toxicity to plants and/or animals consuming such plants, especially sheep. At 120 ppm Cu in the diet (300 g $CuSO_4$/tonne) using manure at 8 tonne/acre, the ground will become contaminated within 10 years. Tranquilizers and anti-hypertensive drugs definitely delay the onset of aortic rupture although their use is greatly limited by various regulatory agencies. From a practical point of view the best prevention is stress reduction, and as such, 12-16 wk-old toms should not be subjected to sudden noise, temperature changes, etc.

Chapter 2

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CHAPTER 3. ASCITES

<u>Other names:</u>	RIGHT VENTRICULAR HYPERTROPHY
	PULMONARY HYPERTENSION
	WATER BELLY
<u>Species:</u>	BROILER CHICKENS
	TURKEYS, DUCKS

1.   <u>COMPENDIUM</u>

Ascites is characterized by accumulation of fluid in the abdomen which is caused by a cascade of events related to the need to supply high levels of oxygen to the tissues. The condition is most prevalent in fast growing male broilers maintained at high altitude and where there is a degree of cold stress. In extreme situations, up to 25% mortality is seen, although 5-12% mortality is more common.

The broiler chicken has a high demand for oxygen necessary to fuel metabolic processes. When such demand is increased by very fast growth rate, or cold conditions, then the lungs must oxygenate increased quantities of blood. At high altitude the situation is made worse by low oxygen tension in inhaled air. In order to meet the demands for metabolism, the bird attempts to pump more blood through the lungs and so this places extra stress on the right ventricle of the heart. Under normal conditions, the right ventricle is relatively small, but in the situation of ascites this ventricle becomes grossly dilated and its size doubles. This weakened ventricle creates back-pressure to the various supply systems, a consequence of which is leakage of plasma from the liver, commonly referred to as ascitic fluid (water belly). Ascites is effectively caused by hypoxia. Upon necropsy the bird is identified by the presence of fluid in the abdomen, marked ventricular hypertrophy, atrial hypertrophy, pulmonary congestion and edema. The lungs may be damaged by environmental contaminants or respiratory infection, and these may precipitate the overall condition. Birds exhibit elevated PCV, RBC and WBC counts. Pulmonary arterial pressure may be doubled. Birds dying of ascites are invariably smaller than non-affected birds, although the condition is undoubtedly precipitated initially by superior growth characteristics.

There is discussion that ascites is problematic because lung volume in commercial broilers is now much less than that seen in wild species, relative to body weight and metabolic needs. Ascites can be prevented by reducing growth rate, and so reducing oxygen demand, although this is not always economical. Under commercial conditions mortality due to ascites can be minimised by providing quality air flow across the birds and preventing night-time cold stress. Where ascites mortality is problematic, then low-energy mash diets should be considered, and under extreme situations broilers can be subjected to a skip-a-day or a limited-access feeding schedule from 7-21 d of age.

2.   OCCURRENCE AND GENERAL SIGNS

Ascites is characterized by the accumulation of fluid in the abdomen, and hence the basis for the common phrase of "water-belly". Fluid in the abdomen is in fact plasma that has seeped from the liver, and this occurs as the end result of a cascade of events ultimately triggered by oxygen inadequacy within the bird. For whatever reason, the need to provide more oxygen to the tissues leads to increased heart stroke-volume, and ultimately to hypertrophy of the right ventricle. Such heart hypertrophy, coupled with malfunction of the heart valve, leads to increased pressure in the venous supply, and so pressure build-up in the liver, and often the characteristic fluid leakage.

Because of the relationship with oxygen demand, ascites is affected and/or precipitated by such factors as growth rate, altitude (hypoxia) and environmental adequacy. Of these factors, hypoxia was the initial trigger, since the condition was first seen as a major problem in birds held at high altitude, where mortality in male broilers of 20-30% was not uncommon. Since this time, ascites now occurs as a problem at reduced incidence in broilers maintained at all altitudes. Today ascites is commonly seen in fast-growing lines of male broilers fed high-nutrient dense diets and where the environment is cool/cold at least for part of each day. Mortality seen with ascites is dictated by the number of "stressors" involved and hence the efficacy of the cardio-pulmonary system to oxygenate tissues.

Ascites is most commonly seen in broiler chickens, although there are reports with ducks (Julian, 1987), guinea fowl (Cowen *et al.,* 1988) and turkey poults (Julian *et al.*, 1993). It is likely that ascites will be a problem in any fast-growing bird with high oxygen demand. Numerous factors can cause liver damage and plasma seepage, for example the ingestion of certain disinfectants. Because classical ascites is associated with hypertrophy of the right ventricle, then an imbalance in the right-left ventricle weight, together with evidence of abdominal fluid accumulation, is necessary for adequate diagnosis. Depending upon environmental conditions, ascites is most commonly diagnosed at 4-5 wks of age, although conditions have been recorded in day-old birds (Muirhead 1987) and there are even implications of the oxygen status of embryos during incubation (Maxwell *et al.,* 1987).

Ascitic birds are often seen panting even though there is no apparent heat stress. Older birds may show cyanosis, especially around the comb and wattles and death is seen to occur spontaneously, especially when birds are excited. The panting is likely the result of abdominal fluid accumulation causing physical restriction of the large abdominal air sacs, and so reduced tidal volume per respiration. Opening of the abdominal cavity reveals amber or clear fluid that resembles plasma. Wideman (1988) suggests the presence of clotted plasma proteins on the surface of the liver indicates this to be the origin of the fluid. For whatever reason, plasma that is normally held within the low-pressure liver venous system is unable to return to the heart in sufficient volume. Classically the right ventricle is grossly dilated, and can reach 40% of total ventricle weight, versus 20% as occurs more normally. Varying degrees of lung damage are seen, most often the lungs appearing pale or grey.

While the events leading up to the onset of clinical signs of ascites have been fairly well established, the exact reasons for cardiopulmonary insufficiency have not been adequately quantified. Because a number of major factors can influence the degree of oxygen demand by the bird, then treatment and/or prevention is not straightforward. The most obvious solution is to reduce growth rate, thereby reducing the major oxygen demand by the tissues, although this has obvious

adverse economic implications, and hopefully will be considered as a short-term solution to this major industry problem.

3.   PATHOLOGY AND METABOLIC CHANGES

Discussion of ascites invariably focuses around the roles of the cardio and pulmonary systems, and obviously their interrelationship. In this section, pathobiology and metabolic changes in the heart and lung are described under separate headings, although this is done merely for convenience and hopefully clarity, and does not imply their independence from one another.

3.1.   General considerations

Wilson *et al.* (1988) in studying a field outbreak of ascites showed afflicted birds to have marked right ventricular dilation and hypertrophy, atrial hypertrophy, pulmonary congestion and edema, hepatic capsular fibrosis and accumulation of ascitic fluid in the abdomen. Microscopic changes in heart tissue were not severe enough to indicate heart failure *per se*. The authors found the lungs exhibited hypertrophy of smooth muscle in the parabronchi and this caused the collapse and apparent loss of associated air capillaries. Wilson *et al.* (1988) showed ascitic birds to have a RV÷TVx100 (right ventricle ÷ total ventricle weights) to be around 50, versus 20 for normal birds. In addition to hypertrophy, the RV also showed dilation because volume was 0.6 ml vs 0.1 ml in control birds.   Maxwell *et al.* (1986a) quantitates in detail the effect of ascites on various physiological parameters *(Table 3.1)*.

Ascitic birds were smaller, and there was an increase in both PCV and hemoglobin status. Maxwell *et al.* (1986a) suggest that the increase in WBC count of ascitic birds may be a consequence of stress, since there was an increase in the heterophil count at the expense of lymphocyte numbers. In a series of studies, Maxwell and co-workers detail the ultrastructure changes seen in various organs of ascitic birds. Hernandez (1987) also quantitated changes seen in birds examined at commercial farms maintained at 2600 m above sea level *(Table 3.2)*. Hernandez (1987) concludes that the severity of ascites is greatly influenced by nutritional program and environmental temperature *(See Section 4)*.

TABLE 3.1 Comparison of 35 day-old ascitic and normal broiler chickens			
	Normal	**Ascitic**	
Body weight (g)	1390	960	**
Heart weight (g)	9.5	9.3	NS
Heart weight (% Bwt)	0.7	1.0	*
Hemoglobin (g/100ml)	9.3	11.6	**
PCV (%)	30	38	**
RBC (10^6/mm^3)	2.6	3.2	**
WBC (10^6/mm^3)	16.1	20.5	*
Heterophils (% WBC)	17.8	32.3	**
Lymphocytes (%WBC)	71.0	52.6	**
			Adapted from Maxwell *et al.* (1986a)

TABLE 3.2 Cardio-pulmonary changes in ascitic birds held at 2600 m altitude					
	Hemoglobin (g/100 ml)	**Hematocrit (%)**	**RV/TV %**	**Pulmonary arterial pressure (mm)**	**Pulmonary arterial thickness (% diam)**
Control	10.8	26	15	21	6.6
Ascites	12.1	44	41	44	10.1
					Adapted from Hernandez (1987)

Maxwell *et al.* (1986b) suggest that the ultrastructure changes seen in the myocardium resemble observations reported in cases of spontaneous cardiomyopathy in turkeys and ducks and hypoxic rabbits. Cardiac myofibril degeneration was the principal change with disruption and subsequent disorganization of sarcomeres and mitochondria. Thickening and damage to capillary walls paralleled that seen in birds at high altitude and this damage resulted in further capillary fragility causing hemorrhage. Maxwell *et al.* (1986b) also described changes in kidney structure and intimated that such dysfunction may be a factor in control of the hormone erythropoietin, output of which may need to be increased during hypoxia in order to stimulate RBC production. However hypoxic and associated changes in heart muscle metabolism are likely still the major factors of concern (Maxwell *et al*., 1993).

Owen *et al.* (1990) recorded similar general findings in ascitic birds held in hypobaric chambers used to simulate oxygen tension at high altitude. While no mortality occurred with birds maintained at an oxygen tension equivalent to 366 m altitude, 24% of the birds died at the equivalent of 3000 m and 47% birds died at 5000 m. Birds maintained at high altitude showed increase PCV and increase in heart and lung weight as a proportion of body weight. Ascitic birds exhibited cardiomyofiber hypertrophy characterized by thickened myocardial fibers that contained moderately enlarged nuclei, while the liver showed cellular necrosis and fibrosis. Owen *et al.* (1990) also observed an increase in hyaline, fibrous and osseous nodules in the lung, the size of which increased with increasing altitude *(See following section)*. These workers attempted to find a predictor of ascites by means of EKG analysis. Mean EKG amplitude of birds subsequently dying of ascites was greater than that of survivors.

Maxwell and Mbugua (1990) also studied the effect of altitude on various tissues. Histopathology seen in heart, lung and kidney of 7 d old birds resembled that of 4 wk old birds with ascites. Almost 50% of the hearts examined had myofibril disorganization and mitochondrial hyperplasia, while affected lungs showed capillary congestion and engorged parabronchi. Maxwell and Mbugua (1990) indicated that 4 d old birds can contain up to 5 ml of abdominal fluid, and cite evidence for such early development of ascites to perhaps be related to hypoxic conditions imposed during late incubation. In yet another study, Maxwell *et al.* (1989) describe myofibril disorientation and degeneration with extensive mitochondrial hyperplasia in the heart of ascitic birds. There was also an increase in myocardial glycogen reserves together with an increase in lipid droplet number. In the lung, the parabronchi were greatly dilated with areas of sub-epithelial fibrosis and the presence of more surfactant material than normal. Ascites is caused by an increase in pulmonary arterial pressure for whatever reason. Julian and Wilson (1986) describe the major factors that can influence pulmonary blood pressure *(Table 3.3)*.

TABLE 3.3 Factors influencing pulmonary arterial pressure	
Condition	**Related Factors**

1. Increased pulmonary blood flow or cardiac output	a. Metabolic rate (growth rate)
	b. Hypoxia - altitude
	             - ammonia, dust
	             - carbon monoxide

2. Organic vascular obstruction	a. Blockage of capillaries
	b. Polycythemia - young birds
	                 - diet sodium
	                 - erythrocyte
	                    flexibility

3. Pulmonary vasoconstriction	a. High altitude
4. Increased pulmonary venous pressure	a. Rarely seen in birds
	Adapted from Julian and Wilson (1986)

Owen *et al.* (1992) describe in some detail the mechanical and physical factors that can influence conditions 1-3 *(Table 3.3)* which are primarily responsible for development of ascites. Owen *et al.* (1992) suggest that the work required by the heart to move blood is described by the equation: W=PV, where P is mean arterial pressure and V the volume of blood moved. Short-term, the heart can compensate for the increased work load by ventricular hypertrophy. However with extensive hypertrophy, there is concomitant loss in the effectiveness of the A-V heart valve, which is merely a muscular flap unlike the complex structure seen in mammals (Julian *et al.,* 1987) and so contraction of the heart causes not only increased pulmonary arterial pressure, but also back pressure to the venous system and especially the liver. Within a specific blood vessel Owen *et al.* (1992) suggest that pressure is proportional to cardiac output and resistance to flow. Cardiac output itself is a product of heart rate and stroke volume, while resistance within a vessel is proportional to blood viscosity and inversely proportional to vessel diameter. Pulmonary hypertension is, therefore, most likely to occur due to either an increase in cardiac output, an increase in blood viscosity (polycythemia) or a decrease in vessel diameter (vascular constriction). Maintaining birds at high altitude influences most of these conditions, because the bird will exhibit increased heart rate, polycythemia and pulmonary vasoconstriction. However as already stated, ascites is now no longer confined to locations at high altitudes, and so we must look elsewhere for other factors inducing hypoxia (relative to tissue needs). The following subsections detail effects of ascites on the cardiopulmonary systems.

3.2.   *Heart and vascular system*

Julian *et al.* (1986) suggest that the right ventricle (RV) of birds has developed as a low-pressure volume pump, because it rarely has to respond to pressure changes. However the RV can respond quickly to increased workload. The RV wall is normally thin and the usual contribution to total ventricular size is usually 1:4, RV:LV. Julian *et al.* (1986) indicate that the A-V valve in birds is formed mainly as a continuation of the muscle of the RV, and when the later hypertrophies for whatever reason, so does the valve. Such thickening of the valve may interfere with its effectiveness leading to valvular insufficiency and this contributes to ascites.

Huchzermeyer and De Ruyck (1986) found ascites in South Africa to occur mainly at higher altitude and at cooler times of the year. These workers measured arterial pressure in normal birds at sea level, at an altitude of 1600 m, and in ascitic birds. Pulmonary arterial pressure was significantly different for each group, with the birds at altitude exhibiting elevated pressure relative to control birds at sea level. The ascitic birds exhibited twice the blood pressure of normal birds at high altitude. Huchzermeyer and De Ruyck (1986) in describing these effects compare ascites in chickens to mountain sickness in humans which is accentuated by cold conditions, and also to the apparently additive effects of hypoxia and cold on pulmonary hypertension in sheep. Burton *et al.* (1968) also measured blood pressure in Leghorn birds maintained at sea level or at 3500 m, *(Table 3.4)*. These data show the dramatic effect of high altitude on pulmonary arterial pressure, and perhaps more importantly how this is most obvious in males. Maxwell *et al.* (1990) studied experimentally induced hypoxia in young birds, showing treatment to increase PCV, RBC count and hemoglobin concentration. Ascitic birds exhibited myofibril and cellular degeneration of heart tissue with infiltration of lipid, while congested lungs contained hemorrhages. The hearts of both ascitic and hypoxic-normal birds were found to contain increased concentrations of lactate dehydrogenase intimating reduced oxygen availability.

TABLE 3.4 Effect of altitude on pulmonary arterial pressure in Leghorns		
	Altitude	
	Sea Level	3500m
Pulmonary arterial pressure (mm Hg) _ _	15.7 10.3	34.2 26.2
RV÷TVx100 _ _	22.7 19.0	32.1 26.2
		Adapted from Burton *et al.* (1968)

Maxwell *et al.* (1987) indicated that such changes in blood parameters could be induced in day-old chicks merely by imposing hypoxic conditions on the developing embryo. Without other stressors being applied, these differences in PCV, RBC count etc. had stabilized by 3 wks of age, and normalized after 5 wks.

Julian and Wilson (1986) hypothesized that increased pulmonary arterial pressure was due to polyalkemia, megalocytosis and/or RBC malleability. The main response to hypoxia is an increase in RBC production, leading to a more viscous blood and so increased resistance to flow (Owen *et al.,* 1992). Megalocytosis, or increase in blood cell size may be a factor with young fast growing animals, because blood cell size is known to decrease with age. Finally Julian (1990) suggests malleability of RBC's to be a potential factor in arterial pressure, because these cells must fold or deform in order to pass through lung capillaries. Factors that impede deformity of the RBC, such as elevated sodium will, therefore, contribute resistance to blood flow.

3.3.   Lung

With insufficient oxygen getting to the bird's tissues, there is an obvious concern regarding the adequacy of the lung structure. It is generally recognized that lung irritants, such as dust and ammonia can accentuate problems with ascites, presumably through interfering with alveolar structure. Julian *et al.* (1989) induced ascites in birds by dosing them with amiodarone, an anti-arrhythmic drug used in human medicine that has the unfortunate side effect of causing alveolar septal thickening and intraalveolor accumulation of macrophages, all of which interfere with oxygen transfer. The drug caused marked phospholipidosis in the lung atria, and consequently a dose-related effect on incidence of ascites.

Julian (1989) suggests that inadequate lung volume, relative to body weight, is a contributing factor to ascites. In this context Vidyadaran *et al.* (1990) indicated that lung volume per unit body weight is 20-30% less in modern broilers compared to non-selected jungle fowl. In

addition to reduced volume, there was also a 30% increase in the thickness of the blood-gas tissue barrier in broilers, leading to a 25% lower anatomical diffusing capacity of O_2. Vidyadaran *et al.* (1990) conclude that these anatomical differences make the modern broiler susceptible to stress factors such as cold, altitude and air pollution that predisposes hypoxia and ultimately ascites. The same authors suggest that the modern fowl has been bred to a point closely approaching pulmonary inadequacy and that the practical solution lies in breeding birds with pulmonary characteristics seen in the more primitive *Gallus* species.

In addition to there perhaps being reduced lung volume, Julian (1989) suggests that development of the lung does not match the bird's overall development during a seven-wk growth period. Julian (1989) measured lung volume using a water displacement technique, and found that lung volume per unit body weight declined from day 1 through day 53. However these calculations may be misleading, because it seems more relevant to relate lung size to metabolic body size, rather than absolute body size. Recalculation of this data reveals a different interpretation *(Table 3.5)*.

TABLE 3.5 Lung volume in relation to broiler growth					
Age (days)	Body wt (g)	Body wt$^{.75}$ (g)	Lung vol (ml)	Volume/100g Bwt (ml)	Volume/100g Bwt$^{.75}$ (ml)
1	40	16	0.82	2.0	5.2
7	140	40	2.72	2.0	6.7
14	350	81	6.57	1.9	8.1
24	800	150	13.85	1.7	9.3
38	1600	250	27.27	1.7	10.7
53	2625	370	42.04	1.6	11.5
					Adapted from Julian (1989)

Metabolic body size is more likely a reflection of the energy needs of birds at different ages, and on this basis, lung volume/100 g Bwt$^{.75}$ is seen to increase with age. However as concluded by Julian (1989) and as previously described by Vidyadaran *et al.* (1990) it is likely that the O_2 diffusion area in the lung of the broiler becomes compromised and/or is inadequate for all metabolic needs. Julian (1993) concludes that future improvements in growth rate will only be possible if the lung and abdominal cavity capacities of the bird are enlarged.

There is some concern over the development of cartilaginous and osseous nodules in the lungs of birds, and their obvious disruption of lung function. These nodules are found in the lungs of most birds, although more cartilaginous nodules are seen in the lungs of ascitic birds (Maxwell *et al.,* 1988). These workers have shown that excessive collagen synthesis is a feature sometimes seen in ascitic birds, and that a similar situation arises during hypoxia in mice. Maxwell (1988) found 88% of ascitic birds to carry these lung nodules while there was a 63% incidence in control birds. However the major difference in ascitic birds was nodule number, since ascitic birds were seen to have an average of around 30 nodules per lung compared to just 3.5 per lung in normal

birds. Maxwell (1988) found that the abnormal accumulation of fibrous tissue in these nodules destroyed normal lung architecture and together with the presence of inflammatory cells and the evidence of thickening of capillary walls, suggest that pulmonary fibrosis is a contributor to ascites. That lung nodules are not commonly seen in ascitic birds at high altitude suggests that dust or other environmental factors may be predisposing factors. In order to test this assumption, Maxwell *et al.* (1989) maintained birds under conditions of minimal air movement where CO_2 levels increased from 0.036% to 0.142%. Between 7-28 d, there was progressive increase in the number of dilated and/or congested parabronchi in birds maintained in the poorly ventilated room. This was accompanied by thickening of smooth muscle and epithelial hyperplasia. Maxwell *et al.* (1989) found almost twice as many lung nodules in poorly ventilated birds, and interestingly most of these appeared in the left lung. The majority of nodules were fibrous although there were also more osseous nodules. The most dramatic increase in nodule number occurred over the 14-21 d period. It is interesting to note that Maxwell and co-workers observed no nodules in any species of wild bird studied. It seems likely that lung damage, as indicated by nodule formation, is aggravated by modern confinement conditions, and this can be a contributing factor to ascites.

4.   RELATED FACTORS

4.1.   *Growth rate and body composition*

There seems little doubt that growth rate is the major factor contributing to oxygen demand, and consequently the potential to induce ascites. Julian *et al.* (1986) indicate birds with defective hearts to be 3-4% larger than normal birds *(Table 3.6)*.

Owen *et al.* (1992) show even more dramatic differences in body weight of normal and ascitic birds, although in this situation ascitic birds are smaller. This apparent dichotomy presumably relates to the fact that fast growth initially precipitates the problem with heart physiology, and that subsequent severe ascites causes slow-down in growth and eventually death.

As described previously in relation to lung volume per unit body weight, metabolic body size rather than actual body size is likely more relevant in discussion of metabolic disorders. The change in metabolic body size is more consistent, and most important the rate of change is greater at early ages. This very rapid rate of increase in metabolic body size probably accounts for earlier appearance of ascites (and other metabolic problems) than can be accounted for by consideration of time of maximum growth rate seen in conventional body weight graphs.

Body composition is another factor that will influence oxygen demand, and so the propensity to ascites. While growth rate *per se* is the major factor contributing to oxygen demand, the composition of growth is also influential, because oxygen need varies for metabolism of fats vs proteins. Oxygen need for nitrogen and protein metabolism is high in relation to that for fat, although it must be remembered that the chicken carcass actually contains little protein or nitrogen. The carcass does contain a great deal of muscle, but 80% of this is water. On the other hand, adipose tissue contains about 90% fat, and so its contribution to oxygen demand is proportionally quite high. Excess fatness in birds will therefore lead to significantly increased oxygen needs for metabolism.

TABLE 3.6. Body weight of normal birds and those with RV/TV imbalance				
	Body weight (g)		Difference	
	Defective heart	Normal heart	g	%
14d	360	350	+10	3.0
28d	1130	1100	+30	2.8
47d	2660	2540	+120	4.4
				Adapted from Julian *et al.* (1986)

4.2.   Altitude

Because oxygen tension decreases with an increase in altitude, then the cardiopulmonary system is under greater stress relative to the reduced oxygen content at the parabronchi. The data of Witzel *et al.* (1990) clearly demonstrates this relationship in terms of incidence of ascites, body weight and related cardiopulmonary criteria *(Table 3.7)*.

TABLE 3.7 Altitude and broiler metabolism						
Altitude (m)	Body wt (g)	Ascites (%)	RV/TV x 100	RBC (10^6/ml)	Hematocrit (%)	Hemoglobin (g/100ml)
100	1760[a]	0	20.4[b]	2.1[b]	29[b]	8.3[c]
1980	1535[b]	13	30.1[a]	2.7[a]	37[a]	9.9[b]
2440	1510[b]	27	34.4[a]	2.5[a]	36[a]	9.9[b]
2900	1230[c]	80	35.5[a]	2.5[a]	36[a]	11.7[a]
						Adapted from Witzel *et al*. (1990)

With an increase in altitude, there is an obvious increase in the incidence of ascites, although such change does not always correlate with changes in cardiac and other blood parameters. There is an increase in the size of the right ventricle, RBC count and hematocrit at all altitudes above 100 m, yet there is no significant increase at higher altitudes corresponding with the

greater incidence of ascites.   The actual incidence of ascites seems best correlated with hemoglobin concentration *(Table 3.7)*.

4.3.   Environment

Environmental temperature will influence oxygen demand, while environmental contaminants may adversely affect lung structure. Al-Mashhadani and Beck (1985) indicate that gaseous NH_4 levels as low as 25 ppm are detrimental to lung structure in young broilers, and although structural damage to the upper respiratory epithelium was slight, there was damage to the lung.   Such damage was evident as thickening of the atrial wall due to cell infiltration and development of scar tissue. In most commercial poultry houses ammonia levels increase as birds age, and this may be a factor in the age-related occurrence of ascites. Obviously with multiple re-use of litter, an increase in ammonia level occurs more quickly.

As environmental temperature changes, there is a change in the bird's oxygen requirement. If one considers the thermoneutral zone following the brooding period to be 20-26°C, then temperatures outside this range cause increase in metabolic rate, and so increased need for oxygen. Low environmental temperatures are most problematic, since they are accompanied by an increase in feed intake with little reduction in growth rate.   While there is an increased oxygen demand at high temperatures due to panting etc, this is usually accompanied by a reduced growth rate, and so overall reduced oxygen demand.   Under commercial farm conditions, cold environmental conditions are probably the major contributing factor to ascites. For example at 10 vs 26°C, the oxygen demand by the bird is almost doubled. This dramatic increase in oxygen need, coupled with the need to metabolize increased quantities of feed, often leads to ascites.   It is interesting to note that birds maintained at high altitude under commercial conditions are often subjected to cool or cold night-time temperatures.

Scheele *et al*. (1992) showed that genetic lines susceptible to ascites have limited thyroid hormone production especially at low environmental temperatures. Such hypothyroidism leads to anoxia and hence ascites. These authors conclude that exposing birds to low temperature and anti-thyroid drugs may be used as a means of identifying genetically susceptible lines. Decuypere *et al*. (1994) also conclude that ascites may be linked to thyroid function and that T3 supplementation could be used in identification of ascites-inducing factors or susceptible lines.

Discussion of factors affecting the incidence of ascites invariably suggests that oxygen levels in commercial poultry barns are low, and so contribute to hypoxia. Julian and Wilson (1992) measured O_2 and CO_2 levels in various commercial buildings, and while O_2 levels were lower than outside air (20.5 vs 20.85%) there was no correlation between gas levels in environments where ascites was quite variable.   It seems unlikely that O_2 or CO_2 levels normally encountered in commercial buildings, are likely factors in the ascites syndrome.

4.4.   Health status

Any infection or contaminant that affects lung or heart condition is likely to affect the incidence of ascites. While severe viral infections may damage lung structure, there is often a concomitant reduction in growth rate. Under commercial conditions, *aspergillus* infection is the most problematic, because it can damage lung structure without there being a major depression in growth rate. Julian and Goryo (1990) experimentally induced aspergillosis in day-old chicks. About 20% of the birds died from right-ventricular failure/ascites, while chicks developing ascites before 16 d of age showed mouth breathing and other signs of hypoxia. When ascites developed later (>16 d) there was no change in rate or depth of respiration, suggesting minimal hypoxia. Microscopic examination of affected birds by Julian and Goryo (1990) revealed lung congestion and fibrosis that effectively restricted blood capillary space.

4.5.   Breeder age and genetics

Any genetic predisposition to ascites and heart failure is most likely to be related to growth rate. However Scheele *et al.* (1991) suggest that modern strains of broilers may have inadvertently been selected for their inability to consume large volumes of oxygen. These workers cite evidence indicating selection for low O_2 consumption to result in a line of birds that has increased growth rate, and that is leaner. Scheele *et al.* (1991) attempted to investigate this concept, suggesting that birds with improved growth and feed efficiency characteristics are more susceptible to ascites. Unfortunately the design of this study was not ideal, because the selection for growth rate obviously increases the occurrence of ascites. It is obviously difficult to separate the effects of genetics *per se* from those of growth rate.

Jones (1994) suggests that modern fast growing strains of broiler may be susceptible to ascites due to failure to exhibit normal slow-down in growth usually seen at extremes of environmental temperature. That broilers continue to grow quickly at low temperatures may, therefore, be a predisposing factor. Jones (1994) also suggests that in ascites-susceptible strains there is greater flux of buffering capacity in response to acid-base disturbance. Julian and Mirsalimi (1992) suggest that measurement of oxygen saturation may be a useful tool in genetic selection studies against ascites, while Odom *et al*. (1992) indicate that it may be possible to screen susceptible birds based on measurement of body weight and electrocardiograms. Interestingly these authors were able to identify susceptible birds as early as 7 d of age, suggesting that pre-hatch or early post-hatch stressors may predispose the condition.

Lopez and Leeson (1993, unpublished data) provide evidence that broilers hatched from young breeders are more prone to ascites than are birds from older breeders, *(Table 3.8)*. There was a very high incidence of ascites from male broilers originating from young breeders, even though these birds were slightly smaller in weight than birds from the older breeders *(Table 3.8)*. Because these broilers were derived from pedigree hatches, Lopez and Leeson (1993) were able to compare both hatching egg size and chick size for normal vs ascitic birds in this study *(Table 3.9)*. In all situations, regardless of the breeder age or the sex of the broiler, ascitic birds came from larger eggs and were larger chicks compared to their contemporaries that did not develop clinical ascites.

TABLE 3.8 Breeder age and ascites					
		% Ascites mortality			48d body
	Breeder age	0-21d	22-35d	36-48d	weight (g)
_ Broiler	30 wks	0	0	6.4	3000
	52 wks	0.4	0	1.7	3020
_ Broiler	30 wks	0	0	1.3	2480
	52 wks	0	0	0	2543
					Lopez and Leeson (1993, unpublished)

TABLE 3.9 Comparison of egg size and chick size with occurrence of ascites			
Breeder age	Broiler	Egg size (g)	Chick size (g)
30 wks	Normal _	57.5	38.4
	Ascitic _	58.9	39.0
	Normal _	56.8	37.7
	Ascitic _	59.3	39.7
50 wks	Normal _	67.9	46.9
	Ascitic _	69.4	48.2
			Lopez and Leeson (1993, unpublished)

4.6.   Feeding program

Manipulation of diet composition and/or feed allocation system can have a major effect on the incidence of ascites. In most instances, such changes to the feeding program influence ascites via their effect on growth rate. However, there is also a concern about the levels of nutrients that influence electrolyte and water balance, the most notable being sodium. Wilson *et al.* (1988) discussed the similarity of a field outbreak of ascites in broilers with that of salt poisoning, and observed that even though feed and water did not appear to provide excessive levels of sodium, a water-softener was being used on-farm. Julian (1987) observed an increase in RV/TV ratio and PCV in birds provided with high-sodium water, although in a subsequent study Julian *et al.* (1992)

found no evidence that diet sodium levels as high as 0.44% had any effect on the incidence of ascites. Turkeys fed 1.85% salt for 5-11 d did however exhibit ascites (Swayne *et al.,* 1986). These affected poults had hydropericardium and hydrothorax which seemed to develop just minutes before death. Swayne *et al.* (1986) observed salt poisoning to cause a flaccid heart with dilated right ventricle, together with swollen and pale kidneys. In a series of studies, Misalimi *et al.* (1992) and Misalimi and Julian (1991) show that sodium in the drinking water can result in up to 30% expansion in blood volume. Of greater concern is the effect of sodium on expansion of RBC's and the fact that less deformable erythrocytes may contribute to increased blood pressure due to resistance to flow through lung capillaries.

Ascites may also develop secondary to rickets, because of poor rib structure. Julian *et al.* (1986) observed that broilers fed diets grossly deficient in phosphorus developed rickets as expected, but that these birds also displayed an increased respiration rate, increased arterial CO_2 and decreased arterial O_2. Most birds showed signs of cardio-pulmonary abnormalities and some birds died of hypoxia and some of RV failure with or without clinical signs of ascites. Julian *et al.* (1986) suggest that RV hypertrophy occurred in response to the pulmonary arterial hypertension due to hypoxia, which itself was caused by abnormal breathing due to poor rib strength and infolding.

Apart from obvious nutrient deficiencies, or excesses as in the situation with sodium, the major involvement of feeding program as it affects ascites revolves around nutrient density and feed restriction. Ascites is more common when high-energy diets are used, especially when these are pelleted. For example, Lamas da Silva *et al.* (1988) found no ascites in birds fed mash rather than pellets, while Hernandez (1987) suggests that hypoxia is aggravated by feeding high-energy diets, and that problems are most evident at time of diet change from starter to grower. Schlosberg *et al.* (1991) also showed reduced incidence with mash vs pelleted feed (1.21 vs 2.32%) while mortality could be reduced even further if birds were subjected to 6-11 d feed restriction (0.9% mortality). Julian *et al.* (1989) found a higher incidence of right ventricle hypertrophy in birds fed diets providing 3100 vs 2700 kcal ME/kg, although this effect was most pronounced when birds were subject to cool temperatures (13 vs 23°C). Dale and Villacres (1988) grew birds on high-energy diets designed to promote rapid growth and likely to induce ascites. There was no correlation between 14 d body weight and propensity to ascites, although birds fed 3000 → 3100 kcal ME/kg rather than 2850 → 2950 kcal ME/kg had twice the incidence of ascites. Unfortunately Dale and Villacres (1988) did not show body weight data, although they concluded that predisposition to ascites is not related to growth rate. In this study, the higher energy diets were produced essentially by increasing the level of supplemental fat. In a previous report, Dale and Villacres (1986) support the concept that feed change *per se* is often the trigger to ascites, but that the condition is also seen in single-diet feeding programs. In formulating diets of varying nutrient density and fat content, these workers again show clear evidence of correlation between ascites and growth rate *(Table 3.10)*. The highest incidence of ascites occurred when the highest energy level was fed, regardless of either energy:protein or fat content of the diet. These data *(Table 3.10)* show little effect of added fat, although at both energy:protein diet series, the greatest incidence of ascites occurred in the fastest growing birds.

TABLE 3.10. Effect of nutrient density and diet composition on incidence of ascites					
Diet ME (kcal/kg)	**Crude protein (%)**	**ME/CP**	**Diet Fat (%)**	**49d body wt (g)**	**Ascites mort. (%)**
2950	23	128	0	1800	8.8
2950	23	128	4	1820	8.7
3100	24	128	4	1830	15.8
2950	21	140	0	1810	9.0
2950	21	140	4	1810	8.5
3100	22	140	4	1860	12.0
Adapted from Dale and Villacres (1986)					

Because feeding program, nutrient density and growth rate are all intimately correlated in affecting severity of ascites, then there is invariably discussion on the possible advantages of feed restriction. Maxwell *et al.* (1991) studied the hematological and the histopathological response of birds to feed restriction from 6-14 d of age. Feed restriction had no effect on the hemoglobin or the hematocrit levels at the time of restriction, although at 49 d birds had reduced RBC and WBC counts.

Arce *et al.* (1992) carried out a series of interesting studies to record bird response to varying nutrient restriction programs. As pointed out by these authors, the goal of such programs is to reduce the incidence of ascites without adversely affecting economics of production. It is expected that nutrient restriction programs will reduce final weight-for-age to some degree, and obviously there is a balance between the degree of feed restriction and commercially acceptable growth characteristics. Arce *et al.* (1992) conducted studies in Mexico at 1940 m or at 2500 m elevation. Birds were either fed on a skip-a-day schedule for varying periods from 7-28 d or allowed access to the feeders for just 8 h per day (7am - 3pm). Results of four separate trials are summarized in *(Table 3.11)*. In all studies, control full-fed birds were the heaviest and exhibited the greatest ascites mortality. As expected, the later in the grow-out cycle that skip-a-day feeding is introduced, the greater the reduction in ascites mortality, although this is accompanied by greater reduction in body weight. By restricting the feed access time to 8 h per day, ascites mortality was greatly reduced *(Table 3.11)* and this seems the most advantageous system, especially where hand-feeding is practised, and feeders can easily be raised during the off-feed period. Restricting feed access time with mechanical feeders is more complicated and may not be practical on-farm. It appears as though ascites mortality can be reduced through limiting feed intake, and depending upon the timing of this restriction program, will be accompanied by some 100-200 g loss in weight-

for-age. In most commercial operations, this will mean a 2-3 d delay in achieving 50-56 d market weight.

TABLE 3.11 Feed restriction and ascites in broilers grown at 1940-2500 m elevation								
	Exp1 (53d)		**Exp 2 (51d)**		**Exp 3 (56d)**		**Exp 4 (56d)**	
Treatment	**B Wt**	**Mort[1]**	**B Wt**	**Mort**	**B Wt**	**Mort**	**B Wt**	**Mort**
1. Full-fed control	1930	15	2150	37	2310	10	2430	28
2. Skip-a-day (7-13d)			2140	15	2280	3	2310	12
3. Skip-a-day (15-21d)			2030	17	2350	5	2320	20
4. Skip-a-day (22-28d)			2060	8	2200	8	2340	17
5. Skip-a-day (15-27d)							2280	9
6. Feed access 7am-3pm	1630	0					2337	7
[1]Mortality due to ascites (%)								
							Adapted from Arce et al. (1992)	

Another factor of concern regarding the effect of the nutritional program on ascites, is the balance and the quality of the protein supply. It is well known that crude protein is supplied as a source of amino acids, and that birds have a minimal requirement for nitrogen *per se*. Excess nitrogen must be removed from the body, and this is an energy (oxygen) demanding process. There is a potential to reduce the oxygen demand through minimizing crude protein supply while maintaining essential amino acid levels in a diet. If we consider two diets providing the same level of available amino acids, but with 20 vs 24% crude protein, then there will be a need for birds to deaminate an extra 4% CP in the high-protein diet. If birds consume 130 g/d, this means an extra 5 g/d of protein for catabolism. Such protein catabolism will likely result in uric acid and fat synthesis, and these are calculated to need 2 and 1 litres of oxygen per day respectively. Therefore catabolism of an extra 5 g crude protein each day means a 3 litre increase in oxygen demand, which represents about an 8% increase relative to the bird's total requirements. There is an obvious incentive to minimize crude protein *per se*, because its catabolism merely imposes another stress on the oxygen demand of the bird.

5.   POTENTIAL TREATMENT AND PREVENTION

There does not seem to be any effective treatment for ascites, and once birds exhibit symptoms, death occurs fairly quickly. Ascites and right ventricular failure can be prevented by a number of methods, although these invariably result in reduced growth rate and so economics of these systems must be calculated locally.

5.1.   *Environmental temperature*

Low environmental temperature leads to an increased oxygen demand, and so is likely a trigger mechanism for ascites. In many commercial farms, open-sided houses are common, and here birds will be subjected to cooler night-time temperatures. If ascites is problematic, then every effort should be made to maintain house temperature through effective use of curtains etc. This is especially important in the first two wks of life, where chicks are especially susceptible to cold stress. Cold environments are most stressful to poorly feathered birds that possess a minimum of insulation.

5.2.   *Environmental conditions*

Contaminants that irritate and damage the lungs will obviously be a factor in ascites. The major contaminants are dust and ammonia. If ammonia can be detected in the house by humans, then levels are such that some lung damage will occur. Litter management, and drinker management are the major factors affecting ammonia levels. It is essential to move adequate quantities of air over the birds, and this becomes most critical in controlled environment buildings.

5.3.   *General health status*

Disease and vaccine reactions that influence the respiratory tract will likely contribute to ascites, and so must be minimized as part of good husbandry conditions.

5.4.   *Diet and feeding programs*

Ascites can be totally prevented through feed restriction or through the use of very low nutrient dense diets. However, there is an obvious economic balance to consider between reduction in mortality due to ascites and reduced growth rate of the entire flock. Economics obviously are affected by age at which birds die of ascites, but as a rule of thumb 10% mortality is equivalent to a 10% reduction in growth rate of the entire flock. In practice therefore, a 10% reduction in mortality must be achieved with less than 10% reduction in weight gain, etc. for the

management system to be economical. The following diet and/or feed program scenarios may be considered in attempts to reduce ascites:

a)   Low energy feeds throughout the entire life cycle *eg:*

> starter 2850 kcal ME/kg
> grower 2950 kcal ME/kg
> finisher 3100 kcal ME/kg

b)   Use mash rather than pelleted feeds. Do not use too fine a mash diet, since this encourages feed wastage and causes dustiness at broiler level.

c)   Consider skip-a-day feeding from 7-20 d of age. Longer periods of restricted feeding may be necessary where ascites levels are very high. Water management becomes more critical with this system.

d)   Consider limit-time feeding, such that birds have access to feed for 8-10 hours each day. Extra care is needed in water management so as to prevent wet litter.

e)   Minimize toxin contamination of feed.

f)   Limit sodium level to 0.19% of diet.

Chapter 3

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CHAPTER 4. HEPATIC HEMORRHAGE

Other names:	LIVER HEMORRHAGIC SYNDROME
Species:	LAYING HENS

1.   COMPENDIUM

Hepatic hemorrhage often occurs in laying hens fed diets containing appreciable quantities of high glucosinolate rapeseed meal. Birds die of massive liver hemorrhage caused by a loss in the reticulin structure of the hepatic tissue. These changes in tissue structure are assumed to be mediated by glucosinolate compounds either directly and/or through the alteration of thyroxine output. There is no major infiltration of lipids in the liver, and so this differentiates the diagnosis relative to FLHS *(Chapter 5)*. There is a major genetic component, with some strains of White Leghorn exhibiting a 50% mortality over a laying cycle when 15-20% rapeseed is used in a diet. Thyroid glands will also be enlarged, although this is a usual response to glucosinolates in rapeseed and should not be considered specific for hepatic hemorrhage. Birds often appear normal and have functional ovaries at the time of death. It is possible that the increased blood pressure associated with oviposition is ultimately responsible for the rupture of the liver capsule, and so the cause for the characteristic massive hemorrhage. The condition can be prevented by removing the high-glucosinolate rapeseed from the diet. When it is necessary to use this ingredient, its inclusion should be limited to 5% of the diet.

2.   OCCURRENCE AND GENERAL SIGNS

Liver hemorrhages are known to occur in laying hens fed rapeseed meal that contain high levels of glucosinolates. The condition seems to be strain specific, because in some flocks the mortality can reach 50%, whereas other strains show only a 5-10% mortality. Affected birds appear normal, and there are no outward signs of morbidity preceding death, other than occasional situations of anemia. Olomu *et al.* (1975) fed layer diets containing up to 10% rapeseed meal for 330 d. Mortality was little affected with up to 5% inclusion of rapeseed, but at 10% there was increased mortality. However these authors showed that the rapeseed meal inclusion level had no effect on the liver fat content which averaged 35% across all treatments. On this basis, Olomu *et al.* (1975) conclude that although the lesions at first appear typical of Fatty Liver Hemorrhagic Syndrome *(Chapter 5)*, there is no major increase in the liver fat content, and so the term "hepatic hemorrhage" is more appropriate. Unaffected birds appear normal, and there seems to be no loss in egg production for these birds. Campbell (1979) observed a marked strain difference in incidence of hepatic hemorrhage in White Leghorn birds. Feeding up to 10% high-glucosinolate rapeseed meal, resulted in a 48% incidence in one strain of bird, while no mortality occurred in another strain. The average mortality was around 5%.

3.   PATHOLOGY AND METABOLIC CHANGES

3.1.   Gross pathology

Hall (1972) described a condition in the UK in the early 1970's that seemed to relate to rapeseed toxicity. Birds died in good condition, often with an egg in the oviduct, and with a functional ovary. In some instances, the birds were anemic with pale combs and wattles. At post-mortem examination, there was invariably a massive hemorrhage on the liver, which itself was pale yellow and friable. Hall (1972) observed little fat infiltration in the liver, and so this precluded FLHS. Jackson (1969) observed up to 50% mortality in one strain of bird fed 20% rapeseed meal, with all affected birds showing a large hemorrhage on the liver. In addition to the single large hemorrhage that probably caused death, there were often smaller hemorrhages that appeared older, and were bordered in some cases by necrotic tissue. Yamashiro *et al.* (1975) observed moderate to severe fatty degeneration, focal necrosis and moderate to severe hemorrhage, in layers fed 10-20% of span rapeseed meal or 20% rapeseed oil.

3.2.   Tissue changes

Yamashiro *et al.* (1975) observed edematous foci and lysis of hepatocytes along with large amounts of lipid droplets within the necrotic lesions. Hemorrhages were not always present in this necrotic tissue. Yamashiro *et al.* (1975) conclude that changes in the structure of hepatic cells and blood vessel walls are predisposing factors to the condition, and that hemorrhage is a secondary occurrence. When this necrotic tissue becomes extensive, along with a loss in the integrity of the blood vessel walls, the elevated blood pressure and stress associated with oviposition (metabolic and physical) could lead to the rupture of the vessels within the liver.

Hall (1972) observed liver hemorrhage in birds from a large number of commercial flocks. In all livers examined, there was a defect of the reticulin that was thought to be due to the presence of some toxin. In extreme cases, the reticulin was virtually absent, with vestiges associated only with the larger vessels and ducts. The condition was specific to birds fed rapeseed meal. In subsequent studies, Hall (1974) carried out a more detailed investigation of reticulin structure. In normal birds, the reticulin was evenly distributed within the liver, the fibres being delineated and sinusoids appearing as narrow tracks permeating the parenchyma. Reticulin was most dense around the blood vessels. In birds showing liver hemorrhage, the reticulin was less extensive and in extreme cases, non-existent. Hall (1974) concludes that if the liver capsule remains intact, the bird likely survives, whereas if it ruptures, the loss of blood is fatal. Again the author indicates the potential predisposition caused by rise in blood pressure associated with oviposition.

Jackson (1969) indicated a large increase in the thyroid size of affected birds. However this may not be useful in diagnosis because most birds fed rapeseed meal, and even low-glucosinolate canola meal, will show some degree of thyroid hypertrophy. Jackson (1969) showed increased thyroid size in all affected birds, but the effect was most evident in the strain showing the highest incidence of hepatic hemorrhage. The thyroid size was 100% larger in the susceptible vs normal strain of bird in response to eating diets containing 20% rapeseed meal for 250 d.

3.3.   Blood profile

There have been no reports of significant changes in the blood profile of birds affected by hepatic hemorrhage. This in itself identifies it as distinct from FLHS because the latter invariably results in major changes in blood lipid profile.

4.   RELATED FACTORS

4.1.   Bird strain

There is obviously a genetic predisposition to the condition, and one strain of commercial White Leghorn is repeatedly shown to be more susceptible. Strain differences have been shown in white vs brown egg birds in terms of the development of fishy-taints in eggs caused by eating rapeseed meal.   However, the situation with hepatic hemorrhage is not likely related to trimethylamine metabolism which is a factor in fish-taint of eggs. Jackson (1969) suggests that susceptible strains may have higher levels of naturally occurring myrosinase enzyme in the gut. Myrosinase enzyme, which is also found in the seed coat of rapeseed, is the catalyst required to convert the glucosinolates into toxic cyanide compounds.

4.2.   Rapeseed meal

The condition is specific to layers fed rapeseed meal containing high levels of glucosinolates. In immature birds, reduced growth rate relates to the depression of thyroid function, although hepatic hemorrhage is seldom seen. For this reason, active lipid metabolism by the liver may be a predisposing factor, although again it must be emphasized that the condition is different to FLHS, because fatty infiltration is minimal in birds dying from rapeseed toxicity. Interestingly, FLHS occurs at a much higher incidence when thyroid metabolism is depressed, a situation that occurs with feeding high levels of glucosinolates. However, FLHS afflicted birds do not usually exhibit enlarged thyroids.

5.   POTENTIAL TREATMENT AND PREVENTION

With the condition specific to high-glucosinolate rapeseed meal, then treatment relates to removing this from the diet. Rapeseed meal should not be fed to laying hens, although where it must necessarily be used under specific local conditions, it should not be used at more than 5% of the diet. Low-glucosinolate canola meals do not cause hepatic hemorrhage, and can be used at higher levels in the diet.

Chapter 4

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CHAPTER 5. FATTY LIVER HEMORRHAGIC SYNDROME
<u>Other names:</u>   FATTY LIVER SYNDROME
<u>Species:</u>   LAYING HENS CONDITIONS REPORTED IN BROILER BREEDERS, TURKEY BREEDERS

1. COMPENDIUM

Fatty liver hemorrhagic syndrome (FLHS) was first described by Couch (1956) as excessive fat in the liver associated with varying degrees of hemorrhage. The condition is almost universally confined to caged birds fed high-energy diets, and is most often seen in summer months. The liver is usually enlarged, a "putty color", and is very friable. The abdominal cavity usually contains large amounts of oily fat. A number of workers have suggested that the affected birds have pale combs. The ovary is usually active, and the metabolic and physical stress as associated with oviposition may be the factors that induce the final fatal hemorrhage.   FLHS only seems to occur when birds are in a positive energy balance, and so the monitoring of body weight is a good diagnostic tool. Through force-feeding techniques, it is shown that FLHS is caused by a surfeit of energy rather than being specific to an excess of any nutrient such as fat or carbohydrate. Experimentally the condition can be induced in layers and even male birds by the administration of estrogen. This reinforces the concept that FLHS occurs more frequently in high-producing birds that presumably are producing estrogen from very active ovaries.

Numerous attempts have been made to prevent or treat the condition through diet modification. Substituting carbohydrate for supplemental fat, while not increasing the energy content of the diet, seems to be beneficial. Presumably such modification means that the liver needs to synthesize less fat for yolk. Replacement of corn with other cereals, such as wheat and barley, is often beneficial. However this substitution may involve a reduction in diet energy level or may necessitate the use of additional fat in order to maintain isoenergetic conditions, and these two factors are known to influence FLHS. There are numerous reports of reduction in FLHS through the use of various by-product feeds such as distillers grains and solubles, fish meal and alfalfa meal. In these situations the mode of action is unclear, although unintentional supplementation of selenium may be involved. FLHS is best prevented by not allowing an excessive positive energy balance in older birds. This can be monitored through body weight, and when potential problems are seen, remedial action taken to limit energy intake through the use of lower energy diets and/or change in feed management. A wide energy:protein ratio in the diet will aggravate FLHS. When a farm has a history of FLHS, the diet should be supplemented with 0.3 ppm selenium, up to 50 IU vitamin E/kg diet, and appropriate levels of an antioxidant such as ethoxyquin.

2.   OCCURRENCE AND GENERAL SIGNS

Fatty liver hemorrhagic syndrome was first described by Couch (1956) being characterized by excessive fat infiltration of the liver accompanied by varying degrees of hemorrhage. Couch (1956) concluded that the condition coincided with the acceptance by the poultry industry of high-energy corn based diets, and as such, FLHS was due to an over supply of energy. Research over the past 35 years has tended to support this intuitive observation. The condition is almost universally confined to caged birds, with very few cases seen in floor-housed flocks. The fact that the liver is the major site of lipid synthesis in the bird means that the liver is naturally high in lipid content. In contrast to the situation in mammals, therefore, it is perhaps not too surprising that slight changes in liver lipid metabolism can have a major effect on the well being of the bird. For the laying hen, this situation is further accentuated due to the almost daily demand for the synthesis of 6-10 g of lipid for the yolk.

The condition is easy to recognize at post-mortem due to the liver hemorrhage and also the fact that the liver is often enlarged and engorged with fat. This makes the liver friable, and it is difficult to remove each lobe in one piece. The liver also appears pale yellow, although this is not always specific to FLHS, as normal layers that are fed appreciable quantities of yellow corn will have a yellow colored liver. Polin and Wolford (1986) suggest liver color to be indicative of diet xanthophylls rather than FLHS, since by force-feeding semi-purified diets devoid of pigment, FLHS can be induced, although birds lack the characteristic yellow liver. The body cavity may also contain excess fat deposits that are often oily and pale yellow in color. Butler (1976) suggests that FLHS birds have at least 40% fat in the liver dry matter and this may reach as high as 70%. However as will be documented later, there is considerable variation in liver lipids found in birds from different geographical locations, and so these values of Butler (1976) may not always apply. However FLHS birds will always exhibit a relative increase in liver lipid content.

In many studies, the degree of FLHS is described as a liver hemorrhage score, which is usually based on a scale from 1 - 5. Wolford and Polin (1974) describe these scores as 1 = no hemorrhage; 2 = 1-5 hemorrhages; 3 = 6-15 hemorrhages; 4 = 16-25 hemorrhages and 5 = >25 hemorrhages, as well as a massive hemorrhage.

3.   PATHOLOGY AND METABOLIC CHANGES

3.1.   Gross pathology

Pearson and Butler (1978a) described the sporadic occurrence of FLHS in three commercial layer flocks. In all situations there was massive hemorrhage from the liver and large amounts of fat were found in the abdominal cavity. The livers were described as "putty colored" and very friable containing large amounts of fat. No other abnormalities were seen. Ugochukwu (1983) described similar symptoms of birds in Nigeria. Again there was excessive subcutaneous and abdominal fat, while the liver was soft, pale and yellowish. In some cases, there were ruptures of the liver capsule, exposing the soft liver parenchyma. Hemorrhage was apparent, and in the liver cells, fat appeared as clear spaces in the cytoplasm. The environmental temperature was not excessively high at $26^{o}C$, although the diet was high in energy at 3000 kcal/kg. Mortality due to FLHS was 20% (Ugochukwu, 1983).

Grimes *et al.* (1991) studied the comb characteristics of layers with FLHS. Layers with FLHS had much paler combs, although comb size was not affected. Grimes *et al.* (1991) did observe that layers with fatty liver were at least 10% heavier than the standard weight-for-age. Harms and Simpson (1979) had previously reported enlarged combs, and such birds had a greater accumulation of greasy abdominal fat compared to birds with smaller combs. Few other gross abnormalities are seen, and most birds have an active ovary (Pearson and Butler 1978a).

3.2.   Tissue changes

Pearson and Butler (1978a) observed varying degrees of fat in the cytoplasm of the hepatic parenchyma cells together with sinusoidal engorgement. As fat deposition increased, the smaller droplets of fat apparently fused together forming large cysts occupying the entire parenchymal cytoplasm.   In other studies, Butler (1976) described FLHS as a disruption of the normal architecture of the liver cells, due to diffuse capillary hemorrhage, blood clots and the breakdown of vascular integrity. Areas of necrosis and fibrosis varied in size but were more prominent around the margins of the lobes. Some hepatocytes may be ruptured while others are grossly distended with fat. Such fat inclusions eventually reach a size that the normal cell structure is disorganized and the nucleus may be displaced and/or degenerated (Butler, 1976). Walzem *et al.* (1993) also showed abnormal liver structure in that the large lipid droplets frequently displaced hepatocyte nuclei. These authors showed abnormalities in liver reticulin to increase in frequency and severity with a score of 2-3 after subjecting hens to 150% normal feed intake. Ivy and Nesheim (1973) found decreased phospholipid content in the liver, as the fat content increased. However phospholipid content per gram of liver changed little, since FLHS was associated with liver hypertrophy. Ivy and Nesheim (1973) also observed an increase in the oleic acid content of liver fat, and since this is found at very low concentrations in the diet, it suggests synthesis of this fatty acid. However Mee and Palafox (1975) indicated that high-producing layers also have more palmitoleic, which is another fatty acid not normally found in the diet. The significance of liver lipid fatty acid profile is unclear at this time. Fuji *et al.* (1984) indicate that avian liver normally contains lipase, but that its activity is directly controlled by an inhibitor that is present in the serum; it is unknown if lipase activity increases during FLHS. Squires and Wu (1992a) showed that a strain of bird susceptible to FLHS was related to increased incidence and susceptibility to hepatic lipid peroxidation.

3.3.   Blood profile

There seems to be an increase in plasma estradiol for birds afflicted with FLHS. This was confirmed by Haghighi-Rad and Polin (1981) who observed an increase in plasma estradiol from 165 to 247 pg/ml indicating a correlation between FLHS and estradiol levels of 0.74. There was no relationship with plasma progesterone. Changes in estradiol may be responsible for observed changes in plasma calcium levels. Harms and Simpson (1979) reported that serum calcium of hens with FLHS was elevated to as high as 74 mg/100 ml in certain birds, and that this result was comparable to that seen in response to injections of estradiol or to feeding high levels of iodine. Miles and Harms (1985) recorded a high correlation between blood calcium level and comb size, and conclude that under field conditions of FLHS, blood calcium levels will be abnormally elevated. In subsequent studies, Harms and Miles (1989) confirmed this relationship while Harms *et al.* (1985) suggest that phosphorus as well as calcium levels may be affected. These authors conclude

that during FLHS, there may be interference with the formation of 1-25 $(OH_2)D_3$, and hence the effect on calcium metabolism.

4.   <u>RELATED FACTORS</u>

4.1.   *Energy balance*

Most researchers agree that FLHS will only occur when birds are in a positive energy balance and that this situation leads to fat accumulation and obesity. Polin and Wolford (1976) force-fed 28 wk old hens with various energy sources at 150% of normal *ad-libitum* intake. Energy intake in excess of requirement induced FLHS regardless of the source being as glucose, starch or corn oil. Interestingly, in response to force-feeding, hens seem to tolerate much higher hepatic lipid levels than do hens undergoing spontaneous FLHS. The authors point out that FLHS occurs only in birds that are in positive energy balance for a prolonged time period, and while mortality may not be excessive, of greater economic impact is the efficiency of diet energy use by the whole flock. Contrary to these results, Ivy and Nesheim (1973) indicated no relationship between energy intake and FLHS. This result is somewhat surprising, although, as will be discussed later in this section, it is the excess energy available for fat synthesis rather than the energy intake *per se* that may be of more significance.

In attempting to quantitate various aspects of energy utilization Polin and Wolford (1976) partitioned the energy intake of layers fed *ad-libitum* and those force-fed additional energy, and that subsequently developed FLHS. Both types of bird recorded similar quantities of energy available for activity, but the force-fed birds naturally had much more energy diverted to body weight gain. The almost 180 kcal/d of available energy to the force-fed bird vs 27 kcal for the *ad-libitum* bird, relates to potential for 20 g vs 3 g fat deposition per d. Over an extended period of time, such positive energy imbalance leads to obesity and fat infiltration of many tissues. Walzem *et al.* (1993) found FLHS to occur in birds within 2-3 wk following force-feeding at the rate of 150% of normal feed intake. These affected birds had significantly elevated liver levels of malic enzyme, glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase.

Wolford and Polin (1972) studied the effect of starvation - refeeding on incidence and development of FLHS. While 25% of control birds developed FLHS to some degree, no liver hemorrhage was seen in birds offered a restricted quantity of feed. The control diet, that was consumed *ad-libitum*, provided 3000 kcal ME/kg. The authors conclude that high liver lipid was not always associated with FLHS, but that FLHS was always associated with high liver lipid. In another comparable study, Wolford and Polin (1974) showed an interesting relationship between the level of feed intake and incidence of FLHS, suggesting that liver hemhorrage score = 0.047X −2.9 where X = feed intake as grams/bird/day. Since the diet contained 3000 kcal/kg, this can be modified to 0.016E - 2.9 where E = energy intake/day. Because liver hemorrhage starts with a score of 2, then if the equation of Wolford and Polin (1974) is valid, it suggests problems with birds consuming much in excess of 305 kcal ME/day. Obviously this value will be influenced by the weight of the bird and environmental temperature, but it does indicate an alarmingly low energy intake at which problems may start to develop.

4.2.   Hormonal balance

The normal rise in fat content of the liver of the mature vs immature bird is a reflection of lipids required for yolk synthesis, and as such, is influenced by estrogen. In their force-feeding studies, Polin and Wolford (1977) indicated that the hemorrhage score was markedly increased when excess energy intake was combined with exogenous estrogen treatments. Pearson and Butler (1978b) used estrogen-treated immature birds as a model for FLHS. In these studies, the liver score was directly related to the level of estrogen administered. Interestingly, with feed withdrawal, there was a dramatic decline in liver hemorrhage suggesting that the associated lipogenesis is related to carbohydrate intake. Akiba *et al.* (1983a) observed an increase in plasma lipids of birds treated with estrogen, although there was no major increase in liver lipids. These authors suggested that estrogens may enhance lipid transport from the liver rather than lipid deposition. Harms *et al.* (1977) observed an increase in liver size, liver lipid content, and serum cholesterol in response to estrogen. Pearce and Johnson (1986) did not observe FLHS in estrogenised birds, even though liver size and plasma lipid levels were increased. Pearce and Johnson (1986) recorded a reduction in the level of certain lipogenic liver enzymes following estrogen treatment, leading to the suggestion that there may be a negative feed-back effect on hepatic lipogenesis caused by the synthesized lipid accumulating in circulation. Akiba *et al.* (1984) tested NKK, an organic compound, that has previously been shown to reduce liver lipids in other animals. This product did in fact reduce liver size and lipid content in estrogenized birds. NKK appeared to reduce plasma transaminase activity suggesting that impaired liver function, caused by estrogen, can be prevented with an appropriate pharmacological treatment. Details of product NKK are given by Akiba *et al.* (1984).

The hyperlipidemic effects of estrogen are thought to be mediated via the stimulation of fatty acid synthetase (Aprahamian *et al.,* 1979), while Akiba *et al.* (1982b) suggest that plasma estrogen levels can be modulated by feeding diets that influence liver fat content. While progesterone administration may temper the effects of estrogen on liver fat content (Leszczynski *et al.,* 1982), Haghighi-Rad and Polin (1981) show no relationship between plasma progesterone and incidence of FLHS. Thyroid hormones are also thought to play a role in liver fat content due to their effect on the metabolic rate and general energy balance. Feeding iodinated casein is known to reduce fat accumulation in young birds and Harms *et al.* (1982) suggested that FLHS can be treated with the addition of up to 220 mg Protamone per kg diet. However this treatment also resulted in reduced egg production. After feeding thiouracil, an anti-thyroid compound, liver fat content is increased and there is increased FLHS mortality (Roberson and Trujillo, 1975). Akiba *et al.* (1983) studied the effects of both thiouracil and estrogen treatment. Thiouracil had more of an adverse effect on liver fat accumulation when birds were held at 21°C, while estrogens were more damaging at 34°C. Akiba *et al.* (1983) indicated an additive effect between thiouracil and estrogen, suggesting different modes of action.

4.3.   Environmental temperature

As mentioned in section 4.2, Akiba *et al.* (1983) showed exogenous estrogen treatment to be more detrimental at higher environmental temperatures. This observation agrees well with general field observations that FLHS is somewhat seasonal, with the highest mortality occurring in the warmer parts of the year. Ivy and Nesheim (1973) made observations from a number of flocks over a two year period, and in New York State observed the highest incidence of FLHS in May -

August which are usually the warmest months of the year. Contrary to these findings, Akiba *et al.* (1982b) showed no difference in liver lipid content for birds held at 13-24°C vs 24-35°C. Jensen *et al.* (1976a) also observed more FLHS in the warmer vs cooler regions of Georgia. It is likely that environmental temperature is a factor in FLHS, since at moderately-high temperatures (25 - 30°C), birds are more likely to be in positive energy balance which seems to be an important prerequisite for the development of a fatty liver.

4.4.   Diet composition

4.4.1. General considerations

Because energy balance is a factor in FLHS, then diet composition has obviously come under close scrutiny.   Without the knowledge of the bird's reaction to various diet energy concentrations, the obvious suspect is the use of high-energy diets. As Couch (1956) mentioned when first describing FLHS, its introduction coincided with the acceptance of corn as a major diet component, and this means the use of high energy diets. Subsequent to this has been the introduction of another high-energy ingredient, namely fat. However, it must be remembered that the bird eats with some precision in order to achieve its daily energy requirement. If there is any imprecision in this system, it seems to be one of a mild overconsumption, although this is accentuated at high temperature *(Table 5.1)*. It is unlikely that birds will be fed diets providing the two highest energy levels as shown in *(Table 5.1)*, although the concept of "overconsumption" of energy at high temperatures is emphasized. There is obviously a fine line between stimulating the energy intake needed for optimum production, and any excess, accumulating as body fat. As will be discussed later, there is possibly the need to phase-feed energy, when birds are held at high temperatures, in order to combat FLHS in older birds.

TABLE 5.1 Effect of diet energy and environmental temperature on feed and energy intake of laying hens				
	18°C		**30°C**	
Diet ME (kcal/kg)	**Feed intake (g/d)**	**ME intake (kcal/d)**	**Feed intake (g/d)**	**ME intake (kcal/d)**
2860	127	363	107	306
3060	118	361	104	318
3250	112	364	102	331
3450	106	366	101	348
				Adpated from Payne, 1967

The balance of protein:energy in the diet should also be seriously considered, because low protein, in relation to energy, leads to excess fat accumulation. In studying field cases of FLHS, Pearson and Butler (1978a), indicated that protein content of the diet is usually low, and therefore conducive to hepatic steatosis, through not providing the adequate amino acids for apolipoprotein synthesis, thereby impeding the lipid transport. Their observation that soluble protein accumulated

in the liver together with lipids, might be important in this context, since the protein may represent incomplete lipoproteins.   Akiba *et al.* (1992) demonstrated that adding up to 1g/kg dietary tryptophan resulted in a significant reduction in hepatic lipid and occurrence of liver hemorrhage. Mode of action may enhance cytochrome b5 which is a component of the mixed function oxidase system.

4.4.2.   Diet fats and oils

Hargis *et al.* (1991) indicated greater hepatic lipid concentration in birds fed diets containing 3% vs 0% fish oil. Haghighi-Rad and Polin (1982b) showed that by force-feeding, FLHS could be induced in layers, but that liver score could be reduced by including 4% fat in the diet. Savage and Rhoades (1989) also observed a reduction in FLHS when a 13% CP diet was supplemented with 3.5% oil together with a number of vitamins and trace minerals. A confounding factor in many of these studies is the method of adding fat to the diet. In general, it seems as though the incidence of FLHS is increased when fat addition results in increased energy content of the diet, while a reduction in FLHS accrues from isoenergetic substitution of fat for other energy yielding ingredients. On this basis, Haghighi-Rad and Polin (1982b) formulated layer diets composed of either corn-soy or wheat-soy. The wheat diets were maintained isoenergetic by supplements of either corn oil, wheat starch or corn starch. Hens fed wheat supplemented with starch had a much greater accumulation of liver fat. The low-fat diets resulted in more FLHS, although apparent energy retention was unaffected. These authors conclude that supplemental fat depresses *de novo* fatty acid synthesis in the liver. This situation is important to remember in subsequent discussion of the role of various ingredients *per se* on FLHS, since invariably their inclusion in diets also involves fat addition necessary to maintain the energy normally contributed by corn.

4.4.3.   By-product ingredients

There has been considerable research conducted on the role of ingredients such as fish meal, alfalfa meal, and various distillery by-products as they affect FLHS. In most of these studies, there is the confounding effect of fat inclusion as detailed previously.   Jensen *et al.* (1976) formulated isoenergetic diets at 2900 kcal ME/kg and 14.3% CP to contain a number of cereals including corn, wheat or barley. Incidence of FLHS followed the ranking corn > wheat > barley. Fish-alfalfa vs corn diets were shown by Akiba *et al.* (1982a) to reduce liver lipid content, while Akiba *et al.* (1982b) showed reduced liver lipid in birds fed diets containing fish meal or distillers dried grains. In this latter situation, the fish diet contained less total diet fat. Jensen *et al.* (1974) substituted distillers grains in wheat and corn based diets while maintaining constant diet fat levels. While distillers grains did result in reduced liver weight, there was no change in g fat/liver.

Maurice and Jensen (1978b) attempted to reduce plasma lipid levels through the inclusion of various ingredients. Using a conventional corn-soybean meal diet resulted in a 50% incidence of hemorrhagic liver, although liver fat was not affected. (On this basis, it is more likely that liver hemorrhage relates to factors such as mycotoxins, rather than FLHS). However, Maurice and Jensen (1978b) did show reduced plasma lipids in response to feeding alfalfa meal, brewer's grains, and other high-fiber mill-feed by-products. The authors conclude that these by-product ingredients contain some unknown protective factor that will reduce the incidence of FLHS. Again,

all by-product diets contain less corn. In a subsequent study Maurice and Jensen (1979a) showed that the feeding of either brewers grains or distillers by-products, reduced liver fat content and also relative liver weight. In one experiment there was also reduced FLHS. In trying to account for these results, Maurice and Jensen (1979a) suggest these fermentation by-products either alter fat metabolism resulting in greater nutrient bioavailability (not specified), or influence the presence and/or balance of trace minerals and yeast metabolites.

In more detailed studies, Akiba *et al.* (1982) did show some beneficial effects from adding fiber to the diet. Using force-fed immature male White Leghorn birds, there was a reduction in liver lipid in response to fiber. While fiber appeared to increase hepatic *in vitro* lipogenesis from acetate and glucose, Akiba *et al.* (1982) equate reduced liver lipid content on the basis of accelerated transport of lipid from the liver. How fiber brings about such a change is however unclear.

4.4.4. Minerals and vitamins

In showing a dramatic reduction in liver weight, hepatic and plasma lipids and incidence of FLHS in response to adding fish meal to the diet, Maurice *et al.* (1979) hinted at an association with diet selenium concentration. Adding 0.3 ppm Se to the diet had the same effect as did fish meal in reducing liver hemorrhage. Maurice *et al.* (1979) conclude that while selenium may be involved in reducing the incidence of liver hemorrhage, this mineral has no effect on liver fat content. In other species at least, selenium is known to counteract the deleterious effects of natural plant estrogens.

Wolford and Polin (1975) showed no beneficial effect to adding inositol to the diet of birds fed either *ad-libitum* or force-fed. There was however an indication of a reduced liver score in force-fed birds when the diet contained an additional mixture of choline, vitamin B_{12} and vitamin E. Leeson *et al.* (1991) showed that adding as much as 132 mg niacin/kg diet had no effect on liver fat score. Harms *et al.* (1977) did however show an increased liver size (2.5 $\rightarrow$ 3.4% body weight) and increased liver fat content of layers fed up to 5,000 ppm iodine. Feeding such high levels of iodine has been shown to stop egg production, and so this will likely influence energy balance.

Due to an apparent effect of geographical location on the incidence of FLHS, Jensen *et al.* (1976a) analyzed water samples from over twenty different farms located in various regions. Water samples from farms reporting problems with FLHS had significantly elevated levels of Ca, Mg, Sr, Na, Fe and Ba. The higher levels of Ca and Mg contribute directly to the hardness of the water. When hardness *per se* was considered, then it was found that FLHS rarely occurred with birds drinking soft water, whereas most farms with a history of FLHS, had moderate to hard water. Jensen *et al.* (1976a) also speculate on the role of manganese in drinking water, because this mineral is known to influence fat metabolism in mammals, and is present at much lower concentrations in corn than in wheat. However water manganese concentration did not correlate with FLHS, and no benefit was seen from adding additional manganese to the corn-based diets.

Roland *et al.* (1985) indicated that birds fed low calcium diets (1.7 vs 3.5%) developed increased liver fat with an increase in the liver hemorrhage score. Birds fed low calcium may have had higher endogenous levels of estrogen since these birds exhibited an enlarged ovary.

5.   POTENTIAL TREATMENT AND PREVENTION

5.1.   General considerations

FLHS is caused by a positive energy balance in high producing hens, especially when held in hot weather conditions. These three major factors suggest that producers should be prepared for potential FLHS mortality in flocks that are post-peak and maintained in hot environments. Because positive energy balance is a predisposing factor, then again the condition is most likely to occur when flocks are overweight.

5.2.   Energy balance

While birds in positive energy balance will not necessarily develop fatty livers, FLHS rarely occurs without excess energy intake. From a practical viewpoint, a positive energy balance is best monitored through the measurement of body weight. At least 1% of the flock should be weighed each 30 d if there is a history of FLHS, or if this is anticipated in late production.

There is obviously a fine line between providing adequate energy intake in order to maintain high and sustained peak egg numbers, and oversupply, leading to obesity. Certainly high-energy diets lead to increased energy intake. While maximising energy intake is often critical during early production (Leeson and Summers, 1991), it is reasonable to assume reduced energy needs once the peak egg mass has been produced. While a reduced intake of protein is often considered at this time, as exemplified by "phase-feeding", unfortunately, little thought is given to energy. The concept of phase-feeding energy will be beneficial at this time, because even if FLHS does not occur, excess energy intake by the layer is an economic loss. Reduced energy intake can be achieved by reduction in energy concentration (nutrient density) of the diet and/or by restricted feeding. With physical feed restriction, it has been shown that after the period of peak egg mass, then depending on breed or strain, feed intake can be reduced by 5-8%. Such restriction should be introduced gradually, over a 3-4 wk period, with the need and effectiveness monitored through measurement of body weight.

Other factors to consider in an attempt to limit energy intake are the use of finer particle size feed, and reducing the number of times that feeders are operated. The balance of energy:protein in a diet is also of importance in affecting body fat accumulation, where low-protein diets will enhance body fat accumulation. A conflicting situation therefore occurs at high environmental temperatures, where a commonly used industry practice is to reduce crude protein while making increased use of synthetic amino acids. This type of diet change may precipitate FLHS, and so the decision becomes a trade off, between alleviating heat stress vs the potential for FLHS.

5.3.   Season/environmental temperatures

In most situations, little can be done to counteract seasonal changes in barn temperature. Heat-stress type conditions certainly precipitate and/or accentuate the occurrence of FLHS, and so the usual management considerations for alleviating such conditions are relevant. Where there is seasonal variation in house temperature, then at least the manager can be prepared for an

increased incidence of FLHS in peak-heat conditions. Within controlled environment buildings, higher environmental temperatures can be used to advantage. After peak egg mass is achieved, then holding birds at 25-28°C vs 20°C will mean reduced energy intake, and so likely reduce the potential for FLHS.   Again such management considerations can be monitored through measurement of feed intake and body weight.

5.4.   Diet ingredients

There have been many attempts at alleviating or preventing FLHS through diet manipulation. Addition of so-called lipotropic agents, such as choline and methionine, are often advocated and sometimes with apparent success. Such diet changes usually involve the addition of up to 0.1% methionine and 500 mg choline/kg diet. The use of distillers grains, solubles and other fermentation products is of dubious benefit. Together with fish meal, these types of product may have enriched the diet with selenium, which was invariably omitted as a supplement from most of the nutrition studies conducted on this topic in the 1970's. High levels of selenium, vitamin E, and synthetic antioxidants such as ethoxyquin, will likely be beneficial, since FLHS is associated with peroxidation of liver lipids (Squires and Wu, 1992). Diets should contain ethoxyquin, 0.3 ppm selenium, and up to 50 IU/kg vitamin E when treatment or prevention of susceptible flocks is considered.

There does seem to be some benefit to adding fat to a diet. This apparently contradictory situation, is based on the premise that birds consuming fat vs carbohydrate will need to synthesise less fat in the liver, and so reduce hepatic metabolism.   An obvious corollary to this recommendation is that when fat is added to a diet, it merely replaces carbohydrate, and that diet energy *per se* is not increased. Higher levels of diet fat may necessitate higher levels of ethoxyquin and vitamin E to prevent peroxidation (Squires and Leeson, 1988).

Chapter 5

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CHAPTER 6. FATTY LIVER AND KIDNEY SYNDROME

Other names:	NONE
Species:	YOUNG BROILER CHICKENS

1. COMPENDIUM

Diets deficient or marginal in biotin can lead to (FLKS) in young broilers. The condition is most commonly seen in 2-4 wk old birds fed wheat-based diets, although it seems as though some environmental stress is necessary to trigger the condition. Onset of symptoms occurs quickly, with apparently healthy birds exhibiting lethargy and general reluctance to move. Prior to death, birds may lay prone on the litter with necks outstretched. Mortality of 5-20% can occur. Gross pathology reveals enlarged and pale kidneys and liver, both of which have extensive fat accumulation. This fat is usually mono-unsaturated, and there are unusually high levels of palmitoleic acid rather than stearate. Staining of the liver reveals depleted glycogen reserves. Death is ultimately caused by hypoglycaemia due to failure of hepatic gluconeogenesis which in turn is triggered by inadequate levels of the key biotin- dependent enzyme pyruvate carboxylase.

Stress is a major contributor to the severity of FLKS, and under experimental conditions onset is most often induced by sudden change in temperature. Stress likely induces an epinephrine induced catabolism of the already low glycogen reserves. The condition is prevented by ensuring adequate levels of available biotin in the diet, and that broiler chicks hatch from eggs with adequate biotin carry-over. Both broilers and breeders should be fed diets containing at least 0.2 mg available biotin/kg diet. This level will ensure adequate biotin reserves, even when low-protein diets are used, or when sulfa drugs are used to treat other infections.

2. OCCURRENCE AND GENERAL SIGNS

FLKS causes accumulation of large amounts of fat in and around the liver and kidneys of young birds. Marthedal and Vellinge (1958) were one of the first groups to describe the condition in Denmark and since that time mortality ranging from 6-20% has been reported. The condition is diet related and is most obvious when using low-protein diets containing large quantities of wheat which contains little available biotin.

FLKS most frequently occurs in birds 17-30 d of age and is characterised by the sudden onset of birds appearing lethargic and being reluctant to move. Birds sometimes show paralysis by lying on their breast with necks extended. Death most often occurs within 2-3 hours of onset of first signs. The condition is often associated with or triggered by some form of stress such as change in temperature, noise etc. Although it is now known that biotin deficiency is the major cause, it is very rare to see classical biotin deficiency, such as dermatitis, in affected birds. For this reason, biotin

deficiency was discounted for a number of years. In measuring tissue biotin levels, Bryden (1991) indicated that within 14 d after hatch, liver biotin reserves were depleted to a level commensurate with potential for FLKS. However liver biotin had to remain low for 3-4 wk before overt deficiency lesions or growth depression were seen. Therefore liver and kidney biotin levels are much more sensitive indicators (due to associated enzyme pathways) than is general circulatory or other tissue status.

Interestingly, the condition seems affected by biotin status of the very young chick, and so alleviation and/or prevention is often confounded by early life nutrition (0-3 d). In fact, there have been a number of reports that suggest that breeder nutrition is another confounding factor. Much of the research into FLKS occurred in the mid-70's. Obviously the condition was prevalent and problematic at this time, although its "sudden" appearance at this time is mysterious. It has been suggested that this period coincided with the general acceptance of including selenium rather than sources of unidentified growth factor, the latter most usually being rich in biotin.

3.   PATHOLOGY AND METABOLIC CHANGES

Birds often have a ketotic odor upon opening the body cavity (Wight and Siller 1975). Examination of dead birds reveals enlarged and pale colored liver and kidneys, while the adipose tissue appears pink likely due to congestion of the capillaries (Butler, 1976). The upper intestine may contain a dark-colored liquid of unknown origin, the description of which bears some resemblance to so-called "black-vomit". The proventriculus is also dough-like and the thymus weight can be markedly reduced. For example, Wight and Siller (1975) recorded thymus weight of 1.71 vs 2.86 g for control birds at 35 d.

Livers and kidneys of affected birds exhibit a 400-500% increase in the amount of triglyceride present, although phospholipid levels are unaffected (Whitehead, 1975). The liver and kidney usually contain increased amounts of mono-unsaturated fatty acids, and particularly palmitoleic, at the expense of stearic acid (Whitehead, 1975). As the fatty infiltration of the liver develops, deposits gradually radiate out from the portal tracts (Wight and Siller, 1975). Hepatocytes also show extensive cytoplasmic vacuolation. The nuclei of these cells are often enlarged and nucleoli prominent while staining reveals depleted glycogen reserves (Wight and Siller, 1975). In the kidney, fat accumulation is invariably confined to the proximal convoluted tubules which are swollen and pale such that renal structures are compressed (Butler, 1976). Fat also accumulates in regions of the digestive tract, while a pale colored heart is again due to fat infiltration of the myocardium. Because birds die within hours of initially exhibiting signs, fat infiltration of the liver must take place very rapidly. In contrast to this rapid deterioration of liver status, birds with early signs of FLKS show increased kidney fat, and so this is likely a more susceptible organ (Whitehead, 1975). Birds severely affected by FLKS have lowered blood glucose as well as the characteristic lipid accumulation, suggesting malfunction in both carbohydrate and lipid metabolism (Hood *et al.,* 1976). It seems as though these abnormalities are due to the impairment in activity of certain biotin-dependent enzymes. Death is ultimately caused by severe hypoglycaemia due to the failure of hepatic gluconeogenesis. Such failure in turn relates to the insufficiency in the key biotin-dependent enzyme, pyruvate carboxylase. Under the conditions of marginal biotin deficiency, the level of liver biotin seems to have a differing effect on two biotin-dependent enzymes, namely acetyl co-enzyme A (CoA) carboxylase and pyruvate carboxylase (Hood *et al.,* 1976; Pearce and Balnave, 1978). It seems as though acetyl CoA activity is increased in situations of biotin deficiency, while if

liver biotin levels are <0.8 μg/g, then pyruvate carboxylase levels may be insufficient to completely metabolize pyruvate via gluconeogenesis. With such impaired pyruvate metabolism, there is a compensatory increase in liver size, as well as an increase in the activities of the enzymes involved in alternate pathways of pyruvate catabolism. Blood lactate accumulates with the increased synthesis of fatty acids (Hood *et al.,* 1976). Why activity of one biotin-dependent enzyme should be depressed, while activity of another is elevated, is still unclear. Whitehead (1975) suggests that in situations of limited biotin supply, cytoplasmic acetyl CoA is more successful in sequestering available biotin, leaving little for pyruvate carboxylase synthesis in the mitochondria. Hood *et al.* (1976) suggests that when liver biotin is < 0.35 μg/g, and the bird is subjected to stress, accumulation of fat occurs in both liver and kidney, and death occurs due to failure in maintaining blood glucose levels. Pearce and Balnave (1978) found remarkably similar results, suggesting that high mortality due to FLKS occurred when liver biotin was <0.33 μg/g. By dosing birds with various carbohydrates, Bannister *et al.* (1975) showed that FLKS does not involve any major impairment to digestion or absorption, and that hypoglycemia is due to a derangement in post-absorptive carbohydrate metabolism. In a subsequent study, Bannister (1976) did show that FLKS birds had virtually no gluconeogenic activity in the liver, and while biotin was without effect in healthy birds, it had a dramatic effect on gluconeogenic activity of FLKS birds both *in vivo* and *in vitro*.

Evans and Bannister (1974) found a marked reduction in the lipoprotein lipase activity in adipose tissue of affected birds, and this was associated with elevated plasma triglyceride. These authors suggest that the reduced lipoprotein lipase activity, together with reduced rate of clearance of plasma triglyceride are probably important factors in the development of fat depots in various organs. Evans and Bannister (1974) also showed that FLKS birds were invariably hypoglycemic and that this was associated with elevated plasma pyruvate and grossly deficient hepatic gluconeogenesis and glycogen stores. Attempts to restore hepatic activity with known co-factors were generally unsuccessful, with the notable exception of biotin.

FLKS is not however related to a "simple" deficiency of biotin, but seems to require the trigger of some external stress. If birds are not subjected to stress, they can perform quite well even with a marginal biotin deficiency (Pearson *et al.,* 1976). Therefore under normal conditions, birds appear able to tolerate low hepatic pyruvate carboxylase activity, since their glucose needs are met from the digestion of carbohydrate or pyruvate carboxylase-independent pathways of gluconeogenesis (Whitehead, 1975). However if these alternate pathways are impaired, or glycogen reserves are low, then hypoglycemia and death quickly occur. This scenario is somewhat similar to the condition known as Addison's disease in man, where cold stress triggers a hypoglycemic condition (Payne *et al.,* 1974). In this situation excitement or cold stress cause increased release of epinephrine from the adrenals, the effect of which is to quickly deplete glycogen reserves in an attempt to stimulate blood glucose levels. In adrenalectomised rats, biotin is almost as effective as cortisone in prolonging survival.

4.   RELATED FACTORS

4.1.   Diet composition

Most problems with FLKS have occurred with birds fed diets containing appreciable quantities of wheat. Payne *et al*. (1974) suggest that symptoms of FLKS are similar to "six-day

chick-disease" described in the 1940's when wheat was the major cereal used in poultry diets. Pearce (1975) observed 5-6% FLKS mortality in birds fed a diet containing 80% wheat, while no mortality was seen in birds fed a corn/barley mixture. Supplementing this wheat diet with a number of B vitamins (but not biotin) was without effect. At that time, Pearce (1975) discounted biotin as a potential factor because birds did not exhibit classical signs of biotin deficiency, and the biotin content of the wheat and corn-barley diets appeared to be comparable. Contrary to these findings, Blair *et al.* (1975) were unable to relate cereal type to incidence of FLKS. However, because biotin status is intimately involved, then diet levels must be a factor, and biotin availability comes under suspicion. Bannister (1976) assumed 60% availability of total biotin in their diet, although many nutritionists now assume biotin in wheat to be totally unavailable. Using Australian wheat, Bryden (1990) suggests 12% availability of biotin.

In addition to the potential problems of cereal type, Bryden (1990) also intimates variable response related to cereal cultivar. So-called low-ME wheats induced more severe classical biotin deficiency symptoms, although incidence of FLKS was reduced. Adding 75 μg biotin/kg diet resolved problems with feeding both types of wheat, which had comparable levels of assayed available biotin. Interestingly the low ME wheat was much higher in CP at 17.7 vs 11%. Bryden (1990) concludes that the variable nutritive composition of wheat, and especially its energy and/or protein contents may explain differences in the development of biotin deficiency and incidence of FLKS.

The effect of diet protein level *per se* has also been suggested as a factor. For example, Whitehead and Blair (1974) recorded 28% mortality due to FLKS when an 18% CP diet was used, whereas only 10% mortality occurred with a 22% CP diet. Also the low-protein diet needed 0.1 mg/kg supplemental biotin to resolve the FLKS problem, while only 0.05 mg/kg was necessary with the higher protein diet. Blair *et al.* (1975) suggested that elevating the protein level of chick diets has some protective effect against FLKS and that high mortality was most evident when low-protein diets were pelleted. These same workers also indicated that elevated diet fat levels were beneficial. Whitehead *et al.* (1975) also showed reduction in FLKS mortality from 19 to 7%, due to substitution of fat for starch in the diet. Work from this laboratory also suggest there to be a beneficial effect of adding saturated fats to the diet versus the harmful effects of using unsaturated oils. However, this latter scenario relates more to effects on growth and dermal lesions rather than FLKS *per se*.

4.2.   Stress

There is no doubt that some form of stress is necessary to induce high mortality due to FLKS. In most research trials, stress has been artificially induced by a sudden change in environmental temperature and/or 24 h starvation. While Balnave and Pearce (1976) showed no effect of a 30 h period of starvation, most other workers have recorded high mortality only when the birds are repeatedly stressed (Whitehead *et al.,* 1975). In another trial, Whitehead *et al.* (1976) recorded 25% FLKS mortality to occur over 24 hrs, and that this followed a day when the birds had been subjected to the stress of individual inspection and manual weighing. Under commercial conditions birds are subjected to a number of stress situations involving temperature fluctuation, noise, ammonia, stocking density, diet change, medications etc.

4.3.   Intestinal status

Under normal conditions, a significant quantity of biotin is expected to be synthesized by the gut microflora. It is not known if cereals like wheat impair such synthesis, although it is known that wheat can change the viscosity of digesta. With mash or pellets vs whole grain diets there is also less evidence of coprophagy or litter eating, and so less chance of recycling any biotin synthesised by gut flora. Intestinal synthesis can be reduced by administering certain antimicrobial drugs, and especially sulfur containing compounds. Whitehead (1978) indicate that these drugs eliminate coliform organisms, and it is assumed that these are responsible for biotin synthesis. There is evidence of increased severity of biotin deficiency when these drugs are added to diets deficient or marginal in biotin. Also if birds eat their own or other eggs then biotin can complex with avidin in the gut, making it virtually unavailable to the bird *(See section 4.4)*.

4.4.   Breeder nutrition

Biotin status of the young chick has a marked effect on susceptibility to or ability to treat FLKS (Whitehead, 1975). Payne *et al.* (1974) also intimate that biotin level of breeder diets is a variable factor in the etiology of FLKS, while Pearson *et al.* (1976) observed greater incidence in chicks produced by young breeders that deposit sub-optimal levels of biotin in the first few eggs that they lay. Also because very few shell-less eggs, or eggs with cracked shells are collected, it is suspected that they are consumed, and so avidin in the albumen will adversely affect the biotin status of these birds. Unless chicks hatch with adequate biotin reserves, then they will be more susceptible to conditions that precipitate onset of FLKS.

4.5.   Genetics

There has been little work aimed at defining a genetical basis for FLKS, although bird's requirements for biotin, like any other nutrient, are likely to be variable. Bannister *et al.* (1985) based on an index of liver size in biotin deficient birds, suggested there to be 2 or 3 possible sub-groups within a population. FLKS susceptible birds may therefore have diverged from normal development and these susceptible birds show marked changes in enzyme activation related to gluconeogenesis.

5.   <u>POTENTIAL TREATMENT AND PREVENTION</u>

Both prevention and/or treatment revolve around adequate biotin status of the diet. Because of variable biotin availability in cereals and other ingredients, diets should contain supplemental biotin. Biotin status of young broilers will be affected by breeder nutrition, as well as protein level of starter and grower diets. Susceptibility to FLKS is likely to be most acute in broilers hatched from young breeders when both are fed wheat as the major cereal source. Because a number of environmental and dietary stress factors can adversely affect the biotin status of broilers, it is suggested that both broilers and broiler breeders be fed a minimum of 0.2 mg supplemental biotin per kg diet. This level may need to be increased when low protein diets are used, if sulpha drugs are administered, or if unstabilized fat is included in the diet.

Chapter 6

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<table>
<tr><td colspan="2" style="text-align:center">CHAPTER 7. GOUT AND KIDNEY UROLITHIASIS</td></tr>
<tr><td><u>Other names:</u></td><td>VISCERAL GOUT
ARTICULAR GOUT
BABY CHICK NEPHROPATHY
BLUE COMB</td></tr>
<tr><td><u>Species:</u></td><td>ALL BIRDS</td></tr>
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1.   COMPENDIUM

Kidney dysfunction often leads to either visceral or articular gout, or urolithiasis. In all situations, increased substrate load to the kidney eventually leads to precipitation of insoluble products within the kidney itself or in other regions of the body.

Gout describes the condition in which high plasma uric acid leads to precipitation of monosodium urates either in the synovial fluid and tendon sheaths of various joints, especially the hock joint, or on the serous surface of various visceral organs. There seems to be a genetical basis for gout, although interestingly the articular and visceral forms are rarely seen together. Baby chick nephropathy and blue comb disease may be forms of gout. Articular gout is most commonly seen in birds fed excess levels of protein, and/or where the balance of K+Cl:Na is <1. Urolithiasis most commonly occurs in Leghorn birds, and is induced by feeding high levels of calcium for a long time prior to sexual maturity. Feeding diets containing 3-5% calcium after 4-6 wk of age invariably leads to a proportion of the flock developing insoluble uroliths in the kidney tubules. These uroliths are usually composed of calcium-sodium-urate.   Often just one kidney is affected, and due to compensatory hypertrophy of the normal kidney, then asymmetry in size and weight can be used during diagnosis. There does not seem to be any problem with urolithiasis if pullets are fed high calcium layer diets for a more conventional period of 3-4 wk prior to sexual maturity. Mycotoxins such as oosporein and citrinin can also adversely affect kidney function, while urolithiasis at least is made worse when birds are infected with infectious bronchitis. Urine acidification can reduce urolith formation and solubilize already formed uroliths in mature birds. Ammonium chloride is an effective urine acidifier although this can cause metabolic acidosis which adversely affects shell quality, especially during hot weather. Urine can be acidified by feeding methionine hydroxy analogue, and this reduces kidney damage and urolith formation without causing acidosis or increased water consumption.

2.   OCCURRENCE AND GENERAL SIGNS

Urolithiasis and gout together are responsible for significant losses in poultry. In each situation, there is kidney dysfunction, and eventually birds most often die from kidney failure. With urolithiasis, there is a urolith build-up in various parts of the kidney that effectively impedes the normal urine flow to the drainage ducts. In both visceral and articular gout there is unusual uric acid metabolism that leads to urate deposits not only in the kidney but also on the surface of many visceral organs. In many situations, kidney damage is precipitated or aggravated by viral infection. For example, Slemons *et al*. (1990) indicate that influenza type A viruses isolated from wild ducks and injected into chickens caused swollen kidneys and visceral gout. Similarly Shirai *et al*. (1990) were able to induce visceral gout through the introduction of avian nephritis virus. In many instances therefore, viral infection may be a co-factor in any problems of kidney dysfunction, and this must always be considered when investigating etiology. If a viral infection is not a factor in the occurrence of kidney problems, then attention is usually focused on diet constituents, and in particular that of protein, minerals and water supply.

Urolithiasis can be one of the major causes of mortality in laying hens, Wideman *et al*. (1983) suggesting that it represents up to 20% of mortality over a normal laying cycle. Cowen *et al*. (1987) described an outbreak of urolithiasis in young pullets, where the mortality averaged 0.5% per wk. Mallinson *et al*. (1984) describe a comparable outbreak in layers that was thought to have multiple causes, the major problems being infectious bronchitis, Newcastle disease and Ca:P imbalance. Despite there being large unilateral uroliths in the kidney and/or severe renal atrophy, the layers maintained normal production up to the time that uroliths eventually blocked the renal tubules. Mallinson *et al*. (1984) suggest that such action produces acute renal failure, rapidly developing visceral gout, uremia and subsequently death.

3.   PATHOLOGY AND METABOLIC CHANGES

3.1.   Urolithiasis

Wideman *et al*. (1983) studied kidney function in 23-24 wk old birds from two commercial farms experiencing an outbreak of suspected urolithiasis. In most affected birds, the right kidney showed extensive damage, while the left kidney appeared normal. The damaged kidney showed a thickened and distended urethral wall, while the lumen was occluded with uroliths. The left kidney was hypertrophied but normal, and these authors cite other similar results reported in the literature. For example Niznik *et al*. (1985) suggest that any significant reduction in the nephron number caused by factors such as high dietary calcium can be masked by compensatory hypertrophy of remaining tissue in pullets showing urolithiasis. In these situations, the hypertrophy most likely results as compensation for the reduced function of the alternate kidney, and as such, asymmetry in kidney weight and appearance can be used as a diagnostic tool. Wideman *et al*. (1983) suggest the outbreak under study related to an increase in diet calcium and crude protein found in layer vs pullet diets, coupled with the stress of physically moving birds and their experiencing a change in watering system. Mallinson *et al*. (1984) state that during histological examination of affected birds, there was evidence of chronic glomerulonephritis, and interstitial nephritis.

Oldroyd and Wideman (1986) collected and analyzed uroliths taken from four commercial layer flocks and also from an experimental flock of immature pullets fed high calcium-low phosphorus diets. With one exception, the stones were found to be composed of compact masses of microcrystalline to fine pleomorphic crystals of calcium-sodium-urate, with a random substitution of magnesium for calcium and potassium for sodium. It is the calcium-magnesium content of these uroliths that distinguishes the condition from gout as described in the following section. Wideman *et al*. (1983) conclude that uroliths are produced by some type of transient insult rather than any progressive degeneration, however this does not explain why mortality often remains high in affected layer flocks long after the diet abnormalities are corrected. If urolithiasis is a progressive condition, then pathogenesis involves discrete destruction of entire tubules rather than there being general damage to specific tubular segments. For example, there could be a blockage in the glomerulus or tubule itself. Wideman *et al*. (1983) conclude that the impact of urolithiasis appears to arise mainly from a reduction in renal mass rather than a dysfunction in the clearance of any specific mineral(s) or nitrogenous compounds. Abrupt changes in the mortality pattern, likely relates to toxin accumulation and hence the reason for live birds within the flock rarely exhibiting urates scattered around the body.

3.2.   Gout

Birds are susceptible to gout because they are uricotelic and lack the enzyme uricase. Gout occurs as two distinct forms and Siller (1981) emphasizes that these conditions never occur concurrently, and so must be considered to be of different etiology. These two forms are articular gout where urate crystals appear in the joints and visceral gout which is characterised by white urate deposits on the surface of various visceral organs and membranes. For whatever reason, kidney dysfunction results in inadequate urate excretion and consequently hyperuricemia.

Chandra (1985) describes various cases of nephritis occurring in commercial poultry flocks. In most cases, the kidneys were swollen and congested, and greyish-white in color with a soft consistency. Apart from the kidney, the proventriculus, myocardium and lungs were the main cites of urate deposition. In the acute form, the kidneys were congested with varying degrees of tubule degeneration. The glomeruli appeared enlarged due to an increased cellularity as a consequence of endothelial cell proliferation and hyperplasia. In the subacute form, the tubules and ureters had excessive urate deposition and these caused tubule dilation. Chandra (1985) observed gout mainly in younger birds. In these cases, the urate deposition on the kidney surface was apparent as small foci with atrophy/aplasia of one or more lobes of the kidney. Severe urate accumulation was seen in the ureters and in the cloacal region. The breast muscle was also congested while the spleen was usually enlarged. Gouty nephritis was characterized by the presence of necrotic foci in the interstitium, and in severe cases, all the parenchymatous tissue was destroyed by urate granulomas. Chandra (1985) concludes that gouty nephritis has multiple etiology.

Austic and Cole (1972) studied the genetic basis of hyperuricemia. For over four generations, lines were selected for high plasma uric acid, and these birds compared with a random bred control line. While the two lines of chickens had vastly different plasma uric acid levels, they secreted about the same amount of urine. The selected line with hyperuricemia obviously had reduced renal clearance of uric acid, and this was determined at 40% of that seen in control birds. These birds developed articular gout, and this was characterized by the accumulation of urates in the synovial fluid and tendon sheaths of various joints, and especially the hock joint. In subsequent

studies, Austic and co-workers showed that the problem with renal clearance of uric acid related in some way to a deficiency within an organic acid transport mechanism. Zmuda and Quebbemann (1975) also developed a line of birds susceptible to gout and used this to study the site within the kidney of defective uric acid transport. In gouty birds, the tubular excretion ratio of preformed uric acid was only 36% of that seen in control birds, a value which agrees well with the 40% previously reported by Austic and Cole (1972). In comparison to this low excretion rate of preformed uric acid, the excretion of uric acid formed from guanine was equal in the two genetic lines. This data suggests that the uric acid transport is located in the pretubular membrane and that this is defective in gouty birds.

Siller (1981) gives perhaps the best description of visceral and articular gout. Visceral gout occurs as the result of a kidney dysfunction that results in progressive hyperuricemia and consequently the characteristic deposition of monosodium urate crystals on the serous surface of such organs as the heart, liver and peritoneum. Siller (1981) refers to this condition as nephrotoxic gout, as a means of differentiation with obstructive visceral gout, as can occur with urolithiasis or vitamin A deficiency. Why such urate crystals appear on the surface of these organs is not clear, since if precipitation *per se* was the trigger, then one would perhaps expect this to occur in the blood vessels. Siller (1981) describes articular gout as urate deposits in the leg joints, although less commonly, there is a deposition in the joints of the wing and vertebrae. Cutting into the joint reveals a semi-solid white urate-containing exudate. Siller (1981) indicates that with advanced articular gout, there may be degeneration of the leg muscles, again due to the influx of the urate crystals. Siller (1981) concludes that although unnaturally high levels of diet protein may be a factor in etiology of articular gout, the most likely cause is genetic predisposition and this is controlled by a simple recessive gene.

Visceral and articular gout are therefore two distinct problems, and it is rare to see urates in both the leg joints and visceral organs. As concluded by Siller (1981), if on rare occasions the urates are seen in the visceral and articular regions, then the latter are always non-inflammatory, probably due to the sudden onset of death associated with visceral urate deposits.

Baby chick nephropathy and pullet disease (blue comb disease) may also be related to gout since in both situations urate deposits are very common. Pullet disease was quite common in the 1950-60's when birds developed a characteristic blue comb and at necropsy revealed enlarged kidneys with urates. Siller (1981) indicates that baby chick nephropathy can cause 2-6% mortality in young chicks where the major clinical finding is renal damage and visceral urate deposits. The most striking macroscopic lesions are the swelling and paleness of the kidney with urates on the serous membranes of the viscera, giving it a white stippled appearance. Siller (1981) cites evidence that IBV is not a factor in chick nephropathy, and that the dam's nutrition, and perhaps her protein intake may be a causative agent.

4.   RELATED FACTORS

4.1.   Dietary minerals

Levels and balance of dietary minerals seem to be a predisposing factor in urolithiasis. Halvorson *et al*. (1982) described a field case in which adult Leghorns were exhibiting

approximately 0.3% daily mortality associated with mild to severe nephrosis and visceral urate deposits. The feed analysis subsequently revealed exceptionally high levels of dietary calcium. Higher than normal levels of phosphorus (0.8 vs 0.2%) have also been recorded to cause calcium deposition in the kidney of rats (Mars *et al*., 1988).

Anasuya (1983) suggests that it is a mineral balance that is the primary factor influencing the urinary calculi deposition in the kidney and associated organs/ducts brought about most commonly by excess of calcium in relation to phosphorus or low intake of phosphorus in relation to calcium. In humans at least, calcium oxalate is a common form of urolith, and this is predisposed by a deficiency of pyridoxine in the diet (Anasuya 1983). However, this form of urolith is rarely seen in chickens, which is most likely due to their being little oxalate naturally consumed in the diet. In poultry, most uroliths are composed of calcium-sodium-urate. Wideman and co-workers have carried out an extensive series of studies involving mineral imbalance and urolithiasis in immature pullets and laying hens. The most severe cases seem to occur when immature pullets are fed high calcium diets (3.5%) for an extended period of time prior to maturity. In field cases, however, the potential confounding effect of IBV infection should always be considered. Wideman and Glahn (1987) describe studies aimed at elucidating the deleterious effects of high-calcium grower diets and/or IBV on urolithiasis. The worst case scenario, in which 30% urolithiasis occurred, was induced by feeding a 3.5% calcium diet from 4 wk of age and subsequently challenging with IBV at 10 wk of age. In affected birds, there was a reduction in the number of glomeruli and tubules per kidney, although because of compensatory hypertrophy, kidney mass *per se* was not useful in a diagnosis. Such hypertrophy was noticed in undamaged tissues within an infected kidney or in the unaffected kidney. These authors suggest asymmetry of kidney weight as being one of the best indicators of urolithiasis in Leghorns, caused by high calcium diets. In subsequent studies, Wideman *et al*. (1985) differentiated effects due to calcium and those due to IBV. At 18 wk of age 14% of pullets fed 3.25% Ca - 0.4% AvP from 7-18 wk, exhibited urolithiasis while only 1% of pullets were affected when the phosphorus level was maintained at 0.6%. However these later birds did exhibit urolith formation during the subsequent layer period. These results suggest that feeding high calcium diets for extended periods of time (uncomplicated by IBV infection) can be detrimental and that problems may not always occur during the time of feeding. The effect of the phosphorus level in the diet is unclear at this time.   Low diet phosphorus stimulates 1,25 dihydroxycholecalciferol production, leading to an increased uptake of both P and Ca from the digesta. Consequently, the parathyroid glands may be inhibited leading to hypercalciuria. Urine flooding with Ca is a common safety valve for attenuating hypercalcemia.

In further studies of the independent roles of diet Ca and IBV, Glahn *et al*. (1988a) fed diets containing 1 or 3.5% calcium to immature pullets with or without IBV challenge. The high calcium diet resulted in increased excretion of calcium and a decreased excretion of PO_4 in a urine that was more alkaline. The high diet calcium caused an 11% incidence of urolithiasis and this was not enhanced by IBV. In this study, IBV was given 2 wk prior to the high calcium diet and the authors conclude that the effects of IBV may be more severe if the infection occurs after the diet challenge since at this time the urine is already alkaline and high in calcium. This same group studied the role of water restriction on urolith formation, as it is assumed that a mild restriction may occur naturally when pullets are initially located in laying cages, especially when a change in the watering system is involved. Surprisingly, depriving birds of water for up to 5 d had no effect on urolithiasis. This was somewhat surprising since *a priori* it was expected that with reduced urine flow there would be greater chance for mineral precipitation. Julian (1982) describes two cases in which water deprivation resulted in urolithiasis. Birds died of renal failure with signs of visceral gout. The author

describes several scenarios for the effect of mild to severe water deprivation as they may influence renal function.

4.2.   Diet electrolytes

Because diet electrolytes can influence water balance and renal function, it is often assumed that an electrolyte excess or deficiency may be predisposing factors in urolithiasis or gout. McNabb and McNabb (1975) suggest that since salts of uric acid are very insoluble, then the excretion of precipitated urate salts could serve as a water conservation mechanism, especially when cations are excreted during salt loading or when water is in short supply. When roosters were given saline water (1% NaCl) and fed high-protein diets, the uric acid excretion rates doubled relative to birds offered the high-protein diet but with non-saline drinking water. Because uric acid colloids are negatively charged, they attract cations such as Na, and so when these are in excess, there is an increased excretion via urates, presumably at the expense of conventional NH_4 compounds (McNabb and McNabb, 1975). Wideman (1985) cites evidence from the UK of an imbalance of Na+K-Cl levels influencing kidney function. When excess Na+K relative to Cl is fed, a small percentage of the birds develop urolithiasis. It is likely that such birds are excreting a more alkaline urine, a condition which encourages mineral precipitation and urate formation.

Condron and Marshall (1985) indicated significant loss of water and net loss of Na^+ and K^+ in birds infected with IBV. In most instances, the birds became dehydrated and the ureters were distended with urates. With IBV infection, there was a 50% increase in Na^+ and K^+ loss in the urine and 33% of ingested Na^+ appeared in the urine, compared with a 10% appearance for non-infected birds. It appears as though IBV influences sodium resorption from the proximal tubules and this influences both water loss and potential for urate formation. Contrary to these findings, Martindale (1975) suggests there is little evidence for a direct effect of high salt diets on renal function in young birds. Similarly, Siller (1981) cites evidence suggesting that diet sodium levels *per se* are probably not an issue in the development of gout. However the balance of anions:cations may be important, because Siller (1981) infers that if K+Cl:Na is <1, then there is a greater chance of visceral gout occurring, and that mortality may be increased. Again this effect may relate to urine pH and urolith/urate solubility.

4.3.   Diet Protein

An increased intake of crude protein will likely result in increased uric acid production, especially in older birds. McNabb and McNabb (1975) cite evidence which suggests that even though increased protein intake results in increased urate N excretion, the proportion of nitrogen excreted as uric acid does not change. Hocking (1989) describes articular gout in male broiler breeders fed very high (40%) protein diets. As diet protein increased from 11% to 40%, there was a linear increase in plasma uric acid. In a subsequent study involving more practical levels of diet protein, Hocking (1990) observed no effect of feeding diets providing 11-16% CP on the occurrence of articular gout, although again high intakes of protein caused elevated plasma uric acid. Hocking (1990) suggests that gout could be misdiagnosed as a bacterial infection, because signs develop slowly and are non-acute until the bird experiences locomotory problems.

Zemel *et al*. (1981) found an increase in urine calcium for humans in response to increased protein intake. When protein intake increased, hypercalciuria developed, and urine calcium levels were almost double to that seen when an equivalent level of sulphur amino acids (SAA) were fed. With an increased intake of SAA, there was still an increase in urine Ca. The authors conclude that there is a decrease in the fractional tubular resorption of calcium due to increased urine levels of SO_4^- and H^+, both of which are end products of SAA catabolism. The fact that proteins *per se* induce greater hypercalciuria, means that other factors are also involved, likely via effects of insulin, GH and/or glucocorticoids (Zemel *et al*., 1981). The protein-calcium-SAA mechanism may be further complicated by water availability, since Ward *et al*. (1975) indicated that water restriction of birds resulted in an increase in the proportion of urinary N excreted as ammonia rather than uric acid. Chandra *et al*. (1984) studied the effect of feeding high levels of diet protein (42%) to young broiler chickens. This level of protein was used in conjunction with high or low levels of calcium, and diets containing regular or deficient levels of vitamin A. Birds fed a vitamin A deficient diet exhibited a slight increase in serum uric acid by 9 wk of age, while diets high in both calcium (3.4%) and protein caused this parameter to increase 10 times. While these plasma levels took some considerable time to plateau, within 2-3 wk of feeding the high protein diet there was a steady increase in levels of serum glutamate oxaloacetate transaminase. Chandra *et al*. (1984) explained these results on their being severe nephritis as diffused uric acid tophi replaced parenchymatous tissue.

4.4.   *Mycotoxins*

Many of the mycotoxins found in poultry feed can directly or indirectly influence kidney function. Ochratoxin, citrinin and aflatoxin are all known to influence kidney metabolism, and most often excretion/resorption of water, electrolytes and/or uric acid are involved. For example, Mollenhauer *et al*. (1989) fed aflatoxin at levels up to 5 ppm of diet, and among other things, examined kidney structure and function. Feeding up to 2.5 ppm aflatoxin had little effect on renal integrity although a number of chicks showed signs seen in birds fed higher levels. At 5 ppm, there was no change within 3 d, but after 21 d a number of cellular changes were evident, the most consistent being the thickening of glomerular basement membranes. While these changes were usually associated with crystalline deposits in the tubules there was only sporadic disruption to their normal function (Mollenhauer *et al*., 1989). Although not measured in this study, aflatoxin B has been recorded to increase the excretion rates of Na and K. There are no reports of aflatoxin influencing uric acid levels in the blood, although ochratoxin at 1 ppm has been reported to increase blood uric acid level by almost 20%. As citrinin level of the diet is increased, there is an increase in water intake and urine output. For example, Gustavson *et al*. (1981) observed that birds fed up to 400 ppm citrinin showed a linear increase in urine output. When water intake was restricted, the urine flow was reduced suggesting that water imbalance *per se* is a factor in citrinin toxicity. Hnatow and Wideman (1985) indicated that citrinin infusion had no effect on the glomerular filtration rate, renal plasma flow, urine pH or Ca and Mg excretion. Citrinin did increase the urine flow, however, and also the fractional clearance of Na, K and PO_4. However water intake increased to a greater extent than mineral secretion, and so citrinin resulted in reduced urine osmolarity. Interestingly, Hnatow and Wideman (1985) recorded normal renal function within 30 minutes of cessation of infusion. Even with infusion concentrates of 200 or 800 ppm citrinin, there was no observable tissue damage. Contrary to this finding, Campbell *et al*. (1981) had previously suggested that birds fed $\geq$ 125 ppm citrinin exhibited enlarged kidneys as well as a 50% increase in water intake and wetter manure. These authors suggest citrinin to be nephrotoxic.

The nephrotoxic properties of oosporein were studied by Pegram and Wyatt (1981). Oosporein is a toxin produced by various *Chaetomium* species of fungi and is found in various feed ingredients. Feeding up to 100 ppm caused no measurable problems, although from 200 -600 ppm there was a closely related effect on mortality, essentially due to visceral and articular gout. Necropsy revealed massive urate deposits in various tissues, swollen and pale kidneys, dehydration and an enlarged proventriculus. Although dehydration occurred, oosporein did stimulate water consumption. The authors suggest the nephrotoxic properties of oosporein to occur through the impaired renal clearance of uric acid. This situation is somewhat unique, since as described previously by Siller (1981) it is uncommon to see visceral and articular gout in the same bird. The hyperuricemia seen in these birds (Pegram and Wyatt, 1981) likely relates to impaired renal clearance, since feed and N intake were reduced in response to this mycotoxin. Because considerable bird to bird variation was seen, it is possible that response relates to genetics in some way.

4.5. Other diet factors

Because kidney dysfunction often involves hyperuricemia it is assumed that feeding high levels of uric acid would be problematic. Martindale and Lee (1976) investigated the effect of feeding birds high levels of urates in the form of dried poultry manure. Surprisingly there was no effect on kidney weight or function by feeding up to 20% manure. There was an increased renal plasma flow, presumably as a consequence of the need to clear urates, although there was no change in tissue integrity. Siller (1981) cites considerable evidence for the involvement of vitamin A deficiency in kidney dysfunction and visceral urate deposition. Siller (1981) suggests that the most characteristic lesions are the dilation and the impaction of the kidney collecting ducts in the medulla, with the accumulation of cell debris, inflamed cells and urates. These changes are accompanied by severe interstitial fibrosis and compression of other medullary tissues. In the cortical tissues vitamin A deficiency resulted in hyperemia and areas of tubular degeneration and necrosis. Deficiency of vitamin A probably leads to the keratinization of tubular epithelium, since this is common to other epithelial tissues in the body. Chandra *et al*. (1984) suggest that hyperuricemia and nephritic lesions occurred only after vitamin A levels in the liver were below 24 IU/g tissue.

5. POTENTIAL TREATMENT AND PREVENTION

Urolithiasis seems to be most problematic in laying hens fed high levels of calcium well in advance of sexual maturity. Although the situation is often confounded with IBV infection, it seems obvious that no more than 1% calcium should be fed to Leghorn birds prior to maturity. Feeding pre-lay (2% Ca) or layer diets containing 3% calcium for 2-3 wk prior to first egg is not problematic, and surprisingly, uroliths rarely form in adult male breeders fed high calcium diets. Visceral and articular gout both seem to have a genetic component, since the occurrence is sporadic within a flock. High levels of crude protein will increase plasma uric acid levels, and so potentially provide conditions conducive to urate formation. Certainly numerous mycotoxins influence kidney function, and so general mill management regarding quality control and/or use of feed additives to suppress their harmful effects would likely be beneficial.

Most forms of kidney dysfunction are associated with an increased loss of water and electrolytes, and so electrolyte therapy is often considered. Condron and Marshall (1975) suggest the addition of potassium and to a lesser extent sodium salts, especially citrates and bicarbonates. However, such therapy is not straightforward because feeding additional sodium will exacerbate problems of water loss. Also death from acute renal failure is frequently related to cardiac arrest caused or induced by high levels of plasma potassium.

In humans at least, urolith formation can be prevented or treated through the use of urine acidifiers. Glahn *et al*. (1988b) studied this possibility in pullets intentionally fed high-calcium diets prior to maturity. At 32 wk of age, birds were fed either 1% NH_4Cl or 1% $NaHCO_3$, and performance monitored for 20 wk. Birds fed NH_4Cl developed increased blood H^+ and reduced HCO_3^- content. At 52 wk these birds had a more acidified urine, and none of these birds had uroliths in the kidney. Urolithiasis (8%) did occur in the birds fed the untreated diet while 13% of birds fed the alkaline diet developed this problem. Unfortunately all treatments resulted in an increased water intake (20%) and manure moisture was increased by 10-14%. Stevens and Salmon (1989) fed up to 3% NH_4Cl to young turkey poults. Diet acidification resulted in a 30% increase in kidney weight together with increased fecal moisture content. A concomitant reduction in tibia ash led Stevens and Salmon (1989) to conclude that the acidified urine resulted in increased calcium solubilization in the urine. Wideman and co-workers have shown that urine acidification can reduce urolith formation in immature birds, and have also shown that such treatment can solubilize uroliths already formed in mature birds. One of the potential problems in using NH_4Cl in laying hens, is that it induces a metabolic acidosis and this is detrimental to eggshell quality especially under conditions of heat stress. Such treatment also assumes the kidney can clear the increased load of H^+, and for a damaged kidney, this may not always occur. As a potential urine acidifier without such undesirable side effects, Wideman *et al*. (1989) investigated the role of methionine hydroxy analogue (MHA) free acid. From 5-17 wk of age, pullets were fed diets containing 1 or 3% calcium in combination with 0, 0.3 or 0.6% MHA. Birds fed the untreated high-calcium diet excreted alkaline urine containing elevated calcium concentrations together with urolith formation and some kidney damage. Feeding 0.6% MHA acidified the urine, but did not cause a general metabolic acidosis. MHA therefore reduced kidney damage and urolith formation without there being acidosis or increased water consumption. In subsequent studies Wideman *et al.* (1993) also showed supplemental DL-methionine to be effective in preventing kidney damage.

It is concluded that urine acidification can be used as a prevention or treatment of urolithiasis, and that this can be accommodated without necessarily inducing a generalized metabolic acidosis. From a nutritional viewpoint, kidney dysfunction can be minimized by not oversupplying nutrients such as calcium, crude protein and electrolytes.

Chapter 7

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CHAPTER 8. OILY BIRD SYNDROME

<u>Other names:</u>	NONE
<u>Species:</u>	ALL BIRDS

1.   COMPENDIUM

Oily bird syndrome (OBS) results in broiler carcasses that are oily and greasy to the touch, and often have pockets of water accumulating in regions beneath the skin. The condition is most prevalent in warm climates and is accentuated by harsh processing conditions and especially scald temperature and pick-time. OBS is most noticeable in female broilers. OBS is not due to the incorporation of unsaturated oils into the diet, and in fact there is an indication of a greater incidence when tallow is fed. Characteristics of OBS are caused by changes in skin collagen structure. The various skin layers separate more easily and oil and/or chilled water accumulates in the discreet pockets, especially in the back region. The skin of affected birds shows a deficiency in collagen crosslinking that has been described as an immature development of this important structural bonding. Collagen maturation can be adversely affected by a deficiency of copper or an excess of vitamin A in the diet. However, because broiler performance is often normal when outbreaks of OBS occur, then diet imbalance *per se* is not likely a major factor.

2.   OCCURRENCE AND GENERAL SIGNS

As its name implies, birds with OBS have skin that is oily or greasy to the touch. Garrett (1975) was one of the first to document the condition, indicating the subcutaneous fat, especially in the back region, to be very oily. At that time, Garrett (1975) suggested that OBS was observed most frequently in older broilers and especially those fed high energy diets in the warmer summer months. The problem also seemed to relate to specific processing plants, where the occurrence was greater with the increased "stress" applied during processing, and especially plucking. Interestingly, the condition is rarely seen in hand-plucked birds. Garrett (1975) also saw the condition associated with increased water retention in the carcass, especially in regions of the carcass where skin "elasticity" had been affected. These pockets of water were most often seen in female birds.

Jensen *et al*. (1980) describes the oily skin of affected carcasses, suggesting that the problem is most noticeable in pockets of the skin that separate in the back region. Because the skin seems more prone to tearing, these pockets rupture and coat the surrounding skin with oil.

3.   PATHOLOGY AND METABOLIC CHANGES

A general finding in situations of OBS is the change in the skin ultrastructure, such that, either the layers of skin separate to allow the pockets of oil and/or chilled water to accumulate, or the skin tears more easily. Apparently, fat saturation is not a factor in OBS (Horvat 1978), rather there being some change in the integrity of the various layers of the skin. Ramshaw *et al*. (1986) observed that the skin from affected carcasses could be easily separated and removed from the underlying musculature. The normal five collagenous layers beneath the epidermis seem less compact than normal, and the deepest layers contain the most fat cells. Ramshaw *et al*. (1986) suggest that in most mammals at least, skin strength is dictated by that of the middle dermal layer. While these workers saw no real change in total skin thickness with OBS, the breaking strength of the skin was markedly reduced. The melting point of fat in these birds at $61.5^{o}C$ was not different to that of control birds, again confirming no marked change in fatty acid profile.

Kafri *et al.* (1986) suggested that male broilers usually have a thinner skin than do females, but that this is usually stronger. The thicker less resistant skin of the OBS female may relate to hypodermal adipose tissue. Weinberg *et al.* (1986) also suggest that the skin of male birds tends to have greater tensile strength than that of females, although in both sexes, strength increased with age. Both Smith *et al.* (1977) and Granot *et al.* (1991b) suggest that the higher rate of skin tearing in females was related to reduced skin collagen content. Smith *et al.* (1977) also showed males to exhibit more insoluble skin collagen, and so this may be important in reducing the problems of solubilization and water uptake, as often occurs with OBS carcasses in chill-water tanks.

In more detailed studies of skin collagen in OBS birds, Ramshaw *et al*. (1986) suggested there to be a change in crosslinking. Interestingly the electrophoretic pattern of collagen was similar in all birds from an OBS flock, and different to that seen in normal flocks, even though all birds in the OBS flock did not exhibit the typical greasy skin condition. Ramshaw *et al.* (1986) showed that the main collagenous layer in the skin of OBS birds was 30% weaker at $36^{o}C$ than that from normal birds. When the temperature was increased to $90^{o}C$, then a 50% reduction in skin strength was observed. These workers observed no real difference in collagen chains, although there appeared to be a difference in intermolecular crosslinking in OBS skin, and Ramshaw *et al.* (1986) hypothesized that the major problem related to collagen crosslink maturation. In mammals at least, and in the formation of eggshell membranes, lysyl oxidase is thought to be the only enzyme involved in crosslink maturation of collagen and elastin, converting lysine and hydroxylysine into aldehydes (Eyre, 1984). Lysyl oxidase is a copper metalloenzyme that requires pyridoxal phosphate as a co-factor, and copper deficiency is known to impair normal collagen crosslink structure (Eyre, 1984).

4.   RELATED FACTORS

4.1.   *Processing conditions*

Scald water temperature and finger settings on plucking equipment seem to affect the severity of OBS. Fletcher and Thomason (1980) indicated that scalding at 60°C for 25 sec. vs 53°C for 60 sec. had little effect on moisture pick-up during subsequent water chilling, but did influence the skin. As scald temperature increased, there was a definite increase in oily skin appearance and severity. Also as picking time was increased, there was increased moisture uptake, and also more noticeable oily and loose skin with more areas of broken skin. Fletcher and Thomason (1980) conclude that higher scald temperatures coupled with a shorter pick-time should result in less subcutaneous chill-water uptake, and less broken skin, although this may be at the expense of a slightly more oily skin. While processing conditions are a major variable in OBS, it seems unlikely that the condition will be seen without there being some prior disruption of collagen crosslinking in the skin. At this time, the exact reason for this abnormal skin development is unknown.

4.2.   *Diet*

Because of the oily nature of the carcass, diet constituents, and especially fat, have been suspected of direct involvement. However there is no good evidence of a relationship with fat levels in the diet or of diet fat saturation. Jensen *et al*. (1980) showed no relationship between OBS and unsaturated fats provided in the diet. Garrett (1975) and Jensen *et al.* (1980) in fact suggested that OBS occurs more commonly in birds fed tallow rather than unsaturated oils, and that such affected birds often have more saturated fatty acids in their adipose tissue. The availability of diet energy *per se* or balance of energy:protein may also be factors in OBS. Granot *et al*. (1991b) showed that by increasing the crude protein content of the diet in relation to energy, then there is a reduced incidence of skin tears, while Kafri *et al*. (1986) conclude that higher energy in relation to protein in the diet produces a weaker and thicker (fatter) skin. Contrary to these findings Weinberg *et al.* (1986) observed no relationship between energy:protein in the diet and skin fat content or skin tensile strength. Smith *et al*. (1977) were also unable to show any effect of feeding broilers diets providing 3200 vs 3400 kcal ME/kg on total skin collagen or collagen crosslinking. However this later study was conducted in a cool environment. There has been some discussion on the role of various feed additives as they may influence OBS. For example, Granot *et al*. (1991a) suggest the anticoccidial halofuginone may suppress skin collagen synthesis and result in a thinner skin that tears more easily. Halofuginone caused skin fibroblasts to direct less proline into extracellular collagen. There have been no reports of other anticoccidials affecting skin development, and it should be emphasized that halofuginone has not been directly associated with OBS.

Because collagen crosslinking is apparently deficient in OBS birds, then diet copper and vitamin A levels have come under some scrutiny. Copper deficiency can reduce collagen and elastin crosslinking (Eyre, 1984; Ramshaw *et al*., 1986). However it is doubtful if simple copper deficiency or a deficiency induced by high levels of zinc are involved in OBS, because one would not expect normal growth and development under such severe nutritional imbalance. Garrett (1975) also cites evidence for potential of excess vitamin A to result in reduced skin keratinization. Lathrytic compounds, as sometimes occur in plant toxins, have also been shown to adversely affect collagen crosslinking (Ramshaw *et al*., 1986).

4.3.   Environmental temperature

Incidence of OBS has invariably been confined to broilers grown in warmer climates. Garrett (1975) indicates environmental temperature to be a major predisposing factor while Jensen *et al.* (1980) were only able to duplicate the condition by using warm growing conditions. With elevated environmental temperature, there is a greater deposition of carcass fat including subcutaneous depots.

5.   <u>POTENTIAL TREATMENT AND PREVENTION</u>

If OBS occurs, then the only immediate practical solution is to modify the processing conditions, and in particular, scald temperature and pick-time. Because the exact cause of impaired collagen crosslinking has not been identified, then other changes to the diet and/or environment are of questionable value. While fat levels in the diet do not seem to be a factor, there is an indication of more problems occurring with saturated fats such as tallow. The diet should contain adequate levels of copper and not contain excessive levels of zinc or vitamin A.

Chapter 8

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CHAPTER 9.   WATER IMBALANCE

<u>Other names:</u>	WET MANURE
	DEHYDRATION
<u>Species:</u>	MAINLY LAYING HENS, BUT ALL SPECIES CAN BE AFFECTED

1.   <u>COMPENDIUM</u>

Expectations of, and factors influencing normal water balance in poultry are discussed. The most common consequence of water imbalance is wet manure, which is problematic in modern poultry houses. Due to increased water:feed intake, wet manure is most commonly associated with elevated levels of sodium and potassium in the diet. Diet sodium levels should not exceed 0.15-0.20% of the diet, and where high-salt water is suspected, both feed and water should be assayed for sodium and chloride levels independently. A number of diet ingredients can stimulate water intake, the most common being barley, molasses and soybean meal. When ingredients such as barley are necessarily used due to economics, and wet (sticky) manure is a problem, then consideration should be given to use of synthetic β-glucanase enzymes. Laying hens also seem to produce a wetter manure, and especially under hot weather conditions when high calcium layer diets are introduced 2-3 wk prior to maturity. Wet manure due to high-calcium intake can be prevented by the use of appropriate pre-lay diets that contain intermediate (2%) levels of calcium.

Dehydration most commonly occurs due to equipment failure on the farm. Depending upon the degree of water restriction, the effect on poultry is usually not too severe. For example, in situations of force-molting, some types of bird are intentionally without water for at least 48 hours, and under these conditions, mortality is very rare.   For birds at peak egg production, an unintentional period of water deprivation will dramatically reduce egg output. Evidence is given of almost total cessation of production in a flock subjected to an unintentional 48 h period of water deprivation. Under these conditions however, a pause-in-lay is induced, and birds will resume normal production in 4-6 wk. Young turkey poults are most susceptible to water deprivation, where death often occurs when water is reintroduced.

2.   <u>OCCURRENCE AND GENERAL SIGNS</u>

Excessive drinking occurs periodically in poultry flocks as a consequence of various environmental stresses, while dehydration can result from mechanical failure of water supply systems. In most instances, birds will modify their water input/output mechanisms in order to obtain a physiological balance, although this sometimes results in management problems at the farm. With intensive mechanized housing systems, the major problem of water imbalance is wet manure, and this occurs most frequently in laying hens. Excessive water intake, for whatever reason, results

in increased manure water content, and this can lead to problems of manure handling, odor and fly control, dirty eggshells and increased barn humidity. The manure of layers will normally contain around 75% water, although it seems that even quite small changes in the water content can have a dramatic effect on its physical appearance, and handling characteristics. Environmental temperature, diet composition, feed texture and drinker design are all known to influence water consumption by poultry. Knowledge of water intake under various conditions is also important due to the fact that vaccines and other medicants can now be administered via the drinking water. Prior to the discussion of specific problems of water imbalance, it is pertinent to consider the normal physiological systems of water intake and output, and how these can be influenced by the commercial farm environment.

3.   NORMAL WATER METABOLISM

Water represents about 70% of total body weight. Of this body water, approximately 70% is intra-cellular and 30% extracellular, whilst 75% of the latter is present in the interstitial space and the remaining 25% is found in plasma (Houpt, 1970). Water balance and metabolism are related to the maintenance of a dynamic equilibrium within and between these compartments. Prior to outlining the processes involved in the maintenance or imbalance of this equilibrium, it is necessary to consider the various routes by which water enters and leaves the body of the fowl as variations in these activities affect the various methods by which an imbalance can occur.

3.1.   Water Intake

3.1.1.   Drinking water

For all poultry, actual water intake increases with age although its consumption per unit weight decreases with age. For growing pullets, water intake decreases from 0.45 g/g body weight at 1 wk of age to 0.13 at 16 wk of age (Medway and Kare, 1959). Brake *et al.* (1992) describe daily water intake in broilers to 21 d by the equation: 9.73 + 6.142 x d age (ml). Because water intake is closely correlated with feed intake, factors affecting feed intake indirectly influence water consumption (Bierer *et al*., 1966b; Zeigler *et al*., 1972). Although the texture of poultry diets does not appear to affect water consumption (Eley and Hoffmann, 1949), high levels of various dietary constituents such as molasses (Ross, 1960) and salt (Herrick, 1971) are known to stimulate water consumption. Differences in environmental temperature significantly affect water consumption by the fowl. Laying birds housed at 31°C will consume twice as much water as birds housed at 15°C (Snetsinger, 1973). Since feed intake decreases with increased environmental temperature, the ratio of water:food ingested by poultry is greatly increased at these temperatures. May and Lott (1992) indicate that with daily cyclic temperatures of 24-35°C, the water intake pattern of broilers closely follows ambient temperature. Compared to birds kept at constant 24°C that consume water at about 4% of body weight depending upon age, during heat stress, this value increases to 6% of body weight. Budgell (1970) describes three hypotheses to explain the relationship between water intake and environmental temperature: (a) stimulation of water intake at high temperatures due to local dryness of oropharyngeal receptors; (b) systemic dehydration, and (c) alteration in brain temperature. At cold environmental temperatures, water intake may be reduced (Parker *et al*.,

1972) although birds usually show little aversion to water as cold as $0^{o}C$. Metabolic body water, created from increased fat metabolism, may be of significance in contributing to the body water pool at low environmental temperatures.

Under commercial-type conditions it is difficult to accurately measure water intake on replicate groups of birds within a house. Lott *et al.* (1992) recently outlined a computerised system for monitoring both feed and water intake.

3.1.2.   Dietary water

The major feedstuffs commonly used in poultry rations contain from 5-15% water, while most complete diets contain about 10% water.

3.1.3. Metabolic water

Water is created in the body as a by-product of the catabolism of various metabolites. Different amounts of water are produced from the oxidation of fat, carbohydrate and protein. The oxidation of one gram of these nutrients yields 1.18, 0.6 and 0.5 g of water respectively. Total metabolic water can be more easily estimated from energy intake because about 0.135 g of water is produced per kilocalorie of energy consumed (Kerstens, 1964). Thus for a bird consuming 300 kcals ME per d, approximately 40 g of metabolic body water is produced and enters the body pool. This water represents about 15% of total water intake by the fowl. Some exotic birds weighing less than 50 g (therefore with high metabolic rates) are known to be capable of surviving without drinking water for many d on a diet of dry seeds (Wagner, 1964).

3.2.   *Water output*

3.2.1. Excreta

The quantities of water excreted in the feces and urine depend upon the water intake. Broiler chickens produce excreta containing about 60-70% moisture (Kerstens, 1964), while that produced by the laying hen contains up to 80% moisture (Anderson and Hill, 1968). Dicker and Haslam (1972) showed that laying hens produce about 12 ml urine daily per kg body weight. The fact that this value is doubled in birds with exteriorized ureters, suggests that considerable water resorption occurs in the large intestine. Two "types" of urine are produced; a fraction of low concentration produced following eating and drinking corresponding to an elevated glomerular filtration rate, and another urine fraction of higher osmolarity occurring when kidney filtration rate is low. Because the lining of the coprodeum and urodeum, as well as the rectum, are suited to water resorption (Clara, 1926) and that retrograde urine flow into the rectum has been demonstrated, (Akester, *et al.*, 1967), water resorption will occur and will be of the order of 25-30 ml/d for the adult fowl as long as urine is hypotonic to extra-cellular fluid (Dicker and Haslam, 1972). For the laying hen at least, the quantity of water excreted in the feces is about four times that excreted as urine
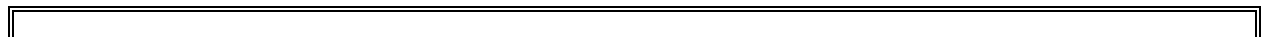
(Dicker and Haslam, 1972). Undoubtedly this loss is subject to considerable variation with the amount and hydrophilic nature of undigesta. Belay and Teeter (1993) indicate considerable variation in urine output in response to increased environmental temperature. Broilers subjected to heat distress at 35°C vs 24°C, showed a 133% increase in water loss that was associated with only a 78% increase in water intake. The increased water loss was due to greater output of a hypo-osmotic urine.

3.2.2. Evaporative loss

Evaporation is one of four physical routes by which the fowl can control its body temperature. Some 575 calories of heat are required to vaporize one gram of water. Evaporative heat loss takes place through the body surface and the respiratory tract. The fowl has no sweat glands, consequently evaporation via the skin is limited. Loss in this manner overwhelmingly occurs via the moist surface layer of the respiratory tract to the inspired air which is "saturated" with water vapor at body temperature. Evaporative rate is therefore proportional to respiratory rate. Heat lost through evaporation represents only about 12% of total heat loss in the broiler chicken housed at 10°C, but this increases dramatically through 26-35°C where it may contribute as much as 50% of total heat loss from the body (Kerstens 1964). High environmental humidity will reduce the efficiency of evaporative heat loss through the skin more so than via the lungs. *Table 9.1* shows the significance of evaporative water loss in broiler chickens at various ages and indicates a loss approximately equivalent to drinking water intake. In addition to promoting heat/water loss, panting can also precipitate other metabolic problems related to electrolyte balance *(See Chapter 10)*.

3.3. *Normal water balance*

Under normal physiological conditions for adult birds, water intake and output are controlled to maintain a constant level of water in the body. A positive water balance is found in the growing bird relative to deposition in developing tissue *(Table 9.1)*. Young birds have a greater proportion of body water than do mature birds. Lopez *et al*. (1973) recorded values of 57 and 76% (of body weight) for 7 year-old hens and 5 month old pullets. This effect may be related to increased fat deposition with age, since body fat and body water are negatively correlated, or due to increased proportions of other tissues of low osmotic activity, such as collagen and bone. Alternatively, a real decrease in intra- and extracellular body water with age may occur (Lopez *et al*., 1973).

		Water input (g/d)			Water retained as growth	Water in excreta	Evaporative loss
		Drinking	**Feed**	**Metabolic**	**(g/d)**	**(g/d)**	**(g/d)**
Age (wk)	**Env. Temp. °C**						
1	31	11	1	3	5	4	6
2	25	15	2	5	9	6	7
3	25	35	3	7	15	12	18
4	23	60	5	12	18	16	43
5	22	90	8	16	22	20	72
6	20	120	10	20	26	24	100
7	20	150	12	25	30	28	129
8	20	180	14	30	30	32	162

TABLE 9.1 Water balance in broiler chickens

(Adapted from Kerstens 1964)

As previously mentioned, water balance is achieved by the maintenance of an equilibria between the three water compartments of the body, *i.e.* intracellular, extracellular and plasma. The total volume of water is relatively constant under the conditions already described, although the fluid in the different compartments constantly interchanges. The concentration of water in these compartments is the same relative to osmotically active electrolytes, and a net shifting of water only occurs when an imbalance in osmolarity between two compartments occurs (Tosteson and Hoffman, 1960). Such changes in osmolarity may be due to loss of either water or electrolytes. Fluid transfer from the capillary to the extracellular fluid will only occur when the hydrostatic pressure in the former is greater than the osmotic pressure of the extracellular fluid (Goodman and Gilman, 1965). Barragry (1974) states that the principal cause of water or fluid shift between the extra-and intra-cellular fluids is a change in composition of the former, although a simple loss of extracellular fluid does not markedly effect the fluid within the cell, because its ionic composition remains relatively unchanged despite the fact its volume is reduced. Water movement across the cell membrane is initiated by changes in the concentration of the intra- and extra-cellular fluids, such that the initial equilibrium with respect to osmolarity is achieved. By using tritiated water, the rate of water turnover in the body in terms of radioactive half-life may be measured. Chapman and Mihai (1972) determined the half-life of body water in laying birds, non-laying birds and roosters to be 3.5, 5 and 7 d respectively. These values suggest that factors other than egg production *per se* influence the rate of water turnover in hens. Chapman and Mihai (1972) showed the laying bird to have a greater body water flux (pool size x turnover rate) than the non-laying bird (419 as opposed to 195 ml/d). Even accounting for the water content of the egg, body water flux in the laying hen is significantly greater than in the non-laying hen and may relate to an increased reservoir of soluble metabolites necessary for egg production (Chapman and Mihai, 1972). This scenario parallels the normal observation that the laying hen consumes more water than does the non-layer, and that this difference is much larger than can be accounted for by the egg and its synthesis. Body water flux in the laying bird is also greater than that recorded for the adult male bird (Chapman and Black, 1967).

Water turnover rates may be affected by age. Lopez *et al*. (1973) recorded values of 8.2 and 6.1 d for the half-life of body water in 7 year old hens and 5 month old pullets, respectively. These authors suggested that this age difference may be due to an increased utilization of body water by the younger birds for their greater anabolic needs.

4.   WATER IMBALANCE

4.1. Dehydration

Dehydration can occur as a consequence of either reduced water intake or increased water output. In commercial situations, the latter is most likely to occur, because increased water output is often a consequence of increased input, and so dehydration *per se* does not occur. Swayne and Radin (1991) describe the renal changes seen in birds subjected to 5 d water deprivation. Diarrhea is the most common reason for excessive water loss from the body. When the body loses water at a rate exceeding intake, circulatory fluid volume is decreased resulting in decreased hydrostatic and increased osmotic pressure. This depletion is compensated for by movement of extracellular fluid into the plasma. The greater susceptibility of the young animal to dehydration may be associated with the fact that older animals contain greater quantities of extracellular fluid in relation to their size.

Acidosis often develops in cases of diarrhea, due to the loss of alkaline digestive secretions. It has already been noted that feed and water intake are closely related. If feed intake is decreased, the accumulation of ketone bodies resulting from fat metabolism may aggravate any existing acidosis. Fisher (1965) suggests that acidosis may contribute to hyperkalemia by stimulating kidney potassium resorption in exchange for hydrogen ions thus elevating circulating levels of this element. Because of the dangers of hyperkalemia to the myocardium (Fisher, 1965), the administration of potassium containing fluids to the dehydrated animal (*e.g.* with excessive diarrhea) is a potentially dangerous procedure.

With drinking water supplied *ad libitum* in commercial poultry units, dehydration due to insufficiency of drinking water should not occur. The drastic effect of partial or complete deprivation of drinking water to the fowl is well documented. Ross (1960) observed that chicks offered water during three 1/2 h periods each day, consumed as much water as control *ad libitum* watered birds, but grew only 90% as well. This difference in growth was attributed to differences in feed intake. Kellrup *et al*. (1965) showed that 10% water restriction did not affect broiler performance, but that 20-50% restriction severely affected the feed intake:weight gain. No difference in chicken broiler performance was noted when growing birds were subjected to 24-72 h periods of water deprivation (Arscott, 1969). With birds of 8, 12 and 18 wk of age, Mulkey and Huston (1967) recorded a 45% loss of body water prior to death due to water deprivation. With 48-72 h periods of water deprivation in the laying bird, Adams (1973) observed a reduction in egg production from 65 to 15% over a two wk period. Under conditions of complete water starvation, Bierer *et al*. (1966a) found laying birds to survive about 8 d, whilst non-laying birds survived some 15 d longer. Early death in the layers was attributed to toxemia. *Fig. 9.1* indicates the layer's production response to an accidental 48 h period of water deprivation. Egg production declined quickly to almost 0%, although interestingly, a few birds maintained almost normal production. Most birds resumed production within 28 d and returned to their normal production level, albeit with improved eggshell quality.

Turkey poults are most susceptible to dehydration and mortality occurs when drinking water is reintroduced. Poults dehydrated from 11-13 d showed 83% mortality following the reintroduction of cold water; in most cases death occurred within 30 minutes (Marsden *et al*., 1965). Poults at 18 d showed less mortality which was somewhat delayed (2-34 hours) whilst turkeys 8 wk or older showed no mortality. Haller and Sunde (1966) observed reduced body temperature in such poults. When water was reintroduced the temperature initially decreased then increased rapidly during the next hour, returning to normal within 12 h. After heavy drinking in the first 30 minutes, poults excreted large quantities of uric acid. Haller and Sunde (1966) suggested that such mortality is a form of water intoxication resulting from dilution of electrolytes and subsequent intracellular changes.

Whatever the reason for dehydration in the bird, the intracellular and extracellular fluids and plasma to some extent share the water deficit. Depending on the severity of water deficit, circulation is affected, resulting in increased body temperature and metabolic acidosis (Barragry, 1974). In extreme cases, subsequent death is often a result of bradycardia, circulatory failure, toxemia, damage to the nervous system or cardiac failure in cases of hyperkalemia (Barragry, 1974).

4.2.   Water temperature

At high environmental temperatures, birds will drink more to meet the demands for evaporative cooling. However as water temperature increases, birds seem to drink less water, and there are reports that birds are able to differentiate water samples that differ by only 2°C. At high environmental temperatures (>28°C), it is conceivable that birds are not consuming enough water in relation to metabolic needs, and so this may contribute to reduced growth or egg production. For example, Deyhim and Teeter (1991) indicate improved performance of heat-stressed broilers by including salt in the drinking water, where survival rate closely paralleled change in water consumption. It is possible that the temperature of water *per se* may limit water intake at high environmental temperatures. Herrick (1971) states that water at body temperature is more readily consumed during cold weather, and cold water increases consumption during warm weather. As water and feed intake are closely correlated, stimulation of water intake may increase feed intake at these temperatures. Consumption of cold water would also increase the birds energy requirement because the temperature of such water must necessarily be raised to body temperature. Lofgreen *et al*. (1975) showed that beef cattle in a hot environment (30°C) consumed more feed and gained more weight when given water cooled to 19°C compared to 30°C. The authors ascribed the beneficial effect of cold water as providing sufficient cooling to allow an increased feed intake, and showed that the cooling effect of this water more than compensated for the increased heat dissipation resulting from increased feed intake.

Bianca (1964) showed dramatic differences in the respiratory rate of oxen housed at 40°C when offered water (after a 4 d water starvation) at temperatures of 14°C or 40°C. Drinking cold water immediately decreased the respiration rate from 130 to 40 respirations/minute, followed by a gradual increase to 80 respirations/minute. Animals drinking hot water (40°C) increased their rates from 130 to 180 respirations/minute. Although these effects in oxen are confounded with prior dehydration, the respiratory rate of animals housed at high environmental temperatures may be reduced through the administration of cold water. This would be of importance in the case of the laying fowl in terms of shell quality as described. Leeson and Summers (1975) showed that birds housed at 32°C consumed more feed and produced more eggs when offered water at 4°C vs 32°C. Similarly by cooling the water of layers by only 5°C, Bell (personal communication) was able to maintain higher egg production and increased feed intake *(Table 9.2)*.

Contrary to these results shown in *Table 9.2*, Damron (1991) found little benefit for layers subjected to 32°C when given water at ambient temperature at 21-10°C. Degen *et al*. (1992) likewise showed no benefit to cooling the water of broiler breeders housed at around 32°C. On the other hand, Spinu *et al*. (1993) did show improved immunological response of broiler breeders housed in a cold environment (5-13°C) by giving birds warm (28°C) rather than cold (13°C) drinking water.

TABLE 9.2   Water temperature and egg production

Age weeks	Environment temperature 32°C	
	Water at 32°C	Water at 27°C
25	64%	74%
27	77%	86%
29	88%	93%
Feed Intake	83 g/b/d	90 g/b/d
		(Bell, 1987 personal communication)

At high environmental temperatures, the respiratory rate of poultry increases due to the significance of evaporative heat loss from the respiratory tract and this eventually leads to a reduced carbon dioxide concentration in the blood. Respiratory alkalosis resulting from thermic hyperventilation may reduce egg shell quality due to the role of plasma bicarbonate in egg shell formation and its dependence on plasma acid:base equilibrium *(Chapter 10)*.

4.3.   Problems with wet manure

Periodically, poultry producers encounter flocks of birds that produce very wet manure. This occurs most frequently in high-producing laying hens, that have increased water flux. Performance and bird health are rarely affected, although more eggs can become soiled by the wetter manure. The problems of wet manure relate to mechanical handling and/or odor and fly control. The situation is more prevalent in hot environments, when birds will naturally drink more water due to the importance of evaporative cooling through panting.

4.3.1.   Diet composition

Both diet nutrients and diet ingredients can have a marked effect on manure water content. Sodium or salt levels are most suspect, as high levels of salt will invariably lead to increased water intake. *Table 9.3* shows the effect of varying dietary salt content on litter moisture content for male broilers. As salt content increased, so litter moisture content increased, and this was especially true when open-type bell drinkers were used. In this study, there was no major improvement in broiler performance related to higher salt inclusion, consequently such high sodium levels as commonly used in N. America, seem questionable. Other electrolytes that can affect water loss in the excreta, are potassium and magnesium. Potassium levels are high in molasses and soybean meal, and so levels of these ingredients may need to be adjusted when wet manure is problematic.

TABLE 9.3 Diet salt and litter moisture (%)

Dietary Salt	Nipple drinker		Bell drinker	
(%)	LITTER MOISTURE (%)			
	21d	49d	21d	49d
0.25	16	18	17	21
0.50	17	20	21	33
0.75	22	23	28	49
				(Leeson, 1994; unpublished data)

There are also some non-nutritive components of various feed ingredients that can influence manure moisture and/or manure water holding capacity. Birds fed barley or rye usually produce wetter manure, and this is accompanied by increased stickiness of the droppings. In large part, these problems relate to β-glucan content of these cereals, and so addition of synthetic β-glucanase enzymes can be used to correct these problems. Another major dietary factor influencing manure moisture is diet protein level. Higher levels of crude protein necessitate increased excretion of uric acid, and so urine flow is increased. *Table 9.4* shows manure moisture of broiler breeder hens fed varying levels of crude protein. All these birds received similar quantities of essential amino acids, and egg production was unaffected by diet.

TABLE 9.4 Diet protein and excreta moisture		
% Diet Protein	Excreta N (g/b/d)	Excreta water %
9	1.2	73
11	1.3	75
13	1.8	76
15	1.6	79
		Lopez, 1994

In excreting the unusable or excess "crude protein" portion of the diet, birds will excrete more water and so develop potential litter problems.

4.3.2. Prelay calcium

Most cases of wet manure occur in laying hens, and while in part this relates to the greater need for water during egg formation, other factors are likely involved. A major variable in management of Leghorn birds is pre-lay nutrition, especially in relation to calcium level. Feeding high calcium (3.5-4.5% Ca) layer diets for up to 4 wk prior to maturity seems ideal in terms of medullary bone deposition, although it may be detrimental to water excretion. Certainly more calcium is retained when higher levels of Ca are fed, although the relationship is far from linear, and so increasing quantities of calcium are necessarily excreted in this prelay period. Leeson and Summers (1987) show increased water intake of pullets fed 4 vs 1% Ca diets prior to maturity. These same pullets then produced wetter manure. While this is problematic in the immediate pre-lay period, there is also an indication of such calcium treatment having a more long-term effect on excreta moisture (*Table 9.5*).

TABLE 9.5 Effect of prelay diet calcium on excreta moisture (%)				
	Bird age (days)			
Diet calcium up to 5% production	147	175	196	245
1%	71	79	75	65
2%	72	77	74	64
3%	72	78	74	64
4%	77	80	76	69
				(Leeson and Summers, 1987)

Certainly the high calcium diets had the most pronounced effect on water output around the time of maturity at 147 d *(Table 9.5)*. However these same birds continued to produce wetter manure even though all birds were fed 4% calcium throughout the laying period.

4.3.3.   General health status

A number of infectious diseases can affect water balance, the most notable being coccidiosis and infectious bronchitis. Carbo-Baptista *et al*. (1976) indicate that birds infected with coccidia often exhibit net loss of water in the duodenum and mid-lower jejunum. With infectious bronchitis, increased water loss likely relates to increased urinary sodium loss due to associated nephritis.

4.3.4.   Water restriction

In certain situations, it is necessary to restrict the quantity of water consumed by birds, usually in an attempt to prevent excessive manure wetness. This most commonly occurs with broiler breeding stock that are fed limited quantities of feed in order to control growth. Under these conditions, high water:feed intake occurs due either to physical satiety needs by the bird and/or boredom. In extreme situations, it may also be necessary to limit the water intake of adult egg layers, although this is usually a last resort situation when all other management attempts at reducing water:feed intake have failed. Poultry seem to adapt readily to reduced water intake, which is usually imposed through limiting time that water is available. As little as two 1 h watering periods each day (8-10 h light) is sufficient to achieve desired growth and there are rarely signs of excessive thirst when water becomes available. It seems important that water always be available before the restricted quantity of feed is allocated to breeder pullets. When feed is given before water, mortality at up to 0.5% per wk can occur. This is seen more frequently as birds approach maturity, and symptoms are not dissimilar to "sudden death syndrome" described for broiler breeders in Australia *(Chapter 3)*. Apparently healthy birds that invariably have a full and distended crop of dry feed, die suddenly after the feeding frenzy associated with restricted feeding. Because the condition can be prevented by allowing birds access to water for up to 1 h prior to feed, suggests that either the distended crop interferes with respiration and/or blood supply in the neck region or perhaps that the sudden uptake of dry feed to an already partially dehydrated bird, adversely affects electrolyte balance. Access time to water should be increased in hot weather conditions so as to encourage evaporative cooling via panting.

As boredom/satiety is often considered to be a factor in the imbalance of water and feed in feed-restricted breeders, it is generally assumed that water intake will be most problematic on the "off-feed" days of a skip-a-day feed program. Recent data by Bennett and Leeson (1989) suggests that most water is voluntarily consumed on feed-days *(Table 9.6)*.

	Water restriction time each day	Water restriction time only on "feed-day"	Free choice water
TABLE 9.6 Water consumption of 13 week old growing breeder pullets (ml/bird/day)			
Water intake on "feed day"	175	182	270
Water intake on "off-feed day"	108	109	36
Average	141	145	153
(Bennett and Leeson, 1989)			

When given free access to water, birds do not consume an excessive quantity of water relative to restricted time-access watering, since average intake is 153 ml/d vs around 140 ml/d. However, it is obvious that birds consume excessive quantities on the feed-day, this being much more than occurs with restricted-time watering, and so water:feed ratio will be increased. Under practical conditions, it is water:feed intake over a relatively short period of time (5-6 hours) that will

likely influence water loss, and so the occurrence of wet manure. This data does show however that water intake on "feed-days" rather than "off-feed days" may contribute most to wet litter. Hocking (1993) suggests that over-drinking of feed-restricted breeders may be an extension of foraging activity and act as a dearousal activity. Hocking (1993) observed that birds subjected to water-restriction spent more time scratching and pecking the litter, but that when given only 2 - 8 h water time each d, they exhibited no unusual signs of thirst when water was reintroduced.

5.   POTENTIAL TREATMENT AND PREVENTION

5.1.   Dehydration

Because dehydration should occur only as a result of mechanical failure of equipment, then prevention obviously equates to the maintenance of all aspects of the watering system. Periodically birds will dehydrate when transferred to new watering equipment, as sometimes occurs when Leghorn pullets are moved to laying cages at 17-19 wk of age. This scenario is most common when birds have been exposed to cup or bell drinkers and are transferred to nipple systems. Often entire cage-groups seem unable to find the nipple system. Once nipples are manually depressed, and at least one bird is drinking, then others in the group quickly adapt. When it is known that nipple drinkers will be novel to pullets, then it may be advantageous to slightly reduce water pressure for the first 2-3 h after moving, so that droplets of water form on the bottom of the nipple, and these are easily seen by the birds. Nipple drinkers *per se* may cause reduced water intake, which in extreme situations may lead to dehydration. Gernat and Adams (1992) indicate significant reduction in water intake of Leghorn pullets as the number of birds per nipple drinker increases. Carpenter *et al*. (1992) also show increased body weight in broilers drinking from nipples when the water flow volume in the system is increased from 0.4 to 2.3 ml/second.

Electrolyte therapy may be considered if dehydration has occurred. However this should not be excessive, because electrolyte imbalance itself can be problematic to the bird. As a rule of thumb, the treatment should provide no more than 50% of the normal daily intake of sodium and potassium.

5.2.   Wet manure

As previously discussed, wet manure can be most problematic in broiler breeders subjected to restricted feeding and for caged layers. In either situation, the first course of action is to ensure that all watering equipment is functioning properly and that birds are not spilling water due to improper height adjustment, etc. Assured that the wet manure is caused by excessive water intake, then the most likely cause is feed. Feed should be assayed for sodium and potassium, and for layers and broiler breeders, sodium levels should not exceed 0.15-0.20% of the diet. Unless there are unusual mitigating circumstances, then the sodium levels can be reduced to 0.15% without a loss in performance. Sodium levels can also be very high in drinking water, and, in fact is the only such element that can dictate the need to adjust feed levels. When high sodium water is used, the diet concentration can be adjusted such that total daily intake equates that normally occurring with diets containing around 0.15% sodium. Under these conditions, if the feed contains less than 2 kg

salt/t, then diet chloride levels may need to be increased. In these situations, both feed and water should be assayed specifically for sodium and chloride, independently of each other.

Ingredients that can cause wet manure are barley, molasses and to a lesser extent wheat. Soybean meal contains high levels of potassium, although this ingredient is not likely to be at high concentration in diets for adult birds. For reasons not fully understood at this time, crumbled diets always produce a wetter manure than an identical diet offered as a mash. Depending upon the mechanical feeding system therefore, problems of wet manure occurring with crumbled/pelleted feed can often be resolved by changing to mash diets.

In hot weather, it will likely be advantageous to use a moderate-calcium prelay diet, rather than using a high-calcium layer diet for 2-3 wk prior to maturity. While early introduction of layer diets are advantageous in terms of calcium mobilization of the early maturing birds, there is need for the bird to excrete the largest proportion of this increased calcium intake, and so wetter manure can be expected.

> ***Caution:*** In a number of situations, farm managers take too extreme a position in trying to prevent wet manure. A high-producing laying hen naturally produces excreta containing 75-77% water, and levels much less than this are usually a result of reduced water flux in the bird. Reduced water intake will result in reduced feed intake and reduced egg output, especially in hot weather situations.

Chapter 9

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CHAPTER 10. ELECTROLYTE IMBALANCE

<u>Other names:</u>	ACIDOSIS, ALKALOSIS
	ALSO AFFECTS TD AND AMINO ACID METABOLISM
<u>Species:</u>	ALL POULTRY

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1.   COMPENDIUM

Electrolyte balance in the body is mainly affected by supply in the diet, but also by endogenous acid production and rates of renal clearance. While requirements for individual elements have been clearly defined, there is now an understanding of the need to achieve a balance between cation and anion supply. Most commonly, electrolyte balance is described by the simple formula of Na+K-Cl expressed as mEq/kg of diet. In most situations it seems as though an overall diet balance of 250 mEq/kg is optimum for normal physiological functions. In reality, electrolyte imbalance does not occur, because the buffering systems in the body ensure the maintenance of near normal physiological pH. In extreme situations the need for buffering capacity seems to adversely affect other physiological conditions, thereby producing or accentuating potentially debilitating conditions.

In young broiler chickens, tibial dyschondroplasia (TD) can be affected by the electrolyte balance of the diet. The unusual development of the cartilage plug at the growth plate of the tibia can be induced by a number of factors, although its incidence can be greatly increased by metabolic acidosis induced by feeding products such as NH_4Cl. It seems as though TD occurs more frequently when the diet contains an excess of sodium relative to potassium when at the same time chloride levels are very high. Unfortunately much of the research involving TD and acid-base balance is confounded with a concomitant effect on body weight. For example, a certain balance of electrolytes may be claimed beneficial in reducing TD, but at the same time the body weight may be greatly reduced and this in itself will reduce TD severity. Great care must be taken in the interpretation of any research data in this area, such that any changes to the diet formulation will hopefully reduce or limit the incidence of TD, while at the same time maintaining normal growth characteristics. TD seems most problematic when high diet chloride levels are used.

Electrolyte balance can also affect the metabolism of a number of basic amino acids, and in particular, that of lysine and arginine. The antagonism known to occur between lysine and arginine can be accentuated or partially alleviated by manipulation of diet cations or anions. Likewise, the bird's growth response to diet electrolytes can be influenced by the crude protein level of the diet.

In laying hens, respiratory alkalosis causes a reduction in blood HCO_3^- and so in order to maintain a normal balance, there is competition for HCO_3^- with the shell secreting glands in the uterus. Severe metabolic acidosis develops from feeding NH_4Cl, and this produces less shell secretion. Again, it seems necessary to minimize chloride levels in the diet, and to definitely avoid adding any readily metabolized chloride salts to the diet. On the other hand, the addition of

$NaHCO_3$, to the feed or water, will not correct respiratory alkalosis, but may improve shell secretion. Respiratory alkalosis in broiler chickens can be partly alleviated by electrolyte treatment. The balance of electrolytes seems less important than the actual levels used because their beneficial effect under extreme heat stress may well be via the stimulation of water intake.

2.   GENERAL ELECTROLYTE BALANCE

While the primary role of electrolytes is in maintenance of body water and ionic balance, the requirements for elements such as sodium, potassium and chlorine cannot be considered individually because it is the overall balance that is important. Electrolyte balance also referred to as acid-base balance, is affected by three major factors, namely the balance and proportion of these electrolytes in the diet, endogenous acid production and the rate of renal clearance.

In most situations an animal will attempt to maintain the balance between cations and anions in the body such that physiological pH is maintained. If conditions in the body result in a shift towards acid or base conditions, the normal physiological defence mechanism is to alter metabolic processes such that normal conditions prevail. In reality, electrolyte imbalance *per se* rarely occurs because these regulatory mechanisms must ensure optimum cellular pH and osmolarity. Electrolyte imbalance can therefore more correctly be described as the mechanisms that must occur in the body so as to achieve normal physiological pH. In extreme situations, such modification in regulatory mechanisms seem to adversely affect other physiological systems, and so produce or accentuate potentially debilitating conditions. In birds, tibial dyschondroplasia and respiratory alkalosis are examples of "electrolyte imbalance". It is the cation:anion balance of the diet that provides the major mechanism for influencing electrolyte balance in the body when feeding poultry. Mongin (1980) describes cellular cation:anion balance as: mEq $(Na^++K^++Ca^{++}+Mg^{++})$ - mEq $(Cl^-+SO_4^=+H_2PO_4^-+HPO_4^=)$

Mongin (1980) rationalizes that in fact only Na, K and Cl are likely to be involved in homeostasis, and this certainly makes accommodation within ration formulation more realistic. In fact, this simplification has been widely accepted since the more complex picture would not likely be considered in practical formulation. As described above by Mongin (1980), ion balance is usually expressed in terms of mEq of the various electrolytes, and for an individual electrolyte this is calculated as Mwt ÷ 1000. This unit is used on the basis that most minerals are present at a relatively low level in feeds. As an example calculation, the mEq for a diet containing 0.17% Na, 0.80%K and 0.22% Cl can be developed as follows:

a) **Sodium**   Mwt = 23.0 ∴ Eq = 23g/kg, ∴ mEq = 23 mg/kg

Diet contains 0.17% Na ≡ 1700 mg/kg ≡ $\frac{1700}{23}$ mEq = 73.9 mEq.

b) **Potassium**   Mwt = 39.1 ∴ Eq = 39.1g/kg, ∴ mEq = 39.1 mg/kg

Diet contains 0.80%K ≡ 8000 mg/kg ≡ $\frac{8,000}{39.1}$ mEq = 204.6 mEq

      c) Chloride   Mwt = 35.5 $\therefore$ Eq = 35.5g/kg, $\therefore$ mEq = 35.5 mg/kg
      Diet contains 0.22% Cl $\equiv$ 2200 mg/kg $\equiv$ $\dfrac{2200}{35.5}$ mEq = 62.0 mEq

$\therefore$ overall diet balance becomes Na + K - Cl = 73.9 + 204.6 - 62.0 = 216.5 mEq.

The normal buffering systems within the body will obviously need to temper any major deviation from physiological pH. Mongin (1980) suggests that:

Diet Na+K-Cl $\equiv$ Diet Cations-Anions + Endogenous acid production + Base excess

The balance of ions in the diet needs to be such that the base excess is close to zero, so that the above equation balances. Alternatively there is a need to produce base excess in order to maintain equilibrium, and this "electrolyte imbalance" can lead to abnormal physiological conditions. Mongin (1980) concludes that when Na+K-Cl is other than 25 mEq/100g of diet, then either acidosis or alkalosis develops, and growth/production will be adversely affected.

Cohen and Hurwitz (1974) indicate that the addition of Na (without Cl) to a diet increases plasma HCO_3^- and pH, while with addition of Cl (without Na) there are reductions in plasma HCO_3^- and pH. Addition of both, as salt, causes little change in pH or plasma HCO_3^-. Diet may also influence the acid-base balance indirectly via an effect on endogenous acid production. Mongin (1981) suggests that this is most evident with protein sources, that normally contain a variety of nitrogen: ion balance. For example; Mongin (1981) cites change from 17.4 to 13.1 mEq/100g when a proportion of soy is replaced by fish meal. Assuming no other changes in formulation, then substitution of soy with fish meal would necessitate the inclusion of ions such as bicarbonate in order to reduce base excess in the body. With animal proteins there may also be the need to consider the contribution of sulfate, since Mongin (1981) states that considerable variation in the growth response to various fish meals could best be accounted for by estimation of Na+K-Cl-SO_4. Ruiz-Lopez and Austic (1993) compared the relative acidogenicities of several anions using chloride as a standard. Chloride increased blood H^+ concentration, although the effect was most noticeable when high levels (160 - 240 mEq/kg) were fed to young birds. Sulfate was also acidogenic, although there was an indication that this effect was most noticeable on the first d of administration. Dibasic phosphate on the other hand was consistently without effect in changing acid-base parameters in the blood. These authors showed the acidogenic properties of sulfate to depend on source, with values relative to mEq of chloride being approximately 58% when $CaSO_4$ and K_2SO_4 were used, but that potency increased to 84% relative to chloride when, sulfate originated from Na_2SO_4. As pointed out by Ruiz-Lopez and Austic (1993) the failure of phosphate to influence acid-base parameters is likely a reflection of the buffering capacity of phosphate, because the pK_2 of phosphoric acid is within the range of normal physiological pH.

In certain situations it may also be necessary to take into account the contribution of divalent ions. For example, feeding calcium chloride will induce acidosis in birds, while feeding NaCl or KCl has little effect (Mongin, 1981). This situation probably develops due to less calcium being absorbed from $CaCl_2$ than occurs with Na from NaCl. Since chloride absorption remains unchanged, and since Ca is excreted as $CaCO_3$, there is the potential net loss of bicarbonate and net gain of Cl^-. Hurwitz and Bar (1968) also indicate the significance of gut lumen buffering capacity as it influences intracellular ion balance. The bird seems to adjust lumen pH in a very short time period, when confronted with either acidic or alkaline conditions imposed via the diet. Within 10 minutes, pH is normalized from 9.0 or 4.0 to around 7.0. Such buffering must obviously be

accomplished by a net shift of electrolytes into the lumen, and so for example with an acidic lumen pH one assumes a net outflux of ions such as bicarbonate. This buffering will obviously influence the electrolyte balance in the bird.

3.   EFFECTS OF ELECTROLYTE IMBALANCE

3.1.   *Tibial dyschondroplasia (TD)*

Electrolyte imbalance is only one of the factors that can influence the incidence and severity of TD. The etiology of TD is complex, and is described in detail in *(Chapter 11)*. TD involves incomplete ossification at the active growth plate, such that the head of the tibiotarsus is composed essentially of a cartilage plug, and this usually leads to bending of the tibiotarsus. Metabolic acidosis induced by feeding NH_4Cl increases the incidence of TD, while additions of either ammonium sulfate or acetate markedly reduces the incidence (Mongin and Sauveur, 1977). It is likely that sulfate and acetate ions reduce the severity of acidosis by promoting H^+ excretion or uptake. The occurrence of TD seems to be related to plasma bicarbonate levels, since Mongin and Sauveur (1977) observed 70% TD in birds with plasma $HCO_3^->26mEq/l$ while TD was lowest in birds with <14mEq/l. In general, excess of sodium relative to potassium increased the incidence of TD, especially when the diet contained high levels of chloride. The exact mechanism by which acid-base balance may affect bone calcification is unclear, although Mongin and Sauveur (1977) suggest involvement with vitamin D metabolism. For example, metabolic acidosis may impair cartilage maturation by alteration of synthesis of 1-25 dihydroxy cholecalciferol [1-25 (OH_2)] from hydroxy cholecalciferol in the kidney. Under conditions of acidosis Mongin and Sauveur (1977) recorded conversion to 24-25 DHCC rather than 1-25 (OH_2), which is a less active metabolite.

The significance of balance between the major electrolytes, rather than their levels *per se*, on incidence of TD is shown in *Table 10.1*. With low diet chloride levels, there is no TD regardless of electrolyte balance from 10 to 40 mEq/100 g. As chloride level increases, there is more TD, especially when birds become acidotic due to excess of chloride in relation to Na+K. The incidence of TD can be reduced by adding Na or K to the diet. When very high levels of diet chloride are used, then incidence of TD is high, and is little affected by electrolyte balance. It must be emphasized that these recommendations of diet electrolyte balance relate solely to effect on TD, since optimum balance in terms of growth rate may be somewhat different. Contrary to these observations, Karunajeewa *et al.* (1986) indicated little effect of electrolyte balance of 150-300 mEq/kg (Na+K-Cl) on general skeletal integrity or incidence of TD. The higher cation contributions did however seem to alleviate growth depression caused by feeding high levels of diet phosphorus. That TD was unaffected may relate to the relatively low chloride levels used (max 0.24% of diet). In other studies, Karunajeewa and Bar (1988) showed no effect of electrolyte balance from 125 to 205 mEq/kg on TD, and in this study more moderate levels of diet chloride (0.3% of diet) were used.

TABLE 10.1 Effects of diet electrolytes (mEq/100g) on incidence of TD in broilers								
Diet Cl	**10**			**25**			**40**		
Diet Na+K	20	35	50	20	35	50	20	35	50
TD(%)	0	0	0	17	8	8	22	18	17
Adapted from Sauveur and Mongin (1974)								

A review of the work of Hulan (1985) and Hulan *et al.* (1987) indicates another problem associated with this type of research, namely the confounding effect of body weight. In these studies, there is an indication of reduced incidence of TD when electrolyte balance is elevated, but at these higher levels of Na+K, there is invariably reduced body weight. As discussed in *Chapter 11*, fast growth rate is a major predisposing factor of TD. Interestingly, Hulan (1985) confirms the general observations of Mongin that optimum growth and feed utilization occurs when diet electrolyte balance is around 250 mEq/kg.

3.2.   *Protein and amino acid metabolism*

Electrolyte balance can affect the metabolism of a number of basic amino acids, particularly that of lysine and arginine. Lysine and other basic amino acids are known to accumulate in the tissues of animals fed potassium deficient diets, where, depending upon the degree of potassium deficit, lysine can become the major amino acid in muscle tissue. Unfortunately, such lysine enrichment is usually associated with dramatically reduced growth due to the potassium deficit. The increase in tissue lysine concentration is approximately equal to the reduction in tissue potassium, suggesting that the basic amino acids are acting in a buffering capacity in order to maintain normal ionic balance. There is known to be a lysine-arginine antagonism in poultry, where excess of lysine can lead to a metabolic deficiency of arginine, most likely brought about by stimulation of kidney arginase. Various researchers have shown that high levels of dietary potassium can alleviate such adverse effects on growth, while Austic and Calvert (1981) clearly show that the imbalance is accentuated when the diet is high in chloride (*Table 10.2*).

High levels of chloride, regardless of amino acid balance seem detrimental to growth. However growth depression is most noticeable for Treatment 3 *(Table 10.2)* where a major lysine:arginine antagonism occurs, and here the effect is accentuated by an increase in diet chloride. Using C^{14} labelled lysine, Austic and Calvert (1981) conclude that chloride does not influence lysine degradation *per se*, and that the mechanism relates in some way to electrolyte balance. This concept is also supported by the observation of Austic (1981) that varying K:Cl levels, while influencing the severity of the effects of a lysine:arginine imbalance has little influence on growth of arginine-deficient birds.

TABLE 10.2 Effect of dietary chloride on growth response of chicks fed various

levels of basic amino acids					
			Weight gain (g/d)		
Treatment	% Lysine	% Arginine	0.5% Cl	1.0% Cl	1.5% Cl
1	0.8	1.0	5.5[bc]	4.8[cd]	4.2[d]
2	1.2	1.0	7.5[a]	7.1[a]	5.3[bc]
3	2.5	1.0	3.3[e]	2.2[f]	1.7[f]
4	2.5	2.3	7.6[a]	7.2[a]	6.0[b]
				Adapted from Austic and Calvert (1981)	

During acidosis there is also an increased NH_4 loss by the kidneys. For example, feeding $NaHCO_3$ reduces the loss of NH_4 in the urine while feeding HCl has the opposite effect (Austic and Calvert, 1981). Because uric acid production is little affected by ion balance, then such changes in NH_4 loss can obviously lead to variability in overall nitrogen balance. In rats, at least, the mechanism is thought to involve acid-base effects on glutaminase activity. Adekunmisi and Robbins (1987) suggest that optimum dietary electrolyte balance varies with diet crude protein level. Growth of chicks fed low protein (14.3%) diets was depressed when electrolyte balance was changed by addition of sodium and potassium. However adding these electrolytes to diets containing 28.6% CP, improved growth rate. As previously suggested by Austic and Calvert (1981) higher levels of cations, stimulate uric acid excretion, a situation observed by Adekunmisi and Robbins (1987). Electrolyte imbalance can therefore be expected to be more detrimental when low protein diets are used since reduced nitrogen balance is more problematic. The effect of acid-base balance on amino acid metabolism warrants further investigation, especially since much of the older work on this subject seems to be confounded in terms of nutrient deficiency vs nutrient balance scenarios. However in general, it seems as though high chloride levels will be detrimental, while higher levels of metabolizable potassium salts may be warranted, especially when higher crude-protein diets are to be considered. Adekunmisi and Robbins (1987) conclude that electrolyte balance in the diet will be different for situations involving low crude protein and high levels of synthetic amino acids, compared with formulations with high crude protein aimed at reducing carcass fat content.

3.3.   Respiratory alkalosis

At high environmental temperatures, birds increase their respiration rate in an attempt to increase the rate of evaporative cooling. Such panting increases CO_2 loss, and consequently a degree of alkalosis will develop. Such changes in electrolyte balance may be involved with reduced growth rate seen in meat birds, and a decline in eggshell quality often seen in high-producing laying hens. El Hadi and Sykes (1982) describe the usual pattern of respiratory alkalosis as it develops in laying hens. Panting first started at $35^{\circ}C$, and although there was no increase in body temperature, mild alkalosis (pH 7.55) developed. At $38^{\circ}C$ there was moderate alkalosis, while at $41^{\circ}C$ the condition was described as severe with blood pH at 7.65. Various attempts have been made to correct this imbalance through the administration of electrolytes via the feed and/or water.

3.3.1. Layers

The availability of both calcium and carbonate ions at the uterine mucosa is important for shell synthesis. Acid-base balance can dramatically influence the process of shell formation. This is most clearly seen when birds exhibit respiratory alkalosis during heat stress resulting in thinner eggshells. However, the effects of acid or alkaline conditions in the uterine extracelluar fluid, while having a major effect on calcium solubility (precipitation), are not so clearly defined in terms of bicarbonate flux. In fact, it is the availability of bicarbonate *per se* that seems to be the major factor influencing eggshell thickness, and to a large extent, this is influenced by acid-base balance, kidney function and respiration rate.

Under normal conditions shell formation induces a renal acidosis related to the total resorption of filtered bicarbonate. At the same time, shell secretion induces a metabolic acidosis because the formation of insoluble $CaCO_3$ from HCO_3^- and Ca^{++} involves the liberation of H^+ ions. Such H^+ release would induce very acidic and physiologically destructive conditions, and is necessarily balanced by the bicarbonate buffer system in the uterine extracelluar fluid. The release of H^+ ions and the mild acidic conditions also aid in the initial cleavage of calcium from protein-bound transport molecules (Mongin, 1968). While a mild metabolic acidosis is therefore normal during shell synthesis, a more severe situation leads to reduced shell production because of intense competition for HCO_3^- as either a buffer or a shell component. A severe metabolic acidosis can be induced by feeding products such as NH_4Cl, and this results in reduced shell strength. In this scenario, it is likely that NH_4^+ rather than Cl^- is problematic because formation of urea in the liver (from NH_4^+) again needs to be buffered with HCO_3^- ions, creating more competition with uterine bicarbonate metabolism. Conversely, feeding sodium bicarbonate, especially when Cl^- levels are minimized, may well improve shell thickness. Under commercial conditions, the need to produce base excess in order to buffer any diet electrolytes must be avoided. Likewise it is important that birds not be subjected to severe respiratory excess, as occurs at high temperatures, because this induces reduced blood bicarbonate levels, and in extreme cases, possibly a metabolic acidosis. Under practical conditions, replacement of part of the supplemental dietary $NaCl$ with $NaHCO_3$ may be beneficial in terms of shell production.

Experimental validation of a metabolic acidosis adversely affecting shell quality is shown by Hamilton and Thompson (1980). By varying the quantity of NaCl and the ratio of Na:Cl in layer diets, a range of electrolyte concentrations and balance was achieved. Blood pH and bicarbonate were decreased as the Na:Cl ratio decreased. However given the large excess of Cl necessary to affect shell quality, these authors relegate its significance under practical conditions. It should be emphasized that birds used by Hamilton and Thompson (1980) were only producing eggs at a rate of 50%, and so results may be different when the requirement for bicarbonate is increased relative to greater egg production, that itself may accentuate any potential deficiency related to respiratory alkalosis. Certainly under commercial conditions, effects of respiratory alkalosis are most pronounced in birds at peak egg production.

Acclimatization to heat stress may well be another factor confounding many research results, since temporary acute conditions are more problematic. For example, pullets grown to 31 wk under constant 35 vs 21^0C conditions exhibit no difference in pattern of plasma electrolytes (Vo *et al.,* 1977). Kohne and Jones (1975) also suggest that if birds are allowed to acclimatize to high

environmental temperatures, then there is little correlation between plasma electrolytes and shell quality. Certainly temporary acute heat stress and cyclic temperature conditions seem most stressful to the bird.

3.3.2. Broiler Chickens

With broiler chickens and other meat birds, increased panting induces respiratory alkalosis by mechanisms comparable to that described for layers. In meat birds however, growth is the major consideration and so there is perhaps less need for emphasis on bicarbonate status. Teeter *et al.* (1985) studied growth and physiological response of broilers to chronic or acute heat stress situations. Blood pH was elevated by chronic heat stress at $32^{o}C$, and further elevated by acute stress to $41^{o}C$ over a 20 minute period. With chronic heat stress there was some respiratory alkalosis, while with acute heat stress all birds suffered from alkalosis. Including 0.5% $NaHCO_3$ in the diet of chronic heat-stressed birds improved weight gain even though blood pH was further elevated. Adding NH_4Cl decreased blood pH, but also improved weight gain, while using both NH_4Cl and $NaHCO_3$ was synergistic in terms of weight gain and slightly reduced severity of alkalosis.

Bottje *et al.* (1989) suggest that treating heat-stressed broilers with NH_4Cl could potentially be deleterious to the bicarbonate buffer system, as any metabolic acidosis associated with NH_4Cl catabolism may accentuate HCO_3^- loss due to increased respiratory rate. Bottje *et al.* (1989) tested this assumption by infusing NH_4Cl solution into the crop of heat-stressed broilers. As a result of this treatment, acidosis developed, and since equimolor intubation of KCl had no effect, it is suggested that acidosis may relate to NH_4 metabolism as previously described (H^+ liberated during uric acid synthesis). Therefore while NH_4Cl may be beneficial in reducing lactate production under heat stress, this seems to be at the expense of the bicarbonate buffer system.

A confounding factor in electrolyte treatment during heat stress, is the influence on water intake. It seems as though mortality and growth depression can be reduced if birds drink more water, and this does occur in response to some electrolytes. Branton *et al.* (1986) studied the response of broilers to NH_4Cl and $NaHCO_3$ administration during a period of heat stress. $NaHCO_3$ stimulated water intake, while NH_4Cl was without effect. These authors concluded that any beneficial effect of electrolyte therapy is via stimulation of water intake, rather than plasma electrolyte balance and/or blood pH *per se*. Teeter and Smith (1986) also showed some alleviation of heat stress in broilers through administration of KCl, but not with K_2CO_3, and that KCl stimulated water consumption, whereas K_2CO_3 depressed water intake. Whiting *et al.* (1991) likewise indicate that adding electrolytes to the drinking water during a period of heat stress is beneficial when water intake is stimulated, and that this effect is independent of cation or anion status of the supplement. Broiler chickens and laying hens obviously differ in their response to, and needs for, electrolyte therapy during heat stress.

4.   POTENTIAL TREATMENT AND PREVENTION

Prevention of electrolyte imbalance should obviously be approached through incorporation of appropriate cations and anions in diet formulations. However it must be accepted that diet is only one factor influencing potential imbalance, and so general bird management and welfare also become of prime importance. As suggested by Mongin (1980) electrolyte balance can be accommodated by consideration of Na+K-Cl balance in the diet, and under most dietary situations this seems a reasonable simplification. A balance of around 250 mEq/kg seems a compromise in terms of optimum performance coupled with minimum of undesirable side effects such as tibial dyschondroplasia or abnormal amino acid metabolism.

While it is true that overall electrolyte balance is of major importance, it appears as though this scenario is most critical when chloride levels are high. With low diet chloride levels, there is often little response to the manipulation of electrolyte balance, but when diet chloride levels are necessarily elevated then it seems critical to make adjustments to the diet cations such that overall balance is maintained. Alternatively chloride levels can be reduced, although it must be remembered that most species of poultry have requirements around 0.12 - 0.15% of the diet, and deficiency signs will develop with diet levels much less than 0.12%. Therefore care must be taken in meeting the minimum chloride requirements when, for example, $NaHCO_3$ replaces NaCl in a diet. *Table 10.3* outlines electrolyte content and electrolyte balance of some major feed ingredients.

TABLE 10.3 Electrolyte content of feed ingredients				
	% of ingredient			
Ingredient	**Na**	**K**	**Cl**	**Na+K-Cl(mEq)**
Corn	0.05	0.38	0.04	108
Wheat	0.09	0.52	0.08	150
Barley	0.02	0.56	0.18	101
Milo	0.04	0.34	0.08	82
Soybean meal	0.05	2.61	0.05	675
Canola meal	0.09	1.47	0.05	400
Meat meal	0.55	1.23	0.90	300
Fish meal	0.47	0.72	0.55	230
Cottonseed meal	0.05	1.20	0.03	320
Sunflower meal	0.02	1.00	0.03	255

Within the cereals, electrolyte balance for milo is low, while wheat is high relative to corn. Major differences occur in the protein-rich ingredients, and relative to soy, all sources are low in electrolyte balance. As shown in *Table 10.3*, this situation develops due to the very high potassium content of soybean meal. Careful consideration to electrolyte balance must therefore be given when changes are made in protein sources used in formulation. For example, the overall balance for a diet containing 60% milo and 25% soy is 210 mEq/kg, while for a diet containing 75% milo and 10% fish meal the balance is only 75 mEq/kg. The milo-fish diet would perhaps need to be supplemented with $NaHCO_3$ if effects of imbalance, such as poor growth or TD, were evident.

Assuming that heat stress cannot be tempered by normal management techniques, then electrolyte manipulation of the diet may be beneficial. However the technique should be different

for immature birds compared to egg-layers. With adult female birds there is need to maintain the bicarbonate buffer system as it relates to eggshell quality. As such, diet or water treatment with sodium bicarbonate may be beneficial, again emphasizing the necessity to meet minimal chloride requirements. For example, Koelkebeck *et al.* (1992) show improvement in shell thickness of layers maintained at 24 - 30°C when water is saturated with CO_2 to give pH of 4.7 vs 7.7 for control. On the other hand, treatment of respiratory alkalosis in layers, with acidifiers such as NH_4Cl, while relieving respiratory distress, may well result in reduced shell quality. For immature birds such as the broiler chicken, treatment with electrolytes is often beneficial, and there seems less need for caution related to bicarbonate buffering. Up to 0.3% dietary NH_4Cl may improve the growth rate of heat-stressed birds, although as detailed previously it is not clear if this beneficial effect is via electrolyte balance/blood pH or simply via the indirect effect of stimulating water intake. Under commercial conditions, adding salt to the drinking water of heat-stressed broilers has been reported to alleviate bird distress and to simulate growth. In this context, Belay and Teeter (1993) show heat distressed broilers to respond well to adding 0.75% KCl to their drinking water, by increasing their water consumption by 91%, their evaporative heat loss by 20% and their apparent respiration efficiency by 27%.

Chapter 10

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CHAPTER 11. SKELETAL DISORDERS

<u>Other names:</u>	TIBIAL DYSCHONDROPLASIA (OSTEOCHONDROSIS)
	CAGE LAYER FATIGUE (OSTEOPOROSIS)
	RICKETS (OSTEODYSTROPHY)
	CHONDRODYSTROPHY (PEROSIS, ANGULAR BONE DEFORMITY)
	SPONDYLOLISTHESIS (KINKY BACK, SCOLIOSIS)
	FEMORAL HEAD NECROSIS (BRITTLE BONE DISEASE)
	FOOD PAD DERMATITIS (PODODERMATITIS)
	TURKEY LEG DISORDERS
<u>Species:</u>	ALL POULTRY

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1.   **COMPENDIUM**

Various skeletal disorders affect most fast-growing meat birds, and also laying hens. Actual leg problems are discussed in *Sections 5-12*, following a review of bone development. Normal bone development is discussed in relation to bone cell types, structure, and the normal growth of bone from the embryo through to maturity. A number of factors are known to influence normal bone development and these are discussed essentially in relation to fast growth rate. Nutritional factors include protein and amino acids, vitamins, minerals and electrolyte balance as well as the role of specific ingredients and mycotoxins. Genetics, the sex of the bird, and the absolute growth rate are also major factors affecting potential bone disorders. A compendium of specific leg disorders are given in *Sections 5-12* of this Chapter.

2.   **GENERAL INTRODUCTION**

Most poultry are afflicted with varying degrees of skeletal disorders at some time during their productive life-cycle. As with most metabolic disorders, these problems are more pronounced when fast-growth rate is involved, and so broilers, turkeys and ducks are most susceptible. Infectious agents such as bacteria, viruses and mycoplasma can also play a role in abnormal bone, cartilage or joint development. Most bones in the body can be adversely affected in some way, although by far the greatest incidence and severity are seen with the leg bones. The leg bones are one of the fastest growing bones in the skeleton, and coupled with their weight-bearing characteristics, it is perhaps not too surprising that femur, tibiotarsus, and tarsometatarsus bone problems are so prevalent. In birds such as the broiler chicken and turkey, leg problems occur early in life, and lead eventually to morbidity with failure to eat and drink, or in extreme cases, mortality. Additionally, there are problems of downgrading during processing. In most countries, skeletal problems, account for 30-40% of overall mortality, morbidity and/or carcass downgrading, and so probably equate to about 1.5-2% of all monetary farm gate receipts from meat birds. In the following

discussions, it is assumed that the skeletal problems described are true metabolic derangements of bone growth, and that infectious agents are not causative or aggravating factors. In field diagnosis, the presence of bacteria, virus and/or mycoplasma should be discounted prior to the consideration of any metabolic derangement.

3.   <u>NORMAL BONE DEVELOPMENT</u>

Bone is a collagenous connective tissue with the unique property of being mineralized to varying degrees, creating rigidity not seen in other tissues. The skeleton as a whole dictates the basic shape and stature of the bird, and almost as importantly, provides support for soft tissues and especially muscle. To some extent, the limit of muscle growth is dictated by skeletal size. The mineral component is essentially calcium phosphate. The ratio of calcium:phosphorus in bone is around 2:1 and this essentially is the reason for the maintenance of this important ratio during feed formulation. Bone is the major mineral reserve in the body, representing about 99% of calcium, 88% of phosphate, 80% of bicarbonate, 50% of magnesium and 35% of total mineral body reserves. The bone cavity is also a major site of fat stored in the body. Skeletal structure is shown in *Fig. 11.1*. Most of the discussion in this chapter deals with problems associated with the three main leg bones, namely the femur, the tibiotarsus and the tarsometatarsus. As will be discussed, most problems relate to bone or ligament integrity at the articulating joints of these three bones, and particularly the proximal and distal ends of the femur and tibiotarsus. For more extensive detail of general bone biology, the reader is directed to Whitehead (1992).

3.1.   *Bone and cell types*

In the context of metabolic disorders, there are two major bone types. Cortical bone is the most common of these, and in the adult bird, this is normally seen as a dense structural bone with characteristic marrow cavity. In the young bird, bone ends contain cartilage that represent areas of growth. The second type of bone is medullary bone which is a specialized spongy bone found in mature female birds, and acts as a reservoir of labile calcium to be used for eggshell formation. The medullary bone essentially replaces the normal marrow cavity, and is most pronounced in the leg bones.

There is essentially only one cell type in bone, the osteocyte, although its appearance and name is altered depending upon its activity. Osteoblasts participate in bone formation (ossification) and are seen at the surface of the organic matrix or already deposited bone surface. The deposition of mineral into the collagen and micropolysaccharide organic "precursor", is directly controlled by the osteoblastic activity. Osteoclasts on the other hand are responsible for the removal of inorganic bone material. Unlike osteoblasts, osteoclasts are multinucleated, leading to the supposition that they are derived from aggregations of osteoblasts. Osteoblastic and osteoclastic activity can occur concurrently, although it is more common to see the dominance of one cell type at any one time. Parathyroid hormone controls osteoclastic activity. Osteoclasts are highly mobile, being capable of extreme migration along the surfaces of resorbing bone and subsequently transferring minerals to the blood stream.

3.2.   Bone structure

Because most metabolic disorders or nutritional deficiencies seem to affect the articulating ends of the long leg bones, it is important to understand the terminology of the structural areas in these regions. *Fig. 11.2* is a schematic representation of a juvenile long bone that is still undergoing development, as may be seen in any young broiler chicken. As shown in *Fig. 11.2*, the long bone can be subdivided into three separate regions, the epiphysis, metaphysis and diaphysis. The diaphysis consists of compact bone surrounding the medullary cavity, and it is this bone shaft that imparts rigidity to the normal "mature" bone. The metaphysis is the area of most active bone growth, and unfortunately where most problems of metabolic derangement originate. The epiphysis consists of an outer articular cartilage that accepts the physical pressure during joint flexure while internally there is a layer of spongy or cancellous bone. The entire bone is surrounded by a thin sheath or periosteum, while the marrow cavity is lined by a comparable endosteum (*Fig. 11.2*).

Bone is composed of a mixture of collagen fibres and mucopolysaccharides that are mineralized by calcium salts in the presence of osteocytes (Hodges, 1974). The important interrelationship between the developing inorganic matrix and the active living phase is via fine lacunae, or channels, that are well endowed with capillary blood vessels. Derangement of these lacunae and/or their capillary invagination seems to be a common factor in many leg abnormalities. This probably accounts for the fact that numerous metabolic derangements may precipitate remarkably similar types of leg disorders. Under ideal conditions, the cortical, or compact bone is deposited by osteoblasts on the inner edge of the subperiosteal boundary and in order to prevent excessive thickening, osteoclasts reabsorb material at the boundary with the marrow cavity. Normal development, therefore, relies on the continual deposition and reabsorption of bone. It is interesting to note that the majority of skeletal abnormalities relate to problems with deposition, rather than the reabsorption of bone at the growth plate. In the young bird, the marrow cavity is composed almost exclusively of myeloid tissue, while in the adult, this is replaced by adipose tissue. In older birds, long bones represent a major store of adipose tissue. Because of this large fat reservoir in the skeleton, it would be important to monitor skeletal integrity in response to any management, nutritional or genetic changes, aimed at reducing fat content of meat birds.

Medullary bone is unique to adult females, where a spongy-type bone replaces the marrow in the cavity of the long bones. It is assumed that this labile calcium reserve is the major skeletal contributor of calcium during shell formation. Presumably the medullary bones role in shell formation is to augment calcium supplies during the dark period, when the hen consumes little feed. This bone is then replaced in the interim between successive shell formations. The interlacing spicules are similar in gross appearance to cancellous bone in the epiphysis of the long bone shown in *Fig. 11.2*. In medullary bone, the spicules are surrounded by red marrow and blood sinuses.

The medullary bone develops under the action of both estrogen and androgen, and this is the reason for its association with sexual maturity. Clunies *et al.* (1992) recently estimated the total skeletal medullary calcium reserve in adult hens at around 0.75 g when birds are fed 3.5% calcium in the diet. Most of this calcium was in the femur and tibiotarsus with much lesser amounts in the humerus. Clunies *et al.* (1992) found that birds fed deficient levels of calcium attempted to maintain medullary calcium integrity, at the expense of cortical and spongy bone.

3.3.   *Bone growth*

Both the longitudinal growth and bone thickness are controlled by the activity at the growth plate in the metaphysis region (*Fig 11.2*). The zone of active hypertrophy is recognized by its hardness and especially its opaqueness. In normal bone, the proliferating zone is a relatively narrow band, but as will be discussed later, certain metabolic derangements lead to the thickening and widening of this zone and associated non-ossified cartilage. Bone growth is accomplished through two basic processes. First, there is the formation of the bone matrix (collagen fibres and

mucopolysaccharides) followed by calcification, mainly as $Ca_3(PO_4)_2$ (Hodges, 1974). Osteoblasts are responsible for the synthesis of basic collagen units, which tend to increase in size with distance from the site of synthesis. Calcification occurs on the matrix parts that are more mature, and once ossification has occurred, the osteoblast reverts to a quiescent osteocyte (Hodges, 1974). There is still some controversy as to the mechanisms with which unmineralized collagen matrices become impregnated with mineral salts. Either nucleation sites exist on the collagen molecules, or so-called matrix residues actively accumulate calcium and phosphorus ions to levels required for precipitation. Berthet-Colominas *et al.,* (1979) suggest that when collagen is calcified, minerals penetrate throughout the fibrils, becoming crystalline in the so-called "hole region" and amorphous between the collagen molecules. These two mineral forms probably account for differences in the labile nature of various minerals within certain bone, and the rate with which they are affected during nutritional inadequacy.

Osteoclasts bring about resorption of bone during growth and/or repair situations. Such osteoclasts invaginate medullary bone during shell calcification, and are responsible for the gradual internal erosion of bone surface as normally occurs with elongation and growth in width of the bone (Hodges, 1974). There are marked differences in the rates at which different bones are ossified. Hogg (1980) suggests the possibility of there being some set biological time limit to normal ossification, and so this may have implications to the industry situation of marketing broilers at ever decreasing ages. Bone formation in the zone of ossification therefore results in gradual replacement of hypertrophic cartilage. The multiplying chondrocytes at the growth plate are arranged in parallel columns aligned with the bone shaft, and this is the basis of the calcifiable matrix. Minerals and other nutrients are supplied by invading blood vessels, originating from the base of the growth plate, such that calcified hypertrophic cartilage is eventually replaced by inorganic bone material. In birds, the chondrocytes at the top end (epiphysis) of the growth plate are supplied with separate blood vessels that enter the articular cartilage in the joint region. There is no joining of these two separate blood supply systems. Growth of bone is therefore dependent upon regular blood supply of nutrients to the active growth plate.

The thickness of the actual growth plate is proportional to the growth rate of the plate. Growth plates of the proximal tibiotarsus and tarsometatarsus have the fastest growth rates, and so consequently these bones are wider at these regions. Interestingly, Riddell (1981) suggests that the number of vascular tunnels penetrating a given area of zone of hypertrophy is less in plates with fast vs slow growth rates. It is not known if this relates to problems seen at the growth plate of these faster growing leg bones. Normal bone growth in the young bird therefore involves continual progression of the hypertrophic growth plates that bring about growth of bone in width and length. Assuming adequate nutrition and normal vascular integrity, osteoblastic activity ensures correct deposition and balance of bone minerals. In the young broiler chicken it must be remembered that leg bones such as the tibiotarsus will increase in length from around 25 mm at hatch to about 100 mm in only 42 d. While this rate of bone growth may be surpassed by some wild bird species, it undoubtedly contributes to a potential for metabolic disorders.

3.4.   Embryology

Because a number of skeletal disorders are seen in the first few days after hatching, it is possible that metabolic disorders are initiated during incubation. Freeman and Vince (1974) indicate that skeletal mineralization starts at around the eighth day of incubation, and at this time

the yolk serves as a source of calcium. These authors suggest that shell calcium is not utilized until about the 12th d of incubation. During the course of embryonic development, the embryo will take up some 120 mg Ca from the shell (Tuan and Lynch, 1983).

Culturing developing embryos in a medium deficient in calcium quickly results in gross skeletal abnormalities. Tuan & Lynch (1983) suggest that calcium deficiency at this age influences the normal progression from collagen type I, to collagen II. Finally ossified bone abnormalities are seen due to the expression of mature collagen II in regions where osteogenesis should normally occur. The problem, therefore, seems to be due to retardation of cellular differentiation in the cartilage-bone transition, a situation that will be described later as being common with broilers and turkeys post-hatch. In addition to maintaining normal calcium metabolism, the embryo must, of course, adjust to balance of other ions during the period of ossification. Freeman and Vince (1974) indicate concurrent increases in skeletal Ca^{2+}, PO_4^{3-} and CO_3^{2-}, with a decrease in HPO_4^{2-}. As the bone matures, the proportion of crystalline CO_3^{2-} increases, with the CO_3^{2-} substituting for HPO_4^{2-} in the acidic $CaHPO_4$ precursor.

4.   POTENTIAL CONTRIBUTORS TO ABNORMAL BONE DEVELOPMENT

4.1.   Nutrition

Deficiencies of most nutrients will adversely affect bone development, and therefore influence skeletal integrity. The major nutritional problems likely to influence skeletal development relate to the metabolism of vitamins, minerals and electrolytes, although an imbalance of major nutrients such as protein and energy can also affect bone growth. The classical nutritional problems relate to the balance of calcium, phosphorus and vitamin D_3 in the diet and their availability to the bird. Deficiencies or inappropriate levels of any of these three critical nutrients will lead to rickets and other bone disorders. Likewise an imbalance of electrolytes will precipitate a condition known as tibial dyschondroplasia, where normal ossification at the growth plate of long bones is replaced by excessive cartilage development. The specific details of nutritional problems as they relate to individual metabolic disorders will be discussed in the following sections. At this time, it is hoped to provide a more general overview of how nutrition can influence bone development.

There seems to be some disparity between the effects on leg problems of (1) limiting the incidence of leg problems by reducing the plane of nutrition and (2) failing to aggravate the problem by artificially increasing body weight. This apparent dichotomy suggests that it is the rate of growth and/or metabolic derangements related to higher levels of nutrient intake rather than body weight *per se* that is important in precipitating any condition.

4.1.1.   Protein and amino acids

There has been recent interest in the effect of diet protein on leg problems. From a more traditional point of view, Hulan *et al.* (1980) report fewer leg defects with lower protein diets, relating this effect to a tempering of initial growth rate. However, as suggested by Summers *et al.* (1984), leg problems today are related to more complex situations, and as such, nutrient interrelationships

may be involved. In this context, it is known that excess protein produces "stress" as evidenced by the increased size of the adrenals. Diets high in protein can interfere with folic acid metabolism and in so doing increase the incidence of leg problems (Creek and Vasaitis, 1962; Wong *et al.,* 1977). Similarly Stoews and Scott (1961) indicated that high protein intakes increase the birds' requirement for vitamin A. However, Summers *et al.* (1984) suggest that the diet protein level (22 vs 30%) when combined with a particular vitamin deficiency, does not appear to enhance any particular leg condition. Contrary to these results, Skinner *et al.* (1991) show that increasing the levels of dietary amino acids from 80 to 120% of standard recommendations resulted in reduced bone ash, and reduced tibiotarsus weight and length. These higher levels of amino acids supported normal growth rate, but decreased the rate of bone calcification, especially when combined with low calcium diets. Working with rats, Weiss *et al.* (1981) found that very high (4 times normal) levels of diet protein adversely affected bone mineralization and that when combined with low calcium in the diet, it resulted in a 62% reduction in bone cell proliferation and chondrogenesis and 98% inhibition of bone formation. The high protein-induced osteoporosis seems due to failure of osteogenesis at the stage of ossification, which is possibly a result of reduced availability of calcium at the site of mineralization. However, in subsequent studies, these same researchers show that adding calcium to high protein diets does not correct the bone formation problem. Deficiences of certain amino acids can also affect bone mineralization. For example Farran and Thomas (1992) show valine deficient birds to have increased incidence of leg problems, and relate this to reduced hydroxyproline availability and also to increased calcium excretion in the urine.

In studying factors influencing the skeletal development in broiler breeders and Leghorns, Leeson & Summers (1984) indicated that while early skeletal development was little influenced by mineral and vitamin fortification, shank and keel lengths could be increased by feeding diets of higher protein content (22 vs 16%). It is also conceivable that the ratio of amino acids:non-protein nitrogen may be of importance in the development of bone organic matrix. Elkin *et al.* (1978) and Giles (1981) showed that replacing soybean meal with purified amino acids resulted in a dramatic increase in leg problems. With a totally synthetic amino acid diet, De Moraes *et al*. (1984) indicated that 5% glutamic acid as the sole dispensable amino acid source resulted in a large number of leg problems. While the addition of 10% glutamic acid was necessary to optimize growth under these conditions, 12.5% glutamic acid was necessary for optimum organic matrix content of the leg bones. Since the organic matrix content alone was influenced, no real difference in bone ash was observed (De Moraes *et al*., 1984). Comparable effects are seen with manganese deficiency (Leach, *et al.,* 1969) while Elkin *et al.* (1978) also reported that leg problems caused by grain sorghum are related to derangement of the inorganic matrix. These data suggest that the common experimental practice of monitoring bone ash content may not always be useful in assessing adequacy of certain treatments.

4.1.2. Vitamins

El Boushy (1974) and Riddell (1975) provide extensive reviews of the classical effect of vitamin deficiencies on leg disorders. As expected, the vitamin D_3 status of birds has been extensively studied as it relates to bone disorders. At certain times a deficiency of D_3 will mimic both Ca and P deficiency situations. While Ca deficient chicks are usually hypocalcaemic and hyperphosphatemic, D_3 deficiency invariably results in hypocalcaemia and hypophosphatemia (Long *et al*., 1984a,b,c). In the D_3 deficient chick a greater relative P deficiency is caused by parathyroid hormone.

Vitamin D_3 is absorbed into the blood and stored in the liver, and so circulating levels of D_3 are usually low. In the liver, vitamin D_3 is converted to a metabolite, 25 $(OH)D_3$ and this is released into circulation. Goff (1990) suggests that circulating 25$(OH)D_3$ levels in broilers should be at least 5 ng/ml while in layers the value should be closer to 10 ng/ml. In vitamin D_3 deficient chicks, bone resorption is inhibited after about 7 d, while the formation of osteoid continues, resulting in an increased total bone mass. This increased bone mass is characteristic of D_3 deficiency, and suggests that osteoclastic activity is in some way D_3 dependent.

Timing of onset of deficiency symptoms for vitamins such as D_3 is variable, because there is variable carry-over of D_3 into the egg related to dam nutrition *(Table 11.1)*.

TABLE 11.1 Maternal D_3 status and bone ash content of offspring		
Maternal dietary Vitamin D_3 (IU/kg)	**Vitamin D_3 in egg yolk (IU/g)**	**7d offspring bone ash (%)**
1700	0.32	39.2
3400	0.54	39.2
5100	0.68	42.1
6800	0.68	43.1
27000	5.40	47.2
		Adapted from Murphy *et al.* (1935)

Huffer and Lacey (1982) studied the time sequence involved in the recovery process of D_3 deficient chicks. Within 12 h of receiving an oral dose of D_3, distal portions of elongated epiphyseal vessels and adjacent maturing chondrocytes underwent necrosis. Within 72 h, calcification had resumed and by 120 h necrotic cartilage had been removed a normal growth plate restored. These authors concluded that in normal birds (but not hypocalcemic birds), since both chondrocyte hypertrophy and calcification occur in the proximity of perforating epiphyseal vessels, it seems likely that these vessels exert their influence by delivery (or not) of calcium to the chondrocytes. Thornton (1970) implied that in situations of D_3 repletion, the skeleton responds much more slowly than does the intestine, since the immediate effect was for better "absorption" of the diet Ca. There is also good evidence to suggest that D_3 is involved with collagen synthesis. Mechanic (1976) indicated that vitamin D_3 status is reflected in the ratio of structural crosslinks of dihydroxylysinonorleucine (DHLNL) to HLNL. The maturation of collagen crosslinks seems D_3 dose related, and Mechanic (1976) suggested that imbalance between DHLNL and HLNL may be one of the first indications of bone disorder.

Controversy still exists with respect to the biopotency of D_3 sources and metabolites. Dickson *et al.* (1984) report that 1,25$(OH)_2$-D_3 is able to prevent rickets and as such has five times the potency of D_3. Although bone growth is greater when chicks receive 1,25$(OH)_2$-D_3, high doses do seem to impair skeletal development. Similarly, while 24,25$(OH)_2$-D_3 corrects plasma Ca homeostatis, it exacerbates cartilage lesions due to apparent stimulation of chondrocyte proliferation with failure of mineralization. Masoni *et al*. (1984) suggest that in terms of longitudinal

growth rate of the tibiotarsus and its Ca and P content, D_3 was more effective than $1,25(OH)_2$-D_3, while $25(OH)$-D_3 was intermediate. However, in subsequent studies this same group indicated the converse sequencing of 1,25; 25; D_3 as being effective in promoting bone strength (Ferretti *et al.*, 1984), leading these workers to suggest that the degree of bone mineralization is not related to mechanical properties. Orban *et al.* (1993) suggest that relatively high levels of ascorbic acid (2 - 3,000 ppm) added to the diet of broiler chickens results in increased femur strength.

The exact involvement of vitamin metabolism in the etiology of leg problems is certainly unclear at this time, the general consensus being that "complex deficiencies" are involved. Summers *et al.* (1978) indicated remarkably similar gross leg problems in broilers fed diets deficient in a range of individual vitamins. In more detailed studies of these affected birds, Ferguson *et al.* (1978) showed no clear trend to suggest a basis for diagnosis although vitamin deficiencies *per se* resulted in less of an effect on intercondyle depth than did manganese deficiency. Summers *et al.* (1984) also omitted a number of B-vitamins from the corn-soy diet of broilers up to 3 wk of age. Only with the omission of supplemental riboflavin and niacin was there an effect on mobility, although "deficiency" of most vitamins resulted in some degree of paralysis, crooked toes or angular bone deformity. Summers *et al.* (1984) conclude that the deletion of supplemental vitamins from corn-soy diets for a short period of time is likely of little consequence in causing major leg problems in chicks. In their earlier work, Summers *et al.* (1978) found that the effect of feeding vitamin "deficient" diets was more problematic to floor rather than cage-reared birds, explaining this difference on the necessary distance travelled by these walking-impaired birds to reach feed and water. This concept raises the general question of locomotion of birds with leg problems, and the rapid deterioration that can occur not only in skeletal developments, but also growth in general, if birds are reluctant to move in order to eat and drink.

While most B-vitamins have been associated with leg problems, pyridoxine has perhaps received most attention. There is overwhelming evidence to suggest that low levels lead to skeletal abnormalities and/or that supplementation reduces the incidence (Cope *et al.,* 1974, 1979; Beirne and Jensen, 1981). Sauveur (1984) hypothesizes that pyridoxine may exert its beneficial effect via involvement with zinc homeostatis and in particular the formation of picolinic acid which is involved in intestinal zinc absorption. Citing evidence for the necessity of B_6 in picolinic acid synthesis from tryptophan, Sauveur (1984) indicates a synergism between zinc, B_6, and tryptophan involved in the prevention of leg weakness. The situation with pyridoxine is further complicated through the effect of diet protein. Gries and Scott (1972) clearly demonstrated a perosis-like condition in birds fed 2.5 mg B_6/kg at 31% CP, while no such effect was seen at 22% CP. As previously described for many other diet situations, pyridoxine deficiency manifests itself through epiphyseal lesions consisting of the uneven invasion of irregular blood vessels into the maturing growth plate. Presumably the higher level of diet protein increases the metabolic requirement for pyridoxine through such processes as transamination and/or deamination. Diets high in protein have also been implicated in the involvement of folic acid with leg problems (Wong *et al.*, 1977) although, as previously mentioned, Summers *et al.* (1984) failed to confirm this effect.

While the deficiencies of many vitamins can, therefore, precipitate leg problems in broilers and turkeys, there is also evidence to suggest that certain vitamin excesses may be detrimental. Stevens *et al.* (1983) indicated that very high levels of vitamin A in the diet increase the incidence of rickets, while March *et al.* (1973) observed impaired bone formation with excess dietary vitamin E. Working with rats, Yang and Desai (1977) correlated excess vitamin E intake with reduced bone ash with little change in Ca and P content, suggesting impaired mineralization. Murphy (1981) also showed reduced bone ash in birds fed excess vitamin E, inferring a derangement of vitamin D

metabolism as the likely cause. Johnson *et al.* (1992) show that high levels of dietary niacin can also lead to reduced bone strength. It must be pointed out, however, that all these reported effects of vitamin excess on bone metabolism relate to dietary levels grossly in excess of normal feeding levels and hence would only be practically encountered under unusual circumstances.

4.1.3. Minerals

As with vitamins, deficiencies or excesses of a vast range of minerals can also influence bone development. The effect of abnormal levels and/or ratios of calcium:phosphorus are well documented (Edwards & Veltmann, 1983). While confusion sometimes exists with respect to the diagnosis of calcium vs phosphorus deficiencies, Lacey and Huffer (1982) cite histological evidence for their differentiation. Long *et al.* (1984) also indicate that the accurate on-farm diagnosis of phosphorus deficiency vs calcium excess is difficult, and initial recommendations of diet change can be misleading prior to complete diet analysis. Long and Britton (1984) observed identical lesions for the two conditions suggesting that excess calcium forms insoluble $Ca_3(PO_4)_2$ in the intestine, so inducing phosphorus deficiency. Ogura (1981) likewise indicated severe leg problems when birds were fed high levels of $CaCO_3$, but that comparable levels of $CaHPO_4$ resulted in no leg abnormalities. In attempting to isolate a similar mechanism between Ca and Mn, Ogura (1981) observed excess diet calcium to have no adverse effect on manganese availability. Guinotte *et al.* (1991) conclude that particle size of calcium source can also have an effect on skeletal development.

Considering birds with a stilted gait or reluctance to move or walk as an indication of weak legs, Nelson *et al.* (1992a) found no meaningful difference in diet treatments involving various "practical" levels of available phosphorus (AvP) and calcium. While diet had no effect on leg problems, birds with weak legs had significantly increased bone volume, reduced bone length and dramatic increase (18.8 to 52.1%) in crooked bones. In a previous study, Nelson *et al.* (1990) concluded that dietary effects of available phosphorus or the ratio of Ca:AvP are inconsistent. Such inconsistency could relate to an interaction of Ca and P with other minerals or other nutrients, and so effectively create different ratios of Ca:AvP at the metabolic level. For example, Portsmouth (1984) concludes that high levels of both zinc and manganese can influence the insolubility of phytate phosphorus, forming complexes even less soluble than Ca-phytase. Hakansson and Svensson (1977) likewise suggest an interaction of diet fat and diet calcium, in that formation of insoluble soaps renders calcium much less soluble. Using diets containing 1.2% calcium, Atteh and Leeson (1984) show the adverse effects of feeding saturated fats to young broilers in terms of fat and calcium retention and bone integrity *(Table 11.2)*.

TABLE 11.2 Effect of fat source on calcium utilization and bone calcium content			
Fat type	**Calcium retention (%)**	**Bone ash (%)**	**Bone Ca (%)**
Unsaturated	57.3	40.7[b]	32.5[b]
Saturated	51.8	39.2[a]	29.3[a]
			Adapted from Atteh and Leeson, 1984

Even the form of calcium, such as large vs small particle size can apparently affect calcium absorption and so skeletal characteristics (Guinotte *et al.,* 1991). The calcium content of bones usually varies little within different regions of the skeleton, and so analysis of a bone such as the tibiotarsus is representative of the skeleton *per se*. Bone ash usually contains 34-37% Ca *(Table 11.3)*.

TABLE 11.3 Normal mineral concentrations in bone ash	
	Bone Ash
Calcium	36.8%
Phosphorus	17.5%
Magnesium	0.58%
Zinc	220 ppm
Copper	20 ppm
Iron	460 ppm
Manganese	3 ppm
Fluorine	20 ppm
Cadmium	<0.01 ppm
Lead	1.5 ppm
	Adapted from Doyle (1979)

Changes in levels of some of the trace minerals have been reported to influence bone integrity. Considerable research has been carried out with diet fluorine levels, because rock phosphates can be heavily contaminated with this mineral. Norberto-Michel *et al.* (1984) fed birds 0 or 250 ppm F from rock phosphate and observed a 3-10 times increase in bone F, although this was not associated with changes in growth or feed intake. After sexual maturity, females concentrated much more F in bone, and at 104 wk of age the bone concentration was 11,000-12,500 ppm F. It seems likely that F is deposited in the bone along with Ca, but is not removed when Ca is mobilized for shell formation. However, even this high level of F in bone had no observable effect on layer performance. Merkely and Miller (1983) in fact observed increased bone ash and breaking strength when birds were fed 100 ppm dietary F. Contrary to these results, Huyghebarert *et al.* (1988) suggest that F supplementation of drinking water does not improve bone strength.

Mineral sources may also be contaminated with magnesium, the classic example being, dolomitic limestone which contains up to 20% Mg. Lee *et al*. (1980) indicate that birds fed high levels of Mg often show unusual skeletal development, and especially that of the tarsometatarsus. In earlier studies, these workers have shown that feeding 0.3% Mg caused shortening, twisting and bowing of the tibiotarsus with a concomitant reduction in bone ash. Microscopic examination revealed rachitic-type lesions, with a widened and lengthened growth plate, excessive osteoid seams on endochondral bone and osteoid or capped metaphyseal blood vessels with few associated osteoblasts. When the diet contained 0.9% Mg, 80% of the birds were affected. These results are of commercial significance, because broiler diets can contain up to 0.4-0.5% Mg through contribution of conventional ingredients. Copper is intimately associated with collagen formation, and as will be detailed later, Cu deficiency produces signs that are similar to some classical leg disorders. Rucker *et al.* (1975) observed increased bone fragility in chicks fed <1ppm Cu, and this

was associated with less collagen crosslinking. This cross-linking defect seems to be the problem, since the introduction of artificial crosslinks by formaldehyde treatment improved bone strength.

In order to combat the effects of mycotoxins and/or to improve shell strength, zeolites are sometimes added to poultry diets. A large range of natural and synthetic zeolite compounds are available, most of which contain high levels of aluminum, and this raises some concern regarding bone integrity. Johnson *et al.* (1992) found 0.3% dietary Al to reduce tibiotarsal length, although levels of 0.1 or 0.05% were without effect. At 0.1% Al there was reduced breaking strength and reduced bone concentrations of Ca, P, Mg and Zn. Watkins and Southern (1992) also found reduced bone breaking strength when Al was fed.

4.1.4. Electrolyte balance

In addition to the actual levels of minerals in the diet, the balance of certain minerals has a direct effect on skeletal development. Of greatest importance is the balance of cations and anions, and especially Na+K:Cl. Such ionic balance can affect the incidence of disorders such as tibial dyschondroplasia as will be subsequently discussed in more detail under this particular leg problem. Electrolyte balance is also discussed later in more detail *(Chapter 10)*.

4.1.5. Mycotoxins

A number of dietary mycotoxins can influence bone development, and again tibial dyschondroplasia is the condition most affected. Mycotoxin effects are detailed in Chapters 12-16 where the reader is particularly directed to discussions on aflatoxin, ochratoxin and various *Fusarium* species toxins.

4.1.6. Dietary ingredients

There has been considerable research conducted on the potential beneficial effects on skeletal development, of products such as brewer's yeast and distiller's grains. Plavnik and Scott (1980) suggest that 10% dietary brewer's yeast significantly reduced the incidence of tibial dyschondroplasia (TD) while at the same time bone strength was increased. Veltmann and Jensen (1979), however, failed to show any response to distiller's grains, while Veltmann and Jensen (1981) likewise showed no effect of fermentation products on the incidence of TD. Of more commercial importance perhaps is the report of Edwards (1985) that source of soybean meal can have a marked effect on the occurrence of TD. In testing three samples of meal, Edwards (1985) indicated that two caused a high incidence of TD, while birds fed the other source of soybean meal exhibited a much lower incidence. Lowering the levels of supplemental chloride and increasing diet calcium concentration seemed to aggravate the condition regardless of soybean source. Comparable to the situation seen with rye, autoclaving of the soybean meal seemed to reduce subsequent leg problems; a correlation was also noted between leg problems and trypsin inhibitor level. Jensen (1985) has also implicated soybean source in the occurrence of foot pad problems with breeders.

The exact cause of leg problems occurring with rye has not been fully elucidated, although the impairment of vitamin D_3 absorption is often implied. The prompt increase in bone ash content

following the removal of rye from the diet led MacAuliffe and McGinnis (1976) to suggest the presence of a factor which interferes with nutrient absorption rather than there being some metabolic antagonism. In addition to the beneficial effect of autoclaving, as previously described with soybean meal, rye seems to respond favourably to both gamma irradiation and water extraction (MacAuliffe *et al.,* 1979).

As some similarities may exist between the deleterious effects of certain soy and rye samples on leg disorders, so similarities exist between sorghum and rapeseed meal. Jurd & Geissman (1956) indicated that tannins which are normal constituents of most rapeseeds and sorghums form complexes with metal ions and so this may affect bone development. Seth and Clandinin (1973) proposed that perotic conditions related to the feeding of rapeseed meal may be due to binding of minerals with tannin. However, in this situation, increasing the level of manganese in the diet did not alleviate the condition. Elkin *et al.* (1978) suggest that tannins in sorghum may influence the degree of collagen solubility in the femur. It is possible that tannins are being absorbed and functioning to increase the amount of crosslinking in collagen. Elkin *et al.* (1978) cite evidence for comparable effects of tannins on the soluble collagen content of tendons in the rat. Seth and Clandinin (1973) suggest that the deleterious effect seen with rapeseed meal may be due to the confounding effect of goitrogenic activity. Briggs and Lillie (1946) indicate a high incidence of perosis due to adding thiouracil to the diet, and that this effect was not corrected with manganese supplementation, although Holmes and Roberts (1963) cite evidence which suggests factors other than goitrogenic activity are involved in such perotic conditions. Regardless of mode of action, it is obvious that a number of feedstuffs can aggravate certain skeletal abnormalities.

4.2. Genetics

While Hulan *et al.* (1980) conclude that genotype: diet interactions are of little importance in meat birds, field observations indicate real differences in the incidence of leg problems for different strains of bird. Sorensen (1992) concludes that the majority of leg disorders have a hereditary background. Mandour *et al.* (1989) showed that it is possible to select for increase in humerus strength in broilers, and that even within three generations, significant change occurred. Interestingly these workers found that while such selection within caged birds resulted in improved skeletal characteristics, bone strength was still less than that of non-selected floor-reared birds. Nestor and Emmerson (1990) selected turkeys for straight vs twisted tibiotarsus-tarsometatarsus joints. From these matings, only 19% of offspring from "straight" legged parents had leg defects, while a 50% incidence of twisted leg was observed in offspring who's parents showed these abnormalities.

Interest in genetic selection against leg defects intensified with the observation of Leach and Nesheim (1972) that incidence of tibial dyschondroplasia was responsive to genetic selection. Over a seven year period, selection for TD increased the general incidence from 2 to 29%, while selection against TD over the same time period, eliminated TD. Tibial dyschondroplasia is affected by acid-base balance *(See Chapter 10)* and Leach and Nesheim (1972) found that the low incidence strain still showed changes in electrolyte balance when fed high Cl diets, although TD did not occur. This data suggests that success in genetic selection against TD does not occur because of a special ability to resist changes to acid-base balance. Nestor and Emmerson (1990) suggest that the rapid response to selection for TD indicates that major gene(s) are involved. Sheridan *et al.* (1978) previously indicated rapid response to selection for TD, suggesting circumstantial evidence

for the presence of a major sex-linked gene, the recessive allele of which is associated with higher occurrence of TD. Because the realized heritability was greater than one, Sheridan *et al.* (1978) conclude that environmental factors are major contributors. In this context there was a high maternal component, or dominance genetic component, or both for inheritance of TD, suggesting environmental factors associated with the female parent likely have a major effect on TD in broilers.

4.3.   Sex effect

Leg problems and skeletal disorders are more common in male rather than in female birds, although it is obviously difficult to separate effects due to sex *per se* from that of growth rate. Bond *et al.* (1991) show a differential growth rate of bones such as the tibiotarsus, in male and female birds. Weight of the tibiotarsus keeps increasing in males up to about 10 wk of age, whereas in females there is a plateau effect around 8 wk of age. To some extent these skeletal characteristics mirror sex differences in body weight. Male birds also usually exhibit greater bone strength, but this obviously does not help prevent more problems from occurring with male birds.

Turner and Schraer (1977) found an increase in organic mass of the femur within 36 h of estrogenizing male quail. A large increase in the uptake of collagen began 36 h after administering estrogen, and this reached a peak at 3.5 times normal after 60 h. Initiation of mineralization of the organic matrix lagged the initiation of collagen synthesis by 24 h (Turner and Schraer, 1977). Taking an alternate approach Pierson *et al.* (1981) showed castrated turkeys to have a higher incidence of leg abnormalities than did intact males or those treated with testosterone. It is suggested that androgens act to fuse the epiphyses and shafts of long bones. In attempting to account for the apparent dichotomy of females not having a higher incidence of leg problems, Pierson *et al.* (1981) suggest there are major sex differences in the hormonal control of skeletal development related to the balance of androgens:estrogens. Ranaweera (1981) also indicated that treatment with anabolic steroids resulted in reduced growth rate of tibiotarsal length, although bone mass was not influenced. However, the effect of androgens:estrogens *per se* on skeletal development in the relatively juvenile broiler of today is perhaps questionable due to the fact that little sex differentiation in tibiotarsal length is seen until after 5 wk of age (Mullen and Swatland, 1979).

4.4.   Body weight/growth rate

It is often assumed that leg problems and skeletal disorders are in large part due to the heavy body weight of commercial meat birds. The statement that "weight is too much for the legs" is often quoted as the simple reason for so many of the leg problems seen today. In reality, body weight *per se* does not seem to be a factor, and this has been clearly demonstrated by harnessing birds with even more weight. In the early 80's researchers at Houghton in the UK reported no effect on incidence of twisted tibiotarsi when birds were artificially loaded with up to 10% of their body weight. Cook *et al.* (1984) also added weights to the back harness of chicks and poults, with loads at 0.3, 4.5 or 8.5% of body weight. The artificial weights had no consistent effect on leg abnormalities. Patterson *et al.* (1986) also loaded poults with artificial weights but also superimposed treatments involving various levels of crude protein. Weight loading had no consistent effect on stress or modulus of elasticity of leg bones. Turkeys fed 28% CP had stronger bones than did those fed lower levels of protein, although this effect was due simply to increased size and weight of bones from heavier birds. Diet effects were independent of weight loading.

These results suggest that body weight *per se* is not a factor in skeletal development. Growth rate *ie.* rate of change of body weight, is therefore the major factor influencing bone disorders, rather than body weight at any one period in time. Certainly slowing down the growth rate of birds has a dramatic effect on leg problems and most metabolic disorders, but of course this is not always an economical solution.

5.   TIBIAL DYSCHONDROPLASIA

5.1.   *Compendium*

Tibial dyschondroplasia (TD) is characterized by an abnormal cartilage mass in the proximal head of the tibiotarsus. TD is seen in all fast-growing meat birds, but it is most common in broiler chickens. Symptoms can occur early, but more usually are first seen at 21-35 d. Birds are reluctant to move, and when forced to walk, do so with a swaying motion or with a stiff gait. TD relates to the disruption of the normal metaphyseal blood supply in the proximal tibiotarsal growth plate,where the resultant disruption in nutrient supply means that the normal process of ossification does not occur. The abnormal cartilage is composed of severely degenerated cells, with cytoplasm and nuclei appearing shrunken. The exact cause of TD is unknown, although incidence can quickly be affected through genetic selection, the condition apparently being affected by a major sex-linked recessive gene. Dietary electrolyte imbalance, and particularly high levels of chloride seem to be a major contributor in many field outbreaks. More TD is also seen when the level of diet calcium is low relative to that of available phosphorus. Treatment involves adjustment of dietary levels of calcium:phosphorus and consideration of dietary electrolyte balance. Diet changes rarely result in complete recovery. TD can be prevented through reducing growth rate, and so programs of light or feed restriction must be considered in relation to economic consequences of reduced growth rate.

5.2.   *Occurrence and general signs*

The most characteristic feature of TD is an abnormal cartilage mass in the proximal head of the tibiotarsus. The condition is reported in all fast growing meat-strain birds, although it is most prominent in the broiler chicken. Most flocks of broiler chickens will have a few affected birds, although in extreme situations the incidence can be as high as 30-40%, especially in male birds. Most affected birds are well-fleshed, and so as with most other leg disorders, it is a condition most frequently seen in fast-growing birds. The condition is similar to osteochondrosis seen in mammals while Siller (1970) was the first to describe the condition in birds as TD. Signs can occur as early as 14 d of age, although most frequently problems are seen to start at 21-35 d of age. Variation in age at the onset of signs likely relates to the fact that initially many birds will show abnormal cartilage growth without there being changes in locomotion. Birds become reluctant to move, and when forced to walk do so with a swaying motion or with a stiff gait. Inactivity leads to reduced feed intake, and so growth rate is quickly affected.

5.3.   *Pathology and metabolic changes*

5.3.1. Gross pathology

In their early interpretation of the causes of TD, Wise and Jennings (1972) correctly concluded that the condition relates to disruption of the metaphyseal blood supply in the proximal tibiotarsal growth plate. These workers concluded that abnormal blood supply was due to excessive pressure on the active growth plate. This assumption was based on work involving application of artificial pressure to the bones of rabbits and large dogs. Although Siller (1970) was the first to use the term TD, the condition was first described by Leach and co-workers in the mid 1960's. More recently Leach (1987) concludes that while normal chondrocytes undergo cell division and maturation, this process is impeded during TD. Rather than the terminal hypertrophic chondrocytes becoming vascularized and eventually being replaced by bone, in TD birds these cartilage cells do not become hypertrophic chondrocytes, are not vascularized and so do not become ossified. A typical cartilage plug therefore develops and as the bone grows there is lateral displacement of the growth plate causing characteristic bowing or bending of the legs.

Riddell (1975c) concludes that body weight *per se* is not a factor in TD, because birds that are artificially weight-loaded are unaffected in this regard. Riddell (1975b) suggests that the occurrence of TD may relate to the fact that the proximal tibiotarsus shows the fastest growth plate development. Between 21-24 d of age, the proximal tibiotarsus showed the greatest development, although interestingly, the growth rate of most bones was higher in high vs low TD incidence strains *(Table 11.4)*.

TABLE 11.4 Growth rate of bone ends in high and low TD incidence bird strains (cm/wk)		
	Low TD	**High TD**
Proximal tibiotarsus	0.69	0.75
Distal tibiotarsus	0.42[b]	0.47[a]
Proximal tarsometatarsus	0.65[b]	0.71[a]
Distal tarsometatarsus	0.20	0.23
Proximal humerus	0.48[b]	0.56[a]
Distal humerus	0.33[b]	0.38[a]
Adapted from Riddell (1975b)		

In comparison to unselected New Hampshire x Barred Rock birds, the ratio of growth rate of the proximal:distal growth plates was greater in broiler strains, regardless of TD incidence suggesting that genetic selection may have disproportionately increased the development of the now susceptible proximal growth plate. However, Riddell (1975b) showed no evidence for difference in development of this plate in high vs low TD strains *(Table 11.4)*, suggesting that growth rate *per se* is a contributing, rather than primary, factor in TD. Riddell (1975) carried out an intriguing study on the development of TD by inserting plastic film into the growth plate of young birds. Examination of the growth plate a few days following surgery indicated the development of a white opaque cartilage plug, similar to that seen in TD, which was overlying the plastic insert. The insert apparently blocked the blood supply in the metaphysis. The insert continued to move away from the zone of proliferation, because this area is supplied by a separate epiphyseal blood supply.

With growth away from the proliferation zone, but with no "removal" of cartilage (because of impaired nutrient supply) the prehypertrophic cartilage remains, and this is the characteristic of the TD plug.

5.3.2   Histology and metabolic changes

Siller (1970) suggests that the most striking abnormality of affected birds is thickening of the layer of hypertrophic cartilage in the proximal extremity of the tibiotarsi. The normal differentiation between the several layers of cartilage is less distinct as is the layer of proliferating cells with their normal columnar arrangement. Siller (1970) found that most of the abnormal cartilage is made up of severely degenerated cells, with cytoplasm and nuclei appearing shrunken in an otherwise vacant cell space. These apparently empty spaces become progressively more numerous towards the distal limit of the abnormal band of cartilage. Also of significance, Siller (1970) found that the few blood vessels that crossed the epiphyseal plate appeared as empty clefts devoid of osteoblasts.

Meinecke *et al.* (1980) described the hyperplastic TD cartilage as being composed of cells with densely staining nuclei surrounded by empty zones. Cells are often arranged in rows with wide spaces between groups of cells that are larger at the distal end of the cartilage zone. These workers maintained birds for as long as 16 wk, and saw no signs of recovery in cartilage structure. Gay and Leach (1985) describe TD cartilage as dead or dying chondrocytes entombed in matrix, whose origin is presumed to be the growth plate and perhaps relates to persistence of embryonic cartilage. Gay and Leach (1985) used tritiated thymidine to assess the life span of cells and to clarify the hypothesis that TD is due to the persistence of chondrocytes that began their existence in the growth plate. These workers found a progressive pattern of labelling over time, first in the proliferative zone, then in the hypertrophic zone, and after 4 d into the TD cartilage zone. This data suggests that TD arises as a result of persistence of growth plate chondrocytes and matrix at the mature face of the growth plate.

In a subsequent study, Leach and Gay (1987) studied the growth of hypertrophic cells in more detail. While early hypertrophic cells had normal ultrastructure, they ceased to grow once attaining a size of about 20 μm, and never attained a more normal size of around 50 μm. Rather than continuing growth to maturity, they show classical signs of necrosis and there is a progressive condensation of cells with size of only 2-9 μm. Leach and Gay (1987) suggest that these dying chondrocytes likely bind less K, and so alter the ionic balance such that insufficient calcification occurs. Because of inadequate calcification of the matrix, there will be less vascularization because the latter seems dependent upon the former. Although Leach and Gay (1987) describe the sequence of necrosis of these cells, the "trigger" mechanism is still to be determined.

Lowther *et al.* (1974) studied the composition of cartilage from normal and TD birds. The proteoglycan and collagen content of TD cartilage differed considerably from that of hyaline articular cartilage, but was similar to that of epiphyseal growth plate cartilage found in normal birds. Also the purified proteoglycan subunits extracted from the abnormal cartilage were identical in molecular weight and composition to those extracted from normal epiphyseal cartilage and there was no change in the proportion of soluble collagen contents. These findings of Lowther *et al.* (1974) confirm the observations of Leach and co-workers that TD cartilage arises through the proliferation of epiphyseal growth plate cartilage during early development. Another interesting observation of Lowther *et al.* (1974) was that degradation rates as well as biosynthesis of proteoglycan was reduced in TD cartilage. It is not clear if this change in proteoglycan turnover is a precursor to, or a

consequence of, reduced vascularization. Chen *et al*. (1993) also describe abnormal crosslink development in cartilage, and suggest the presence of a secondary growth plate in TD affected birds, while Orth *et al.* (1991, 1994) describe comparable changes in intermolecular collagen bonding in dyschondroplastic cartilage.

Freedman *et al*. (1985) suggest that TD cartilage cells do not undergo events necessary for the development of hypertrophic cartilage which is a prerequisite for vascularization and mineralization. The reduced protein and DNA contents *(Table 11.5)* suggest necrosis and a loss of P correlates with inability to synthesize protein. The authors suggest that an increase in the Na content at the expense of K is a classical change associated with cell injury caused by acute shortage of nutrients and/or oxygen. Interestingly these workers also showed a decline in cAMP in TD cartilage, a condition that they quote as being caused by continuous pressure application, at least in other species.

TABLE 11.5 Composition of normal and TD cartilage							
Cartilage	Dry Matter	DNA	Protein	Ash	% Ash		
	(%)	(µg/g)	(mg/g)	(% DM)	Na	Mg	P	K
Normal	13.6	1249	29	11.6	19.7	.85	7.2	9.0
TD	10.6	373	22	10.6	25.6	.53	3.8	4.0
					Adapted from Freedman *et al.* (1985)		

The histological changes occurring in the development of TD have been well documented, as have changes in cartilage constituents. Reduced protein synthesis may well be a consequence of reduced nutrient supply, but the trigger mechanism of this change in vascularization is still not clear.

5.4.   *Related Factors*

5.4.1. Genetics

There is obviously a genetic susceptibility to TD, because high vs low incidence strains can quickly be established. Leach and Nesheim (1965) confirmed the heritable nature of what was to become known as TD, by developing high and low incidence strains showing on average 40 and 16% TD respectively. Riddell (1976) also selected strains, ending up with an incidence of 50 vs 0%. Sheridan *et al.* (1978) suggested that TD may be related to a major sex-linked recessive gene, a concept later confirmed by Leach (1987). Veltmann and Jensen (1981) however showed no difference in incidence of TD for 9 commercial broiler strains, while Nelson *et al*. (1992b) showed neither breeder age nor strain to have any effect. In dwarf breeders Triyuwanta *et al*. (1992) give

evidence that maternal weight and maternal feed intake to influence bone development in broiler offspring.

5.4.2.   Electrolyte balance

Field studies have clearly shown that TD can be greatly influenced by dietary electrolyte balance, and in particular the level of chloride. A problem in the interpretation of the research results however is that changes in diet electrolyte balance often changes growth rate which in itself is a major contributor to TD. The relationship between TD and electrolyte balance is discussed more fully in *Chapter 10.*

Riddell (1975) studied the development of TD in high and low-incidence strains, as affected by levels of diet chloride. The control diet contained 0.04% Cl, while the high-chloride diet was at 0.75%. Lesions of TD were mild in the low incidence strain regardless of diet, while for the genetically susceptible birds there was greater incidence and severity in birds fed the high-chloride diet. Birds fed the high chloride diet exhibited elevated serum chloride and reduced blood pH and bicarbonate level. Halley *et al.* (1987) found TD in 3 wk old birds when the dietary ratio of cations:anions was reduced. For example 29% TD was observed in birds fed 54:60 meq/100g cations:anions, while no TD was seen when the cation concentration was increased to bring the ratio to 78:60 meq/100g. In these studies, the cation balance was improved by adding calcium or magnesium. Halley *et al.* (1987) did show however that different strains of bird differ in their susceptibility to acid:base related TD. Adding high levels of ammonium chloride (1.5-3%) has also been shown to increase the incidence of TD (Veltmann and Jensen, 1981) while Lilburn *et al.* (1989) showed that diets containing more chloride (0.36%) and high available phosphorus (0.65%) caused most problems to birds. In this later study, the incidence of TD was independent of diet calcium.

Assessing the interaction of Na, K, Cl and Ca on bird performance and incidence of TD, Hulan *et al.* (1987) showed that TD was reduced by increasing the levels of Ca, Na or K, but that these effects were independent. The lowest incidence of TD was seen in birds fed 0.44% Cl and 1.4% K, although these birds were also very small. Lui *et al.* (1992) describe a problem of TD in China where indigenous feed ingredients are naturally high in chloride content. TD in certain regions of China can be as high as 34-69% while in other areas incidence is a more moderate 14-28%. Lui *et al*. (1992) relate this difference to variation in composition of soybean meal used in different geographical locations. Data in *Table 11.6* indicate that the deleterious effect of using high chloride soybean meal can to some extent be corrected by adding magnesium to the diet.

TABLE 11.6 Occurrence of TD in birds fed variable levels of chloride and magnesium

Diet levels (g/kg)		Weight gain	TD
Chloride	Magnesium	(g/d)	% Incidence
1.5	2.0	20.7	16.7
1.5	4.0	22.5	6.7
1.5	6.0	15.1	6.7
3.5	2.0	25.6	60.0
3.5	4.0	26.6	32.2
3.5	6.0	19.9	3.3
			Adapted from Lui *et al.* (1992)

These authors conclude that magnesium plays a role in improving the deposition of Cu and Zn in bone.   Birds with TD had depressed collagen synthesis, and Cu, as a co-factor in lysyloxidase, is necessary for collagen biosynthesis. Data from Hulan *et al.* (1986) outline some of the complexities and interactions between ions as they may affect TD. Only chloride exerted an independent effect on TD.   With Na and K, the effects were dependent on levels of other electrolytes. For example, TD was highest with an intermediate level of K (1.1%), but the TD incidence increased when K was increased to 1.4%, only when moderate levels of Na (0.3%) but not low levels (0.17%) were used. These authors conclude that in trying to prevent TD, levels of Ca and P should be considered in addition to the more usual concern over balance of Na+K-Cl.

5.4.3.   Calcium, phosphorus and other minerals

Edwards (1984) shows high incidence and severity of TD in birds fed diets containing low calcium in combination with moderate levels of phosphorus. Using levels of calcium that are applicable to commercial use (0.94%) for young birds resulted in the lowest incidence of TD. Kling (1985) concludes that Ca:P balance, rather than absolute levels, is of significance in affecting TD. As expected, P levels as high or higher than diet Ca levels produced most TD, and the optimum ratio seems to be around 2:1 for Ca: AvP in young broilers. Riddell and Pass (1987) also found more TD by increasing the P content of diets, but that TD could not be eliminated by feeding 1.5% Ca and 0.5% AvP. TD was seen by one of these authors in Australia, where due to the high usage rate of meat meal, diets contain high levels of phosphorus *(Table 11.7)*. In this same study a thickened growth plate was most commonly seen in birds fed 1.4% Av P. Birds fed 1.4 vs 0.5% AvP had a doubling of growth plate thickness from 1.36 to 2.87 mm. Riddell and Pass (1987) suggest that growth plate lesions caused by high dietary P are characteristics of calcium-deficient birds with rickets. Such rickets can lead to TD, possibly as a result of an increased hypertrophy of chondrocytes as the birds attempt to adapt to the Ca deficiency.

TABLE 11.7 Effect of diet Ca:P on TD in 28d-old broilers			

% of diet				
Calcium	Av. Phosphorus	Body wt (g)	% TD	TD severity[1]
0.8	0.75	955[a]	70	2.5[b]
1.1	0.55	1010[b]	40	1.5[a]
1.4	0.55	1005[b]	27	1.5[a]
[1]Score 1-3	**Adapted from Riddell and Pass (1987)**			

Hulan and co-workers have carried out an extensive series of studies involving the incidence of TD in response to variable levels of diet minerals. Hulan *et al*. (1985) found that while the highest levels of Ca and AvP gave better bird performance, this was associated with the highest incidence of TD. The best bird performance was achieved with Ca:AvP of around 1.9, while TD was lowest when the ratio widened to 2.8 (although performance deteriorated). In another study, Hulan and Proudfoot (1987) studied the practice of allowing birds free-choice dicalcium phosphate, in a separate feeder, as a means of preventing TD. In fact this resulted in an increase in both the incidence and the severity of TD. Adding zeolites to the diets of broilers has been shown to reduce the incidence of TD (Edwards, 1988). However such improvement was only seen when high P diets were fed, and so presumably the zeolites were complexing with P, and so normalizing the Ca:P ratio.

Similarities between the cartilage from birds with TD and those fed Cu-deficient diets has led to interest in the role of this mineral in the TD disorder. Lilburn and Leach (1980) found that Cu-deficient and TD cartilage both oxidized less glucose to CO_2 compared to normal cartilage. Also abnormal cartilage showed a significant decrease in the activity of the Cu containing enzyme, cytochrome oxidase. However, these authors do not think that Cu is involved in the development of TD because the affected birds show normal plasma ceruloplasmin and tissue Cu levels. They conclude that some mechanism causes abnormal cells to metabolize substrate at a much slower rate, so enabling cells to persist in an area where degeneration and vascularization normally occur. Alternatively it could be argued that slower metabolism may simply be due to lack of substrates for whatever reason.

Adding thiram to the diet of birds induces lesions similar to those seen in classical TD. Wu *et al.* (1990) show that such treated birds do have unusual cartilage formation, reduced ceruloplasmin activity and reduced bone strength. The incidence and severity of the TD-like lesions were much less when $CuSO_4$ was added to the diet but not when $ZnSO_4$ or $MnSO_4$ were added, suggesting an involvement with copper metabolism. Even though Lilburn and Leach (1980) think that simple Cu deficiency is not a factor in TD, they do not preclude the possibility that TD is a result of a genetic defect related to copper metabolism and cite evidence for this from other species where Cu deficiency symptoms appear in the presence of adequate diet Cu.

Bai *et al*. (1994) recently suggested that cysteine-induced TD could be counteracted by dietary supplements of molybdenum. High levels of cysteine can cause TD, and molybdenum is a co-factor necessary for formation of enzymes necessary for cysteine catabolism via taurine. High

levels of dietary cysteine (150-300 mg/kg) caused TD, and molybdenum at 10 ppm counteracted this negative effect.

5.4.4.   Other diet factors

As previously mentioned in *Section 5.4.2*, the composition of soybean meals is suspected to be a contributing factor to TD. Edwards (1985) in trying to account for considerable trial to trial variation in the incidence of TD also investigated the source of soybean meal. One sample of soy consistently produced more TD (34-62%) compared to other samples (14-28%). Edwards (1985) found that TD correlated with increase in urease and trypsin inhibitor. Edwards (1990) also studied the role of vitamin D_3 and its metabolites. With birds fed a diet known to induce TD and containing a basal level of 1,000 IU D_3/kg, supplementation of 1,25(OH)$_2$ D_3 at 10 μg/kg diet resulted in reduced incidence and severity of TD. Because TD lesions somewhat resemble those seen in wild birds and guinea pigs fed vitamin C deficient diets, Leach and Burdette (1985) studied the role of this nutrient in various genetic lines. Adding 0.1 or 0.25% ascorbic acid to the diet had no effect on TD, and high and low incidence strains seemed to have the same levels of circulating ascorbic acid.

5.4.5.   Molds and mycotoxins

Mycotoxins produced by various *Fusarium* molds are known to affect TD *(Chapter 12)*. Lee *et al.* (1985) isolated *Fusarium roseum* in oats and tested various fractions of the mycotoxins produced, as they affected TD. The water-soluble fraction was found to be most problematic, and of the six major components of this fraction, one known as TDP-1 was found to be lethal to embryos and caused 100% TD when fed at 75 ppm. TDP-1 has since been isolated as fusarochromanone. Lee *et al.* (1985) thought that TDP-1 may be the cause of Kashin-Beck disease, a degenerative bone condition seen in humans in China. Walser *et al.* (1988) studied the role of Se in TDP-1 induced TD, again because of a potential role in Kashin-Beck disease in humans. While mortality was less in Se-fortified birds treated with TDP-1, there was no effect on the incidence or severity of induced TD. Krough *et al.* (1989) reported what they claimed to be the first direct evidence of TD due to naturally occurring fusarochromanone. The feed tested at 4-60 ppb of this mycotoxin, inducing a TD incidence of 50%. The morphological characteristics of the cartilage of affected birds was classical to TD in that the typical cartilage plug was not penetrated by the metaphyseal vascular system. Krough *et al*. (1989) showed that while these changes were most pronounced in the tibiotarsus, lesions also occurred in the humerus, femur and tarsometatarsus. An ultrastructure study of TDP-1 treated chicks was carried out by Haynes and Walser (1987). After treatment for 2-6 d, no lesions were seen, although between 8-12 d there were moderate to severe lesions. Intracellular lipid accumulation and necrosis of chondrocytes was seen within the retained cartilage. More recently Wu *et al*. (1993) indicated that moderately high levels (75 ppm) of fusarochromanone caused 100% incidence of TD in broilers, and that the minimum dietary level of this toxin needed to produce leg problems, was 20 ppm.

Certain fungicide compounds have also been shown to induce TD. Veltman *et al*. (1985) fed tetramethylthiuram disulfide, an organic fungicide, at 30 ppm. The characteristic opaque cartilage plug was observed in the proximal tibiotarsal growth plate of 3-4 wk old Leghorn birds. The plug was not mineralized, containing partly hypertrophied chondrocytes with shrunken eosinophilic cytoplasm and round pyknotic nuclei. Both the incidence and the severity of TD increased with bird

age. In a subsequent study Veltmann and Linton (1986) showed higher levels of this fungicide to be even more problematic.

5.5. Potential treatment and prevention

5.5.1   Reduced growth rate

As with most metabolic disorders, the incidence and severity of TD is greatly reduced when birds grow more slowly. This can best be accomplished by modifications to the lighting program as described with SDS *(Chapter 1),* and/or the feed program as described for ascites *(Chapter 3).* Lilburn *et al.* (1989) conclude that decreasing the growth rate of broilers after 14 d of age results in a significant reduction in incidence of TD, although severity of lesions is little affected. Edwards and Sorensen (1987) also showed that fasting birds for 8-10 h each day, had a dramatic effect on the incidence of TD *(Table 11.8)*.

	20 d body wt	Bone ash	TD	
Treatment	**(g)**	**(%)**	**% incidence**	**Severity score (0 - 3)**
Ad-libitum	560[a]	38.2[b]	59[a]	2.3[a]
10 h fast	503[b]	40.1[a]	11[b]	1.4[b]
Ad-libitum	509[a]	39.2[b]	68[a]	2.4[a]
8 h fast	435[b]	40.5[a]	5[b]	0.3[b]

TABLE 11.8 Incidence of TD in fasted broilers

Adapted from Edwards and Sorensen (1987)

Fasting did reduce TD problems, although growth rate was reduced by at least 10%. Using data from other workers, Edwards and Sorensen (1987) concluded that the incidence of TD could be reduced by about 6% for each 1 h of fasting imposed each day.

5.5.2. Dietary electrolytes

High levels of dietary chloride seem to cause more problems with TD. Chloride levels should be considered during formulation and especially in relation to levels of dietary cations. High chloride levels, and unusual electrolyte balance are most commonly seen when diets contain more meat and/or fish meal, rather than soybean meal. Animal proteins in general are concentrated sources of chloride, although cation levels are quite low. If high levels of chloride are inevitable, due to ingredient availability, then other sources of chloride must be minimised, or cation levels in the diet should be increased. Where chloride levels are problematic, it is essential to consider all sources of chloride during formulation, and especially the potential contribution of synthetic choline and lysine.

5.5.3. General diet effect

Diet modification, and especially levels of minerals and electrolytes can be used to reduce the problem of TD. It is generally accepted that TD is perhaps most greatly affected by the diet fed during the 14 d immediately prior to bird examination. Changing from high to low incidence diets (and vice versa under experimental conditions) does affect the occurrence of TD, although never to the degree that the problem is fully resolved. Diet modification should therefore be considered as a method of tempering the problems of TD. Rennie *et al*. (1993) conclude that under experimental conditions TD can be prevented by supplementing the diet with $1,25(OH)_2D_3$, although the authors were unsure of mode of action of this metabolite. Thorp *et al*. (1993) conclude that $1,25(OH)_2D_3$ plays a major role in chondrocyte differentiation, and that this is the reason for such treated birds to show much less TD.

6.   CAGE LAYER FATIGUE AND BONE BREAKAGE IN LAYERS

6.1.   Compendium

High-producing laying hens maintained in cages sometimes show paralysis around the time of peak egg production. The condition is caused by a fracture of the vertebrae that subsequently affects the spinal cord. The fracture is due to an impaired calcium flux related to the high output of calcium in the eggshell. Because of depletion of medullary bone reserves the bird utilizes cortical bone as a source of calcium for the eggshell. The condition is rarely seen in floor-housed birds, suggesting that reduced activity/exercise is a predisposing factor. Affected birds are invariably found on their sides in the back portion of the laying cage. At the time of initial paralysis, birds appear healthy and will often have a shelled egg in the oviduct and an active ovary. Death often occurs from starvation or dehydration, due to the failure of the birds to reach feed or water. Affected birds will recover if moved to the floor. A high incidence of cage layer fatigue can be prevented by ensuring the normal weight-for-age of pullets at sexual maturity, and that such pullets receive a high-calcium diet (minimum 3.5% Ca) at least 14 d prior to first oviposition. Older caged layers are also very susceptible to bone breakage, especially during their removal from the cage and during transport to processing. It is not known if CLF and bone breakage are related. Bone strength cannot practically be improved without adverse consequences to other economically important traits because in general, these diet manipulations lead to reduced eggshell quality.

6.2.   Occurrence and general signs

As its name implies, cage layer fatigue (CLF) is a syndrome most commonly associated with laying hens held in cages, and so its first description by Couch (1955) coincides with commercial acceptance of this housing system. Apart from the cage environment, CLF also seems to need a high egg output to trigger the condition, and for this reason, it has been most obvious in White Leghorn strain birds. At around the time of peak egg output birds become lame, and are reluctant to stand in the cage. Because of the competitive nature of the cage environment, affected birds usually move to the back area of the cage, and death can occur due to dehydration/starvation

because of their reluctance to drink or feed. The condition is rarely seen in litter floor managed birds and this leads to the assumption that exercise may be a factor. In fact removing CLF birds from the cage during the early stage of lameness and placing them on the floor, usually results in a complete recovery. However this practice is usually not possible in large commercial operations. In the 1960-70's up to 10% mortality was common, although now the incidence is considered problematic if 0.5% of the flock are affected. There is no good evidence to suggest an association of CLF to general bone breakage in layers, although the two conditions are often described as part of the same general syndrome. Today general bone breakage of older birds, especially during handling at the end of the laying cycle, is now more problematic than CLF.

6.3.   Pathology and metabolic changes

6.3.1.   Gross pathology

Birds are usually found on their sides, with legs outstretched if they are in a non-competitive environment. In a cage, birds often crouch in the back corner away from general activity. If birds are identified early, they seem alert and are still producing eggs. The bones seem fragile, and there may be broken bones. Dead birds may be dehydrated or emaciated, simply due to the failure of these birds being able to eat or drink. The ribs may show some beading (Riddell, 1975) although the most obvious abnormality is a reduction in the density of the medullary bone trabeculae. There is also significant evidence of osteoblast activity, although little osteoid is present. Riddell *et al*. (1968) suggest that paralysis *per se* is often due to fractures of the fourth and fifth thoracic vertebrae causing compression and degeneration of the spinal cord. If birds are examined immediately after the paralysis is first observed, then there is invariably a partly shelled egg in the oviduct, and the ovary contains a rich hierarchy of follicles. If birds are examined some time after the onset of paralysis, then the ovary is often regressed, due to reduced nutrient intake. Rowland and Foutz (1990) describe CLF as either osteoporosis of the cortical bone or osteomalacia of the medullary bone. If this is correct, then it is assumed that hens will respond rapidly to the treatment of osteomalacia but slowly to that for osteoporosis. Likewise, Rowland and Foutz (1990) suggest that an osteoporotic lesion strongly implies that the skeletal mass prior to maturity is critical in preventative nutrition.

6.3.2.   Bone histology

Bone composition is consistent with birds being deficient in calcium, and so mineralization is abnormal. There is a dilation of the Haversian canals, a cavity formation in the compact bone of the femur, and an increase in the reticular tissue (Riddell, 1975). Depending upon the stage of any eggshell formation, there may be considerable osteoclastic activity in the medullary bone. In extreme cases, there may be an erosion of the cortical bone, since it is assumed that this acts as a reservoir to help supplement the mobile medullary reserves when extreme calcium deficiency occurs.

6.4.   Related factors

Cage layer fatigue obviously relates to immobility within the cage environment and metabolic calcium deficiency.

6.4.1   Cage environment

Because CLF is rarely seen in non-caged birds, it is assumed that lack of exercise within the cage environment is a predisposing factor. While birds will rarely recover once paralysis occurs within the competitive cage environment, birds will often recover when placed on a solid floor and are given easy access to feed and water. King (1965) placed birds in various sizes of cages and found that those in very small cages developed CLF. Riddell (1975) describes histological changes in birds that had their legs wrapped in a restrictive bandage to be comparable to changes previously described for CLF. The fact that there is less incidence of CLF seen today, compared to 10-20 years ago, confirms the report of Bell and Siller (1962) suggesting there to be a genetic predisposition to the condition, and so there is a basis for improvement through breeding strategies.

6.4.2.   Diet composition

CLF is obviously due to an inadequate supply of calcium available for shell calcification, and the bird's plundering of unconventional areas of its skeleton for such calcium. Because calcium metabolism is affected by the availability of other nutrients, the status of phosphorus and vitamin D_3 in the diet, and their availability, are also critical. Birds fed diets deficient in calcium, phosphorus, or vitamin D_3 will show cage layer fatigue assuming there is a high egg output.

Scott *et al.* (1977) fed diets containing 1 or 3.5% Ca for 4 wks prior to expected maturity, and found that 1% Ca was inadequate for maximum bone mineralization or general bone ash content. In this later context, birds fed just 1% pre-lay calcium had 35% bone ash, while those birds fed 3.5% Ca had 40% bone ash. Feeding a more extensive series of diets Leeson *et al.* (1986) showed only a small increase in Ca retention of pre-lay birds when fed diets containing 0.9 up to 3.5% Ca from 19 wk of age up to the time of the 4th egg. Low calcium diets resulted in reduced early eggshell quality, although there were no apparent changes in the bone histology of birds examined after producing their 15th egg. Keshavarz (1989) however suggests that the reappearance of CLF in some commercial flocks may be a result of too early a sexual maturity due to the genetic selection for this trait coupled with early light stimulation. Feeding a layer diet containing 3.5% Ca vs a grower diet at 1% Ca, as early as 14 wk of age, was beneficial in terms of an increase in the ash and calcium content of the tibiotarsus *(Table 11.9)*.

TABLE 11.9 Diet calcium and bone characteristics of young layers in response to pre-lay diet calcium

Time of change to 3.5% Ca	Tibiotarsus Ash (%)	Tibiotarsus Ca (mg/g)
20 wk	53.5[c]	182[b]
18 wk	55.7[b]	187[b]
17 wk	59.3[a]	202[a]
16 wk	58.9[a]	199[a]
15 wk	58.4[a]	197[a]
14 wk	57.9[a]	196[a]
		Adapted from Keshavarz (1989)

Feeding a high calcium diet as early as 14 wk of age seems unnecessary, and in fact, may be detrimental in terms of kidney urolithiasis *(Chapter 7)*. As suggested by Keshavarz (1989) changing from a low to a high calcium diet should coincide with the observation of secondary sexual characteristics, and especially comb development which usually precedes first oviposition by 14-16 d.

There have been surprisingly few reports on the effect of vitamin D_3 on CLF in young birds. It is assumed that D_3 deficiency will impair calcium utilization, although there are no reports of testing graded levels of this nutrient as a possible preventative treatment. The other major nutrient concerned with skeletal integrity is phosphorus, and as expected, phosphorus deficiency can accentuate effects of CLF. While P is not directly required for shell formation, it is essential for the replenishment of Ca, as $CaPO_4$, in medullary bone during successive periods of active bone calcification. Without adequate phosphorus in the diet, there is a failure to replenish the medullary Ca reserves, and this situation can accelerate or precipitate the onset of CLF and other skeletal problems. Garlich *et al*. (1982) found the femur of P-deficient birds to have a reduced mineral content, although the organic matrix was little affected. For this reason, they suggested that the measurement of bone ash expressed per unit of external bone volume could be used as an indicator of osteoporosis in layers.

6.4.3.   Bone breakage in older hens

CLF may relate to bone breakage in older hens, although a definitive relationship has never been quantitated. It is suspected that like the CLF situation with young birds, bone breakage in older birds results as a consequence of impaired calcification of the skeleton over time, again related to a high egg output coupled with the restricted activity within the cage environment. Few live birds have broken bones in the cage, the major problem occurring when these birds are removed from their cages and transported to processing units. Apart from the obvious welfare implications, broken bones prove problematic during the mechanical deboning of the muscles. Wilson (1991) recently studied preconditioning with various levels of calcium and phosphorus as it influences bone strength in older hens. Birds that did not lay for more than 3 wks, prior to examination, had an increased radius bone strength. Bone shear strength increased with the increased bone ash content, but unfortunately diet manipulation had no effect on bone ash. Bastien *et al*. (1979) fed various types of trace minerals with varying degrees of purity, but showed no

improvement of practical significance. Ruff and Hughes (1985) did however show that humerus strength could be increased by increasing levels of both Ca and P provided that the ratio of Ca:P was maintained at 1.3:1 (immature birds). Merkley (1981) concludes that there is no evidence to suggest that any diet nutrients fed in excess of those required for optimum shell quality are in any way beneficial to the skeletal integrity of older hens. There is an indication however that fluorine given at up to 300 ppm in the water of growing pullets can significantly improve bone breaking strength and increase the bone ash of younger birds, although this treatment has not been followed through for older birds. As with CLF, bone breakage in older hens is much worse for cage rather than floor managed birds. Harms and Arafa (1986) found bone breaking strength to gradually decrease throughout early egg production, and that this was most evident for cage rather than floor housed birds. However these authors showed that moving birds from cages to floor and vice-versa at 26 wk of age had no effect on breaking strength. It would be interesting to repeat this study with older birds.

6.5.   *Potential treatment and prevention*

6.5.1.   Age at sexual maturity

The most severe cases of CLF occur when birds mature early and there is a lag with the introduction of high calcium layer diets. These early maturing birds are usually underweight and their smaller appetite leads to a reduced nutrient intake. Underweight pullets should not be light-stimulated, and all birds should receive high calcium layer diets at least 10 d prior to the anticipated first egg.

6.5.2.   Cage environment

For small-scale producers, the incidence and severity of both CLF and bone breakage can be reduced if birds are removed from cages and temporarily placed on the floor.

6.5.3.   Diet composition

Neither CLF nor bone breakage in older hens can be totally prevented by diet manipulation, and there are practical limits to the concentrations of calcium that can be added to the diet. Birds should not receive a diet containing less than 3.5% Ca on the day that they produce their first egg. In practice, this means that the whole flock must be fed a layer diet preceding the maturity of the earliest maturing birds. Sexual maturity can be anticipated from an observation of comb and wattle size and the width between the pelvic bones. There is little advantage to providing diets with more than 4.5% calcium at this time, unless the feed intake is so low that the birds are not consuming 3 g Ca/day. Feeding levels of available phosphorus much in excess of 0.42%, will likely lead to a reduced shell quality, although the effects of low-P diets on CLF and subsequent bone breakage are not known at this time. Depending upon the level of feed intake, CLF will be minimized if layers are fed a diet providing >3.5% Ca, 0.4% available phosphorus, and 1500 IU Vitamin D_3/kg for at least 14 d prior to first oviposition. Once CLF is observed, there is little advantage to diet manipulation of the whole flock, because the immediate nutrient needs of affected birds are different

from the remainder (majority) of the flock. Birds will likely recover if they can gain access to feed and water.

At this time, it is not known how to improve the bone integrity of older high producing hens, without adversely affecting other traits of economic significance. For example, Roland (1987) clearly shows that the bone breaking strength in older birds can be increased by feeding high levels of vitamin D_3. Unfortunately this treatment also results in an excessive pimpling of the eggshells, and these extra calcium deposits on the shell surface readily break off causing a leakage of the egg contents. It may be possible to improve the skeletal integrity of older birds by causing cessation of ovulation for some time prior to slaughter. Presumably the associated reduction in the drain of body calcium reserves would allow re-establishment of the integrity of the susceptible medullary bones. Currently such a feeding strategy is uneconomical, although consideration for bird welfare may provide the impetus for research in this area.

7.   RICKETS

7.1.   Compendium

Rickets most commonly occurs in young meat birds, the main characteristic being inadequate bone mineralization. Calcium deficiency at the cellular level is the main problem, although this can be induced by feeding diets deficient or imbalanced in calcium, phosphorus or vitamin D_3. Young broilers and poults exhibit lameness, usually around 10-14 d of age. Their bones are rubbery and the rib cage is flattened and beaded at the attachment to the vertebrae. Rachitic birds exhibit a very disorganized cartilage matrix, with an irregular penetration of vascular canals. Rickets is not caused by a failure in the initiation of bone mineralization, but rather in the early maturation of this process. There is often an enlargement of the ends of the long bones, with a widening of the epiphyseal plate. Differential diagnosis of rickets due to deficiencies of calcium, or phosphorus, or vitamin D_3, or an excess of calcium (which induces a phosphorus deficiency) is found in blood phosphorus levels and parathyroid activity.

In most field cases of rickets, a deficiency of vitamin D_3 is often suspected. This can be due to a simple dietary deficiency, inadequate potency of the D_3 supplement, or other factors that reduce the absorption of vitamin D_3. For example, adding rye to the diet seems to induce rickets simply through the production of a viscous digesta that impairs absorption of D_3. Rickets is often more problematic when diets contain mycotoxins and especially aflatoxin. However it is unclear if the mycotoxins create a specific metabolic deficiency of vitamin D_3 and of other nutrients, or if they simply affect the bird by reducing the feed intake. Rickets can best be prevented by ensuring adequate levels and potency of vitamin D_3 supplements, and by ensuring that the diet is formulated to provide the optimum utilization of fat-soluble compounds. Diets must also provide a correct balance of calcium:available phosphorus. For this reason, ingredients that are notoriously variable in their content of these minerals should be used with some caution.

7.2.   Occurrence and general signs

Rickets is one of the classical forms of leg weakness that occurs due to unusually reduced bone mineralization. Calcium deficiency is the ultimate cause of the problem, although these signs can also be induced by inadequate levels of phosphorus or vitamin D_3. The usual signs are soft rubbery bones and a soft, pliable beak. The rib cage is often flattened and there is often beading of the ribs at their attachment to the vertebrae. Rickets can occur at any age but it is most common in young birds, and the young turkey poult is currently most susceptible. Rickets is easily diagnosed if the diet is deficient in either calcium, phosphorus or vitamin D_3, although so-called field rickets often occurs when the diet analysis appears normal. In this context however, it should be pointed out that it is very difficult to assay vitamin D_3 in complete feeds, and so adequacy of diets is often extrapolated from assays of the vitamin premixes with the assumption that the diet was mixed correctly.

In most field outbreaks, abnormal bird behaviour is seen between 7-10 d of age. Broilers and poults appear listless, reluctant to eat, and to move around the pen. Characteristic weak bones and rib-beading can be seen at 10-14 d, where 10-100% of the flock is affected. The higher incidence outbreaks occur due to gross errors in diet formulation. The more common lower incidence situation is likely the result of an induced nutrient deficiency. Riddell (1983) studied 15 outbreaks of field rickets in poults and could only find obvious diet deficiencies in 5 of these cases. In the other outbreaks it is assumed that other dietary factors induced the deficiencies of calcium, phosphorus, or vitamin D_3, and exogenous agents such as mycotoxins or pathogens in some way impaired the normal absorption of these nutrients.

7.3.   *Pathology and metabolic changes*

The characteristic features are soft bones and a beak that can be easily bent or twisted. Bones of young birds are more pliable than those of older birds, although with rickets, the bones can be bent without breakage occurring.   There is also widening and disorganization of the epiphyseal cartilage of the long bones that is accompanied by reduced vascularization. Lameness occurs due to an enlargement of the bone ends coupled with a loss of bone rigidity, which often causes bowing of the legs. Boyde and Shapiro (1987) studied the microanatomy of the growth plate in 6-8 wk old birds that were rachitic due to inadequate vitamin D_3. At the mineralization front, calcification is usually most advanced close to the canals. Even though there was little matrix adjacent to the canal lumen, a diffusion of nutrients, metabolites and dissolved gases can still occur. Rachitic birds on the other hand exhibit a very disorganized cartilage matrix, with an irregular penetration of vascular canals with a greatly expanded hypertrophic region. Boyde and Shapiro (1987) suggest that vitamin D_3^- induced rickets is not caused from a failure of the cartilage matrix to mineralize, but rather from a failure of the initial cartilage matrix to mature.   This failure of maturation goes some way to explaining bone fragility in rachitic birds. Itakura *et al.* (1978) indicate the main gross lesions to be an enlargement of the ends of the long bones, a widening of the epiphyseal plates, a thickening of the cortical bone and the enlargement of parathyroids. The enlarged growth plate was associated with widening of both the proliferating and hypertrophic zones.   The porosity of the cortical bone was due to a dilation of the canal system and a demineralization on the walls of the Haversian canals. In more detailed studies of the parathyroids, Itakura *et al.* (1978) showed that D_3 deficiency caused swelling and hyperplasia of the parenchymal cells. During rickets, proleoglycan and hyaluronate content of cortical bone may be affected (Dickson and Roughley, 1993). Bone hyaluronate levels were elevated in rachitic chicks, although this was not directly related either to the absence of vitamin D_3 or to abnormal blood calcium levels.

These authors conclude that bone hyaluronate levels are regulated by as yet unidentified factors that are responsive to changes in D_3 and general mineral metabolism.

Long *et al.* (1984b) described some of the problems involved in a diagnosis due to the changes associated with bird age. In 14 d old birds, the growth plate was variably lengthened and disorganized in the prehypertrophied zone. However these lesions changed over time, such that at 28 d, the prehypertrophied zone decreased in length and TD like lesions were present. Long *et al.* (1984b) conclude that the lesions of calcium deficient rachitic birds are different from those caused by either calcium excess or phosphorus deficiency, and that lesions were variable over time for each type of rickets. Therefore care must be taken in advising a corrective treatment. These authors suggest that lesions of calcium deficiency represent a primary defect in cell maturation. Failure of proliferating chondrocytes to increase in size supports this concept, and this may result from failure to accumulate adequate intracellular calcium. In other studies, Long *et al.* (1984a) attempted to define a basis for the differentiation of ricket lesions caused by either phosphorus, calcium, or vitamin D_3 deficiency, or by calcium excess. One view is that phosphorus deficient diets result in rickets without there being any obvious histological signs, while others suggest that a calcium excess and phosphorus deficiency operate through similar mechanisms. While calcium deficient birds are usually hypocalcemic and hyperphosphatemic, vitamin D_3 deficiency invariably results in hypocalcemia and hypophosphatemia (Long *et al.,* 1984a). In the D_3 deficient bird, a greater relative phosphorus deficiency is apparently caused by parathyroid hormone. Long *et al.* (1984a) suggest that because diets deficient in phosphorus and those containing an excess of calcium produce similar gross, microscopic and radiographic lesions, that the ratio of Ca:P is probably the important underlying factor. An excess of calcium likely exerts its affect by causing a metabolic phosphorus deficiency via a stimulation of excretion of $CaPO_4$. This assumes that diet phosphorus levels are not excessive. Riddell (1981) also provides a basis for the differentiation of rickets due to a diet imbalance of calcium vs phosphorus. Riddell (1981) suggests that both calcium and vitamin D_3 deficiency retard bone growth, reduce bone ash and cause parathyroid hyperplasia. With phosphorus deficiency however, there is an atrophy of the parathyroids. Presumably a diet containing high levels of calcium, relative to phosphorus, would also cause atrophy of the parathyroids.

7.4.   Related factors

7.4.1.   Calcium, phosphorus and Vitamin D_3

Diet content and the balance of these three critical nutrients is obviously essential in preventing rickets. Calcium level should be the easiest to control, although problems most often occur when diets contain excessive levels of calcium in relation to available phosphorus. Formulation to specific levels of available phosphorus is more difficult, and often open to interpretation, especially for some ingredients. For turkey prestarter diets, this single factor alone is grounds for placing upper limits to the inclusion of such ingredients as meat meal and other animal proteins that are notoriously variable in phosphorus content. Diets low in calcium can cause rickets, although Skinner *et al.* (1991) suggest that signs such as an irregular capillary penetration of the cartilage are somewhat similar to that seen in TD. In most instances, rickets seems to be associated with inadequate levels, or the low potency of vitamin D_3. Itakura *et al.* (1978) conclude that rickets observed over large areas of Japan in 1974-75 was due to a simple deficiency of

vitamin D_3. In this respect, it is worth considering the phosphorus status of the birds because a D_3 deficiency causes hypophosphatemia, while calcium deficiency, usually results in hyperphosphatemia (Long *et al.,* 1984c). Potency of vitamin D_3 is obviously difficult to control, and one assumes adequate bioavailability when adding synthetics to the diet. Waldroup (1986) cites evidence of significant numbers of vitamin D_3 samples assayed as having a low biopotency, and that in order to protect against this, nutritionists can use two sources of D_3 within a given premix. Today, gelatin coated beadlets of vitamin D_3, impregnated with antioxidant, are now more stable under most mixing and holding conditions.

7.4.2.   Other dietary vitamins

Diet content of vitamins A, E and C can affect the utilization of vitamin D_3. High levels of vitamin E and vitamin A can interfere with vitamin D_3 utilization. Ricket-type lesions, including soft bones, have been reported in birds fed high levels of vitamin A (Whitehead, 1989). In these instances, the toxicity of vitamin A at 16,000 IU/kg diet has been recorded, and while this is high, it is not too much higher than values of 8-10,000 IU/kg recommended commercially. This problem can be overcome by adding more vitamin D_3, therefore a balance between the vitamins is very critical. Stevens *et al.* (1983) induced rickets in poults by feeding high levels of vitamin A, although these diets also contained significant levels of tallow that is poorly digested by the poults, and so this itself may lead to problems of utilization of fat and fat soluble nutrients such as vitamin D_3. There is also growing evidence of an involvement of vitamin C in the metabolism of vitamin D_3 and subsequent bone development. While birds usually synthesize vitamin C, stress situations lead to a response to exogenous supply. Volker and Weiser (1993) give evidence of an increase in $1,25(OH)_2D_3$ in birds fed 100 ppm ascorbic acid and that levels of calcium binding protein and bone weight were also improved. Volker and Weiser (1993) conclude that an activation of the $25(OH)D_3$-1-hydroxylase by vitamin C is a decisive step leading to optimum levels of $1,25(OH)_2 D_3$, while this in itself is a prerequisite to bone growth.

7.4.3. Bird age

Rickets most commonly occurs in young birds and this lends support to the involvement of vitamin D_3 because its hydroxylation is age dependent. The activity of hydroxylase enzyme in the kidney is low and this means that breeder as well as starter diets must contain adequate levels of vitamin D_3.

7.4.4.   Electrolyte balance

Whitehead (1989) cites evidence that metabolic acidosis, caused by high chloride levels in the feed, can reduce the production of $1,25(OH)_2 D_3$ in the kidney. Electrolyte balance is well known for its effect on TD, and so again there is evidence for similarities in metabolic pathways for these two conditions, suggesting the need for caution in the diagnosis by gross pathology.

7.4.5.   Mycotoxins

When diets are known to contain mycotoxins, and especially those produced by *Fusarium* molds, it is common to recommend increased levels of vitamin D_3. Zearalenone, produced by *Fusarium roseum*, is known to impair shell calcification in layers, and because this situation can sometimes be corrected with water-soluble vitamin D_3, this suggests that mycotoxins can impair calcium mobilization. Waldroup (1986) cites evidence of outbreaks of rickets in young birds fed diets containing marginal levels of vitamin D_3 together with aflatoxin. Bone strength has been reported to be reduced in birds fed aflatoxin or ochratoxin. An outbreak of rickets in Germany was related to the contamination of feed with metabolites of *Fusarium moniliform* (Riddell, 1981). This mold has since been shown to produce fumonisin, a potent mycotoxin. Because of their hepatotoxic or nephrotoxic effects, it is not too surprising that these mycotoxins can impair vitamin D_3 metabolism and perhaps also calcium mobilization. In addition to these direct metabolic effects, it must be remembered that birds eating mycotoxin contaminated feeds will invariably have other major signs, one of which is often reduced feed intake. The role of mycotoxins in affecting rickets may therefore occur simply due to a reduced intake of vitamin D_3 and calcium.

7.4.6. Intestinal status

Rickets can occur due to impaired absorption of digested nutrients. For example, Campbell *et al.* (1983) show ricket-like conditions in birds fed rye. Because fat retention is reduced in birds fed rye, it is assumed that vitamin D_3 absorption and possibly calcium absorption are also both reduced. When feeding rye, fat retention and tibiotarsus ash were improved by supplementing with a synthetic bile salt. Tibiotarsus ash was improved by adding extra vitamin D_3 or a detergent to the diet. As suggested by Campbell *et al.* (1983), the deleterious effects of rye are due to gums interfering with the normal absorptive processes. High β-glucan barley may have the same effect on young birds.

There is always the possibility of infectious agents causing malabsorption, and rickets may be one of the first symptoms to appear, especially in poults. Hurwitz *et al.* (1973) in comparing control birds and those with rickets, found that the affected birds had reduced intestinal mucosa, and also a reduction in mucosa calcium binding protein. Because of this, it is assumed that vitamin D_3 was not absorbed even though the diet contained apparently adequate levels. Hurwitz *et al.* (1973) observed some response in calcium binding protein activity following intramuscular injection with very high levels of vitamin D_3. It would be interesting to study the response of young birds to addition of $1,25 (OH)_2 D_3$ to the diet rather than vitamin D_3, therefore negating all potential factors that impair the conversion of D_3 to this active metabolite.

7.5.   *Potential treatment and prevention*

Both prevention and treatment rely on feeding diets containing optimum levels of calcium, available phosphorus, and vitamin D_3. Birds with clinical signs of rickets often respond to a top-dressing of the feed with dicalcium phosphate, and administering vitamin D_3 in the water. Because of the potential interference of D_3 utilization with vitamin A, it is not advisable to include this vitamin in a water soluble therapy solution.

It is difficult to assay vitamin D_3 in complete feeds, and often, accurate results can only be obtained from the assay of the more concentrated premix. However such an assay assumes an

adequate incorporation of the premix in the feed. For this reason, it is useful to assay the complete feed for another vitamin, such as vitamin A, because this will give an indication of the effective incorporation of premix into the diet. It is also difficult to assay the available phosphorus content of the diet. In general, the phosphorus present in animal protein ingredients such as meat meal, is considered 100% available, whereas that in vegetable proteins and cereals may only be 30% available for very young birds. Assay of total phosphorus level can only give an indication of potential gross deficiencies of available phosphorus content. For this reason, it is essential to practice strict quality control on ingredients such as meat meal, and especially if any single ingredient provides more than 25% of the available phosphorus in a diet. This situation is most critical for very young poults, and so the ingredient selection for prestarter diets should not always be based on the "least-cost" supply of major nutrients. Likewise, prestarter and starter diets should only contain ingredients that are "easily" digested, and not ingredients such as rye or high β-glucan barley that can adversely affect overall nutrient absorption. If fats are added to these diets, they should be unsaturated oils rather than saturated tallows, and be protected by antioxidants such as ethoxyquin.

While the consideration of these nutritional factors is often adequate to prevent a large-scale outbreak of rickets, it is realized that certain farms have consistent recurrence of this problem. Where the occurrence of rickets is a real possibility (for reasons unknown) then the diet should be modified so as to ensure optimum bone mineralization (*Table 11.10*).

TABLE 11.10 Suggested diet specifications for broilers and turkey poults at high-risk to rickets

Nutrients	Turkey prestarter	Broiler starter
Calcium (%)	1.50	1.00
Available phosphorus (%)	0.80	0.48
Vitamin D_3 (added IU/kg)	6,000	4,000
Vitamin C (added mg/kg)	300	200
Vitamin A (added IU/kg)	5,000	5,000

Ingredients	Turkey prestarter	Broiler starter
Added fat	Soybean oil	Soybean oil
Corn	_	_
Soybean meal	Max 20%	_
Meat meal	Max 5%	Max 5%
Fish meal	Max 8%	Max 8%
Corn gluten meal	Max 10%	Max 10%
Wheat (by-products)	Max 10%	Max 20%

8.   **CHONDRODYSTROPHY**

7.1.   Compendium

Wise (1975) defined chondrodystrophy as a general disorder of the growth plate in long bones that eventually impairs linear but not appositional growth. The condition was originally termed perosis when the gastrocnemius tendon slipped off the condyles, and it is now often referred to as angular or valgus-varus bone deformity. It is probably the major cause of leg problems in broiler chickens, affecting many birds to some degree, and 2-3% of birds may be severely affected. Chondrodystrophy can simply be induced by feeding diets deficient in manganese or choline, although similar bone characteristics are also seen in birds deficient in zinc and most of other B vitamins. The general impairment of normal cartilage proliferation results in the loss of columnar arrangement of cells, which in turn affects normal vascularization. Failure in normal cartilage maturity results in an impaired linear growth of bone, although the bone width continues to increase. It is possible that chondrodystrophy occurs as a result of a selective slowing in the mitotic rate of proliferating chondrocytes at the growth plate. Prevention or treatment relies on an optimum dietary inclusion of choline, manganese and other B vitamins. However chondrodystrophy still occurs in diets that are apparently well fortified, and so nutrient bioavailability and/or antagonists are likely involved.

8.2.   Occurrence and general signs

There is considerable confusion and disparity concerning the terminology of what is thought to be the major cause of leg weakness in broilers. Most often termed perosis in the past, it is now more common to refer to the condition as angular bone deformity, twisted leg, valgus-varus deformity, bowed legs or chondrodystrophy. With classical perosis, the tendon slips out of the condyles on the distal end of the tibiotarsus, and the bird loses control over the lower leg and foot. Wise (1975) defined chondrodystrophy as a general disorder of the growth plate in the long bones that eventually impaired linear growth while mineralization and appositional bone growth are unaffected. This disproportionate increase in bone width leads to the enlargement of the hock joint and often deviation of the end of the tibiotarsus. This abnormal bone growth can lead to a valgus (knock-kneed) or varus (bow-legged) stance. If the deformities become severe, the gastrocnemius tendon may become displaced from the condyle groove at the distal tibiotarsus, leading to a slipped tendon.

Chondrodystrophy is probably the major cause of economic loss within the leg problem syndrome. As many as 2-3% of males will be affected up to 40 d, while for older roaster birds, a 1% incidence per wk, after 7 wks of age is not uncommon. Most affected birds are reluctant to walk and have difficulty reaching feed and water within the competitive environment of the barn. Mortality sometimes occurs, although more often birds are culled due to low body weight or the obvious leg deformity. Birds will often be downgraded at processing due to either the severe leg deformity, or skin blemishes, bruising, etc.

8.3.   Pathology and metabolic changes

Chondrodystrophy can simply be induced by feeding diets deficient in manganese or choline. In these birds with classical perosis, the leg bones are short and thickened with a swollen hock joint, and in severe cases the gastrocnemius tendon is displaced from the condyles. Both valgus and varus deformities occur, although the latter is most common. Riddell (1981) indicates there to be a reduction in the capacity of the epiphyseal cartilage for growth that is associated with a lack of columnar arrangement of cells and an increase in the quantity of the proliferating zone matrix. Also there is no easily defined differentiation between the zones of proliferation and hypertrophy, where the latter is often smaller than usual. The general impairment of normal cartilage proliferation results in a loss of the columnar arrangement of cells, which in turn affects normal vascularization. Failure of normal cartilage maturity results in an impaired linear growth of bone, although bone width continues to increase. It is possible that chondrodystrophy occurs due to a selective slowing of the mitotic rate of proliferating chondrocytes in the active growth plate. There have been reports of inducing chondrodystrophic type lesions by irradiating the proliferating zone and consequently stopping mitosis.

8.4.   *Related Factors*

8.4.1.   Diet manganese

Since the mid 1930's it has been known that a deficiency of manganese will lead to classical perosis. Affected birds show a characteristic shortening of the bones together with reduced bone ash. Thomas and Lowther (1976) compared the epiphyseal cartilage of birds from various ages showing a slipped tendon as the result of either a manganese deficiency or a natural field occurrence. Broilers with slipped tendons showed an impaired tibiotarsal-tarsometatarsal articulation due mainly to the lateral rotation of the distal tibiotarsal epiphyses. Slipping of the tendon prevents normal flex or muscle operation and an immobilization of the tarsometatarsus. At 26 d of age, Thomas and Lowther (1976) showed 42% of manganese deficient birds to have a slipped tendon, although the Mn content of the liver and cartilage of these birds was not different. These authors suggest that field cases of slipped tendon are not due to a growth plate disturbance, but rather to an abnormal rotation for whatever reason. In an extensive series of studies, Leach and co-workers have shown the role of manganese to be in the normal formation of the chondroitin sulfate in the cartilage, due to its role in galactotransferase and polymerase enzyme systems.

Under modern commercial conditions, it seems unlikely that manganese would be deficient in a diet, and because even when corn-soy diets do not contain added manganese, Summers *et al.* (1978) did not observe classical perosis. If manganese deficiency is in any way causative in field outbreaks of chondrodystrophy, then it is likely that an induced Mn deficiency is involved. Halpin and Baker (1986) suggest that an imbalance of Ca and P in a diet may affect manganese utilization. While a Mn deficient diet caused chondrodystrophy, adding Mn up to 14 mg/kg diet corrected the situation only when there was no fish meal in the diet. Birds fed fish meal exhibited more severe perosis, and while there was no change in tibia Mn level there was reduced tibia ash. In subsequent studies, these workers showed that the P content of fish meal may be involved in this interference, because "excess" P seems to be antagonistic to Mn bioavailability, reducing its uptake by as much as 50%. Smith and Kabaija (1985) also studied the role of diet Ca and P on the incidence of chondrodystrophy. At normal levels of diet P (0.6%) a high level of diet calcium (2%)

disrupted Mn metabolism and caused perosis. Increasing diet P to 1.2%, while correcting the apparent Ca:P imbalance, attenuated the disruptive effect on Mn metabolism. Smith and Kabaija (1985) indicated that when the diet contained a high level of Ca (2-3%) then chondrodystrophy was controlled with supplements of 200 mg Mn/kg diet. Rapaka *et al.* (1977) suggest that diet iron levels may also be a confounding factor in Mn utilization. These workers showed that the administration of hydralazine, a manganese sequestering agent, causes leg defects very similar to those seen in classical manganese deficiency, and in fact successful Mn treatment has been recorded in these situations. Hydralazine blocks collagen secretion and this can be restored by the administration of Fe^{2+} or Fe^{2+} and Mn^{2+}, but not by Mn^{2+} alone. The agent seems to block the synthesis of hydroxylysine and within this mechanism there seems to be a step requiring Fe^{2+}.

8.4.2. Choline

As shown by Lipstein *et al.* (1977) the severity of perosis can be directly related to the choline content of the diet and that no perosis occurred when the diet contained 800 mg choline/kg. Riddell (1975) cites evidence for histopathological differentiation of epiphyseal cartilage changes due to a Mn or choline deficiency. With choline deficiency, there is again an irregular vascularization of the growth plate, with changes being most evident 10-14 d after the initial feeding of a choline deficient diet. Unlike the situation with manganese, there are minor changes in the cell distribution within the matrix. It seems unlikely that chondrodystrophy is currently related to choline deficiency because soy contains high levels of this vitamin, and an unsupplemented corn-soy diet usually meets the birds minimal requirements for choline. However variability in soy processing and other integrating factors dictate the need for choline supplementation.

8.4.3. Zinc

Zinc deficiency also results in the shortening of the leg bones which is usually associated with enlarged and thickened hocks. Again, there is an irregular penetration of the matrix by the invading blood vessels. Riddell (1975) cites evidence of an impairment of sulphur uptake into the epiphyseal growth plate, and suggests that this effect can to some extent be ameliorated by adding histamine, histidine or aspirin to the diet.

8.4.4. Vitamins

Deficiencies of most vitamins seem to affect the incidence of leg problems, and many of these are characteristic of chondrodystrophy to some degree. The classical effects of vitamin (and mineral) deficiencies are well documented, although few of these clinical abnormalities are observed in field situations. This may be due to the fact that absolute deficiencies are seldom encountered because of the vitamins contributed by the natural ingredients, and also because of the relatively short commercial life-span of meat birds that makes deficiencies of fat-soluble vitamins less likely. *Table 11.11* indicates the levels of vitamins found in an unsupplemented corn-soy broiler diet, and represents the base level of deficiency likely to be encountered under commercial conditions. Vitamin B_{12} is in greatest deficit, since there are no animal by-products in this diet, although most other vitamins are within the 70-80% of requirement. However this calculation assumes 100% availability of vitamins in the natural ingredients, and even a 20% deficiency is likely to be critical in todays very fast growing meat birds. Summers *et al.* (1978)

studied the effect of these practical vitamin deficiencies by feeding corn-soy diets devoid of selected synthetic vitamins.

TABLE 11.11 Natural vitamin content of corn-soy broiler diet			
	Content	**NRC (1994) Broiler Requirements**	**% NRC Supply by corn-soy**
Pantothenate, mg/kg	8.0	10.0	80*
Choline, mg/kg	1370	1300	105
Thiamine, mg/kg	3.5	1.80	194
Folate, mg/kg	0.4	0.55	72*
Vitamin B_{12}, µg/kg	0.0	10	0*
Riboflavin, mg/kg	1.8	3.6	50*
Biotin, mg/kg	0.18	0.15	120
Niacin, mg/kg	20.0	35	57*
Pyridoxine, mg/kg	5.0	3.5	142
Vitamin A, IU/kg	1450	1500	97*
Vitamin E, mg/kg	12.1	10	121
Vitamin K, mg/kg	0.40	0.50	80*

Birds fed these experimental diets were smaller than conventionally fed birds, but still exhibited an increased incidence of leg disorders. Birds were generally reluctant to move, although chondrodystrophy was not overtly different for the various vitamin "deficiencies". Tarsometatarsal length was reduced significantly with all vitamin treatments. Ferguson *et al*. (1978) examined the tibiotarsal cartilage from these birds together with the degree of bending and the enlargement at the end of the tibiotarsal. Again, regardless of the vitamin deleted from the diet, all birds exhibited unusual tibiotarsal cartilage development and deviation of the tibiotarsal head. The authors conclude that it is interesting to note the compatibility and similarity of histopathological findings for the various nutrient deficiencies with the non-differentiation of leg abnormalities and locomotion seen for these same birds by Summers *et al.* (1978).

8.5.   Treatment and prevention

It is obvious that chondrodystrophy can be triggered by a number of nutrient deficiencies and so there is likely no simple treatment or prevention. Certainly ensuring that adequate levels of all B vitamins, manganese and zinc are present in the diet will prevent simple deficiencies. However, problems with mineral interactions, antagonists, vitamin stability etc. mean that

chondrodystrophy in its many forms will likely be a significant factor in poultry production in the foreseeable future. Because chondrodystrophy causes a shortening and thickening of the bone, then once the clinical symptoms are evident, it is unlikely that treatment will totally correct the problem. During the early stages, when an abnormal gait is observed, it may be beneficial to provide supplemental B vitamins to at least 2 times NRC (1994) requirement levels.

9.   SPINAL COLUMN DEFECTS (SPONDYLOLISTHESIS)

9.1.   Compendium

Spondylolisthesis is a locomotor disturbance caused by unusual changes in the development of the 5-7th thoracic vertebrae that causes a constriction of the spinal cord and eventual paralysis. There is apparently a genetic component because an incidence as high as 50% can be quickly achieved through selection. In commercial flocks of broiler chickens an incidence of 1-2% is more commonly described as being problematic, and often referred to as kinky-back. The condition is rarely seen in slower growing Leghorn birds, and in broilers it can be prevented by inducing a slower growth rate. Some birds with kinky-back also show twisted legs, although many birds with twisted leg show no signs of disrupted thoracic vertebrae. Wry-neck is sometimes seen in male broiler breeders. Unlike spondylolisthesis, wry-neck causes no major locomotory problems, and as long as the birds can eat, they grow and develop normally. The twisted-neck condition is likely hereditary, therefore affected breeders should not be placed in breeding pens.

9.2.   Occurrence and general signs

Spondylolisthesis, or kinky-back as it is commonly referred to on-farm, is a deformity of the thoracic vertebra that can cause varying degrees of paralysis in young broiler chickens. Reports of up to 15% occurrence are known, but more commonly, problem flocks may contain 1-2% of affected birds. Birds are usually found sitting on their hocks and are unable to stand or may be found lying on their sides (Rowland, 1989). The commercial problem therefore relates to the failure of these birds to eat or drink, and they must be culled from the flock. There seems to be a major genetic component to the problem that is aggravated by fast growth rate. Spondylolisthesis is rarely seen in birds growing at less than their genetic potential.   There may be an association between spondylolisthesis and the incidence of twisted legs. It has been suggested that twisted legs may be due to subclinical spondylolisthesis, although Riddell (1981) indicates that most birds with twisted leg show no sign of spondylolisthesis, inferring that the latter is but one of numerous predisposing factors.   Another spinal cord defect sometimes seen in broilers and male broiler breeders is a wry-neck type condition in which 1 - 2% of the flock have a typical lateral twist to the neck. Birds do not seem to be unduly affected by the condition and are seen to eat and drink normally. Male breeders sometimes have difficulty feeding during competitive restricted feeding, and may be seen to lose weight after 8-10 wk of age. These males are unlikely to be effective breeders, and so are culled early from the flock. Crawford and Glick (1974) found that the birds developed an unusual neck curvature (40% incidence) following ablation of specific regions of the hypothalamus. As with wry-neck in breeders, these surgically modified birds had no difficulty in standing or walking, and became adept at eating.

9.3.   *Pathology and metabolic changes*

There have been few recent reports of spondylolisthesis, and so pathological findings relate to more dated observations. Kinky-back was first observed in the late 1960's and the most active research was conducted over the next 10 years. According to Wise (1975), the condition results from a neural impairment incurred through the positional changes of the 5th, 6th and possibly 7th thoracic vertebrae. Rowland (1989) suggests the condition is most commonly associated with a downward tilting of the front end of the 6th thoracic vertebra with the caudal end proportionately displaced upwards. These changes in the placement of the vertebra result in a compression of the spinal cord. The condition does not seem to be caused by the initial failure of musculature or ligaments attached to the vertebrae in this region, although degenerative changes to the leg muscles have been attributed to the denervation (Khan *et al.* 1977). Wise (1973) followed the age-related sequence of changes to the thoracic vertebrae. No abnormalities were seen in chicks at hatching, although by 14 d of age there was considerable curvature to the thoracic region of the spinal column. These changes were most notable in fast growing broiler strains. Wise (1975) concludes that spondylolisthesis is a developmental problem occurring after hatch, and presumably related to growth rate. Contrary to this observation, Khan *et al.* (1977) suggests that the condition may start much earlier because spinal defects can be seen in 20 d embryos. Sullivan (1975) also suggests that the wry-neck condition sometimes seen in male broiler breeders starts during the incubation process.

9.4.   *Related factors*

9.4.1   Growth rate

Spondylolisthesis seems to occur only in the fast growing broiler chicken. Few spinal cord defects are seen in Leghorns or broilers that are grown on programs intended to reduce growth rate. Because of relatively low incidence, it is unlikely to be uneconomical to reduce the growth rate of the vast majority of the flock merely to reduce the incidence of kinky-back in 1-2% of birds.

9.4.2. Genetics

Kinky-back is less problematic than it was 10-20 years ago, suggesting that there has been genetic selection against this condition.   Riddell (1973) raised the incidence of clinical spondylolisthesis to 9% by breeding birds that had recovered from the condition. Khan *et al.* (1977) maintained over 50% incidence in a flock through four generations of selection.

9.4.3. Diet composition

Spondylolisthesis is obviously not the consequence of a simple nutrient deficiency, and in general, diet manipulation is without effect. Waldroup (1986) cites evidence for a reduced incidence of scoliosis within a susceptible line of birds after feeding elevated levels of copper. It is not known if this was a direct effect or if this was simply due to the known association between copper and collagen cross-linking. High nutrient dense diets allow for higher incidence, and prevention through

the use of low nutrient dense diets and associated slower growth rate needs the consideration of local economic conditions.

Wry-neck as sometimes seen in broiler breeders may relate to the metabolism of tryptophan or niacin (Sullivan 1975). During incubation, a wry-neck condition can arise when abnormal tryptophan metabolism causes a change to the neck musculature, creating a greater pull on one side of the neck. This uneven pressure, together with the pressure from the amnion, can cause a deformity to the neck vertebrae (Sullivan, 1975).

9.5.   *Treatment and prevention*

9.5.1   Genetic selection

Because there is an obvious genetic component, the selection against spondylolisthesis should reduce its incidence over time. It seems inadvisable to use male broiler breeders with wry-neck within a breeding program.

9.5.2.   Diet composition

If the incidence is greater than 15%, then it may be necessary to consider a lower nutrient dense diet.

9.5.3.   General management

Affected broilers cannot practically be treated with any modified diet or special equipment that allows easier access to feed and water. Therefore birds with clinical abnormalities should be removed from the flock as soon as possible.

10.   FEMORAL HEAD NECROSIS

10.1.   *Compendium*

Femoral head necrosis (FHN) can occur in fast growing birds such as the broiler and turkey, and is characterized by the head of the femur separating from the bone shank. During examination or commercial processing, the femur head is often found in the acetabulum of the hip. The affected bone is often ochre colored and porous, and its brittle nature leads to the alternate name of Brittle Bone Disease. As many as 30-40% of a flock can be affected to some degree, although for reasons unknown, the condition occurs sporadically in certain locations. FHN is often associated with a condition known as scabby-hip. Julian (1985) in a survey of 18,000 tom turkeys ascribed FHN as due to either osteochondrosis, dyschondroplasia or osteomyelitis. Treatment and prevention relate to optimizing the birds nutrient intake, although this action is not always

successful. The condition is sometimes associated with enteritis and malabsorption and so the vitamin status of these birds is often suspect. Once lameness and reluctance to move is noted, a treatment with water soluble vitamins may help in preventing an increased severity of FHN, but complete recovery is seldom observed.

10.2.   Occurrence and general signs

Femoral head necrosis most often occurs in 3-4 wk old birds, although problems are sometimes seen as early as 2 wks of age (Qureshi, 1990). Birds that are initially quite healthy suddenly start to walk with an unsteady gait with a degree of lameness, and occasionally will be reluctant to move. Younger birds lie down as if paralyzed. Usually just one leg is affected, although, if both legs are involved then the bird is seen lying on its breast. The condition is often associated with scabby-hip, which apparently involves self-inflicted lacerations in the hip region. Birds are seen pecking themselves in the hip area, and it is thought that this may relate to some degree of pain associated with problems in the head of the femur. Birds with femoral head necrosis/scabby-hip invariably have feathers in the gizzard, while skin lacerations themselves are a cause for downgrading of the carcass and can also lead to bacterial infection. During processing, the femoral head invariably breaks off the femur, and is often found in the acetabulum of the hip. A 30-40% incidence is not uncommon, although it appears to be a recurring problem in specific geographical regions for short periods of time.

10.3.   Pathology and metabolic changes

Meens and Litjens (1978) describe the affected femoral head as ochre colored rather than white, with the cartilage often being found still attached within the acetabulum of the hip. The broken femur head has a soft-porous appearance, and often can be crushed between the fingers. Qureshi (1990) also describes the femur head as porous and soft, and that microscopy reveals osteoporosis with a reduction in the number and size of trabeculae and an increase in osteoclastic activity. Riddell (1981) suggests that changes in the femur associated with osteomyelitis are similar to those seen in femoral head necrosis, although infection is rarely discussed in the latter diagnosis. Julian (1985) describes a survey of 18,000 tom turkeys in which 15% died or were culled due to lameness. A significant number of birds had FHN and Julian (1985) segregated these into one of three categories: 25% had FHN associated with osteochondrosis, a similar percentage related to dyschondroplasia, and almost 40% had osteomyelitis. For birds showing osteochondrosis, there was hemorrhage and necrosis between the growth plate and articular cartilage, or between the growth plate and metaphyseal bone. With dyschondroplasia, there was degeneration around the mass of the prehypertrophic cartilage with a fracture of the femur head. Julian (1985) describes the majority of birds with osteomyelitis to exhibit a yellow linear foci of necrosis in the subchondral bone and diaphyses that occasionally caused a collapse of the femur head, and staphylococcus was usually present. Julian (1985) concludes that FHN related to osteochondrosis is the result of focal avascular necrosis resulting from an overgrowth of epiphyseal and articular cartilage in fast growing birds. Dyschondroplasia as a cause of FHN is more common in chickens than in turkeys and is the result of the usual problem of irregular penetration of the blood vessels and persistence of the cartilage plug. Riddell (1981) also describes femoral head degeneration in turkeys, suggesting this to be related to the shaky-leg syndrome. Riddell (1981) cites evidence for erosion of the femur head to be caused by an early resorption of cortical bone and its replacement by dense fibrous tissue, and compares these lesions to comparable conditions seen in dogs and pigs.

Although femoral head necrosis is not too common, it does occur sporadically in certain areas and then disappears very quickly. At post-mortem, or during processing, the femur head can break off very easily in apparently healthy and well-fleshed birds. Femoral head necrosis should only be suspected if this dislocated femur head is porous and brittle and/or associated with hemorrhages or infection.

10.4.   Treatment and prevention

Because FHN is sometimes associated with stunting and abnormal feathering, there is a concern about general nutrient digestibility. When associated with enteritis and diarrhea, for whatever reason, there is reason to suspect the vitamin D_3 status of the bird, and the potential inability to metabolize calcium. Supplementing the diet with additional vitamins, especially D_3 can be considered, or vitamins given via the drinking water. These same vitamins, given as treatment once the condition is noticed, are not likely to correct the problem in affected birds although they may help to prevent an increase in the severity of the lesions. There has been one report of using 1 ppm of dietary molybdenum to prevent FHN (Payne and Barnes, 1975) although this work has never been confirmed.

11.   <u>FOOD PAD DERMATITIS</u>

11.1.   Compendium

Lesions to the footpads of birds cause problems with locomotion and provide a route of infection for bacteria. Birds fed biotin-deficient diets show a characteristic foot pad dermatitis and the condition is obviously responsive to biotin. However even in diets well fortified with all vitamins, foot pad dermatitis still occurs sporadically, and seems to be largely a factor of litter condition. With wet and caked litter, regardless of the diet, some birds will develop foot pad lesions. There is some concern over the use of ingredients such as soybean meal, where manure composition or its consistency seems to accentuate the foot pad problems. Soybean meal contains high levels of potassium, and so this effect may simply relate to litter moisture, or alternatively, undigested oligosaccharides may be causative agents. Caged birds show a much higher incidence than do floor-managed birds, therefore physical abrasion to the foot pad seems to be a factor.

11.2.   Occurrence and general signs

Lesions to the foot pads of birds are a problem because they cause locomotory problems and allow the entry of micro-organisms from the litter. Foot pad lesions are quite common in turkey poults and it is thought that such problems occurring in young birds may precipitate general leg disorders in older market weight birds. In turkeys and older broiler breeders, depending upon the management conditions, 30-50% of a flock can be affected. A major predisposing factor is litter condition, and so even within a single poultry house, the occurrence of dermatitis can vary from one end of the building to other depending on the ventilation system etc. Initially, the foot pad becomes cracked and encrusted with manure. Swelling of the footpad and reluctance to move are likely a result of secondary bacterial infection.

11.3. *Pathology*

Changes in skin condition have been most completely documented for dermatitis induced by biotin deficiency. Dryness and flakiness of the skin first appears on the feet with an abnormal papillary growth on the undersurfaces of the toes and foot pad (Whitehead, 1988). As the severity of the cracks increases, they often become hemorrhagic and secondary bacterial infections can occur. The footpad may become swollen and ulcerous, which is sometimes referred to as bumblefoot.

11.4. *Related factors*

11.4.1. Diet

The level of certain ingredients and of individual vitamins has been implicated in foot pad dermatitis. Harms and Simpson (1975) showed that foot pad lesions in broilers were associated with biotin deficiency. These workers found a higher incidence in male vs female birds, and related this to the faster growing male having a higher biotin requirement. In subsequent work, Harms *et al.* (1979) showed that foot pad lesions were reduced in 2 of 3 experiments when breeder diets were supplemented with biotin at 200 mg/kg. Murillo and Jensen (1976) reported foot pad lesions to be accentuated by a deficiency of methionine, but that the addition of cystine to the diet, actually aggrevated the condition. A high incidence (50% vs 3%) of foot pad dermatitis was seen in 14 d old poults fed corn-soy diets providing 0.44% methionine vs 0.74% methionine, while a supplement of sulfate and cystine aggrevated the condition. Burger *et al.* (1984) studied the role of a number of vitamins, trace minerals and selected ingredients on the incidence of foot pad dermatitis in caged Leghorns. None of the dietary treatments affected foot pad lesions, although a much higher incidence was seen in a dwarf strain compared to a normal sized layer. In a subsequent study, these same workers showed reduced foot pad problems when these dwarf birds were housed in cages with a plastic-coated floor. There has been some discussion concerning the role of soybean meal, and in particular the nature of undigesta from high soy diets as they affect foot pad condition. Abbott *et al.* (1969) studied the role of soybean meal in inducing foot pad dermatitis in turkeys up to 8 wks of age. Using up to 58% inclusion of 44% CP soy in the diet had no consistent effect on foot pad condition. However Waldroup (1986) cites evidence of reduced foot pad integrity in poults fed soybean meal. The condition was apparently not due to any single vitamin deficiency, but rather the composition or consistency of the excreta voided by these poults. This excreta more readily adhered to the feet and caused an irritation leading to footpad lesions. The actual components in soybean that could cause this condition are unknown, although the major culprits are either undigested oligosaccharides or simply the high potassium content of soy which leads to wetter manure.

11.4.2. Litter condition

The major culprit with footpad lesions is litter condition. Abbott *et al.* (1969) clearly showed that litter moisture content was the only factor that consistently correlated with foot pad lesions. These authors concluded that if the litter was damp and/or crusty, then foot pad dermatitis was likely to occur. Conversely with dry litter, assuming no overt vitamin deficiency, foot pad condition is not likely to be compromised.

11.5.   *Treatment and prevention*

Chicks and poults must obviously be fed adequate quantities of vitamins and biotin in particular. Birds showing signs of foot pad dermatitis will respond quite rapidly to the administration of biotin when this is the sole cause. In most situations however, the condition is triggered by wet and caked litter, and so the cause of this poor litter must be established. Foot pad lesions will likely occur to some degree for birds kept on wet litter, regardless of their nutritional status.

12.   <u>**TURKEY LEG DISORDERS**</u>

12.1.   *Compendium*

Regardless of management techniques, a significant proportion of turkeys exhibit leg disorders at some stage of commercial grow-out. Of particular significance, are field rickets and "shaky-leg" although all common poultry leg disorders as detailed in previous sections, occur with turkeys. Field rickets occur in 8-12 d old poults and seem to result from an imbalance or deficiency of either Ca, P or vitamin D_3. Undigested fat may complex with calcium and D_3 while high levels of vitamin A are also antagonistic to D_3 utilization. Field rickets is best treated by providing vitamin D_3 in the drinking water, and by top-dressing feed with small quantities of dicalcium phosphate. Shaky-leg is commonly seen in 9-12 wk old toms, where the birds seem reluctant to move and when standing, are seen to quiver and tremble. The condition is often associated with diarrhea, and the major problem is breast blisters caused by the increased contact with moist litter. General lighting programs, litter management and diet levels of vitamins and minerals can all affect leg development.   Initial slow growth induced by low protein diets is beneficial in preventing leg problems in strains that exhibit very fast early growth rate.

12.2.   *Introduction*

Of all the classes of domestic poultry used in commercial meat production, the turkey is the most prone to leg problems. Most of the specific leg problems described in previous sections of this Chapter apply to the turkey, yet there seem to be more problems with the turkey and especially the heavy-weight toms. Field rickets and shaky-leg are specific to turkeys, and these are discussed in the following sections. Following these descriptions is a general overview of factors affecting leg problems in turkeys.

12.3.   *Field rickets*

As detailed in *Section 7* of this Chapter, rickets is caused by an imbalance of calcium, phosphorus, and/or vitamin D_3. However the condition is most prevalent in young turkeys, and many farms have a chronic history of this problem, with poults inevitably experiencing leg problems at around 14-19 d of age.

Bar *et al.* (1987) studied 32 cases of field rickets over a four year period. Most poults exhibited reduced plasma calcium and inorganic phosphorus, while levels of 24 (OH)D_3 and calcium

binding protein were less than expected. Surprisingly the diets were all found to be adequate in vitamin D_3, containing at least eight times the minimum requirement. Riddell (1983) also studied a number of field outbreaks, again indicating adequate diet levels of all suspect nutrients. Riddell (1983) concluded that inadequate feed distribution was the reason for the early 7 d onset of rickets and also for the reason that vitamin D_3 treatment in drinking water is usually effective. Hurwitz *et al.* (1973) were somewhat less pedantic in their diagnosis, although they made the interesting observation that field rickets was associated with significantly reduced levels of calcium binding protein in the intestinal mucosa. Electrophoretic analysis subsequently indicated that the protein banding was similar to that seen in D_3 deficient chicks. There is still considerable controversy as to the age of the onset of rickets. Riddell (1983) indicated occurrence as early as 7 d while Bar *et al.* (1982) suggested that up to 17 d was required for rickets to show with D_3 deficient poults. This implies an effect of maternal nutrition, although unfortunately this avenue has not been adequately investigated. Stevens *et al.* (1984) did however show that increasing the vitamin D_3 level in a turkey breeder diet resulted in increased bone ash and breaking strength of their offspring at 2 wk of age.

Walser *et al.* (1980) could find few distinctive features during necropsy or histologic examination of poults with rickets. The most consistent finding ocurred during radiology, where reduced mineralization in the proximal metaphysis of the tibiotarsus was seen as a wide less-dense band. A similar pattern was seen in the tarsometatarsus, and bones in general had less contrast with adjacent soft tissue, compared to the situation seen in unaffected birds from within the same flock. Cantor *et al.* (1980) suggest bone mineral mass to be a good indicator of D_3 status in young poults, and that highest breaking strength is seen in poults fed diets with 1200 IU D_3/kg. Because rickets occurs in poults fed apparently well-fortified diets, there is the potential of antinutrients or nutrient interaction reducing the bioavailability of critical nutrients. Stevens *et al.* (1983) studied the role of fat type as it affects skeletal integrity, because saturated fats are known to bind with calcium to form soaps, and such undigested fat may well reduce the availability of fat soluble vitamins such as D_3. Poults fed corn oil had higher tibiotarsus ash levels than those fed saturated tallow. Likewise high levels of vitamin A in the diet were found to be detrimental to D_3 availability. Stevens *et al.* (1983) showed that by 26 d of age, either 7% dietary tallow or 44,000 IU vitamin A/kg diet caused severe rickets. On this basis, it is suggested that poults be fed diets containing unsaturated fats and these diets contain no more than 10 - 12,000 IU vitamin A/kg. In an uncorroborated report, Smith (1983) claimed to correct leg problems in young poults by adding 1 ppm fluorine to the drinking water.

Another potential confounding factor in the development of field rickets, is the conversion of D_3 to its various metabolites. Failure of such conversion would lead to impaired calcium utilization, even though dietary D_3 assays would appear normal. Sanders and Edwards (1991) studied this problem by giving poults the active metabolite of D_3, namely $1,25(OH)_2 D_3$. Increasing the levels of conventional vitamin D_3 from 450 to 3600 IU/kg resulted in increased bone ash. Adding the $1,25(OH)_2$ metabolite was most effective in diets containing the lower level of D_3, and did help to prevent rickets in older birds. Currently however it is uneconomical to use such D_3 metabolites.

12.4. *Shaky leg and foot pad lesions*

Male turkeys between 9 - 12 wk of age often seem reluctant to move, and when standing are seen to tremble and quiver. This shaky-leg condition is often associated with diarrhea. The signs often disappear with age, although in the meantime, affected birds may develop breast lesions due to increased contact time with the litter, and may also show reduced growth rate due to

their reluctance to move to feeders and drinkers. Histological examination of the nervous system reveals few abnormalities.

Researchers at Houghton studied shaky-leg and foot pad dermatitis in fast growing turkeys, and suggested both conditions to be greatly affected by litter quality. Turkeys raised on wet litter (65% moisture) to 6 wk of age rapidly developed foot pad lesions, eventually showing 80% incidence, compared to only 17% occurrence in birds on dry (25% moisture) litter. Many of these affected birds showed a stiff trembling gait, spending much of their time sitting on the litter. By 8 wk of age, these affected birds were 9% smaller in weight. The metatarsal pad was the main site of the foot lesions, although some birds had scabby hocks. Histologically, the chronic lesions appeared as an ulcer covered with fibrin scab. The surface epithelium was lost abruptly at the edge of the lesion and dense inflammatory exudate was seen adjacent to the fibrin scab. Because shaky-leg appears to relate to nerve incoordination, Ranaweera (1981) studied flux of calcium ions across various membranes. Turkeys treated with trienbolone acetate, which is an anabolic steroid, showed a shaky-leg type condition, leading Ranaweera (1981) to suggest that the abnormality is connected to calcium metabolism, and in particular calcium transport. The author hypothesized that "twitching" may be due to the failure of calcium ions being able to rapidly move across the nerve cell membranes. While shaky-leg was not associated with changes in plasma calcium, Ranaweera (1981) suggests the condition likely relates to an impaired flux across the cell membranes, caused by, as yet unknown causes.

Currently the only potential preventative measure is to ensure optimum litter condition, and the prevention of wet and caked litter from developing around drinkers etc.

12.5. *Non-specific leg disorders*

For some strains of turkey, it is generally assumed that high protein/amino acid levels result in an increased incidence of leg disorders. The exact reasons for this effect are unknown because often body weight is not affected. For example, data provided by Ferket (1992) clearly shows a positive correlation between diet protein and leg disorders while market weight is little affected *(Table 11.12)*.

TABLE 11.12. Diet protein and leg disorders in market weight toms					
	Protein level 0 - 20 wks (% NRC, 1984)				
	82	91	100	109	118
Body wt (kg)	13.3	13.7	13.7	13.8	13.5
Leg disorders (%)	11.7[c]	16.8[b]	20.8[b]	19.7[b]	26.7[a]
Adapted from Ferket (1992)					

In this study, diets providing less than 100% NRC (1984) levels of amino acids were fortified with appropriate synthetic sources, and so the effect on leg disorders seen in *Table 11.12* relates to protein *per se*. It is unclear why high crude protein causes these problems, although it may relate to metabolic acidosis resulting from the catabolism of excess amino acids or the fact that increased uric acid excretion usually results in increased calcium excretion. Certainly in strains of turkey most susceptible to leg problems, there is an advantage to using lower nutrient dense starter diets and

relying on compensatory growth to achieve near normal market weight for-age. In general, compensatory gain-type growth patterns seem to reduce the incidence of certain leg problems in turkeys, but do not totally prevent their occurrence. For example, Ferket and Sell (1989) showed that feeding low protein diets to 6 wk of age, reduced leg problems in 18 wk old toms from a control level of 7.5% down to only 5.2%.

In comparable studies, Turner and Lilburn (1992) indicated that reduced amino acid intake influences femur and tibiotarsus width more so than the length of these bones. Contrary to these results Waldroup *et al*. (1993) observed no difference in leg disorders of turkeys allowed variable intakes of amino acids at various times during rearing.

Because there are obvious strain differences in leg problems, Leblanc *et al.* (1986) compared bone structure in two such extreme strains. There was a reduced ossification density and reduced metaphyseal trabecular bone volume in the strain showing more leg problems. Even though there was a difference in body weight for the two strains, Leblanc *et al.* (1986) found there to be no difference in the tibiotarsal cortical thickness, and so thought this may be a predisposing factor in this heavier strain. It is also generally assumed that tom turkeys will show a higher incidence of leg problems than do hens, and this may relate to factors other than the obvious difference in growth rate. Pierson *et al.* (1981) studied the role of male hormone in males that were either caponized or treated with testosterone. Caponized birds surprisingly had the most leg abnormalities and the authors related this to a deficiency of androgens. However this situation does not correspond to the fact that females have fewer leg problems, and the authors discuss this situation on the basis of estrogen being a dominant factor in ossification.

Light intensity and frequency of light/dark cycles also seems to affect turkey performance. Hester *et al.* (1985) found that toms grown on a step-up daylength had a shorter tarsometatarsus, although the length relative to body weight was increased. Klingsmith *et al.* (1986) compared the growth characteristics of turkeys maintained on a high intensity (20 1ux) step-up program vs a low intensity (2.5 lux) step down lighting program. There was no real effect of lighting on bone mineral content although there was an indication of earlier closure of the tarsometatarsal growth plate with birds under the high-intensity lighting. These birds on the high intensity light were more active, and also had larger testes and so bone maturity could have been influenced by hormonal balance. Hester *et al.* (1987) also showed that males grown under high intensity light (20 lux) were heavier and had shorter tarsometatarsal bones, and earlier closure of the growth plate. Currently most flocks of heavy male commercial turkeys are subjected to an intermittent light program in order to improve leg condition. It seems as though cycles of activity:inactivity induced by light programs such as 8L:4D:8L:4D, drastically reduce morbidity associated with locomotory problems in these heavy birds. It is generally assumed that litter condition will have a major effect on leg disorders, although surprisingly this topic has received little detailed attention. Hester *et al.* (1987) were unable to show any effect of so-called hard vs soft litter or by practicing good vs no litter management. It is often thought that shaky-leg can be precipitated by poor litter, but there is little documented evidence of its effect on general bone development.

In recent years there has been some interest in re-evaluating the vitamin needs of poults, especially in terms of skeletal development. The role of vitamin D_3 and its metabolites as they affect rickets has been described in *Section 12.3* of this Chapter. Regardless of the level of D_3 used, there is some concern about the potential toxicity of vitamin A, especially when the two vitamins are included during therapy. For example, Dorr and Balloun (1976) indicate reduced plasma P levels in poults fed 16,000 IU vitamin A/kg diet. Because vitamin A is relatively inexpensive, water-soluble premix packages often contain generous levels that could compete with

vitamin D_3 for sites of absorption. If high levels of vitamin A are used during therapy, then vitamin D_3 levels should be increased accordingly, or more logically low-vitamin A supplements used.

Vitamin C may also be important for optimum skeletal development in young poults. Under ideal conditions, it is expected that birds will synthesize adequate levels of vitamin C for normal metabolism, although under various stress conditions synthesis may be suboptimal. Vitamin C plays a role in collagen synthesis and in key enzymes such as $25(OH)D_3$-hydroxylase. This vitamin C dependent enzyme is therefore essential in the synthesis of $1,25(OH)_2D_3$ and in stimulation of Ca-binding protein. Ferket (1992) recommends that starter diets be supplemented with 250 ppm vitamin C. Contrary to this suggestion, Dorr and Balloun (1976) were unable to show any response in the femur development of poults fed 300 ppm vitamin C. In more recent studies we have shown variable response of poults to vitamin C. It seems as though when poult quality is sub-optimal, or when the poult is subjected to stressors such as early debeaking, de-snooding etc., then there may be some benefit to adding Vitamin C *(Table 11.13)*.

TABLE 11.13 Vitmain C and skeletal development in poults					
Vitamin C (ppm)	**16 d B.wt**	**0-16 d F:G**	**Bone strength**	**Bone mineral**	
	(g)		**(Newtons)**	**Ca%**	**P%**
0	440	1.40	88.6[b]	35.6[b]	19.5[b]
100	470	1.36	105.5[a]	36.3[ab]	19.7[b]
300	460	1.42	93.3[ab]	37.9[a]	21.3[a]
					Leeson, 1994, unpublished

The greatest breaking strength was observed with 100 ppm vitamin C, although bone Ca and P levels continued to increase with up to 300 ppm vitamin C.

Chapter 11

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CHAPTER 12.   TRICHOTHECENES

<u>Other names:</u>	T2, DAS, DON
<u>Species:</u>	ALL POULTRY

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1.   COMPENDIUM

The trichothecene mycotoxins (TCT) comprise a vast group of over 100 fungal metabolites with the same basic structure. Although several fungal genera are capable of producing TCT, most of them have been isolated from *Fusarium* spp. Chemically, TCT are divided into two groups, namely, macrocyclic and non-macrocyclic. The toxicology of macrocyclic TCT (*i.e.,* verrucarins, roridins, satratoxins, and baccharins) has not been studied in poultry species. However, non-macrocyclic TCT are common contaminants of poultry feeds and feedstuffs and their adverse effects on poultry health and productivity have been studied extensively. Non-macrocyclic TCT are subdivided into type A and type B, A type being more toxic for poultry species than type B. Examples of type A TCT include T-2 toxin, HT-2 toxin, and diacetoxyscirpenol (DAS). Fusarenone-X, deoxynivalenol (DON) and nivalenol (NIV) are some of the common naturally occurring type B TCT.

At the cellular level, the main toxic effect of TCT mycotoxins appears to be a primary inhibition of protein synthesis followed by a secondary disruption of DNA and RNA synthesis. TCT affect actively dividing cells such as those lining the gastrointestinal tract, the skin, and lymphoid and erythroid cells. The toxic action of TCT results in extensive necrosis of the oral mucosa and skin in contact with the toxin, acute effects on the digestive tract, and decreased bone marrow and immune system function. The clinical signs and pathology observed in chickens exposed to TCT are the result of the action of the toxins on their target organs. Typical oral lesions caused by TCT in chickens are circumscribed proliferative yellow caseous plaques occurring at the margin of the beak, mucosa of the hard palate, angle of the mouth, and the tongue. The severity of the lesions increases with a longer feeding period and with higher dietary levels. Growth retardation, abnormal feathering, regression of the bursa of Fabricius, and anemia are also observed in chickens exposed to toxic levels of TCT. Laying hens show oral lesions, and decreased feed intake, egg production, and egg shell quality.

2.   CHEMISTRY AND NATURAL OCCURRENCE

The trichothecene mycotoxins (TCT) are a chemical group of fungal metabolites with the same basic structure, produced by various genera of fungi growing on plants. The reason for their name lies in the basic chemical ring described as a tetracyclic 12,13-epoxytrichothecene skeleton (*Fig. 12.1 and 12.2*).

This skeleton can be produced by different genera of fungi whichadd to it different side chains, thus producing the diverse TCT. The known genera of fungi producing TCT are *Fusarium, Stachybotrys, Myrothecium, Trichothecium, Trichoderma, Cylindrocarpon, Verticimonosporium, Cephalosporium,* and *Phomopsis* (Buck and Côté, 1991). The TCT show antifungal, cytotoxic, and phytotoxic effects (Ueno, 1983). Most of the TCT that have been isolated and characterized chemically are from *Fusarium* species, which are distributed worldwide and are important pathogens to plants that produce cereal grains (Scott, 1989). *Fusarium* spp. can also produce zearalenone (Marks and Bacon, 1976), fusaric acid (Smith, 1992), fusarochromanone (Mirocha *et al*., 1985; Wright *et al*., 1987; Wu *et al*., 1993), fumonisins (Weibking *et al*., 1993a, 1993b) and other mycotoxins with deleterious effects on poultry production. Although the number of known TCT is now over 100, information on natural occurrence in agricultural products indicates that the most important are T-2 toxin, HT-2 toxin, diacetoxyscirpenol (DAS), 15-monoacetoxyscirpenol (15-MAS) and deoxynivalenol (DON), which are all *Fusarium* produced toxins (Bauer *et al*., 1989). The chemical structure of T-2 toxin (Fig. 12.1) was revealed by Bamburg *et al*. (1968). These authors gave T-2 toxin its name from the *Fusarium tricinctum* T-2 strain, the strain originally used for the isolation and characterization of the compound. In addition to T-2 toxin, *F. tricinctum* strain T-2 was also found to produce DAS and HT-2 toxin (Bamburg and Strong, 1969). *Fusarium graminearum* is the main species responsible for the natural occurrence of DON and nivalenol (NIV) in contaminated cereals (Mirocha *et al*., 1985; Ueno and Ishii, 1985). *F. sporotrichioides* (synonym of *F. tricinctum*) is the major producer of T-2 and HT-2, while *F. poae* is a minor T-2 producer that can produce DAS. Other important DAS-producing species are *F. sulphureum, F. roseum,* and *F. equiseti* (Scott, 1989).

Chemically, the TCT can be divided into two groups depending on the presence of a macrocyclic ring between C-4 and C-15. Macrocyclic TCT possess an ester or an ether-ester bridge connecting C-4 and C-15 while non-macrocyclic TCT lack this ring. Macrocyclic TCT are distinguishable from the non-macrocyclic by thin layer chromatography because of their intense fluorescence when viewed by ultraviolet illumination (Mirocha *et al.,* 1985). Non-macrocyclic TCT can be subdivided into type A and type B. Type B TCT have a conjugated carbonyl group on position C-8 that is absent in type A compounds (*Fig 12.1 and 12.2*). Type A TCT include T-2 toxin (3α-hydroxy-4β,15-diacetoxy-8α-isovaleroxy-12,13-epoxytrichothec-9-ene) and its derivatives, and the members of the scirpene group, namely, scirpentriol and the mono-, di-, and tri-acetoxyscirpenols (Mirocha *et al*., 1985). Examples of type A TCT naturally occurring in contaminated feedstuffs include T-2 toxin, HT-toxin, and DAS (Stratton *et al*., 1993). Fusarenone-X, DON and NIV are some of the common naturally occurring type B TCT (Ueno and Ishii, 1985). The macrocyclic TCT are, in general, more toxic than the non-macrocyclic TCT and some of them have been implicated in stachybotryotoxicosis, an often fatal disease in farm animals that have ingested contaminated feedstuffs (Eppley and Bailey, 1973). Verrucarins, roridins, satratoxins, and baccharins are the main groups of macrocyclic TCT.

The TCT occur worldwide in grains and other commodities. Toxin production is greatest with high humidity and temperature of 6-24°C. The presence of the fungi does not necessarily mean presence of the TCT but is a sensitive indicator of potential contamination. Natural occurrence of TCT has been reported in Asia, Africa, South America, Europe, and North America (Scott, 1989). TCT have been detected in corn, wheat, barley, oats, rice, rye, vegetables, and other crops (Buck and Côté, 1991). Worldwide DON is the most common contaminant of cereal grains, accompanied in certain regions by NIV. DON may also occur in grains and feeds with other TCT and other *Fusarium* toxins like zearalenone, NIV and T-2 toxin (Müller and Schwadorf, 1993). Combinations of DON and T-2, T-2 and HT-2 and T-2 and DAS, have been reported to occur in

mixed feed and grains (Bata *et al*., 1983b). Natural levels of non-macroclyclic TCT such as DON, DAS, T-2, and NIV range from near zero to 10 μg/g, with few exceptions showing levels of 15-40 μg/g (NRC, 1983).

3.   <u>**TOXICOKINETICS**</u>

The kinetics of absorption, tissue and organ distribution, metabolism, and elimination of the trichothecene mycotoxins in animal species has been studied principally using radiolabeled toxins. Tritium (^{3}H) and ^{14}C have been the most commonly used markers. Only the metabolism of T-2 toxin and DON have been studied in chickens. Studies on the metabolic fate of the highly toxic naturally occurring trichothecenes of the scirpene group (*eg.* DAS, 15-MAS) are needed in order to determine whether there is risk of mycotoxin exposure for humans consuming poultry products.

3.1.   *Absorption and tissue exposure*

Animal studies have been performed primarily using three routes of administration namely oral, dermal, and parenteral. Oral dosing studies conducted in several species indicate that maximum amounts of radiolabeled T-2 appear in blood 1 h after administration (Feinberg and McLaughlin, 1989). After the blood concentration has peaked, a slower phase in which TCT and their metabolites are distributed to different tissues is described (Matsumoto *et al*., 1978). Laying hens intubated with a single oral dose of 2.2 mg DON showed poor absorption from the gastrointestinal tract, as peak plasma levels accounted for less than 1% of the administered dose (Prelusky *et al*., 1986). In laying hens intubated with DON contaminated food, the toxin largely disappears from the gastrointestinal tract somewhere between the crop and jejunum. This disappearance is presumed to occur due to the absorption of the toxin by the enterocytes and its conversion to another metabolite (Lun *et al*., 1988).

The results from absorption studies in animals indicate that there is a cellular mechanism capable of quickly transporting quantities of TCT through the cell membrane to the cytoplasmic- and membrane-bound ribosomes (NRC, 1983). Biological responses to TCT occur within a few minutes suggesting a rapid absorption from the exposed areas, which under natural circumstances of exposure are mainly the gastrointestinal tract and the skin. Dermally administered T-2 toxin is absorbed much more slowly. The skin and subcutaneous fat apparently act as a reservoir for the toxin, delaying absorption and sustaining metabolism and excretion (Swanson and Corley, 1989). In chickens, absorption of T-2 toxin appears to be much higher than the absorption of DON (Chi *et al*., 1978; Prelusky *et al*., 1986) which may account for its greater toxicity.

3.2.   *Distribution*

Matsumoto *et al*. (1978), studying the tissue distribution of a single oral dose of radiolabelled T-2 in mice, found that 3.5 h after dosing most of the T-2 and its metabolites were present in the liver, kidney, stomach, and bile. Mice injected subcutaneously with 4-acetylnivalenol showed distribution throughout the body within 30 minutes with liver and kidneys containing the largest amounts of TCT (Ueno *et al*., 1971).

Chi *et al*. (1978b) examined the distribution of orally administered radiolabelled T-2 toxin in 6 week-old broiler chickens. The toxin and its metabolites moved rapidly to organs and most tissues other than the gastrointestinal tract, reaching maximum concentrations within 4 h. However, in muscle, skin, and bile, maximum concentrations were found 12 h after dosing. In a similar trial, rapid distribution to several tissues was observed in laying hens intubated with radiolabeled DON (Prelusky *et al*., 1986). Maximum tissue concentrations were seen at 3 h postdosing in liver, kidney, brain, heart, spleen, proventriculus, gizzard, and small intestine while maximum levels in fat, muscle, and oviduct occurred at 6 h after dosing. Few studies have been conducted to determine the distribution of TCT in different tissues over time. Experimental results indicate that, in general, the toxins are distributed throughout the body within 24 hours. The highest concentration of radiolabeled T-2 are found in tissues and fluids involved with the excretion of the toxin, especially bile, gall bladder, liver, kidneys, and intestine.

3.3.   *Biotransformation*

Biotransformation is the sum of processes by which a foreign chemical or xenobiotic is subjected to chemical change by living organisms. The end result of biotransformation is that the metabolites formed are chemically distinct from the parent compound and usually more polar (hydrophillic). The enhanced water solubility reduces the ability of the metabolite to partition into biological membranes and thus restricts its distribution to the various tissues, decreases its renal tubular and intestinal reabsorption, and ultimately promotes its excretion via the urine and feces. Biotransformation reactions are accomplished by several enzyme systems located in the cytosol and smooth endoplasmic reticulum of many cells, the most important being the hepatocytes. However, cells in the lung, kidney, and intestine are also capable of chemical biotransformation.

The basic enzymatic reactions involved in the biotransformation of toxicants are of two types: phase I reactions, which involve oxidation, reduction, and hydrolysis, and phase II reactions, which consist of conjugation or synthetic reactions. Phase I metabolism generally converts xenobiotics to derivatives that are more polar by adding or exposing functional groups *eg*., hydroxyl, thiol, amino, carboxyl. These groups allow the compound to undergo phase II reactions which involve covalent linkage to an endogenous molecule, producing a conjugate that is then eliminated. All trichothecene mycotoxins have a basic tetracyclic sesquiterpene structure with a six-membered oxygen-containing ring, an epoxide group in the C12-C13 position, and a double bond in the C9-C10 position. The TCT may also have functional groups like hydroxyl (-OH), esterified hydroxyl, keto (-C=O), or epoxide groups in various combinations.

Phase I reactions known to occur in the metabolism of TCT include the hydrolysis of ester linkages (which exposes an -OH group), the aliphatic hydroxylation (oxidation) of carbon atoms at positions C-3 or C-7 of T-2, and the reduction of the 12,13-epoxide to yield a carbon-carbon double bond. Conjugation with glucuronic acid to form a glucuronide conjugate is the only phase II reaction known to occur in TCT metabolism. The reduction of the 12,13-epoxide is likely to occur primarily through the action of microorganisms in the anaerobic environment of the gastrointestinal tract. Such biotransformation products of T-2, DAS, and DON have been detected, indicating that this is an important pathway in the metabolism of TCT (Swanson and Corley, 1989).

Esterase activity which selectively hydrolyses the C-4 acetyl group in T-2 to yield HT-2 is found mainly in the microsomal fraction of liver, kidney, and spleen of laboratory animals, while *in vitro* incubation of T-2 toxin with microsomal fractions from a variety of animals and organs

produced HT-2 as the sole metabolite of T-2 (Ohta *et al*., 1977). The substrate specificities of liver microsomal nonspecific carboxyesterases were determined in several TCT (Ohta *et al*., 1978). TCT containing a C-4 acetyl group (DAS, T-2, fusarenone-X, diacetyl NIV) were selectively hydrolysed to yield their respective C-4 deacylated products (monoacetoxyscirpenol [MAS], HT-2, NIV, 15-acetyl NIV). Deacylation also occurred at the C-3 position of 3-acetyl DON and at the C-8 position of tetraacetoxyscirpenol. Acetyl groups present at the C-15 position resisted attack by hepatic esterases. Fusarenone-X is also deacetylated *in vivo* at the C-4 position to yield NIV, which is then eliminated in the feces and urine of animals (Ueno, 1977).

Incubation of T-2 with liver S-9 homogenates yield HT-2, T-2 tetraol, 4-deacetylneosolaniol (4-DN), and an unknown metabolite designated TMR-2 (Yoshisawa *et al*., 1980a). The same metabolites were obtained using HT-2 as a substrate which leads to the conclusion that T-2 was preferentially hydrolysed at the C-4 position to yield HT-2, which was subsequently metabolized to T-2 tetraol via 4-DN. Mouse or monkey liver homogenates biotransformed T-2 toxin to produce HT-2, NEO, 4-DN, 15-DN, and T-2 tetraol (Yoshisawa *et al*., 1984). A similar biotransformation pattern of T-2 toxin was observed in chicken embryos. Bata *et al*. (1983) injected the yolk sac of embryonated hen's eggs with either T-2 toxin or DAS. When T-2 toxin was injected, HT-2 toxin, NEO, T-2 triol, T-2 tetraol, and the parent T-2 toxin were detected. DAS, MAS, and scirpentriol were identified in the case of DAS injection. The half-life of T-2 toxin was 48 - 60 h and after 8 - 9 d it was hydrolysed to T-2 tetraol. The half-life of DAS was only 36 - 48 h and by 7 - 8 d almost all the toxin was metabolized to scirpentriol.

Kiessling *et al*. (1984) demonstrated that intact rumen fluid can convert T-2 to HT-2 and DAS to 15-MAS, although rumen fluid had no effect on DON. However, under anaerobic conditions, bovine rumen microorganisms can reduce the epoxide group of DON to a carbon-carbon double bond to yield a deepoxy product called DOM-1 or deepoxy DON (King *et al*., 1984). Rumen microorganisms are also capable of reducing T-2 and DAS metabolites to their corresponding deepoxydes. DOM-1 was detected also in *in vivo* metabolism of DON in rats (Yoshisawa *et al*., 1983). *In vitro* incubation of DON with contents of the large intestines and ceca of chicken causes complete biotransformation of the toxin into deepoxy DON (He *et al*., 1992). When laying hens were fed 83 ppm DON only 5% of the compound was detected unchanged in the excreta (Lun *et al*., 1986) which suggests an almost complete biotransformation of the toxin before it is eliminated. Four DAS metabolites were detected in the excreta of rats given multiple oral doses of DAS at 2.8 mg/kg body weight (Sakamoto *et al*., 1986). The metabolites detected were MAS, scirpentriol, and their corresponding deepoxy derivatives, deepoxy MAS and deepoxyscirpentriol. Incubation of DAS with bovine rumen microorganisms under anaerobic conditions also yield deepoxy MAS and deepoxyscirpentriol (Swanson *et al*., 1987), suggesting that deepoxidation of DAS occurs through the action of microorganisms present both in the rumen and in the gastrointestinal tract of monogastric animals.

Chickens fed ^{3}H-labeled T-2, biotransformed the toxin into metabolites including HT-2, NEO, T-2 tetraol, and 8 unknown derivatives (TB-1 to TB-8) that were quantitatively more significant than the known metabolites. TB-6 was identified as 4-DN and represented 1.5% of the total dose (Yoshisawa *et al*., 1980b). Later studies determined that TB-1 and TB-2 were the same compound: 3'-hydroxy T-2 toxin. TB-3, the major metabolite present, corresponded to 3'-hydroxy HT-2. TB-4 was identified as 8-acetoxy T-2 tetraol, and TB-5 and TB-6 as 4-DN (Visconti and Mirocha, 1985). Visconti and Mirocha (1985) injected T-2 toxin (3.5 mg/kg) intraperitoneally to 5-week-old chickens. Residues were found in excreta, liver, and lung, while no residues were detected in the heart or kidney. Quantitatively, the most important metabolites were 3'-hydroxy HT-2 toxin, HT-2 toxin, 3'-

hydroxy T-2 toxin, 15-acetoxy T-2 tetraol, and 14-acetoxy T-2 tetraol. Low levels of the parent T-2 toxin were found in feces and liver. In a similar trial, NEO, T-2 tetraol, and HT-2 toxin were reported as metabolites present in the excreta of broiler chicks dosed with T-2 toxin; the concentration of HT-2 toxin in the excreta was less than that of NEO or T-2 tetraol (Chi *et al*., 1978a).

In summary, trichothecene mycotoxins are metabolized biphasically as are many xenobiotics. Three major reactions occur during phase I: deacylation (hydrolysis), hydroxylation (oxidation), and deepoxidation (reduction). Hydrolysis of esters seems to be the major pathway in the metabolism of TCT containing esterified side chains (T-2, DAS) with the hydrolysis of the C-4 ester being the primary site of attack. The initial hydrolysis, however, produces metabolites with similar toxicity to the parent compounds (HT-2, MAS) and cannot be considered as significant detoxification. Further hydrolysis by esterases produces the corresponding and less toxic parent alcohols T-2 tetraol and scirpentriol. Oxidation reactions have been reported only in T-2 toxin (hydroxylation of C-3', C-4' at the isovaleroxy side chain, or C-7 positions). The reduction of the 12,13-epoxide ring by anaerobic microflora present in the gastrointestinal tract is an important detoxification reaction since the epoxide is considered essential for toxicity (Ueno, 1986). Contents of the large intestine of chickens readily reduce the epoxy ring of DON (He *et al*., 1992) but Swanson *et al*. (1988) failed to observe deepoxidation of DAS incubated with chicken excreta. The inability of the chicken gut microflora to reduce the DAS epoxy ring may account for the high toxicity to chickens of DAS compared to DON. From the phase II biotransformation reactions, only glucuronide conjugation has been reported for DAS, T-2, DON, and/or their corresponding metabolites. Glucuronides are excreted mainly through the bile into the gastrointestinal tract where they may be cleaved by intestinal microflora liberating the trichothecene which may then be reabsorbed. This process of enterohepatic recirculation may potentially delay excretion and ultimately increase toxicity.

3.4. Elimination

Toxicokinetic studies have shown that poultry are capable of eliminating almost completely a single oral dose of TCT within 48 h. White Leghorn hens intubated with radiolabeled DON eliminated 78% of the dose in the feces at 24 h after dosing, and over 90% of the original label was found in the excreta by 48 h; clearance of radioactivity from tissue had an average half-life of 16.8 h (Prelusky *et al*., 1986). Administration of ^{3}H-DON to colostomized hens shows that elimination of radioactivity occurs almost exclusively through the kidneys as compared to feces. The greatest proportion appears between 1 and 3 h after dosing with over 75% of the total occurring by 24 h. Renal excretion was sufficient to keep pace with gastrointestinal absorption because radioactivity of blood samples was low and changed with time such that the highest level preceded appearance in the urine (Lun *et al*., 1989). In lactating cows fed T-2 toxin at low doses, 30% of the dose is eliminated in the first 24 h, mainly through the kidneys (Yoshisawa *et al*., 1981). After 24 h the remaining toxin is eliminated mostly in the feces, presumably by bile excretion.

Yoshisawa *et al*. (1980b) fed ^{3}H-labelled T-2 toxin as a single oral dose of 1.6 mg/kg to 47-day-old broiler chickens that had received a diet contaminated with 10 ppm DON for 5 d. A total of 19, 29, and 80% of the administered label was recovered from the excreta at 4, 12, and 48 h after dosing, respectively. In a similar study, Giroir *et al*. (1991) investigated the comparative fate of tritiated T-2 toxin in White Pekin ducks and broiler chickens. After a single oral dose of 0.5 mg/kg, early elimination was evident by the rapid appearance of radioactive residues in the excreta. Within

6 h after dosing, more than 25% of the label had been eliminated by both chickens and ducks, and by 24 h, about 60% was found in the excreta of each species. Data from 48-h cumulative excreta samples showed no increase in label relative to the 24 h samples, indicating that no appreciable elimination of ^{3}H residues occurred after 24 h. Chi *et al*. (1978b) fed broilers non-radioactive T-2 toxin to 42 d and then intubated into the crop with tritium-labelled toxin. T-2 and/or its metabolites were almost totally eliminated in the excreta within 48 h.

3.5.   *Residues*

Orally or parenterally administered TCT do not accumulate in the body of animals to any significant extent and residues are rapidly eliminated within a few d after exposure (Swanson and Corley, 1989). In chickens, DON is not significantly distributed as a parent compound into edible tissues. El-Banna *et al*. (1983) demonstrated that broilers fed about 4 ppm DON-contaminated feed for 28 d and laying hens receiving a diet containing 5 ppm DON for 190 d did not have detectable levels of DON in eggs, muscle, liver, or gizzard when detection limits were reported as 10 ng/g tissue. In a similar study, broiler chickens fed diets with 9 or 18 ppm DON for 35 d, did not have residues of DON in liver, heart, kidney or muscle (Kubena *et al*., 1985). Lun *et al*. (1986) fed laying hens a diet containing 83 ppm DON for 27 d and no residues could be detected in eggs or edible tissues. Similarly, laying hens fed 2.2 mg unlabeled DON for 6 d followed by 2.2 mg of radiolabeled DON for 6 more d, did not show tissue accumulation. (Prelusky *et al*., 1986). However, Prelusky *et al*. (1987) observed an accumulation of DON and/or its metabolites in the eggs of laying hens fed a [^{14}C] DON-contaminated diet for 12 d. Radioactivity levels increased with each subsequent egg laid up until the last exposure to the toxin; the maximum levels accounted were 4.2 μg DON equivalents/60 g egg and the residues declined quickly when the birds were fed a non-contaminated diet. DOM-1, the deepoxy metabolite of DON, was detected in the milk of dairy cows given a diet containing 66 ppm DON for 5 d (Côté *et al*., 1986). The toxicity of DOM-1 to birds is not known.

Chi *et al*. (1978b) administered tritium-labeled T-2 toxin to broiler chickens showing that T-2 and/or its metabolites were completely excreted without significant accumulation of residues in the body. Similarly, very small amounts of radioactive residues were observed in tissues of young White Pekin ducks and broiler chickens fed a single dose of ^{3}H-labeled T-2 toxin (Giroir *et al*., 1991); radioactive residues were detected in liver, kidney, and muscle samples at 6 and 12 h after dosing but had declined to almost undetectable levels by 24 h and no residues were detected in the blood, brain, or fat of either species at any sampling period up to 48 h. Investigations on the transmission of ^{3}H-labeled T-2 toxin into the eggs of laying hens showed that, in both single and multiple dosed birds, the specific radioactivity of the albumen was greater than that of the yolk (Chi *et al*., 1978c). In single dosed birds, the maximum radioactivity occurred at 24 h postdosing; the yolk and the albumen contained 0.04 and 0.13% of the dose, respectively. The amount of residue transmitted into an egg in hens intubated daily with 1 mg T-2 toxin per kg for 8 consecutive d was equivalent to about 0.9 μg T-2 toxin or metabolites.

4.   TOXICODYNAMICS: MODE OF ACTION OF TRICHOTHECENES

The adverse effects of a mycotoxicosis result from interactions of the original (or metabolically modified) mycotoxin with functional molecules and subcellular organelles in the animal

cell. The main effect of trichothecene mycotoxins appears to be a primary inhibition of protein synthesis followed by a secondary disruption of DNA and RNA synthesis. The mechanism of toxicity has been focused on protein and macromolecular synthesis, membrane function, enzyme activities, and immune function.

Trichothecenes are the most potent small molecule inhibitors of eukariotic protein synthesis known, and are effective inhibitors in a wide spectrum of organisms including fungi, plants, and animals (Feinberg and McLaughlin, 1989). Trichothecenes can also inhibit the synthesis of polynucleotides but the patterns of inhibition of DNA and RNA synthesis observed, are those of a secondary effect caused by a primary block in cell protein synthesis. In yeast, RNA synthesis is inhibited by more than 80% and polysaccharide synthesis by 60% at concentrations of 34 μM trichodermin. This inhibition of macromolecular synthesis is typical of protein synthesis inhibitors. Eukaryotic cells require newly synthesized protein to enter the S phase (DNA replication phase) of the cell cycle. All inhibitors of protein synthesis prevent cells from entering this phase and replicating their genomes; however, in those cells that have already begun their S phase when the toxin is added, residual DNA synthesis continues due to repair functions. Because this pattern of inhibition of DNA synthesis is observed in the presence of trichothecenes, the inhibitory effect on DNA replication can be fully explained by a primary effect on protein synthesis (Feinberg and McLaughlin, 1989). Protein synthesis can be inhibited at any of its steps of translation, i.e., initiation, elongation or termination. Trichothecenes can be functionally divided into two broad groups: initiation inhibitors and elongation/termination inhibitors; however, their inhibitory effects depend on the concentration of the toxin and other environmental factors, and this classification is not absolute for the evaluation of the toxins (Ueno, 1983). Trichothecenes known to affect the initial step of protein synthesis include scirpentriol, T-2 toxin, DAS, NIV, 4-Ac-NIV, HT-2 toxin and fusarenone-X. Those toxins affecting the elongation or termination steps include trichodermin, crotocin, DON, and verrucarol.

Ribosomal enzyme peptidyl transferase is presumed to be blocked by the binding of one molecule of trichothecene. One toxin binding site per ribosome corresponds to one peptidyl transferase catalytic center (Ueno, 1983) and the stoichiometry of the reaction is one toxin molecule bound per ribosome (Coulombe, 1993). Peptidyl transferase is an integral part of the 60S ribosomal subunit and is involved in elongation and termination. However, the enzyme is not needed for the initiation step which involves met-tRNA and a 40S ribosomal subunit binding. All trichothecenes appear to inhibit peptidyl transferase. Those that inhibit peptide bond formation between the first and second amino acid result in build-up of single ribosome polysomes. Although they contain mRNA and the initiator met-tRNA they appear the same as monosomes lacking both mRNA and met-tRNA when observed on sucrose gradients. Since the initiation complex has been formed, the initiation inhibiting trichothecenes could be more correctly termed "initiation-like" inhibitors (Feinberg and McLaughlin, 1989). It is not clear why trichothecenes inhibit the same enzyme at different points during the process of translation.

Cells most susceptible to the action of trichothecenes are those having a high rate of regeneration and different types of these cells can be distinguished. The first group or type constitutes cells that regenerate from proliferating undifferentiated germinal and/or blast cells, such as lymphoid, erythroid, and intestinal cryptal cells. These cells are characterized by the presence of numerous cytoplasmic free polysomes. The second type includes cells of parenchymal organs like liver, kidney and pancreas, which do not have special proliferating undifferentiated cells. Free polysomes in the cytoplasm of newly formed cells of this kind are not as prominent as in the former type. The susceptibility of the cell to trichothecene mycotoxins depends mainly on the amount of

free polysomes in the cytoplasm. Undifferentiated germinal and blast cells, with a large number of free polysomes are much more susceptible than differentiated cells. Presumably, damage to membrane-bound polysomes is more easily repaired than in free polysomes and the block in protein synthesis may then be removed without serious consequences (Terao, 1983).

T-2 toxin can be incorporated into the lipid or protein components of the cell membranes due to its amphipathic character. Interference with cell membrane function has been demonstrated *in vitro* with erythrocytes, myoblasts, and fibroblasts (Coulombe, 1993). Several reports indicate that T-2 is able to induce a dose-dependent stimulation of lipid peroxidation in rat and mice liver (Tsuchida *et al.*, 1984; Ahmed & Ram, 1986; Chang and Mar, 1988; Karppanen *et al.*, 1989; Suneja *et al.*, 1989). Lipid peroxidation is believed to be caused by depletion of hepatic reduced glutathione (GSH) and/or production of free radicals. Ueno and Matsumoto (1975) demonstrated that several trichothecenes can inactivate thiol (SH)-containing enzymes through binding with SH-residues of the active center of the molecule. Rats receiving T-2 toxin show decreased activity of liver GSH-S-transferase (Ahmed and Ram, 1986) and decreased activities of hepatic NADPH-cytochrome c reductase and NADH-cytochrome b_5 reductase (Suneja *et al.*, 1989). However, this was accompanied by increased activities of enzymes of the GSH biotransformation system (GSH peroxidase, GSH reductase and glucose-6-phosphate dehydrogenase). Rats administered intragastric T-2 toxin showed a marked decrease in the activity of liver lysosomal enzymes and enzymes of the phase I biotransformation system concomitant with increased activities of epoxide hydrolase and UDP-glucuronosyl transferase (Kravchenko *et al.*, 1986). Biochemical changes produced by T-2 toxin in chickens include increases in plasma aspartate transaminase, alanine transaminase and lactate dehydrogenase activity with concomitant reduction in the activities of plasma uric acid and alkaline phosphatase (Pearson, 1978).

Fungal producers of T-2 toxin have been associated with hemorrhagic disorders in man and poultry. In chickens, T-2 toxin had no effect on blood clotting but caused a significant increase in prothrombin times (Doerr *et al.*, 1974). The coagulopathy caused by T-2 toxin in chickens is characterized by a primary effect in coagulation Factor VII and secondary effects on prothrombin and fibrinogen (Doerr *et al.*, 1981).

The immunosuppressive activity of trichothecenes has been recently reviewed by Corrier (1991). The major immunosuppressive toxins appear to be the type A trichothecenes, T-2 toxin and DAS, the type B DON and fusarenone-X, and the macrocyclic trichothecene stachybotriotoxin. T-2 toxin has been shown to cause necrosis and lymphoid depletion in the thymus, spleen, and lymph nodes of laboratory animals (Ueno, 1977). Necrosis and depletion of lymphocytes in the thymus, bursa of Fabricius, and spleen have been reported in T-2 toxin-treated chickens and turkey poults (Wyatt *et al.*, 1973b; Boonchuvit *et al.*, 1975; Richard *et al.*, 1978; Hoerr *et al.*, 1981b). Necrosis of lymphoid tissue has also been reported in chickens after administration of DAS (Hoerr *et al.*, 1981a, b). Trichothecenes have been associated with alterations in serum proteins and immunoglobulin profiles, reduced antibody formation, thymic aplasia, reduced cell mediated immunity but with enhanced delayed cutaneous hypersensitivity, and impairment of bacterial clearance and acquired immunity (Pier and McLoughlin, 1985). The immunosuppressive effects of trichothecenes on both cellular and antibody-mediated immunity decrease host resistance to infectious agents. Mortality caused by paratyphoid infection was reported to be increased in chickens exposed to T-2 toxin (Boochuvit *et al.*, 1975; Ziprin and Elissalde, 1990). In addition to immunosuppression, trichothecenes may induce immunomodulating effects including increased spontaneous antibody producing cells in the spleen, increased IgA production in isolated splenocytes, enhanced delayed hypersensitivity, and blastogenic responses of T and B lymphocytes, (Corrier, 1991).

In summary, trichothecenes affect actively dividing cells such as those lining the gastrointestinal tract, the skin, and lymphoid and erythroid cells. Cytomorphological studies have shown karyorrhexis, in a manner similar to radiomimetic injury (Ueno, 1983). The cytotoxic-radiomimetic-like effects of trichothecenes on rapidly dividing cells are caused by impaired DNA, RNA, and protein synthesis. The inhibition of protein synthesis results from binding to peptidyl transferase, and the DNA and RNA synthesis effects are secondary to impaired protein synthesis.

5.   <u>TOXICITY</u>

The LD_{50} values of the most important trichothecenes for poultry are shown in *Table 12.1*. In general, trichothecene mycotoxins can be considered as highly toxic compounds, type A being more toxic than type B trichothecenes. The LD_{50} values of the trichothecenes vary with the particular toxin and the type and age of the bird. In terms of LD_{50}, the type A trichothecenes T-2 toxin, DAS, and 15-MAS are more toxic than aflatoxin and comparable in toxicity to ochratoxin A which have LD_{50} values of 6.8 and 2.1 mg/kg for chickens, respectively (Smith and Hamilton, 1970; Huff *et al*., 1974). DON, with an LD_{50} value of 140 mg/kg, is the least toxic trichothecene for chickens (Huff *et al*., 1981).

Chi *et al*. (1978) tested the acute toxicity of seven trichothecenes in one day old broiler chickens. The descending order of acute LD_{50} toxicity of the trichothecenes administered, was 8-acetylneosolaniol, DAS, T-2 toxin, HT-2 toxin, NEO, deacetyl-HT-2 toxin, and T-2 tetraol (*Table 12.1*). The toxic potency of 12,13-epoxytrichothecenes appears to change by modifications of the side chains in the molecule. The hydrolysis of the C-8 isovaleroxy residue in T-2 toxin to the hydroxyl group (yielding NEO) decreases toxicity, whereas its modification to the acetate group (8-acetylneosolaniol) increases the toxicity for broiler chicks. The toxicity of T-2 toxin is also reduced by deacetylation of the C-4 ester to yield HT-2 toxin. The toxicity is further decreased by deacetylation, which results in deacetyl-HT-2 toxin and T-2 tetraol. The toxicities of NEO and T-2 tetraol are considerably less than the toxicity of T-2 toxin. HT-2 toxin, however, is comparable in toxicity to T-2 toxin. A lethal dose of dietary T-2 toxin in chickens was estimated to be about 10 mg/kg for 7 d (Hoerr *et al*., 1982).

Hoerr *et al*. (1981a) reported that T-2 toxin was more toxic than DAS in single- and multiple-dose tests conducted with male broiler chicks. The 72-h single oral LD_{50} for T-2 toxin was 4.0 mg/kg body weight and for DAS 5.0 mg/kg. T-2 toxin administered as 14 daily oral doses had an LD_{50} of 2.90 mg/kg and DAS had an LD_{50} of 4.15 mg/kg. The "chronicity factors" (ratio of the acute to chronic LD_{50} doses) for these two compounds are 0.73 and 0.83 for T-2 toxin and DAS, respectively. Chronicity factors near to one are indicative of compounds that are rapidly eliminated and tend not to accumulate (Osweiller *et al*., 1984). These findings agree with the results obtained from the toxicokinetic studies of trichothecenes which have demonstrated a rapid metabolism and excretion of these mycotoxins in poultry (Chi *et al*., 1978; Lun *et al*., 1988; Prelusky *et al*., 1986). Richardson and Hamilton (1990) investigated the toxicity of the eight trichothecenes of the scirpenol family. These toxins include the parent alcohol scirpentriol (STO), and its derivatives, which may have one, two, or three acetylated hydroxyl groups. The monoacetylated scirpenols are 3-MAS, 4-MAS, and 15-MAS; the diacetylated compounds are 3,4-DAS, 3,15-DAS, and 4,15-DAS, while triacetoxyscirpenol is the only compound with the three positions acetylated. All compounds had a high acute oral toxicity in one day old broiler chicks. The 24-h single oral LD_{50} value for 4,15-DAS

and 15-MAS were 2.0 and 2.5 mg/kg respectively. The LD_{50} values for the other scirpenols ranged from 7.2 to >32 mg/kg (*Table 12.1*). The authors suggested that a free hydroxy group at the C-3 position was a primary determinant of toxicity. The scirpenol group did not follow the pattern reported previously for the T-2 toxin family of trichothecenes (Chi *et al.*, 1978) in which a decrease in the number of acyl groups was accompanied by a decrease in toxicity.

TABLE 12.1   LD$_{50}$ Values of trichothecene mycotoxins in broiler chickens after single oral administration

Trichothecene	Chick age (days)	LD$_{50}$ (mg/kg)	Reference
Scirpene group			
Scirpentriol	one	9.3	Richardson & Hamilton, (1990)
3-MAS	one	8.7	Richardson & Hamilton, (1990)
4-MAS	one	9.6	Richardson & Hamilton, (1990)
15-MAS	one	3.4	Mirocha et al., (1985)
	one	2.5	Richardson & Hamilton, (1990)
DAS(4,15-DAS)	one	3.82	Chi et al., (1978a)
	seven	5.0	Hoerr et al., (1981b)
	one	5.9	Mirocha et al., (1985)
	one	2.0	Richardson & Hamilton, (1990)
3,4-DAS	one	>32.0	Richardson & Hamilton, (1990)
3,15-DAS	one	10.1	Richardson & Hamilton, (1990)
TAS	one	8.0	Mirocha et al., (1985)
	one	7.2	Richardson & Hamilton, (1990)
T-2 toxin group			
T-2 toxin	one	5.25, 5.03	Chi et al., (1977a)
	one	4.97	Chi et al., (1978a)
	seven	4.0	Hoerr et al., (1981b)
	?	3.6	Sato & Ueno, (1977)
HT-2 toxin	one	7.22	Chi et al., (1978a)
Deacetyl HT-2 toxin	one	30.18	Chi et al., (1978a)
T-2 tetraol	one	33.79	Chi et al., (1978a)
NEO	one	24.87	Chi et al., (1978a)
8-Acetyl NEO	one	3.22	Chi et al., (1978a)
Nivalenol group			
Deoxynivalenol	one	140.0	Huff et al., (1981)
Abbreviations: MAS = monoacetoxyscirpenol, DAS = diacetoxyscirpenol, TAS = triacetoxyscirpenol, NEO = neosolaniol			

In general, type B trichothecenes are less toxic than type A for poultry. However, lethal toxicity of NIV and fusarenone-X in adult mice, is about 10 times higher than that of DON or 3-Ac-DON and comparable to T-2 toxin (Ueno, 1984). There are no reported toxicological studies involving the type B compounds NIV and fusarenone-X in poultry.

6.   **CLINICAL SIGNS AND LESIONS**

The effects of non macrocyclic trichothecenes in poultry have been studied using several methodological approaches that have included the dietary incorporation of crude fungal cultures with known levels of mycotoxins, the preparation of rations with naturally contaminated feedstuffs containing a known toxic concentration, or the addition of pure mycotoxins to uncontaminated diets. Crude organic extracts of fungal cultures have also been used. Administration of crude extracts and purified mycotoxins by parenteral routes have also been reported. The use of crude fungal culture material, crude extracts and naturally contaminated grains involve the possibility of adding unknown metabolites with potential toxic effects that usually lead to confounding results. Administration of high doses of toxins by parenteral injection or crop gavage in order to detect gross and microscopical lesions or biochemical changes, is of questionable significance because it is not truly representative of natural exposure. Perhaps the most representative methodological approach is the incorporation of known amounts of highly purified mycotoxins into uncontaminated diets.

Early and recent accounts of TCT mycotoxicosis reflect their caustic and radiomimetic effects manifested by extensive necrosis of the oral mucosa and skin in contact with the toxin, acute effects on the digestive tract, and decreased bone marrow and immune system function. The deleterious effects of trichothecene mycotoxins such as T-2 toxin on performance of broiler chickens and laying hens are dose- and time-dependant. Neural disturbances, feather alterations, hemorrhages, and regression of the bursa of Fabricius are some other effects of experimental and natural mycotoxicosis caused by type A TCT.

6.1.   *Type A trichothecenes in broiler chickens*

The toxic effects of dietary TCT mycotoxins in broilers are summarised in Table 12.2. The primary effect of T-2 toxin in chickens is an inflammatory response in the mouth that progresses to necrosis and invasion by normal microbial flora. Wyatt *et al.* (1972, 1973ab, 1975) fed one day old broiler chicks with graded concentrations of dietary T-2 toxin (0, 1, 2, 4, 8, 16 ppm) for 3 weeks. Growth rate was decreased significantly and in a dose-related fashion by concentrations of 4 ppm and above, but feed efficiency was not affected. The relative weight of the liver and its lipid and dry matter content were unaffected but there was a dose-related increase in liver hematomas (Wyatt *et al.*, 1973b). Abnormal feathering was observed in chickens receiving dietary T-2 toxin levels of 4 ppm or higher (Wyatt *et al.*, 1975). The oral lesions characteristic of T-2 toxicosis first appeared when the chicks were about 1 week old, as raised caseous yellow-white plaques. By 2 weeks, the lesions increased in size and invaded the lingual papillae at the root of the tongue. At 3 weeks, the size of the lesions increased to the extent that some of the birds on the higher dose levels were unable to close their mouth completely.

TABLE 12.2   Toxic effects of dietary trichothecene mycotoxins in broiler chickens

lycotoxin	Dietary level (mg/kg)	Time of exposure	Effects	Referer
2 toxin	4	3 weeks	Decreased growth rate	Wyatt *et al*., (1972)
	4	1 week	Oral lesions	Wyatt *et al*., (1972)
	4	3 weeks	Neural disturbances	Wyatt *et al*., (1973a
	4	3 weeks	Reduced weight gain, hepatic hematomas	Wyatt *et al*., (1973b
	4	3 weeks	Abnormal feathering	Wyatt *et al*., (1975)
	16	3 weeks	Regression of the bursa of Fabricius	Boonchuvit *et al*., (1
	0.4	7 weeks	Oral lesions	Chi *et al*., (1977b)
	1-4	3 weeks	Oral lesions; decreased feed intake and weight	Chi *et al*., (1977b)
	8-16	11 days	Decreased weight gain; oral lesions	Joffe and Yagen, (1
	4-16	3 weeks	Oral necrosis; reduced feed consumption	Hoerr *et al*., (1982b
	50-300	3 weeks	Reduced hematocrit, lymphoid atrophy, anemia;	Hoerr *et al*., (1990)
	8	3 weeks	Oral lesions, decreased body weight	Kubena *et al*., (1990
AS	5	3 weeks	Oral lesions, decreased feed intake and weight Oral	Chi & Mirocha, (197
	4-16	3 weeks	necrosis; growth retardation	Hoerr *et al*., (1982b
	1-2	3 weeks	Oral lesions, growth retardation	Ademoyero & Hami
	4-8	3 weeks	Decreased body weight	Ademoyero & Hami
AS	4-8	3 weeks	Oral lesions; growth retardation	Ademoyero & Hami
AS	5-2	3 weeks	Oral lesions; growth retardation;	Ademoyero & Hami
TO	2-4	3 weeks	Oral lesions; growth retardation	Ademoyero & Hami
ON	1.87	4 weeks	No adverse effects	Hulan and Proudfoc
	116	5 days	Decreased growth rate and feed intake;	Moran *et al*., (1982)
	16	3 weeks	Reduced body weight gain and feed efficiency	Kubena *et al*., (1988

Lesions occurred without exception in all birds given T-2 toxin (Wyatt *et al*., 1972). Dietary T-2 toxin also caused abnormal positioning of the wings and hysteroid seizures, (Wyatt *et al*., 1973a); the incidence of the neural signs was dependent on the length of exposure to T-2 toxin and its dietary concentration. Neural signs were observed at dietary concentrations of 4 - 16 ppm, which were the same levels causing growth retardation. However, neural disturbances were only seen in a few chickens and even at 16 ppm T-2 caused visible neural signs in only 37.5% of the chickens while 100% of the chickens receiving T-2 toxin developed oral lesions.

Chi *et al*. (1977) fed one day old broiler chickens with dietary T-2 levels up to 4 ppm for 3, 6, or 9 weeks. Feed consumption and weight gain of chicks fed the diet containing 4 ppm T-2 toxin decreased immediately and in general, chicks were most sensitive to dietary T-2 toxin during the first 3 weeks of age. Oral lesions were caused by T-2 toxin at a concentration as low as 0.4 ppm. These lesions were circumscribed proliferative yellow caseous plaques that occurred at the margin of the beak, mucosa of the hard palate and angle of the mouth and the tongue. The severity increased with a longer feeding period, and with higher dietary levels. Few birds fed the diet containing 4 ppm T-2 toxin had necrotic lesions on the mucosal lining of the gizzard. The authors concluded that a concentration of 1 ppm T-2 toxin in the diet will cause a considerable decrease in weight gain in young chicks kept on the diet for 3 weeks and 0.4 ppm will have a similar effect when the birds are kept on this diet for 7-9 weeks. In another study, Chi and Mirocha (1977) fed one day old broiler chicks a diet containing either 5 ppm DAS, 5 ppm T-2 toxin, or a control diet for 3 weeks. All chicks fed DAS or T-2 showed oral lesions at the end of the experiment. The oral lesions were seen as early as 5 d and consisted of yellow, proliferative plaques on the upper and lower beak, tongue and bilateral angle of the mouth. The severity of the lesions was greater in chicks fed DAS as compared to T-2 toxin. Body weight gain of chicks fed DAS were 75% of the control while that of chicks fed T-2 toxin were 86%. DAS appeared to be more caustic than T-2 toxin in terms of oral lesion formation and the authors concluded that more than one TCT can cause mouth lesions in chickens. Chi *et al*. (1978) administered one-day-old chicks a single oral dose of either DAS, T-2 toxin, HT-2 toxin, NEO, 8-acetyl-NEO, deacetyl HT-2 toxin, or T-2 tetraol, at different levels. Birds treated with each mycotoxin developed similar clinical signs that included asthenia, inappetence, and diarrhea within 4 to 10 h after dosing. Birds treated with high doses of each toxin were in coma before death. Oral lesions and an 18% reduction in body weight gain were observed in broiler chicks fed 8 ppm dietary T-2 toxin from 1 to 21 d of age (Kubena *et al*., 1990) although feed utilization was not affected. Microscopic examination of the bursa of Fabricius revealed an increased number of pyknotic nuclei in the lymphoid follicles.

T-2 toxin and DAS can cause rapid necrosis of lymphoid and myeloid tissue, alimentary and extrahepatic biliary tract mucosa, and of feather epithelium in young chickens. Hoerr *et al*. (1981b) described the sequential gross and microscopic alterations in 7 day old broiler chickens following a single dose of either 2.5 mg/kg T-2 toxin or 2.7 mg/kg DAS. Necrosis of the thymic lymphocytes was present 6 h after dosing and was initially more severe in the medulla of chicks given T-2 toxin. Necrosis with rapid depletion of lymphocytes occurred in both cortex and medulla. In the bursa of Fabricius, necrosis was present 1 h after dosing and was more severe in the medulla. Necrosis of the epithelial tuft (follicular epithelium) accompanied that of follicular necrosis, but the zone of undifferentiated epithelium, which separates the cortex and the medulla, remained normal. Necrosis of the erythroid and granulocytic regions of the bone marrow was first seen at 6 h and was most severe at 24 h. Hoerr *et al*. (1981b) indicated that hepatic lesions were more severe in chicks given T-2 toxin being found within 1 h of administration as disseminated foci of coagulation necrosis, mainly in the portal region. In the proventriculus, necrosis of superficial epithelium was accompanied by exfoliation of epithelial cells from the mucosal folds. Feather lesions, found 12 through 24 h after dosing, appeared as necrosis of the layer of regenerative cells in the feather base and of the basilar layer of the ramus and of the barb ridges. Seven day old male broiler

chickens fed either T-2 toxin or DAS at 4 and 16 ppm for 21 d showed reductions in feed intake and weight gain (Hoerr *et al.*, 1982b). Focal, yellow, oral plaques, located around salivary duct openings on the palate, tongue, and buccal floor, appeared by day two. The plaques progressed to raised, yellow-grey crusts covering ulcers. Microscopically the ulcers had a base of granulation tissue and inflammatory cells, and the crusts were made of exudate, bacterial colonies, and feed components.

Hoerr *et al.* (1982c) administered T-2 toxin (1.5-3.0 mg/kg) or DAS (2.5-3.5 mg/kg) by crop gavage to 7 day-old broiler chicks for 2 weeks. Some chickens became dehydrated and emaciated and died. Body weight and hematocrit were reduced, the feathers were malformed and beak and legs were pale in survivors. At necropsy the lymphoid organs were atrophic. Microscopic lesions included necrosis and cell depletion of lymphocytic and hematopoietic tissues, and necrosis of hepatocytes, bile ductule epithelium, enteric mucosa, and germinal regions of feather barbs. T-2 toxin was more detrimental than DAS affecting lymphocytic and hematopoietic tissues. In other studies Hoerr *et al.* (1982a) fed 7 d broiler chickens a diet containing 1, 5 or 10% *Fusarium* culture for 17 d (Hoerr *et al.*, 1982a). The culture provided a dietary concentration of 5 ppm T-2 toxin, 0.5 ppm NEO, and possibly other fungal metabolites. Half of the chickens on the highest level of fungal culture died during the 17 d and were dehydrated, had necrosis and depletion of lymphocytic and hematopoietic tissues and necrosis of the hepatobiliary system, gastroenteric mucosa, feather epidermis, and renal tubular epithelium. Surviving chickens had anemia, and were smaller. These birds also had atrophied lymphoid tissues, reduced hematopoietic cellularity of the bone marrow, necrosis of oral and crop mucosa, vacuolated hepatocytes, hyperplastic bile ductules, and reduction of thyroid follicular diameter. Chickens fed diets containing 1 or 5% fungal culture had reduced weight gain and feed intake but no mortality.

Joffe and Yagen (1978) fed broiler chickens with diets containing different levels of either *F. poae* and *F. sporotrichioides* culture material (0.5-10%), crude methanolic extracts of the same cultures, or purified T-2 toxin (8 and 16 ppm). Diets with 1% culture material contained 46 ppm T-2 toxin in the case of *F. sporotrichioides* and 60 ppm in the case of *F. poae*. Small amounts of NEO and HT-2 toxin were identified in the *F. sporotrichioides* culture. Chickens given pure T-2 toxin developed only oral lesions. Chickens receiving crude extracts or mixed diets showed a marked reduction in circulating blood cells, including severe depression especially of the leucocyte and thrombocyte counts, and decreases in RBC count and hemoglobin values. Pure T-2 toxin caused mild decrease on RBC and thrombocyte levels and also decreased the leucocyte count. High mortality was seen in chickens fed culture material or crude extracts although no mortality was seen in birds fed purified T-2 toxin. An outbreak of T-2 toxin mycotoxicosis in a commercial flock of broiler chickens was reported by Bitay *et al.* (1981). Altered feathering, depression, necrosis of the oral and oesophageal mucosa and visible atrophy of the lymphoid organs were observed. Feed analysis revealed 2.5 ppm T-2 toxin.

Refusal of feed, the classic sign of trichothecene mycotoxicosis in swine, has also been reported to occur in chickens. Burditt *et al.* (1983a), studying experimental models for feed refusal in broiler chickens, demonstrated that incorporation of a *Fusarium* culture filtrate containing DAS (87 µg DAS/g feed) reduced feed consumption by 77% when added to feed and reduced liquid consumption by about 92% when substituted for drinking water. Similarly, T-2 toxin added to fed or water, produced a dose-related refusal in broiler chickens (Burditt *et al.*, 1983b); presentation in water caused a more sensitive refusal since the minimum effective dose was 0.31 ppm in water compared with 5.0 ppm in feed. In other studies graded dietary levels of scirpentriol (STO), MAS, DAS, and triacetoxyscirpenol (TAS) were fed to male broiler chickens for 21 d after hatching

(Ademoyero and Hamilton, 1991; Parkhurst *et al*., 1991). All the scirpenol mycotoxins tested provoked mouth lesions in a dose-related manner. The minimum effective dose (MED) for mouth lesions were 4, 2, 1, and 0.5 ppm for TAS, STO, DAS, and MAS, respectively. The lesions were clearly visible after feeding for one week and the numbers of affected mouth parts almost tripled after 2 weeks of exposure. The minimum growth inhibitory concentrations were 2 ppm for STO, MAS, and DAS, and 8 ppm for TAS. Feather abnormalities were observed with STO, MAS, and DAS but not with TAS. The most severe feather abnormalities were caused by MAS, which altered feather structure even at the lowest concentration tested (0.5 ppm). The main alteration observed was a frayed and missing web on the medial side of the distal half of the feather, although the shafts of the feather appeared thinner and weaker with an accentuated medial curve.

Richardson and Hamilton (1990) gave 1 d broiler chickens a single oral dose of different trichothecenes of the scirpenol family (STO, MAS, DAS, TAS) and observed petechial hemorrhaging, primarily in the gastrointestinal tract and the vascular beds of the beaks and the toe nails. Reddening of the skeletal muscle was also observed but only in chicks receiving high doses of DAS. Ademoyero and Hamilton (1991) fed broiler chicks to 21 d, with graded dietary levels of STO, the parent alcohol of the scirpene group of TCT. Concentrations of 4 ppm and higher caused significant growth inhibition while higher concentrations (8-16 ppm) induced regression of the bursa of Fabricius and decreased the activities of serum lactic dehydrogenase and aspartate amino transferase.

6.2.   Type A trichothecenes in laying hens

The toxic effects of TCT mycotoxins in layers are summarised in Table 12.3.   The deleterious effects of feeding trichothecene mycotoxins to laying hen performance were first reported by Speers *et al*. (1972, 1973). They added 1 - 5% corn contaminated with *F. tricinctum* and *F. oxysporum* to the diet of hens. There was a marked decrease in feed intake, body weight and egg production and a high incidence of mouth and tongue lesions in birds fed the *F. tricinctum* treated corn. In a second set of trials (Speers *et al*., 1973), SCWL hens were fed corn containing *F. tricinctum, F. roseum* "oxyrose" (2.5% and 5%), and pure T-2 toxin (4, 8, 16 ppm). Feeding 5% *F. tricinctum*, providing 14-15 ppm T-2, reduced feed consumption, body weight, egg production and egg quality. *F. roseum* contaminated corn caused an immediate drop in feed consumption, while egg production stopped within 3 d and there was a marked body weight loss. Pure T-2 toxin at 16 ppm resulted in reduced feed intake and egg production. Lesions in the mouth and tongue appeared as raised, yellow necrotic plaques. Wyatt *et al*. (1975) fed mature laying hens a diet containing 20 ppm purified T-2 toxin for 3 weeks.

TABLE 12.3 Toxic effects of dietary trichothecene mycotoxins in laying hens

toxin	tary vel (g/kg)	Time of exposure	Effects	
kin	16	4 weeks	Decreased feed intake and egg production; oral lesions	Speers e
	20	3 weeks	Decreased feed intake and egg production; oral lesions	Wyatt et a
	8	8 weeks	Decreased feed consumption, egg production, and shell thickness; decreased hatchability; oral, crop, and gizzard lesions	Chi et al.,
	-8	4 weeks	Egg production slightly decreased (not statistically significant)	Speers e
	16	4 weeks	Reduced feed intake, body weight, and egg production	Speers e
	12	18 days	Decreased feed intake and egg production; decreased egg weight; oral lesions	Wyatt et a
	-10	4 weeks	Decreased egg production and hatchability	Tobias et
	2	24 days	Oral lesions; decreased feed intake and egg production	Diaz et a
	.5	4 weeks	Decreased hatchability of fertile eggs	Allen et a
	2	24 days	Oral lesions; decreased feed intake and egg production; increased incidence of soft-shelled eggs	Diaz et a
	-50	28 days	Decreased feed intake and body weight; cessation of egg laying	Speers e
	5-0.7	70 days	Decreased egg and shell weight, shell thickness and percent shell No adversed effects on health or productivity	Hamilton
	-5	24 weeks	No adverse effects	Hamilton
	20	12 days	Increased incidence of chick developmental abnormalities	Prelusky
	-4.9	70 days		Bergsjo e

There was an immediate 25% drop in feed consumption. Oral lesions characteristic of T-2 toxicosis appeared during the first week, although there was no effect on blood parameters, plasma glucose or the size of most organs. The most important economic effect was a lowered egg production that declined by about 20%, and this was accompanied by a thinner egg shell.

Feed consumption, egg production and shell thickness were significantly decreased in hens fed 8 ppm T-2 toxin (Chi *et al*., 1977c). Fertility was not affected by feeding T-2 toxin but the hatchability of fertile eggs of hens fed 2 and 8 ppm was significantly lower. Again, organ weights were not affected by T-2 toxin, although hens fed 8 ppm T-2 toxin had increased serum levels of alkaline phosphatase, LDH, SGPT, and uric acid. Oral lesions were observed from the second week in hens fed 4 and 8 ppm T-2 toxin and after 3 weeks in hens fed as little as 0.5 ppm. At 8 ppm nearly all of the hens developed oral lesions. No significant lesions were seen during gross and histopathologic examination apart from necrotic lesions in the crop and gizzard of birds fed 4 and 8 ppm T-2. Speers *et al*. (1977) fed laying hens with rations containing 2.5 or 5.0% of corn containing *F. tricinctum* (with 8 and 16 ppm T-2 toxin, respectively), 2.5 or 5.0% of corn with *F. roseum* "Gibbosum" (with 25 and 50 ppm MAS), and diets containing 0, 4, 8 or 16 ppm purified T-2 toxin. The diet containing 2.5% of corn with *F. tricinctum* had no significant effect on performance, but the diets with 5.0% corn invaded with *F. tricinctum* and those with 2.5% and 5.0% of corn with *F. roseum* resulted in drastically reduced feed consumption, body weight, egg production, and egg quality. Hens fed ≥ 16 ppm purified T-2 toxin had reduced feed consumption, body weight and egg production.

A drastic and sudden fall in egg production in a flock of hens was associated with the feeding of 3.5 ppm T-2 toxin and 0.7 ppm HT-2 toxin in the hen's ration (Shlosberg *et al*., 1984) although the field condition could not be reproduced under more controlled conditions. Recently Tobias *et al*. (1992) reported a dose-related reduction in egg production and impaired hatchability in laying hens fed diets containing up to 10 ppm purified T-2 toxin for 28 d. Levels of 1, 5 and 10 ppm dietary T-2 toxin reduced egg production by 12.5, 68.0 and 78.9%, respectively.

The recovery of laying hens from T-2 toxicosis was studied by Wyatt *et al*. (1978) by feeding hens a ration containing 12 ppm T-2 toxin for 18 d followed by an uncontaminated diet for an additional 18 d. During T-2 toxin feeding, oral lesions appeared in various parts of the mouth, feed consumption decreased by 22%, egg weight decreased, and all birds appeared morbid. When the diet was switched, a gradual healing process of the oral lesions was evident and after 7 d the lesions had disappeared. Egg production returned to normal by the end of the recovery period but feed consumption increased by 11% over the control value. The authors suggested that during recovery from T-2 toxicosis feed intake may increase even though oral lesions and decreased egg production indicate the active stage of the toxicosis.

Allen *et al*. (1982) also studied recovery of WL birds after feeding diets containing a 1 or 2% culture of *F. roseum*. DAS and another unidentified trichothecene were found in the crude culture at concentrations of 15 and 30 ppm, respectively. Egg production was depressed by both the 1 and 2% levels whereas feed consumption, hatchability and fertility were reduced only by the 2% level of crude culture. All production levels returned to normal when the toxins were removed during the final 6 weeks. In the same experiment (Allen *et al*., 1982), laying hens fed 0.5 ppm pure DAS for 28 d were not adversely affected. However, feeding 0.5 ppm DAS resulted in a gradual decrease in hatchability that returned to normal upon withdrawing the toxic feed.

Recently, Diaz *et al*. (1994) investigated the individual and combined effects of T-2 toxin and DAS in laying hens. Both toxins induced oral lesions when administered singly or combined at dietary levels of 2 ppm each. Oral lesions affecting primarily the palate, tongue, and margins of the

beak were observed as early as 24 h after the administration of the toxic diets. A significant reduction in feed intake and egg production was observed. The effects of T-2 toxin and DAS were additive for reduced feed consumption and incidence of oral lesions. However, a toxic synergism for reduced egg production was observed. Interestingly, hens receiving DAS alone or combined with T-2 toxin showed an increased incidence of soft-shelled eggs.

6.3.   Type B trichothecenes in broiler chickens

Feeding trials with DON have demonstrated that poultry can tolerate up to 5 ppm dietary DON without deleterious effects (Trenholm *et al*., 1984). Feeding one day old broiler chickens diets containing up to 1.87 ppm DON had no significant effect on mortality, weight gain, body weight, feed conversion, or feed consumption (Hulan and Proudfoot, 1982). *F. graminearum*-contaminated corn containing 800 to 900 ppm DON was substituted for control corn at 0-24% in a broiler ration. Growth and feed intake were not significantly reduced until the contaminated corn exceeded 12% of the ration (116 ppm DON). Chickens receiving contaminated corn exhibited plaques in the mouth and gizzard erosions in a dose-related manner although alertness, coordination, and feathering appeared normal regardless of the treatment. Low levels (<1 ppm) of T-2 toxin and DAS were detected in all feeds and so the presence of these type A TCT in the feed could have accounted for the oral lesions observed. Male Leghorn chickens fed diets containing up to 18 ppm DON to 35 d of age (Kubena *et al*., 1985) exhibited decreased hemoglobin and hematocrit levels although again no adverse effects on body weight gain or feed utilization were seen.   Administration of extraordinary high doses (140-1120 mg/kg) of DON to broiler chicks does however cause problems including gasping, lethargy, dropping of the wings and head from the normal upright position, loss of balance, and death within 14 hours (Huff *et al*., 1981). Post-mortem examination of the birds revealed ecchymotic hemorrhages throughout the gastrointestinal tract, liver, and musculature, visceral gout, and necrotic lesions of the gizzard lining. However, these findings may not be truly representative of more natural levels of exposure.

Studies on feeding laying hens and growing chickens with contaminated corn showed that 2-3 ppm DON do not produce any adverse effects on performance. It was suggested that the safe ceiling level of DON in the United States should be increased from 0.5 to 2-3 ppm for poultry feed (Muirhead, 1992).

6.4.   Type B trichothecenes in laying hens

The concentrations of DON most frequently found in corn (up to 20 ppm) are unlikely to cause production problems in hens or turkeys (Young *et al*., 1986). Hamilton *et al*. (1981a, 1981b) fed laying pullets wheat-soybean diets containing 0.35 and 0.7 ppm DON for 70 d observing no meaningful change in performance. However, egg and shell weight, shell thickness and percent shell decreased linearly with increasing levels of dietary DON. No organ damage was related to DON ingestion. Laying hens fed diets for 24 wk containing up to 5 ppm DON from naturally contaminated wheat did not show any adverse effects on health or productivity (Hamilton *et al*., 1985). No overt adverse effects were noted in laying hens receiving DON, either as a single oral dose (2.2 mg DON/bird) or in their feed (2.2 mg/hen/day) over an extended 12 day period (Prelusky *et al*., 1986). All hens appeared healthy, even during the continuous dosing of the toxin whose diet equivalent was 20 ppm.

Branton *et al*. (1989) however, reported decreased egg production and oral and crop lesions in commercial laying hens that consumed grain sorghum-based diets naturally contaminated with 0.3 ppm DON and 1.1 ppm zearalenone. The authors postulated the involvement of unknown *Fusarium* toxins present with DON in order to explain the adverse effects observed because dietary concentrations of up to 20 ppm DON or 100 ppm zearalenone did not cause any adverse effects in laying hens (Prelusky *et al*., 1986; Marks and Bacon, 1976). The administration of DON from naturally contaminated oats at dietary concentrations of 2.5, 3.1 and 4.9 ppm to laying hens for 70 d did not produce any effects on feed intake, egg production, fertility, hatchability or perinatal mortality (Bergsjo *et al*., 1993). However, the incidence of chick developmental abnormalities was increased in birds fed DON and especially unabsorbed yolk sac and delayed ossification. The experimental diets also contained low levels of 3-Ac-DON, NIV, ochratoxin A, and zearalenone whose possible toxic contributions were regarded as negligible.

6.5.   Interactions

Under field conditions, there is the possibility of feed being contaminated with more than one mycotoxin. Fungal strains are often capable of synthesizing several mycotoxins, and some *Fusarium spp*. can produce more than 8 mycotoxins (Ueno and Ishii, 1985). On the other hand, the use of multiple grain sources in poultry diets can lead to mixtures of mycotoxins in the feed. Chemical interactions between such toxins may then occur through several mechanisms, including alterations in absorption, protein binding, and biotransformation or excretion of one or both of the interacting toxins. The response of the organism to combinations of chemicals may be increased or decreased because of the different toxicological responses at the site of action. Therefore, the effects of two compounds given simultaneously may produce additive or synergistic effects. Terms used to describe toxicologic interactions include addition, synergism, potentiation, and antagonism (Klaasen and Eaton, 1991).

Toxic synergism between T-2 toxin and aflatoxin was reported in broiler chicks fed diets containing 2.5 ppm aflatoxin and 4 ppm T-2 toxin, (Huff *et al*., 1988). The combination of both mycotoxins produced a significant interactive effect observed as decrease in body weight, increase in the relative weights of the kidney, gizzard, and heart, and decrease in mean corpuscular volume and serum levels of potassium. The authors concluded that T-2 toxin and aflatoxin can interact to produce synergistic toxicity and that this synergism is a threat to poultry production due to the prevalence of these mycotoxins and the severity of their interactive toxicity. Additive effects of dietary T-2 toxin and aflatoxin were also observed in broiler chicks receiving 8 ppm T-2 toxin and 3.5 ppm aflatoxin, (Kubena *et al*., 1990). Body weight gains were significantly depressed by either aflatoxin or T-2 toxin, and further decreased by a combination of the two toxins. Sato and Ueno (1977) also observed a synergistic interaction between T-2 and ochratoxin A (OA) in broiler chickens. The lethal toxicities of OA and T-2 were synergistically enhanced upon simultaneous oral administration to one day old broiler chicks, as measured by the LD_{50} values. Furthermore, the combination of T-2 and OA produced a marked growth inhibition and high mortality with dietary levels of 20 ppm T-2 and 8 ppm OA. Additive, synergistic, and antagonistic interactions were described in broiler chicks fed dietary T-2 toxin (4 ppm) or ochratoxin A (2 ppm) (Kubena *et al*., 1989b). The effects of T-2 and ochratoxin were additive in their effects on reducing body weight gain, corpuscular volume, serum protein, and lactate dehydrogenase activity. The interaction for serum calcium levels and activity of gamma glutamyl transferase was characterized as antagonistic, while synergism occurred in terms of elevated serum triglyceride levels.

A synergistic interaction between T-2 toxin and DON was observed in chickens fed diets containing DON-contaminated wheat (16 ppm) and pure T-2 toxin at 4 ppm, (Kubena *et al*., 1989a). Final body weights were significantly reduced by the DON/T-2 combination but not by the toxins singly. In a similar trial, broiler chicks were fed diets containing ochratoxin A (2 ppm) or DON, (Kubena *et al*., 1988) where for most of the parameters evaluated there were significant interactions that were described as "less than additive" or in some cases antagonistic. Rotter *et al*. (1991) employed the Chick Embryotoxicity Screening Test (CHEST) to detect possible interactions between DON, 15-acetyl-DON, and HT-2 toxin. The toxins were tested at different concentrations and the effects were based on percent mortality. The combined toxicity of any two trichothecenes was found to be additive.

Interactions between T-2 toxin and some of the anticoccidial ionophorous antibiotics commonly added to poultry diets have been reported (Ványi *et al*., 1989; Varga and Ványi, 1989). Body weight gain of chicks fed a diet containing monensin and T-2 toxin was less than that of birds fed diets containing either compound alone (Ványi *et al*., 1989). Broiler chicks fed diets containing different amounts of T-2 toxin (1-4 ppm) supplemented with 100 ppm monensin were infected experimentally with viable oocysts of *Eimeria tenella*. Groups receiving 2 or 4 ppm dietary T-2 toxin developed clinical coccidiosis in spite of supplementation with monensin. In the same trial, the LD_{50} value of narasin, another ionophore compound, was determined to be much lower for chickens fed diets containing T-2 toxin (102 mg/kg) than for control birds (176 mg/kg). Varga and Ványi (1992) found that the anticoccidial effect of lasalocid at 75 ppm was significantly reduced in cockerels fed dietary T-2 toxin at levels of 1.25-6.0 ppm which were experimentally infected with oocysts of *E. tenella* and *E. mitis*. The authors concluded that in outbreaks of coccidiosis, it is important to be aware of the interactions between anticoccidials and mycotoxins.

Interactions of T-2 toxin with *Salmonella spp* have been also described. Boonchuvit *et al*. (1975) reported a significant interaction resulting in increased mortality in chickens fed 16 ppm dietary T-2 toxin and infected with either *Salmonella worthington*, *S. thompson*, *S. derby*, or *S. typhimurium* var. *copenhagen.* Similarly, T-2 toxin caused a severe negative effect on the ability of chicks to resist inoculation with *S. typhimurium*, as measured by survival (Ziprin and Elissalde, 1990).

Trichothecenes may also affect utilization of other dietary components. Ademoyero and Hamilton (1991) reported a significant interaction between dietary fat and DAS. At dietary levels of 4 and 8 ppm DAS, a greater decrease in body weight was observed with a high-fat diet (12% fat) than with a low-fat diet (6%). Increased micellar absorption of the toxin when administered in the high-fat diet was thought to be the cause of this particular response. Young pullets fed up to 15 ppm T-2 toxin for 21 d had consistently depressed concentrations of plasma vitamin E (Coffin and Combs, 1981). Addition of micelle-promoting compounds (taurocolic, monooleic, and oleic acids) alleviated depressions in both plasma vitamin E and growth.

In summary, the toxicological effects of trichothecene mycotoxins in poultry must be considered not only for their individual effects but they must be evaluated for the possibility of interactions with other mycotoxins, anticoccidial drugs, infectious agents, and various diet constituents.

6.6.   *Trichothecene toxicosis in other avian species*

Experimental and field outbreaks of trichothecene mycotoxicosis in turkeys, geese, ducks, pigeons and game birds, have also been reported. Richard *et al*. (1978) fed 8 day old poults diets containing up to 10 ppm T-2 toxin for 4 weeks. The thymus of the poults given 10 ppm T-2 was markedly decreased in size compared to controls, but no effect was seen on the size of spleen or bursa. Histopathologic examination of the thymus revealed depletion of cortical lymphocytes. Chicks appeared less sensitive to T-2 toxin than did the poults. Oral lesions occurred at numerous foci through the mouth and were seen in the poults but not chicks fed the 10 ppm dietary T-2 toxin after 2 weeks. In this experiment, groups of poults and chicks were pair-fed a control ration equating that consumed by the birds given 10 ppm T-2 toxin the previous day. These control birds had a higher body weight gain suggesting that the effect of T-2 toxin is due to factors other than simple reduction in feed consumption. Allen *et al*. (1983) incorporated a 0.1% *F. tricinctum* culture containing at least 10 different trichothecenes into the diet of turkey breeders. T-2 toxin (1.1 ppm), NEO (0.22 ppm) and HT-2 toxin (0.074 ppm) were among the type A trichothecenes present. This feed caused oral lesions, 78% decrease in hatchability of fertile eggs and a more drastic reduction in feed intake and egg production compared to a diet containing 5 ppm pure T-2 toxin. The authors suggested that feeding crude *Fusarium* cultures providing even low concentrations of unidentified mycotoxins can produce severe effects on birds, and that the presence of mycotoxins like zearalenone, T-2 and DAS should serve as warning of contaminated rations. A field outbreak of T-2 toxicosis was reported in laying turkey hens receiving feed contaminated with 0.75-0.83 ppm T-2 toxin (Fazekas *et al*., 1993); main effects included 20% decrease in hatchability, doubling of embryo mortality and 16% mortality in turkey poults within 8 d after hatching. Egg production was not reduced but the quality of the egg shell was impaired. The same authors fed laying turkey hens a diet containing 1.5 ppm T-2 from a *F. poae* culture, in an attempt to reproduce the outbreak. No clinical signs, mortality or effects on egg production were observed but a decrease in hatchability comparable to the field case was noticed (Fazekas *et al*., 1993).

As previously described with chickens, DON has very low toxicity for turkeys. Feeding 75 ppm dietary DON to turkey poults from 7 to 14 d of age had no effect on feed consumption or growth rate (McMillan and Moran, 1985). In another trial, feed containing 4.4 ppm DON was fed to day-old poults without effect on performance or mortality (Manley *et al*., 1988).

The adverse effects of dietary trichothecenes in laying geese was investigated by feeding diets artificially inoculated with *F. sporotrichioides* (Palyusik and Koplik-Kovács, 1975). The toxic feed contained 3 ppm T-2 toxin as well as other unidentified fungal metabolites. Oral lesions, feed refusal, and marked reduction in egg production that caused complete cessation of laying within 10 d were observed. The ovary and oviduct of geese sacrificed after 14 d showed marked regression. The authors concluded that other toxins, apart from T-2 toxin, were responsible for the severe effects observed. Ducklings have been shown to be particularly sensitive to trichothecene mycotoxins. Steyn *et al*. (1978) inoculated corn with different isolates of *F. sulphureum* and then incorporated this at 50% of the diet for day-old ducklings. The diets, which contained the scirpenol toxins 4-MAS, 15-MAS, 4,15-DAS and TAS, caused 100% mortality within 5 d. In another experiment, young Mallard ducks fed diets containing 20-30 ppm pure T-2 toxin for 2-3 weeks developed necrotizing upper alimentary tract lesions, oral and esophageal lesions, ulcerative proventriculitis, and severe depletion of lymphoid tissues, characterized by thymic, bursal, and splenic atrophy, (Hayes and Wobeser, 1983). Day-old Muscovy ducklings receiving dietary T-2 toxin or DAS at 0.25-1.0 ppm for 7 d developed dose-related lesions in the roof of the oral cavity (Shlosberg *et al*., 1986). Muscovy ducklings showed these signs in a shorter period of time (less

than 16 h in some cases) and at a lower feed concentration of these mycotoxins than did other avian species in similar studies. For this reason, the authors proposed to use this species as a bioassay to detect mycotoxins in contaminated grain. T-2 toxin has also been demonstrated to be toxic for pigeons and chukar partridges. The LD_{50} value of T-2 toxin for pigeons was determined to be 1.7 mg/kg (Fairhurst *et al.*, 1987), which is about one third of the reported LD_{50} value for broiler chickens (Chi *et al.*, 1977a); acute toxicity was manifested by vomiting, ataxia, and abnormal wing positioning (Fairhurst *et al.*, 1987). Chukar partridges receiving 4-16 ppm dietary T-2 toxin for 3 weeks showed dose-related mouth lesions at all levels tested and decreased body weight at 8 and 16 ppm (Ruff *et al.*, 1990).

7.   POTENTIAL TREATMENT AND PREVENTION

Specific therapies against mycotoxins are not currently available. The only effective treatment is removal of the source of toxin coupled with symptomatic and supportive therapy. In the case of TCT mycotoxicosis, the approach for chronically exposed birds should be different from that for acutely intoxicated birds. Administration of a highly nutritious diet (which is free from any mycotoxin), avoidance of stresses, and control of secondary infectious diseases is the usual approach in the case of chronically exposed animals. Acutely exposed animals can be treated with oral adsorbents (*eg.* highly activated charcoal or bentonite) in order to minimize absorption of the toxin from the GI tract and to short-circuit the enterohepatic recirculation of the toxins and/or their metabolites. Pretreatment with antioxidants such as vitamin C, vitamin E, and ethoxyquin have proven effective in decreasing lethality of T-2 toxin in experimental animals. However, antioxidants are of no benefit after T-2 toxin exposure. It would be of interest to investigate whether supplementation with dietary antioxidants could be of some benefit in decreasing the toxicity of TCT in poultry species.

Chapter 12

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CHAPTER 13.   OCHRATOXINS
Other names: NONE
Species: ALL POULTRY

1.   COMPENDIUM

The ochratoxins are a group of 7 fungal metabolites composed by an isocoumarin moiety linked to the amino acid L-β-phenylalanine. Ochratoxins were first isolated from *Aspergillus ochraceus* (now referred to as A. *alutaceus*), but can be produced by several *Aspergillus* and *Penicillium* fungi. Although the ochratoxin group comprises 7 compounds, only ochratoxin A (OA) has been found widespread as a natural contaminant of cereal grains such as barley, wheat, oats, rye, and maize. OA is the most toxic mycotoxin for domestic fowl. It has a lower LD_{50} value and a lower minimum dietary growth inhibitory concentration than aflatoxin, DAS or T-2 toxin. The main target organ for the toxic action of OA is the kidney. At the molecular level, OA interferes with DNA, RNA, and protein synthesis by inhibiting the enzyme phenylalanine-tRNA synthetase. Additionally, OA affects renal carbohydrate metabolism through an effect on the renal mRNA coding for phosphoenolpyruvate carboxykinase (PEPCK), which is a key enzyme in gluconeogenesis. OA-induced alteration in these metabolic pathways results in damage to the epithelium of renal proximal convoluted tubules, decreased electrolyte absorption, and increased water excretion through an osmotic diuresis. Dietary OA levels of 0.3-16 ppm have been associated with field outbreaks of ochratoxicosis in broilers, layers, and turkeys. In broilers, major clinical signs of ochratoxicosis are poor growth, reduced feed efficiency, increased water consumption, and increased manure moisture. At post-mortem examination the kidneys are swollen, enlarged, and pale. Secondary visceral gout can be present. The major effects of OA in laying hens are decreased feed intake and egg production, reduced egg weight and egg specific gravity, and increased incidence of shell stains and blood and meat spots. Increasing dietary protein levels and supplementing the diet with vitamin C appear to reduce some of the adverse effects of OA in poultry.

2.   INTRODUCTION

Ochratoxins are the second major group of mycotoxins to be characterized after the discovery of aflatoxins. Ochratoxin A (OA) was discovered in 1965 in South Africa during laboratory studies in a search for new toxic metabolites from molds and at the time of discovery there was no connection with human and animal disease. The chemical structure of OA was elucidated by Van der Merwe *et al.* (1965) who extracted and purified the toxin from a strain of *Aspergillus ochraceus*, hence the name. Subsequent studies revealed involvement of ochratoxins with human or animal disease. While *A. ochraceus* is the most common producer of ochratoxin within the aspergilli, its

production has also been reported from *A. alliaceus, A. malleus, A. ostianus, A. petrakki, A. sclerotiorum,* and *A. sulphureus*. *Penicillium verrucosum* and *P. purpurescens* are the major OA producers within the *Penicillium* genus. It is important to note that *A. ochraceus* is now referred to as *A. alutaceus* (Marquardt and Frohlich, 1992).

Ochratoxin A is able to cause adverse effects on animal health and productivity and also appears to play a role in certain human diseases. Ochratoxin A induces gross and microscopic lesions in the kidney and liver of livestock and is a potent renal carcinogen in rats (Boorman *et al*., 1992). In pigs, OA causes the disease known as mycotoxic porcine nephropathy. In humans, there is evidence suggesting that OA is a causal agent of Balkan endemic nephropathy, a degenerative, irreversible, and highly fatal renal disease that occurs in certain areas of Eastern Europe.

3.   CHEMISTRY, NATURAL OCCURRENCE, AND TOXICITY OF OCHRATOXINS

3.1.   Chemistry and natural occurrence

The ochratoxins are a group of fungal metabolites composed of an isocoumarin moiety (7-carboxy-5-chloro-8-hydroxy-3,4-dihydro-3R-methylisocoumarin or ochratoxin α) linked through the 7-carboxy group to the amino acid L-ß-phenylalanine by an amide bond. Acid hydrolysis of OA produces L-ß-phenylalanine and ochratoxin α (Oα) (Van der Merwe *et al*., 1965). Also, OA can be hydrolyzed to its nontoxic alpha form by microorganisms in the rumen, cecum, and large intestine (Marquardt and Frohlich, 1992). Although seven metabolites are included in the ochratoxin group (*Fig. 13.1*), only OA has been found widespread as a natural contaminant. According to Krogh (1987), ochratoxin B is rarely found as a natural contaminant and the remaining ochratoxin metabolites have never been observed in naturally contaminated cereal grains. However, Hamilton *et al*. (1982) reported the co-occurrence of ochratoxins A, B and C in seven independent samples of feed and corn. In a sample that contained 16 ppm of OA, the ratios of ochratoxins A:B:C were 90:8:2 respectively.

The natural occurrence of OA in plant products was first reported in 1969 in a sample of corn that contained approximately 150 ppb OA (Shotwell *et al*., 1969). At that time, no adverse effects of OA on human or animal health were known. Subsequent surveys established that OA occurs in cereal grains, moldy green coffeebeans, beans, peanuts, and hay in many areas of the world. The levels of OA in animal feeds are generally higher than in human foods and are usually below 200 ppb, however, in moldy feeds associated with nephropathy in swine or poultry, levels up to 27,500 ppb have been reported (Krogh, 1987). An extensive German survey showed that 12.9% of 984 samples of cereals and mixed feeds were contaminated with OA with levels up to 206 ppb (Bauer and Gareis, 1987). In the United States, levels of OA ranging from 300 to 16,000 ppb have been recorded in feeds associated with field outbreaks of ochratoxicosis in broilers, layers, and turkeys (Morehouse, 1985).

Contamination with OA is most common in cereal grains such as barley, wheat, oats, rye and corn. Ochratoxin production can occur at environmental temperatures as low as 4°C in grains with a moisture content of 18.5-40.4%. Water activity (a_w) and temperature of the stored grains are the main factors controlling OA formation. Laboratory trials showed that the minimum a_w values for OA production were 0.83-0.90 depending on the toxigenic strain, whereas optimum temperatures

for OA production ranged from 4 to 37°C, depending on the a_w value and toxigenic strain involved (Krogh, 1987).

3.2.   Toxicity

Ochratoxin A is considered as the most toxic mycotoxin for domestic fowl. In terms of lethality, which is the simplest measure of toxicity, OA is more toxic than aflatoxin and comparable in toxicity to the trichothecene mycotoxin DAS. In broilers, LD_{50} values for aflatoxin, DAS and OA have been reported to be 6.8 (Smith and Hamilton, 1970), 2.0 (Richardson and Hamilton, 1990), and 2.1 mg/kg (Huff *et al*., 1974), respectively. The higher toxicity of OA compared with other mycotoxins is also reflected on the more severe adverse effect of OA on chicken performance. The minimum dietary growth inhibitory concentration of OA for the young broiler chick is 2.0 ppm, whereas 2.5 and 4.0 ppm are required for growth inhibition by aflatoxin and T-2 toxin, respectively (Morehouse, 1985). The LD_{50} values for OA in several avian species are shown in (*Table 13.1*). Younger birds appear to be more susceptible to OA than are older birds because 3 wk old broilers have higher LD_{50} values than day-old chicks (3.6 and 2.14 mg/kg, respectively; Huff *et al*., 1974). Similarly, 10 day old Leghorn chicks were more resistant than 3 day old Leghorn chicks, showing LD_{50} values of 10.67 (Galtier *et al*., 1976) and 3.4 mg/kg (Prior *et al*., 1976), respectively. In experiments carried out by Golinski *et al*. (1983), male broiler chickens were found to be more sensitive to OA than were females. Differences in susceptibility to OA among different avian species are also observed. In terms of LD_{50}, ducklings appear to be the most sensitive species to OA, followed by broiler chicks, Leghorn chicks, turkey poults, and Japanese quail chicks (*Table 13.1*).

4.   <u>TOXICOKINETICS</u>

The toxicokinetics of OA has recently been reviewed by Krogh (1991) and Marquardt and Frohlich (1992).

4.1.   Absorption

Ochratoxin A is highly soluble in organic solvents and only slightly soluble in water, therefore, absorption of OA through biological membranes is expected to occur easily. Ochratoxin A is absorbed in the upper sections of the gastrointestinal tract (GIT) in a passive manner in the nonionized form and is subjected to secretion and reabsorption via enterohepatic recycling. OA is also reabsorbed in an active manner via the organic anion transport system in the proximal and distal tubules of the kidney. In mammals, OA is absorbed primarily from the stomach and proximal jejunum. The toxin enters the circulation through the portal vein, although some of it can be absorbed by lymphatic vessels. The rate of absorption is faster in

those sections of the GIT that have a low rather than a high pH. Once absorbed, OA is transported bound to plasma proteins, especially albumin. Binding of OA to the serum albumin and recycling in the bile and kidney contributes to its long half-life in animals (Krogh, 1991; Marquardt and Frohlich, 1992). In chickens, Galtier et al. (1981) determined that 40% of a dose of OA is absorbed following oral administration of a dose of 2 mg OA/kg body weight. Studies have shown that OA can also be absorbed through the lungs into the systemic circulation. Di Paolo *et al*. (1993) reported a case in which one farmer developed temporary respiratory distress and acute renal failure as a result of OA inhalation.

TABLE 13.1 LD$_{50}$ values of ochratoxin A for different avian species (after single oral administration)			
Type of bird	**Bird age (days)**	**LD$_{50}$ (mg/kg Bwt)**	**Reference**
Ducklings	3	0.5	Van der Merwe *et al.,* 1965
Broiler chicks	1	2.14	Huff *et al.,* 1974
Leghorn chicks	1	3.3/3.9	Peckham *et al.,* 1971
Leghorn chicks	3	3.4	Prior *et al.,* 1976
Broiler chickens	21	3.6	Huff *et al.,* 1974
Turkey poults	1	4.63	Chang *et al.,* 1981
Turkey poults	3	5.9	Prior *et al.,* 1976
Turkeys	21	7.84	Chang *et al.,* 1981
Leghorn chicks	10	10.67	Galtier *et al.,* 1976
Japanese quail chicks	3	16.5	Prior *et al.,* 1976

4.2.   Distribution

Ochratoxin A appears to deposit in all soft tissues, with the largest levels found in the kidney and to a lesser extent in the liver. Frye and Chu (1977) investigated the distribution of OA in chicken tissues by intubating 5 wk old chickens with 50 μg radiolabelled OA. The highest level of radioactivity was found in kidney and liver 8 h after intubation. Peak levels of OA in kidney, liver, and breast muscle were found to be 12, 4, and 0.2 ppb, respectively. In the same study, when laying hens were fed a diet containing 5.0 ppm OA for 2 wk, the highest levels of OA were also detected in kidney (124 ppb) and liver (80.2 ppb). The levels of OA in breast and leg muscles were 8.4, and 7.2 ppb, respectively. This low distribution of OA to muscle tissue was also reported by Fuchs *et al*. (1988), who investigated the distribution of radiolabelled OA in quails for 8 d after intravenous administration.   The red muscle, possibly due to a higher metabolic activity,

accumulated four times as much radioactivity as the white muscle. However, 6 h after the injection, there were no detectable levels of OA in either red or white muscle.

4.3. Biotransformation

During *in vivo* studies, the major identified biotransformation reaction of OA is its hydrolysis into Oα and L-phenylalanine. Ochratoxin α has been detected in the urine and feces of rats that have been intraperitoneally injected with OA, indicating the cleavage of the toxin (Krogh, 1991). Studies with radiolabelled OA indicated that another, not yet identified, metabolite is formed *in vivo*. In addition to being converted to Oα, a small percentage of absorbed OA is converted to 4-hydroxy-OA (*Fig. 13.1*). In rats given OA *per os* or intraperitoneally, 25 to 27% of the dose was excreted in the urine as Oα and only 1 to 1.5% ecreted as (4R)-4-hydroxy-ochratoxin A. This reaction is considered to be a detoxification process since 4-hydroxy-OA is nontoxic to rats at doses of 40 mg/kg. In *in vitro* experiments using liver microsomes from pig, man, and rat, both (4S)- and (4R)-4-hydroxy-OA were produced in a hydroxylation process involving cytochrome P-450 enzymes (Krogh, 1991; Marquardt and Frohlich, 1992).

4.4. Elimination

In mammals, OA and its metabolites are excreted primarily in the urine, although fecal excretion also occurs to some extent. In rats receiving a single oral dose of 15 mg/kg radiolabelled OA, the cumulative excretion after 120 h was: 11% OA and 23% Oα in feces; 11% OA and 12% Oα in urine; and 33% OA in the bile (Suzuki *et al*., 1977). The disappearance rates of OA from pig kidney, liver, and muscle range from 4 to 5 d; when the level of OA in the feed is 1 ppm, use of OA-free diet for one month prior to slaughter may prevent OA-contaminated meat from reaching the food chain (Hald, 1989). Poultry species appear to eliminate OA faster than do mammals. In chickens receiving a single oral dose of radiolabelled OA, over 90% of the radioactivity was eliminated 48 h after intubation (Frye and Chu, 1977). Elimination of OA via the egg yolk has been reported in laying Japanese quail (Fuchs *et al*., 1988).

4.5. Residues

Residues of OA are generally not detected in ruminants due to the ruminal hydrolysis of the molecule into Oα and L-ß-phenylalanine. However, OA is found in tissues of swine and poultry having consumed contaminated feed. In pigs, OA is present at slaughter at decreasing levels in blood, kidney, lean meat, liver, and fat. Prior *et al*. (1980) fed broiler chickens up to 2ppm OA for 8 wk, showing that birds fed 2 ppm OA had detectable levels in the liver and kidney but no residues in either muscle or fat. In this trial, residues disappeared from the liver within 24 h and from the kidney within 48 h after withdrawal of the mycotoxin from the feed. In a similar study, broilers were fed up to 2.0 ppm OA for 8 wk (Golinski *et al*., 1983) and residues were detected in the livers and white muscles of birds fed ≥1.0 ppm OA and in red muscles of birds receiving ≥1.5 ppm. Four days after the withdrawal of OA from the feed no residues could be detected. More recently, Micco *et al*. (1987) investigated the effect of long term administration of 50 ppb dietary OA on the occurrence of residues in broilers and layers. Residues in liver were higher in broilers (up to 11.0 ppb) than in hens (1.5 ppb), whereas the converse situation occurred in kidney (up to 0.8 and 5.8 ppb in broilers and layers, respectively). Small amounts of OA (0.8 ppb) were detected in hen thigh muscle but not in breast muscle and the authors concluded that residues of OA in poultry appear to be a public health concern. In fact, the presence of OA in chicken meat at a processing plant was reported by

Elling *et al.*, (1975). From 14 birds condemned for showing macroscopic renal lesions (enlarged and pale kidneys), 5 had OA residues in muscle. Residue levels in the positive birds ranged from 4.3 to 29.2 ppb. In another study, kidneys, liver, and muscle from birds receiving diets containing either 0.3 or 1.0 ppm for 341 d contained residues of OA of ≤50 ppm; however, these birds did not have gross renal lesions and would have passed the meat inspection at the processing plant, which confirms the potential public health problem of OA-contaminated poultry products reaching the human food chain (Krogh *et al.*, 1976).

Residues of OA have also been reported in humans. In a German study, the frequent contamination of human blood sera (56.6% positive out of 306 tested) and the detection of OA residues in human kidneys and milk draw attention to a continuous exposure by food of plant or animal origin (Bauer and Gareis, 1987). Human intake of hen's eggs may be one of the routes of exposure to the toxin because a permanent intake of OA in laying hens can lead to accumulation of the toxin in the egg (Fuchs *et al.*, 1988).

5.   TOXICODYNAMICS: MODE OF ACTION OF OCHRATOXINS

Ochratoxin A interferes with DNA, RNA, and protein synthesis and affects carbohydrate metabolism, particularly gluconeogenesis. The effects on DNA, RNA, and protein synthesis are presumably due to an effect by the phenylalanine moiety of the toxin (Marquardt and Frohlich, 1992). Ochratoxin A inhibits specifically and competitively the activity of phenylalanine-tRNA synthetase, an enzyme involved in the initial step of protein synthesis. The inhibition of this enzyme is due to the recognition of the L-phenylalanine moiety of OA, and is reversed when the cells are supplied with sufficient substrate (L-phenylalanine); the lethal effect of OA in mice is largely reduced by pretreatment with phenylalanine, suggesting that the inhibition of protein synthesis is the primary cause of the acute toxicity of OA (Hsieh, 1987). Ochratoxin A reduces the renal mRNA coding for phosphoenolpyruvate carboxykinase (PEPCK), which is a key enzyme in gluconeogenesis. PEPCK catalyzes the decarboxylation of oxaloacetate to phosphoenolpyruvate, GTP (or ITP) acting as the source of high-energy phosphate. PEPCK is the link between the citric acid cycle and gluconeogenesis and makes possible the transformation of citric acid cycle intermediates and their precursors into glucose and glycogen. Interference with this rate-limiting step in gluconeogenesis plays a key role in the development of functional damage to the kidney because one of the most important pathways of carbohydrate metabolism in kidney cortex is gluconeogenesis; in the starved or acutely diabetic state, this pathway accounts for 50-60% of the blood glucose (Ueno, 1991).

6.   TOXICITY IN BROILER AND IMMATURE LEGHORN CHICKENS

6.1.   Clinical signs

Broiler chickens acutely intoxicated with a massive dose of OA appear listless and exhibit huddling, diarrhea, tremors (and other neural abnormalities) and prostration leading to death within 22 to 25 h after administration of a single oral dose of 16 mg/kg (Huff *et al.*, 1974). Listlessness, emaciation, dehydration, diarrhea, and increased mortality were observed in chickens fed a diet containing 3 ppm OA (Manning and Wyatt, 1984). However, under field conditions with relatively

low dietary levels and chronic or subchronic exposure to the toxin, the major clinical signs are poor growth, increased water consumption and increased manure moisture (Huff *et al*., 1974, 1975).

6.2.   Natural outbreaks

Hamilton *et al*. (1982) reported several natural episodes of ochratoxicosis affecting broiler chickens, laying hens, and turkeys in the United States. The level of OA in suspect feed and ingredients ranged from <0.2 to 16 ppm.   The episodes involving broiler chickens were characterized by reductions in growth rate, feed conversion, and pigmentation and increases in nephropathy, and air sacculitis. Layers show reduced egg production, reduced egg shell quality, and nephropathy. Turkeys exhibit increased mortality (up to 59%), renal lesions (pale and enlarged kidneys), decreased feed consumption (as low as 20% of the normal intake) and secondary air sacculitis.

6.3.   Progression of ochratoxicosis in broilers

Huff *et al*. (1988) investigated the progression of ochratoxicosis in broilers by feeding young male broiler chickens graded levels of dietary OA, up to 4.0 ppm, for 21 d. A significant growth depression was observed after 6 d in the chickens receiving 4.0 ppm OA. Dietary OA significantly increased the relative weights of the liver, kidney, spleen, pancreas, and gizzard. Anemia, characterized by a significant reduction in packed cell volume (PCV) and hemoglobin (Hb) levels was observed. Hepatotoxicity was indicated by a significant reduction in serum levels of total protein, albumin, globulin, cholesterol, triglycerides, and blood urea nitrogen, and a significant increase in the serum activities of γ-glutamyl transferase (GGT) and cholinesterase. A significant increase in serum uric acid and creatinine levels was indicative of nephrotoxicity.

6.4.   Effect of ochratoxin A on renal function

Broilers receiving dietary levels of 4.0 and 8.0 ppm OA, showed a reduction in the renal clearance of phenol red (15 and 31%, respectively, as compared with control chickens). The same dietary levels caused an increase in uric acid of 38 and 48% over control values, respectively (Huff *et al*., 1975). Glahn *et al*. (1989) injected 10 wk old pullets intramuscularly with OA at doses of 0.25 or 0.5 mg/kg; OA increased urine flow rate, decreased urine osmolarity, increased ion excretion (Na, K. Ca, P), and caused a relative alkalosis when measured after 10 d of injection. These effects were not detected 2 wk later. The results suggest that OA may cause an osmotic diuresis by inhibiting tubular reabsorption of electrolytes and that the effects may be reversed simply by discontinuing toxin administration.

The effect of OA on avian renal function was recently reviewed by Glahn (1993). In general, acute exposure to OA causes an acute increase in the PCV value, which reflects extracellular fluid volume contraction (*ie.* hemoconcentration and dehydration). Ochratoxin A causes an acute diuretic effect that results in increases in the urine flow rate, urine flow rate to GFR (glomerular filtration rate) ratio, and free water clearance (Glahn *et al*., 1988). Chronic exposure to OA causes increased diuresis in the avian kidney. The OA-induced diuresis results primarily from damage to the proximal tubular epithelium as indicated by increased urine flow, increased electrolyte secretion, and only a mild decrease in urine osmolality (Glahn *et al*., 1989). Damage to the proximal epithelium resulting in decreased electrolyte reabsorption appears to promote an osmotic diuresis because the proximal epithelium reabsorbs the bulk of most electrolytes (Glahn, 1993).

6.5.   Effect of ochratoxin A on broiler performance

Ochratoxin A causes an increase in energy intake and heat production in chickens (Koh and Han, 1991). The minimum dietary level of OA capable of reducing weight gain in growing chickens appears to be 2.0 ppm. Huff *et al*. (1974, 1975) fed graded dietary levels of OA up to 8.0 ppm to day-old broilers for 3 wk and growth was significantly depressed by dietary levels of ≥2.0 ppm. However, Niemiec and Scholtyssek (1989) reported reduced body weight, poor feed conversion, and increased mortality in broilers receiving a diet containing 1.5 ppm OA for 6 wk. Grain sorghum naturally contaminated with 5 ppm OA caused reduced weight gain and decreased feed efficiency when it was incorporated into the diet of Leghorn chicks. The mean concentration of OA in the mixed chicken feed was found to be 3.5 ppm (Harvey *et al*., 1987). In addition to the adverse effects of OA on growth and feed efficiency, broiler chickens receiving dietary OA are less well pigmented, which is an undesirable feature in many marketing areas. Ochratoxin A induces a hypocarotenoidemia more severe than that caused by aflatoxin and impairs the ability of chickens to utilize dietary carotenoids for carcass pigmentation (Osborne *et al*., 1982; Schaeffer *et al*., 1987).

6.6.   Gross pathology

In growing chicks, post-mortem lesions observed after OA administration include emaciation, dehydration, a dry and firm gizzard sometimes with erosions on the koilin layer, proventricular mucosal hemorrhages and catarrhal enteritis. The kidneys are pale, swollen and enlarged and change in colour from the normal mahogany to tan. The liver can be enlarged, pale and friable or hemorrhagic while the gall bladder may be distended with bile (Burns and Dwivedi, 1986). After acute oral administration of OA, extensive accumulation of urates on the serosal surface of several organs can occur (Peckham *et al*., 1971). The breaking strength and diameter of the tibiotarsal bones are significantly reduced by dietary OA levels of 2 and 4 ppm, respectively (Huff *et al*., 1980). The breaking strength of the large intestines is also significantly decreased by dietary levels of ≥2 ppm OA; intestinal fragility is accompanied by an increase in the weight of the large intestine relative to body weight and an increase in lipid content (Warren and Hamilton, 1980). Increased intestinal fragility can cause increased carcass condemnations due to intestinal ruptures on the processing line.

The relative size of major organs has been commonly evaluated during ochratoxicosis in chickens. Graded levels of OA incorporated into the feed of broiler chickens from hatching until three wk of age resulted in enlarged kidney, proventriculus, gizzard, and liver, while the bursa of Fabricius was smaller than normal (Huff *et al*., 1974). In a similar trial, dietary levels of 2-4 ppm OA administered to day-old broilers for 20 d caused a significant enlargement of the kidney, liver, and proventriculus, whereas the thymus and bursa of Fabricius were reduced in size (Dwivedi and Burns, 1984).

6.7.   Microscopic pathology

Microscopic lesions in ochratoxicosis are most prominent in the kidney and liver. On light microscopy, severe distension, enlargement and hypertrophy of the renal proximal convoluted tubules (PCT), and thickening of the glomerular basement membrane are seen in kidney sections of

broilers receiving 2-4 ppm dietary OA to 20 d (Dwivedi and Burns, 1984). Broiler chickens fed a diet containing 4.0 or 8.0 ppm OA had significantly elevated liver glucagon levels and the histopathological examination of the liver revealed intracytoplasmic deposits of glycogen especially in the hepatocytes located at the periphery of the liver lobes (Huff *et al*., 1979a). Histological changes that could account for the reduced bone breaking strength that has been observed in broilers exposed to OA (Huff *et al*., 1980) were reported by Duff *et al*. (1987). Lesions included generalized osteopenia and disturbed endochondral and intramembranous bone formation.

On electron microscopy, abnormal mitochondrial ring forms in the kidney and accumulation of glycogen in the liver are the major lesions observed during ochratoxicosis. The mitochondria of the PCT appear to be the most sensitive to OA toxicity. The ultrastructural changes in the kidney of day-old chickens receiving dietary OA at levels of 2 and 4 ppm for 20 d were reported by Dwivedi *et al*. (1984). There were abnormally shaped mitochondria in the PCT and an increase in the size and number of mitochondrial dense granules and cytoplasmic peroxisomes. Intranuclear and cytoplasmic lipid droplets and electron dense round bodies in the dilated smooth endoplasmic reticulum were also observed. The livers of these birds showed increased accumulation of cytoplasmic glycogen in the hepatocytes. Brown *et al*. (1986) evaluated the renal ultrastructural changes in Leghorn chicks exposed to 3 ppm dietary OA from 1 to 21 days-of-age. Proximal tubular intranuclear membrane-bound inclusions, elongated tortuous and ring-shaped mitochondria, enlarged mitochondrial matrix granules with hyaline centres, and an increase in the number and size of peroxisomes and secondary lysosomes were observed.

6.8. *Clinical pathology and biochemistry*

Several hematological parameters have been reported to be affected by OA in chickens. In studies by Huff *et al*. (1979b), dietary levels of 8 ppm OA administered to chickens up to 3 wk of age caused a significant decrease in PCV and Hb concentration, without affecting the number of circulating red blood cells (RBC). The anemia was categorized as a hypochromic-microcytic anemia of the iron deficiency type. Reductions in total leucocyte count and in lymphocyte and heterophil counts were also reported in broilers receiving diets containing 0.5-8 ppm OA (Chang *et al*., 1979; El-Karim *et al*., 1991). In another trial, Hb, PCV, RBC count, and white blood cell (WBC) count were significantly depressed by 0.5 ppm dietary OA in broiler chicks (Ayed *et al*., 1991). Similarly, administration of 3 ppm dietary OA to broiler chickens from 4 wk to 8 wk of age caused a reduction in PCV and numbers of RBC's, WBC's and heterophils (Mohiuddin *et al*., 1992). Doerr *et al*. (1981) reported a severe coagulopathy in broilers fed dietary OA at levels ≥2 ppm. OA caused an impairment in both the extrinsic and common clotting pathways, primarily due to a hypofibrinogenemia, in contrast to the coagulopathy of aflatoxicosis which has been reported to be primarily a hypoprothrombinemia.

Administration of OA to day-old chicks raised the blood glucose levels and reduced the activities of hexokinase and aldolase (Subramanian and Govindasamy, 1985) with these effects caused by a combination of reduced glucose oxidation and glycogen synthesis, and enhanced gluconeogenesis. Warren and Hamilton (1981) reported that chickens receiving a diet containing 8 ppm OA had a fourfold increase in the levels of muscle glycogen. The authors categorized the hyperglycogenation of ochratoxicosis as a type X glycogen storage disease. Sreemannarayana *et al*. (1989) fed broiler chickens diets containing 0, 1, 5, or 10 ppm OA over a 4-wk period. Concentration of liver RNA, DNA, and protein were decreased while glycogen was increased. Serum alkaline phosphatase, GGT, uric acid and creatinine concentrations were elevated while

serum proteins, albumin, phosphorus, potassium, and cholesterol were depressed. The effects of OA were time- and dose-dependent. In broiler chicks, 0.5 ppm dietary OA caused an increase in the activity of serum sorbitol dehydrogenase (SDH) and glutamic dehydrogenase (GDH) and in the concentration of uric acid (Ayed *et al*., 1991).   Increased activity of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and acid phosphatase was also reported in experimental ochratoxicosis in broilers (Raina *et al*., 1991).

7.   TOXICITY IN LAYING HENS

Decreased feed intake and egg production, reduced egg weight and egg specific gravity, and increased incidence of shell stains and blood and meat spots are the major effects of OA in layers. The minimum dietary level of OA capable of affecting egg production appears to be 0.5 ppm. Ochratoxin A contaminated feed is instinctively rejected by layers. In an organoleptic trial, hens were offered a choice of layer feed either with or without OA added and a significant reduction in the consumption of OA-containing feed was observed (Prior *et al*., 1981). Choudhury *et al*. (1971) fed SCWL pullets diets containing up to 4 ppm OA from 14 wk to one year of age. All levels of OA caused a delay in sexual maturity and a dose-dependent decrease in egg production. Mortality was high in hens fed 4 ppm OA and most of the survivors did not lay eggs. In another trial, Prior and Sisodia (1978) fed SCWL layers 0, 0.5, 1, or 4 ppm dietary OA for 6 wk. Egg production and feed consumption declined in all groups given OA, while egg and body weight were depressed at dietary levels of 1 and 4 ppm OA. In this trial, no effect on fertility or hatchability was observed. However, in another study, an increased incidence of embryo mortality and anomalies was observed from hens receiving varying levels of OA for 4 wk (Niemiec *et al*., 1990). Increased incidence of egg stains was observed in layers receiving diets containing 0.5 or 1 ppm OA (Page *et al*., 1980); the majority of these stains were produced from fecal material. Many eggs had focal areas of urate deposition on the shell, and a yellow stain developed when these eggs came into contact with dust and desquamated feather epithelium. Tohala (1983) fed diets containing up to 2 ppm OA to layers for 12 wk. There was a numerical but not significant reduction in egg production in hens receiving OA. Egg weights were reduced significantly between 8 and 12 wk for the 2 ppm treatment and during wk 11 and 12 at 1 ppm. A dose-dependent reduction in egg specific gravity was observed. Percentages of blood and meat spots were significantly increased by OA levels of 0.5-2 ppm. Body weight was significantly reduced by 0.5-2 ppm OA in a dose-dependent manner. Water intake, mortality and liver weight were significantly increased only in hens receiving 2 ppm OA.

8.   TOXICITY IN OTHER AVIAN SPECIES

Chang *et al*. (1981) investigated the effect of OA in growing turkeys. Levels of 4 and 8 ppm OA resulted in a decreased growth rate, enlarged proventriculus and gizzard, and a regressed thymus, whereas the sizes of the liver, spleen, pancreas, kidneys, and bursa of Fabricius were unaffected. These levels also caused a significant increase in water intake and in plasma uric acid levels. Mortality was significantly increased in poults fed 8 ppm OA. Dwivedi and Burns (1985) investigated the effects of 4 ppm dietary OA in young turkeys. OA caused a significant retardation of growth in both male and female poults, regression of the thymus and bursa of Fabricius, and

lymphoid depletion in these and other lymphoid organs. Increased relative liver weight was observed after 10 wk of exposure to the toxin.

The effects of OA on Japanese quail have also been investigated. Severe depression of growth and feed intake, and high mortality were observed in growing Japanese quail receiving diets containing 4, 8, or 16 ppm (Doster *et al*., 1973). However, in another trial, levels of 4 and 8 ppm OA in the feed did not impair growth in Japanese quail receiving the toxic diets for 11 wk from hatching, although egg production and fertility were reduced (Burns and Dwivedi, 1985; Dwivedi and Burns, 1986). The same dietary levels of OA given to newly hatched quails for the same period of time induced ultrastructural changes in kidney and liver. Pathological changes in the kidneys were limited to the PCT and glomeruli. In the PCT, abnormal mitochondria and excessive numbers of lipid droplets were the major findings with glomeruli showing thickened basement membranes. Swollen mitochondria and variable glycogen deposits were the major findings in the liver and the authors suggested that OA is more hepatotoxic in quails than in broilers (Maxwell *et al*., 1987). Increased relative kidney weight was reported in Japanese quails fed diets containing 2 or 4 ppm OA for 3 wk (Ruff *et al*., 1992).

Male crossbred ducks (*Anas platyrhynchos*) injected daily with 25 μg OA for 90 d developed marked renal tubular degeneration, particularly of the PCT. Decreased testicular weight and histologic lesions of the testes (degeneration and decreased spermatogenesis) were also observed (Jayakumar *et al*., 1988). When OA was fed at 2 ppm to Khaki Campbell ducklings from hatch to 18 d of age it caused retarded growth, enlargement of the kidneys and liver, and regression of the thymus. Microscopic changes in the liver included accumulation of glycogen and the presence of abnormal mitochondria in the hepatocytes while thickening of the glomerular basement membrane and infiltration of lymphoid cells were observed in the kidneys. These microscopic changes were similar to, but less pronounced than those seen in fowl and turkey (Burns and Maxwell, 1987).

Huff *et al*. (1992a) fed ringneck pheasants diets containing up to 4 ppm OA for three wk. Pheasants were more resistant to OA than chickens in that OA had no effect on body weight, even at 4 ppm, whereas 2 ppm is the minimum growth inhibiting level in chickens under most conditions (Huff *et al*., 1974, 1975). However, a significant increase in relative kidney weight was seen at all levels tested, and 4 ppm OA, caused 5% mortality.

9.   EFFECTS OF OCHRATOXIN A ON THE AVIAN IMMUNE SYSTEM

Administration of OA causes regression and cellular depletion of lymphoid organs and exerts an adverse effect on cell-mediated immunity (CMI) but not on humoral immunity in poultry. The effects of OA on the immune response of poultry species were reviewed by Burns and Dwivedi (1986).   In general, immune responses are depressed in a dose-related manner during ochratoxicosis. Cell-mediated immunity is most affected in broilers and least depressed in quail. All immune mechanisms investigated are reduced more in broilers than in turkeys. The regression of the lymphoid organs and the lymphocyte depletion that occurs during ochratoxicosis appears to result in reduced immunity in broilers and turkeys. However OA has only a mild effect on the lymphoid organs of quails and ducks and therefore immune responses are little affected in these species (Burns and Dwivedi, 1986). Chang and Hamilton (1980) investigated the effects of OA on some CMI parameters in broilers. Heterophils from chickens exposed to dietary OA had decreased phagocytic and locomotory capacities. Both directed and undirected locomotion of heterophils were

significantly impaired.   In another experiment, however, a lower dietary level of OA (2 ppm) administered to broiler chicks from hatch to 3 wk of age did not alter the phagocytic activity of heterophils or affect antibody titres or complement activity (Campbell *et al*., 1983). Cell-mediated responses, as measured by delayed hypersensitivity reactions to avian tuberculin and to bovine serum albumin (BSA) in pre-sensitized birds, were significantly depressed in growing turkeys fed 4 ppm dietary OA (Dwivedi and Burns, 1985).   Singh *et al*. (1990) characterized the immunosuppression caused by 0.5 and 2.0 ppm dietary OA in broilers. CMI was assessed by skin sensitivity testing, graft versus host (GVH) reaction and T-lymphocyte count, whereas humoral immunity was evaluated by measuring the hemagglutinin (HA) response to sheep RBC (SRBC). In addition, the phagocytic activity of splenic macrophages was measured in the nitroblue tetrazolium test (NBT), and the weights of lymphoid organs were recorded. Highly significant reductions in CMI were indicated by diminished skin sensitivity, GVH reactions, and T-lymphocyte counts. The number of NBT-positive cells was markedly reduced by both levels of OA compared with controls and the weights of thymus, bursa of Fabricius and spleen of intoxicated birds were reduced (Singh *et al*. 1990)

10.   INTERACTIONS OF OCHRATOXIN A WITH OTHER MYCOTOXINS

Additive, antagonistic, and synergistic interactions have been reported to occur between OA and other mycotoxins in chickens. Huff *et al*. (1984) fed broiler chickens diets containing aflatoxin (2.5 ppm) and/or OA (2 ppm) for three or six wk after hatching. Chickens receiving the toxic diets for 3 wk were fed a control diet for an additional 3 wk recovery period. Body weights were significantly decreased by aflatoxin, OA, and the combination treatment. The effects of OA appeared to be longer lasting than those of aflatoxin. After the 3 wk recovery period, the birds exposed to aflatoxin alone showed a partial recovery of higher magnitude than those fed OA. In this experiment, an antagonistic interaction was observed in liver lipid accumulation. Liver lipid significantly increased during aflatoxicosis (24.3% vs 16.2% in controls), was not altered during ochratoxicosis (15.2%), and was slightly spared by OA in the combination treatment (21.2%). In another trial, no additive or synergistic interactions were observed in broiler chickens fed OA (3 ppm) and citrinin (300 ppm), alone or in combination. Interestingly however, an antagonistic interaction occurred because the severe growth depression resulting from OA and the increased water intake associated with citrinin toxicosis were ameliorated when the toxins were fed in combination (Manning *et al*., 1985). The individual and combined effects of OA (2 ppm) and DON (16 ppm) in growing broilers were investigated by Kubena *et al*. (1988). For a few parameters such as feed efficiency and relative weights of the liver, gizzard and spleen, OA and DON appeared to act synergistically. However, many of the parameters such as body weight gain, BUN, serum total protein, and AST activity showed significant interactions that were described as less than additive or in some cases antagonistic. Kubena *et al*. (1989) evaluated the individual and combined effects of OA (2 ppm) and T-2 toxin (4 ppm) in broilers showing them to be additive for reduced body weight gain, reduced RBC mean corpuscular volume, decreased serum levels of total protein, and for reduced LDH activity. Micco *et al*. (1991) reported a synergistic interaction between OA and penicillic acid in chickens. Significantly higher levels of OA in kidney and liver were detected in chickens exposed to both toxins as compared with those receiving only OA at the same dose. The synergistic effect was caused by the inhibition by penicillic acid of the enzyme carboxypeptidase A, responsible for the transformation of OA to its non-toxic form.

11. <u>POTENTIAL TREATMENT AND PREVENTION</u>

Increasing dietary protein levels and supplementing with vitamin C appear to reduce the toxic effects of OA in poultry. Even though parenteral administration of L-phenylalanine (Phe) has protected against the lethal effects of OA in rodents (Hsieh, 1987), no beneficial effects are seen when Phe is supplemented to OA-contaminated chicken diets. Rotter *et al*. (1989b) studied the possible protective effect of Phe on ochratoxicosis in chickens by supplementing diets containing 0 or 4 ppm OA with either 0, 0.75 or 1.5% Phe. Supplementation of Phe to an OA-contaminated diet had no beneficial effects and supplementation of Phe alone was detrimental on chicken performance. In other studies (Bailey *et al*., 1990; Gibson *et al*., 1990) supplementations of 0.8 or 2.4% Phe to broiler diets containing 4 ppm OA decreased the mortality rate from 42.5% in non-supplemented chickens to around 14%. However, supplemental Phe decreased the concentrations of Hb and serum glucose. In contrast, supplementation of vitamin C (ascorbic acid) has produced beneficial effects in layers exposed to dietary OA (0, 1.7, or 3.1 ppm) under normal (25°C) and high (33°C) ambient temperatures (Haazele *et al*., 1993). OA decreased egg weight and egg mass and increased shell elasticity at both temperatures, and the addition of the vitamin C tended to counteract these negative effects. The OA-induced reduction in feed intake was counteracted by vitamin C at 25°C. In addition, all the effects of OA on plasma electrolyte concentrations and plasma AST activity were moderated by the supplementation of vitamin C to the diet. The effect of the dietary protein on OA toxicity in broilers has also been investigated (Bailey *et al*., 1989; Gibson *et al*., 1989). In the presence of 4 ppm OA in the diet, feed efficiency was improved at protein levels of 22 and 26% compared with broilers fed 14 and 18% protein. Raising the protein levels also counteracted the OA effect on the relative weights of kidney, liver, pancreas, and gizzard, and on some hematological and clinical chemistry parameters.

The addition of adsorbents to poultry diets contaminated with aflatoxin has been shown to reduce the intestinal absorption of the toxin and therefore to reduce its toxic effects. However, adsorption therapy appears to be of no practical use in the case of ochratoxins. Rotter *et al*. (1989a) investigated the influence of dietary charcoal on OA toxicity in Leghorn chicks. The addition of 10,000 ppm charcoal to a diet containing 4 ppm OA did not reduce the toxicity of OA, increased feed consumption by 8.5%, and also had the disadvantage of blackening the feed, the birds, and their environment. In another experiment, the addition of 0.5% sodium calcium aluminosilicate had little effect on the toxicity of 2 ppm OA in the diet of broiler chickens (Huff *et al*., 1992b).

Since recycling of OA in the enterohepatic cycle contributes to its long half-life and high toxicity, selective adsorbents for OA should be investigated as a potential therapy. Even though non-specific adsorbent compounds such as charcoal and HSCAS have not been effective in reducing OA toxicity, the use of more selective adsorbents could prevent the reabsorption occurring via the enterohepatic recycling and potentially reduced the toxic effects of OA. Another factor contributing to the long half-life of OA is the active reabsorption of the toxin via the organic anion transport system in the proximal and distal tubules of the kidney. Administration of drugs capable of making the urine more alkaline could potentially induce ionization of the OA in the renal tubules and prevent its reabsorption into the blood. Finally, binding of OA to serum albumin, which is also responsible for its long half-life, could potentially be counteracted by the administration of drugs with high albumin affinity. Substances with high affinity for serum albumin (e.g. phenylbutazone) could

compete with OA for the albumin binding sites and increase the serum level of unbound OA, which, in turn, would help to excrete the toxin.

Chapter 13

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CHAPTER 14.   AFLATOXINS

Other names:	TURKEY X DISEASE
Species:	ALL POULTRY

1.   COMPENDIUM

Aflatoxins are a group of heterocyclic metabolites produced by storage fungi of the genus Aspergillus, particularly *A. flavus* and *A. parasiticus*. Even though 18 different aflatoxins have been identified, only aflatoxins B1, B2, G1 and G2 have been detected as natural contaminants of feeds and feedstuffs. Aflatoxins were first identified as the causative agent of "Turkey X" disease, a toxicosis that killed over 100,000 turkey poults in England in 1960. Since their isolation and identification, aflatoxins have been a major concern as human hepatocarcinogens and as substances with potential deleterious effects on livestock health and productivity. The target organ for the toxic action of aflatoxins is the liver. Poultry species vary in their susceptibility to aflatoxins with ducklings being the most sensitive and chickens the most resistant. The adverse effects of aflatoxin on broiler performance are both dose- and time-dependent. In broilers, toxic levels of aflatoxin cause decreased body weight and feed intake, poor skin pigmentation, depletion of lymphoid organs such as thymus and bursa of Fabricius, and macroscopic and histologic lesions in the liver. Reduced egg production and egg weight, increased liver fat, and alterations in some serum biochemical parameters are the most prominent manifestations of aflatoxicosis in layers. Several strategies have been developed in order to minimize the adverse effects of aflatoxin in poultry. The addition of adsorbents (eg. hydrated sodium calcium aluminosilicate, HSCAS) to aflatoxin-contaminated diets significantly reduces the adverse effects of the toxin on chicken performance.

2.   INTRODUCTION

In 1960, an apparently new disease occurred in turkey poults in England causing an estimated loss of 100,000 birds. Post-mortem examination of dead poults revealed acute necrosis, associated with generalized bile duct proliferation (Lancaster *et al*., 1961). As the etiology of the disease was unknown, it was called "Turkey X" disease, but within a short time outbreaks of a similar condition were also reported in ducklings and pheasants (Sargeant *et al*. 1961a). The common factor was the presence of a certain Brazilian groundnut meal in the rations. Afterwards, outbreaks of disease associated with feeding of Brazilian groundnut meal were reported in cattle, pigs, sheep, and chickens (Sargeant *et al*., 1961b; Archibald *et al*., 1962) and groundnut meals from other countries were also shown to be toxic (Sargeant *et al*., 1961a).

Sargeant *et al*. (1961b) purified toxic extracts from groundnut meal and noted that the toxic component emitted a bright blue fluorescence under ultraviolet light. This was an important finding for the subsequent development of analytical thin layer chromatography. Sargeant *et al*. (1961b) suspected that the toxic substance might be a fungal metabolite since a highly toxic sample of nuts from Uganda, which had been associated with the deaths of ducklings in Kenya, was seen to be heavily contaminated with fungi. Dead fungal hyphae were also observed in the cotyledon tissue of groundnut kernels associated with the Turkey X disease outbreak (Austick and Ayerst, 1963). The causative role of a fungal metabolite was finally proved when chloroform extracts from pure cultures of the fungus *Aspergillus flavus*, isolated from toxic Ugandan kernels were fed to ducklings and found to reproduce the histologic liver lesions of toxic groundnut poisoning (Sargeant *et al*., 1963). The toxic material derived from the fungus *A. flavus* was given the name "aflatoxin" in 1962 (Sargeant *et al*., 1963). Initially, two toxic components of aflatoxin were identified on thin layer chromatographic plates and were named aflatoxin "B" and aflatoxin "G", due to their blue or green fluorescence under ultraviolet light, respectively (Sargeant *et al*., 1963).

Allcroft and Carnaghan (1963) described the comparative pathology of groundnut poisoning in farm and laboratory animals. While the target organ of aflatoxin was shown to be the liver, there was considerable difference in susceptibility among different animal species. Ducklings seemed most susceptible, and within only three to four d of feeding toxic meal extensive proliferation of bile-duct epithelial cells was clearly visible and was marked by seven d (Asplin and Carnaghan, 1961). This formed the basis of a biological test that enabled samples of ingredients to be tested on a routine basis before the development of instrumental analytical techniques. Chickens proved to be more resistant to aflatoxins compared with turkey poults and ducklings. The main toxic effect of aflatoxicosis in chickens was retardation in growth (Asplin and Carnaghan, 1961). Mice were shown to be resistant to experimental poisoning in the short term (Allcroft and Carnaghan, 1963) but rats receiving 20% Brazilian groundnut meal in a purified diet developed multiple liver tumours, some of them with lung metastases (Lancaster et al., 1961). This was the first report demonstrating the potent hepatocarcinogenic action of aflatoxins.

During the past 30 years extensive research on the characterization of aflatoxicosis in farm and laboratory animals has been conducted. Aflatoxins are a major concern as human hepatocarcinogens and are considered to play an important role in the high incidence of human hepatocellular carcinoma in certain areas of the world.

3.   CHEMISTRY, NATURAL OCCURRENCE, AND TOXICITY OF AFLATOXINS

3.1.   Chemistry and natural occurrence

Aflatoxins constitute a closely related group of heterocyclic metabolites synthesized predominantly by the fungi *Aspergillus flavus* Link and *Aspergillus parasiticus* Speare. Apart from *A. flavus* and *A. parasiticus*, an aflatoxigenic species phenotypically similar to *A. flavus* has been isolated and named *A. nomius* (Ellis *et al*., 1991). It is important to note that *A. flavus* is also a fungal pathogen for avian species and is capable of causing aspergillosis in chickens and turkeys (Richard, 1991). At present, 18 different aflatoxins have been identified. However, aflatoxins B1, B2, G1 and G2, are the only naturally occurring compounds (*Fig. 14.1*). The other aflatoxins (M1, M2,

P1, Q1, aflatoxicol, etc) occur as metabolic products of microbial or animal systems (Smith and Ross, 1991).

Chemically, aflatoxins are difurocoumarolactones (difurocoumarin derivatives, Buchi and Rae, 1969). Their structure consists of a bifuran ring fused to a coumarin nucleus with a pentenone ring (in B and M aflatoxins) or a six-membered lactone ring (in G aflatoxins) (*Fig. 14.1*). The predominant aflatoxin synthesized by *A. flavus* and *A. parasiticus* is B1, which is also the most toxic compound of the group. *A. flavus* produces mainly aflatoxin B1 and B2, while *A. parasiticus* produces aflatoxin B1, B2, G1, and G2 (Ellis *et al*., 1991). However, not every fungal strain of *Aspergillus sp.* is capable of producing aflatoxins, rather the particular genotype of each strain determines aflatoxigenicity (Bayman and Cotty, 1993; Takahashi, 1993.) Furthermore, several biological, chemical, and environmental factors will ultimately determine the amount of aflatoxin produced by aflatoxigenic *Aspergillus spp.* on a given substrate. The relative importance of each of the factors involved in aflatoxin biosynthesis has been reviewed by Ellis *et al*. (1991).

Aspergillus spp. can grow on a variety of substrates and most foods and feeds are susceptible to invasion by aflatoxigenic strains at any stage of production, processing, transportation, and/or storage. However, the presence of aflatoxigenic molds on grains does not necessarily mean presence of aflatoxin just as the absence of molds does not mean absence of aflatoxins. Aflatoxins are extremely stable in grains and may persist long after the mold growth has stopped. For example, the groundnut meal associated with the outbreak of turkey "X" disease was sterile, although it contained large amounts of aflatoxins (Austick and Ayerst, 1963).

In stored grains, the most important factors influencing growth of *Aspergillus spp.* and aflatoxin production are relative humidity around and in the substrate, and storage temperature. Equilibrium relative humidity of 80-85%, equilibrium moisture concentration of 17%, and temperatures of 24-35°C are the optimal conditions for aflatoxin production (Osweiller *et al*., 1985). Mold growth generally does not occur in grains dried below 12% moisture (Reddy, 1992). *Aspergillus spp.* are mainly storage fungi which generally do not contaminate grains prior to harvest. However, conditions such as drought stress and insect damage may allow infection by toxigenic *Aspergilli* and production of aflatoxins in the field before the crop is harvested (Anderson *et al*., 1975; Fennell *et al*., 1975; Lillehoj *et al*., 1976). In feed mills, optimum conditions for aflatoxin formation are reported to be 19-27°C, 79-89% relative humidity, and 10 - 13% moisture (Jones *et al*., 1982).

Aflatoxigenic strains of *A. flavus* have been isolated worldwide (Subrahmanyam and Rao, 1974; Moreno-Romo and Suarez-Fernandez, 1986; Bayman and Cotty, 1993; Takahashi, 1993) and the worldwide occurrence of aflatoxins in food and feeds has been well documented (Jelinek *et al*., 1989; Wood, 1992). The results of the Contamination Monitoring Program for mycotoxins conducted by the Food and Agriculture Organization (FAO), World Health Organization (WHO), and United Nations Environmental Program (UNEP) from 1976 to 1983, were reported by Jelinek et al. (1989). The survey showed that much of the monitored grain contained aflatoxins above 5-20 µg/kg, the regulatory levels for food in most countries, or 20-50

μg/kg, the regulatory limits in feeds in most countries. Of interest, was the observation that aflatoxin levels in corn may vary from year to year and from region to region. The median values for aflatoxins in corn and corn products ranged from <0.1 to <80 μg/kg (Jelinek *et al*., 1989).

Few surveys on the occurrence of aflatoxins in poultry feeds have been conducted. Recently, Jindal *et al*. (1993) analyzed 240 poultry feeds from India. All samples were positive for aflatoxins with levels ranging from 7 to 11,600 μg/kg (ppb). Levels higher than 30 ppb were detected in 76% of the samples. On the other hand, aflatoxin levels of 30-1610 ppb were found in 19% of 31 samples of mixed poultry feed in Nigeria (Shetty *et al*., 1987), while 91% of 34 samples of poultry feed in Indonesia contained aflatoxin levels ranging from 22 to 6171 ppb (Purwoko *et al*., 1991). Hegazy *et al*. (1991) reported that 30.7% of 1175 poultry feed samples collected from Egyptian farms were contaminated with aflatoxins. The concentration of aflatoxin in the positive samples ranged from 1 to 2000 ppb.

3.2.   Toxicity

Aflatoxin B1 (AFB1) can be classified as a highly toxic compound (LD_{50} 1-50 mg/kg) for most animal species, although it is extremely toxic (LD_{50} <1 mg/kg) for some highly susceptible species such as rainbow trout, cats, and ducklings. The toxic effects of aflatoxin exposure are both dose and time dependent and two distinct forms of aflatoxicosis, namely acute and chronic, can be distinguished depending on the level and length of time of aflatoxin exposure. Acute poisoning is most readily recognized as an acute hepatotoxic disease characterized clinically by depression, anorexia, icterus, and hemorrhages (Osweiller *et al*., 1985). Histologic hepatic lesions include periportal necrosis associated with bile duct proliferation and oval cell hyperplasia. Chronic aflatoxicosis resulting from regular low-level dietary intake of aflatoxins causes reduced weight gain in cattle, pigs, and poultry, reduced milk yield in cows, and reduced feed intake and feed conversion in pigs and poultry (Smith and Ross, 1991). Aflatoxin-induced hepatocellular carcinomas, bile duct hyperplasia and hepatic steatosis (fatty liver) are the major hepatic effects of chronic aflatoxin exposure.

Aflatoxin B1 causes acute hepatotoxicity at levels less than one half of the LD_{50} level; the toxicity of aflatoxins G1, B2, and G2 is approximately 50, 20, and 10%, respectively, that of aflatoxin B1 when tested against various animal species and mammalian cells in culture (Smith and Ross, 1991).

Differences in the susceptibility to acute aflatoxin poisoning have been observed among animals of different species (*Table 14.1*). Animals having the highest sensitivity to aflatoxin B1 are the duckling, rabbit, and cat, while chick, mouse, hamster, and rat are relatively resistant. Within poultry the susceptibility ranges from ducklings > turkey poults > goslings > pheasant chicks > chickens (Muller *et al*., 1970). When various poultry species were fed different dietary levels of AFB1 (0.7, 1.4 and 2.1 ppm) poults and goslings appeared to be the most sensitive, quail were intermediate while domestic chicks were most resistant since there were no significant effects of AFB1 in broiler or Leghorn chicks (Arafa *et al*., 1981). Aflatoxin B1 at 0.7 ppm decreased the body weight of turkey poults and at 1.4 ppm feed intake, body weight and weight gain of goslings were adversely affected. Liver damage occurred in goslings and quail chicks at all levels tested and was extensive at 2.1 ppm (Arafa *et al*., 1981).

Young animals are the most sensitive to aflatoxicosis (Patterson, 1973) and male and female animals also differ in their sensitivity to AFB1. In general, female rats are less susceptible to acute toxicity, and tumors induced by aflatoxin AFB1 tend to develop slower in female than in male rats (Terao and Ohtsubo, 1991).

TABLE 14.1 Single dose oral LD_{50} values for aflatoxin B1 in various species[1]	
Species	LD_{50} (mg/kg body wt)
Rabbit	0.3-0.5
Duckling	0.34-0.56
Cat	0.55
Pig	0.62
Rainbow trout	0.81 (Intraperitoneal)
Dog	1.0
Guinea pig	1.4-2.0
Sheep	2.0
Monkey	2.2
Chick	6.5-16.5
Mouse	9.0
Hamster	10.2
Rat	5.5-17.9
	[1]From Patterson, 1973

4.   TOXICOKINETICS

4.1.   Absorption

Absorption is the process whereby toxicants cross body membranes and enter the bloodstream. The main sites of absorption of xenobiotics are the gastrointestinal tract (GIT), lungs and skin. The rate of transport of chemicals across cell membranes correlates well with their lipid solubility, which is usually expressed as hexane/water or chloroform/water partition coefficients (Klaassen and Rozman, 1991). Because aflatoxins are very liposoluble compounds, they are readily absorbed from the site of exposure (usually the GIT) into the bloodstream. Absorption of ^{14}C-labelled aflatoxin administered orally to laying hens was observed to occur very rapidly (Sawhney *et al.*, 1973b) and the fast absorption of aflatoxin administered orally to Rhesus monkeys was evidenced by the appearance of aflatoxin in the blood immediately after ingestion of the toxin (Dalezios *et al.*, 1973). Absorption of aflatoxin from the respiratory system has been reported in

workers at feed mills (Autrup *et al*., 1993), although there have been no studies to determine the quantitative importance of this route of absorption of aflatoxins in poultry.

4.2.   Distribution

Aflatoxins tend to infiltrate most of the soft tissues and fat depots of the chicken. However, major accumulation occurs in organs involved in the biotransformation of the toxin such as liver and kidney. One day after the administration of a single oral dose of ^{14}C-labelled AFB1 to laying hens, the highest concentration of ^{14}C activity was detected in the liver, followed by muscle, pancreas, skin, adipose tissue, lungs and spleen (Sawhney *et al*., 1973ab). In another study using ^{14}C-labelled aflatoxin, Harland and Cardeilhac (1975) determined that the liver, kidney and bone marrow of chickens concentrated aflatoxin more readily than did brain, muscles or body fat. Chen *et al*. (1984) fed broiler chickens a diet containing 2057 ppb AFB1 and 1323 ppb AFB2 for 35 d. Three hours after feeding, measurable amounts of AFB1 and AFB2 were found in all tissues of the birds, the highest levels being found in the gizzard and liver. In a trial with laying hens, analysis of tissues following administration of aflatoxin for 7 d indicated twice as much aflatoxin in the liver than in the kidney and six times as much as in muscle and blood (Trucksess *et al*., 1983). Obioha *et al*. (1986) studied the distribution of ^{14}C-aflatoxin administered as a single oral dose of 1 mg to 3-week old chickens. At 12 h, the highest ^{14}C activity was detected in gizzard content, followed by the liver and intestine and this pattern persisted for at least 24 h. The intestinal tract and its content had the largest amount of aflatoxin of any tissue after the feed had passed through the gizzard. The relatively large amount of radioactivity detected in the liver demonstrates the accumulation of aflatoxin in the liver.

4.3.   Biotransformation

The basic enzymatic biotransformation reactions are conceptually divided into Phase I reactions (consisting of oxidation, reduction and hydrolysis) and Phase II reactions in which the metabolites produced in the first phase are conjugated with endogenous substances in order to facilitate excretion. Conjugation can occur with several compounds including glucuronic acid, sulfate, amino acids, glutathione and methyl or acetyl groups. During Phase I metabolism, AFB1 is biotransformed by cytochrome P-450 enzymes into several water-soluble metabolites including aflatoxins M1, Q1, P1, and aflatoxicol (Ro) (*Fig. 14.2*). Ueno *et al*. (1984) identified 5 cytochrome P-450 isoenzymes responsible for AFB1 metabolism. Aflatoxin M1 formation is strictly mediated by P-448 microsomal enzymes, while Q1 formation is catalyzed by both P-448 and P-450 where the specific activity of the former is 2 to 3 times higher than for the latter (Yoshisawa *et al*., 1982). Both broilers and layers can metabolize the majority of aflatoxin B1 when administered at relatively low levels and aflatoxin conjugates are the predominant form of metabolite produced (Chipley et al., 1974).

4.3.1. Phase I reactions

Fig. 14.2 summarizes the major Phase I metabolic biotransformations of AFB1 reported to occur in living organisms. Smooth endoplasmic reticulum enzymes of the cytochrome P-450 family are involved in the hydroxylation, *O*-demethylation, and epoxidation of AFB1, whereas cytosolic reductases are responsible for the reduction of AFB1, which results in the formation of a hydroxyl derivative known as aflatoxicol or Ro.

Hydroxylation

Hydroxylated derivatives of AFB1 are in general much less toxic than the parent compound. Crude or isolated microsomal preparations of livers from many species have been found to be capable of transforming AFB1 to its hydroxy derivative aflatoxin M1, and the analogous product, aflatoxin GM1, may be formed from G1 (Patterson, 1973). Chen *et al*. (1984) detected aflatoxins M1 and M2 (the hydroxylated derivatives of aflatoxins B1 and B2, respectively) in most of the tissues of chickens receiving a diet containing 2057 ppb AFB1 and 1323 ppb AFB2 for 35 d. The highest levels of M1 and M2 were found in the liver and kidney, which is likely associated with the important role of these organs in the biotransformation and elimination of xenobiotics. Aflatoxin Q1, another hydroxylated derivative of AFB1 has been detected as a minor metabolite of chicken and duck microsomal preparations (Hsieh *et al*., 1977). Also, aflatoxins AFB1 and G1 can be hydroxylated at the vinyl ether double bond (C8-C9) to form 8-hydroxy derivatives or hemiacetals (*Fig. 14.2*), also known as aflatoxins B_{2a} and G_{2a}, respectively (Patterson, 1973). Livers of chicks, guinea pigs and mice metabolize AFB1 into small amounts (5-10%) of M1, whereas the major metabolite produced is the hemiacetal B_{2a} (Patterson and Roberts, 1970). The ability of certain species to metabolize AFB1 to its hemiacetal is an important aspect of resistance to the toxin because the toxicity of the hemiacetal is much lower than that of the parent compound. Aflatoxin B_{2a} has been shown to be non toxic to chicken embryos at levels 100 times the LD_{50} of B1 (Ashoor and Chu, 1975).

O-demethylation

Another microsomal enzyme-mediated reaction of rat, mouse, guinea pig and rabbit liver is the *O*-demethylation of aflatoxins. The phenolic product formed from AFB1 and isolated from monkey urine has been named aflatoxin P1. However, there is no evidence that this biotransformation reaction occurs in avian species (Patterson, 1973; Hsieh *et al*., 1977).

Epoxidation

Another metabolic pathway of the vinyl ether double bond present in the AFB1 bifuran ring is its epoxidation. The resultant product, aflatoxin 8,9-epoxide, which is highly reactive with nucleophilic sites of DNA, is presumed to be the active form responsible for the carcinogenicity and mutagenicity of AFB1. The importance of this metabolite is discussed under toxicodynamics.

Reduction

The C1 carbonyl group present in aflatoxins B1 and B2 can be reduced to a hydroxy group to form the corresponding cyclopentols aflatoxicol and dihydroaflatoxicol. This reaction is not affected by microsomal enzymes but by a cytosolic NADPH-dependent enzyme. Reduction of AFB1 to the cyclopentol aflatoxicol by a cytoplasmic form of reduced NADPH has been observed in duck liver (Patterson and Roberts, 1970). Aflatoxicol can be re-oxidized to AFB1 by a microsomal dehydrogenase, and therefore has been considered by some workers as a "storage" form of AFB1 (Hsieh *et al*., 1977).

4.3.2. Conjugation (Phase II reactions)

Several conjugation reactions of aflatoxin metabolites have been reported to occur in poultry species. Patterson (1973) suggested that aflatoxin M1 and P1 were excreted in the bile as taurocholic and glucuronide conjugates. Chipley *et al*. (1974) observed that in chickens receiving [^{14}C]aflatoxin B1 the major metabolite produced was a peptide or amino acid conjugate of B$_{2a}$ and, to a lesser extent, a glucuronide conjugate of aflatoxin M1. Evidence of a water-soluble aflatoxin M1 glucuronide which comprised about 30% of the total aflatoxin metabolized in poultry species dosed with [^{14}C]aflatoxin was reported (Mabee and Chipley, 1973). In monkeys, sulfate and glucuronide conjugates of several aflatoxin metabolites have been detected (Dalezios *et al*., 1973). The B1-8,9-epoxide can be inactivated by GSH conjugation, in a reaction catalyzed by GSH *S*-transferase, or may be detoxified via conjugation with sulfates and glucuronic acid (Coulombe, 1993). Another detoxification reaction of the AFB1-8,9-epoxide is its conversion to AFB1-8,9-dihydrodiol, which may occur either spontaneously or by enzymatic hydrolysis (Choy, 1993). The marked differences in species susceptibility to the carcinogenic effects of B1 are presumably related to these mechanisms of defense. Rats and rainbow trout are extremely susceptible to AFB1-induced liver cancer while mouse and salmon are almost resistant. The relative resistance of the mouse can be partially explained by the lower covalent binding of AFB1 to liver DNA in mouse compared with rats where binding of B1 to DNA is lower by a factor of 40, (Lutz *et al*., 1980).

4.4. Elimination

Using radiolabeled aflatoxin in chickens has shown that the toxin and its metabolites are excreted mainly through bile and to a lesser extent the kidney and GIT. Sawhney *et al*. (1973) administered a single oral dose of ^{14}C-labelled aflatoxins to White Leghorn hens and found that aflatoxins or their metabolites were eliminated fairly rapidly through the combined urinary-fecal excretion route. Some 28% of the dose was eliminated in the excreta in 24 h while 71% of the dose was recovered from the excreta after 7 d. In this study, the specific activity of the bile was greater than for any other tissue when measured at one and four d, indicating that excretion of the toxin via bile is quantitatively very significant. The rate of depletion of aflatoxins from the body followed first order kinetics and the half-life of total aflatoxins was about 67 hours. In another experiment, ^{14}C-labelled AFB1 was rapidly cleared from the plasma after intravenous injection of egg-type chickens and 70% of the excretory products were found in the bile (Harland and Cardeilhac, 1975). The ^{14}C had a calculated plasma half-life of 1.5 minutes and rapidly appeared in the bile. The ^{14}C concentration in bile reached values approximately 7 times higher than plasma values which indicated that metabolites of the ^{14}C-labelled aflatoxin had been excreted against a concentration gradient into the bile. The label was excreted via bile, urine and intestinal contents at a fairly constant ratio of 70:15:15, respectively (Harland and Cardeilhac 1975).

Gregory *et al*. (1983) evaluated the rate of elimination of AFB1 and its metabolites when the toxin was removed from the diet. Turkey poults were fed a diet with 500 ppb AFB1 for 18 d and had highest levels of aflatoxin in the liver. Free and conjugated aflatoxins B1 and M1 were the principal tissue residues although Ro was detected in some samples. All aflatoxin residues were rapidly cleared following removal of the AFB1 contaminated diet. The half-life for total aflatoxin clearance from the liver was 1.4 d.

4.5.   Residues

Although aflatoxin can be found in the liver and muscle of chickens receiving contaminated diets, results from withdrawal trials show that poultry can metabolize and eliminate aflatoxin from their tissues in a relatively short time (72-96 h) after the withdrawal of the toxin from the diet. The levels of aflatoxin contamination normally found in poultry feeds and feedstuffs probably have little significance for human health from the viewpoint of aflatoxin residues in poultry products.

Aflatoxin M1 is secreted in the milk of cows receiving dietary AFB1 (Veldman *et al*., 1992). Although no evidence of AFM1 excretion in hen's eggs has been reported, other aflatoxin metabolites can be excreted with the egg. Sawhney *et al*. (1973b) gave an oral dose of radiolabelled aflatoxins, and found different concentrations of radioactivity in all components of the egg and edible parts of the carcass. Aflatoxins or metabolites were detected in all components of the egg as early as 10 h after ovulation and 14 h after oviposition. The concentration of label declined in albumen after 48 h while levels in the yolk and shell membranes increased (Sawhney *et al*., 1973b). The carry-over of AFB1 from layer feed to eggs was also demonstrated in hens where dietary levels of 100-400 ppb AFB1 resulted in AFB1 levels of 0.2-3.3 ppb in eggs (Jacobson and Wiseman, 1974).

Trucksess *et al*. (1983) fed 18 laying hens a AFB1-contaminated diet (8,000 ppb) for 7 d after which half of the group were sacrificed, while the remainder were sacrificed after feeding an aflatoxin-free diet for 7 more d. AFB1 and its metabolite Ro were detected in the eggs and edible tissues of all hens sacrificed 7 d after exposure. Liver and ova contained the highest levels of B1 and Ro, while AFM1 was only found in kidney tissue. Seven d after withdrawal, only trace amounts of Ro were detected in eggs and no aflatoxin residues were found in edible tissues. The level of aflatoxin residues in eggs increased steadily for 4 or 5 d to a plateau and decreased after AFB1 withdrawal at about the same rate as its initial accumulation. In another experiment, turkeys fed an aflatoxin-free diet for 7 or 14 d after having received a diet containing 150 ppb AFB1 for 11 wks did not have residues of aflatoxin in the feces or tissues, except for some AFB1 remaining in detectable amounts in the gizzard (Richard *et al*., 1987). Micco *et al*. (1988) investigated the residue levels of B1 and its metabolites (B_{2a}, M1 and Ro) in tissue and organs of male broiler chickens and laying hens after long-term administration of a diet contaminated with 50 ppb AFB1. Residue levels of aflatoxins B1, M1 and Ro were detected in liver, kidney and thigh muscle of both male broilers and hens. The highest levels found were for Ro in liver (1.1 and 0.6 ppb for male broilers and hens, respectively). No B_{2a} could be found in any of the tissues analyzed and no detectable amounts of aflatoxins were found in any tissue after withdrawal periods of 14 and 33 d for male broilers and laying hens, respectively. Obioha *et al*. (1986) administered 1 mg ^{14}C-aflatoxin to 3-week old chickens as a single oral dose and concluded that it would require 72 h to significantly reduce aflatoxin residues from edible tissues.

5.   TOXICODYNAMICS: MECHANISM OF ACTION OF AFLATOXINS

Aflatoxin B1 is the most potent hepatocarcinogen known for the rat and rainbow trout, and is also capable of inducing liver cancer in other animal species (Hsieh, 1985). Aflatoxins can interact with DNA, RNA and intracellular proteins in primary human and chicken hepatocyte cultures (Iwaki *et al*., 1990). Studies *in vitro* with chicken chondrocytes have shown that aflatoxin B1 causes a dose-dependent inhibition of DNA synthesis and, to a lesser extent, a decrease in proteoglycan synthesis (Kichou and Walser, 1994). The effect of AFB1 on DNA is the result of interaction of the toxin with reactive sites of the macromolecule. Two types of interaction are known to occur between aflatoxins and nucleic acids. One results from a weak, reversible, non-covalent binding, the other is an irreversible covalent binding that leads to the formation of aflatoxin-DNA adducts (Kiessling, 1986). Formation of B1-DNA adducts requires metabolic activation of the parent compound, by hepatic or extrahepatic microsomal cytochrome P450 enzymes, to form reactive metabolites. Biotransformation of AFB1 leads to the formation of a number of metabolic products, particularly hydroxylated derivatives. From these metabolites the B1-8,9-oxide (B1-epoxide) is considered to be responsible for the carcinogenic effect, due to its high ability to react with nucleophilic sites in macromolecular components. Presumably because of its extreme reactivity, the B1-8,9-epoxide has been isolated only indirectly from biological systems as adducts of glutathione (GSH), protein or DNA bases (Coulombe, 1993). Hydroxylated derivatives of AFB1 are in general much less toxic than the parent compound. Formation of the 8,9-epoxide ring requires the presence of a C8-C9 unsaturated bond, which means that aflatoxins B2 and G2 are practically atoxic when compared to AFB1 and G1 (Kiessling, 1986). On the other hand, aflatoxin M1, a hydroxylated metabolite of AFB1, is two orders of magnitude less potent than AFB1 as a hepatocarcinogen (Hsieh, 1985). Activated B1 binds to DNA guanyl residues both *in vivo* and *in vitro*. The B1-N7-guanine adduct, [8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B1] has been identified as the most predominant (Croy *et al*., 1978). Other adducts, such as the "ring-opened' derivative of B1-N7-Gua, B1-formamidopyrimidine [8,9-dihydro-8-(N5-formyl-2,5,6-triamino-4-oxopyrimidin-N5-yl)-9-hydroxy-aflatoxin B1] occur in smaller amounts. For instance, in hepatic DNA from livers of rats injected with AFB1, approximately 80% of the adducts present are B1-N7-Gua, whereas the B1-FAPyr constitutes approximately 7% (Coulombe, 1993). Although the B1-FAPyr adduct exists in smaller amounts, it is more stable and persistent than is the B1-N7-Gua adduct. Due to its persistence it has been suggested that the B1-FAPyr adduct is responsible for B1 carcinogenesis, although no evidence for this hypothesis exists. Aflatoxin B1-N7-Gua and B1-FAPyr adducts and total B1 binding are commonly used as indices of DNA adduct formation in quantitative cancer risk studies (Choy *et al*., 1993).

A strong correlation between carcinogenicity, mutagenicity and the extent of covalent DNA binding to aflatoxins (and their metabolites) has been observed (Kiessling, 1986). The hepatocytes are the major target cells for the toxic action of AFB1, which probably relates to the high content of cytochrome P450 enzymes present in these liver cells, as compared with other organs. The specific organelle affected by aflatoxin metabolites is the nucleus of the hepatocyte. Due to the known hepatocarcinogenic effects of AFB1 in experimental animals, chronic human exposure to the toxin has been proposed as one of the possible factors implicated in the development of hepatocellular carcinoma (HCC), one of the most common cancers in the world (Chen *et al*., 1992).

Although a definitive explanation for the carcinogenic effect of AFB1 has not yet been found, recent findings indicate that it may relate to proto-oncogene and anti-oncogene mutations. Carcinogenesis is generally accepted to be a multistage process involving three main steps,

namely, initiation, promotion, and progression. The interaction of electrophilic forms of chemical carcinogens with DNA plays an essential role in the generation of cell variants, the first cellular event in the path towards cancer (Ueno, 1991). DNA adducts formed by initiating carcinogens such as B1-8,9-epoxide can induce point mutations at critical genomic sites. Normal cells contain proto-oncogenes, *i.e.,* highly conserved genes that code for proteins involved in cell growth (growth factors) or hormone signalling. Because these genes are important in regulating developmental, hyperplastic, and regenerative proliferation, alterations in their expression causes increased cell proliferation and eventually progression to cancer (Weinberg, 1989). Proto-oncogenes can be activated by single base mutations and recent studies have revealed activation of a proto-oncogene of the c-Ki-ras family in liver tumors of rats chronically exposed to AFB1 (McMahon, 1986, 1987). It was determined that a single base transition in the codon 12 (GAA) for glycine resulting in a GGA codon for glutamic acid is associated with the activation of the c-Ki-ras gene (McMahon *et al*., 1986, 1987). Besides the c-Ki-ras oncogene, high expression of both c-Ha-ras and c-myc oncogenes has been demonstrated in hepatocellular carcinoma of AFB1 treated rats although expression of N-ras was not elevated in these hepatoma cells (Tashiro *et al*., 1986). Recently, it was reported that AFB1 induces the transversion of G → T in codon 249 of the p53 tumor suppressor gene in human hepatocytes (Aguilar *et al*., 1993). This point mutation leads to a substitution of the basic amino acid arginine by the neutral amino acid serine and is responsible for the altered functionality of the mutant gene product (Gerbes and Caselman, 1993). This is an important piece of information because for the expression of an oncogene to occur, inactivation of the anti-oncogene p53 must also occur.

Although the liver is known to be a target organ of AFB1, respiratory exposure to AFB1 contaminated dust has been linked with increased incidence of tumor in the respiratory tract of both animals and humans. Bioactivation of AFB1 by lung cells and by nasal mucosal epithelial cells, with subsequent formation of B1-DNA adducts, has been reported (Daniels and Massey, 1992; Tjälve *et al*., 1992). Rabbit lung microsomes have been shown to contain a high proportion of cytochrome P450 isoforms that are efficient in the activation of AFB1 (Daniels & Massey, 1992). Bovine olfactory mucosa has a high B1-bioactivation capacity and it has been suggested that AFB1 plays a role in the etiology of nasal tumors in cattle (Tjälve *et al*., 1992). Occupational exposure to aflatoxins through respiration was associated with an unusual increased incidence of lung cancer in Dutch workers (Autrup et al., 1993).

6.   **AFLATOXICOSIS IN CHICKENS**

Experimental trials with aflatoxin in chickens have been done using either naturally contaminated substrates (such as groundnut meal or cereal grains) or using clean cereal grains inoculated with aflatoxigenic strains of *A. flavus*. In the later case, the level of aflatoxin produced after inoculation is measured usually by chromatographic techniques and the contaminated substrate is mixed with clean feed in order to obtain the desired aflatoxin level. Crude extracts of aflatoxin contaminated grains and, to a lesser extent, purified toxins have also been used. The generic term "aflatoxin" is often used when crude extracts or naturally contaminated grains are employed, without specifying which of the four naturally occurring aflatoxins are present in the contaminated feed. That is the case, for example, of the most cited reference of the LD_{50} value of "aflatoxin" (Smith and Hamilton, 1970) where no specification on the composition of the aflatoxin was given. Aflatoxins differ greatly in their toxicity (B1 is the most toxic) and therefore identification

of the specific toxin is needed when reporting experimental trials or natural outbreaks of aflatoxicosis. Only a few studies have been conducted by adding purified aflatoxins to clean diets.

The clinical signs, gross and histopathological lesions, clinical pathology, and effects on performance parameters from experimental and natural aflatoxicosis in broilers have been reported worldwide. The first trials on experimental aflatoxicosis in chickens were conducted using toxic groundnut meals as the source of aflatoxins. Asplin and Carnaghan (1961) fed graded levels of a groundnut meal (found to be highly toxic for ducklings) to chickens for 6 wks or 9 months. Results showed that chickens were more susceptible to the toxic effects of aflatoxin when they were young and that the main adverse effect was retardation of growth. In a later study, Carnaghan *et al*. (1966) investigated the biochemical and pathological aspects of groundnut poisoning in chickens. Day-old chicks were fed a ration containing 15% of a highly toxic groundnut meal with AFB1 content of approximately 10 ppm for 8 wks (AFB1 content of the ration was therefore 1.5 ppm). Chicks on the toxic ration were smaller at 7 d of age, and this effect was significant after 4 wks. Although the mean weights of the livers for aflatoxin-fed chickens and controls were similar throughout the experiment, the liver/body weight ratio was significantly greater for the chickens fed AFB1. No differences in hepatic DNA content were observed between groups but a consistently smaller RNA content was found in B1-fed chickens. Decreased levels of hepatic vitamin A and increased fat content in the liver of chickens receiving AFB1 was detected. At postmortem examination the livers were friable, enlarged, putty-coloured, and with a reticulated appearance. Petechial hemorrhages were frequently present. Histologically, marked vacuolation of the cytoplasm of the hepatocytes and slight proliferation of the bile epithelial cells was detected as early as 86 hours after feeding the toxic meal. Interestingly, the hepatic fat content increased up to 21 d but, after this time, the lipid content of the liver fell sharply and was associated with hepatic cellular regeneration. After 7-8 wks there was little difference in liver fat content.

One of the classic papers on aflatoxicosis in poultry was written by Smith and Hamilton (1970) and was recently commented on by Hamilton (1992). This report was the first convincing evidence that mycotoxicosis (and particularly aflatoxicosis) could be economically important in farm animals in the absence of overwhelming mortality. Also, signs useful in the diagnosis and procedures for the prevention of aflatoxicosis were presented. The authors incorporated graded doses of aflatoxin (from aflatoxin-containing rice powder) into the feed of broiler chickens. Aflatoxins caused decreased growth rate, enlarged spleen and pancreas, and regressed bursae of Fabricius. Although previous trials had demonstrated that young chickens were more sensitive to aflatoxin, sensitivity of day-old chicks was about the same as for market weight broilers in terms of acute LD_{50} values.

7.   <u>AFLATOXICOSIS IN BROILERS</u>

Huff *et al.* (1986a) studied the progression of aflatoxicosis in broilers by feeding day-old chicks four dietary levels of aflatoxin (0, 1.25, 2.5, and 5.0 ppm) for three wks. The toxic diets were prepared by incorporating rice powder containing known amounts of aflatoxin into portions of corn/soybean basal diets, although the specific aflatoxins present in the rice powder were not analyzed. Body weights were significantly decreased by 5.0 ppm aflatoxin at 6 d of age and by 2.5 ppm at 17 d. Aflatoxin induced a significant increase in the relative weight of the proventriculus, gizzard, spleen, and kidney. Liver atrophy was indicated in the early stages of aflatoxicosis by a decrease in the relative weight of this organ. As aflatoxicosis progressed, hepatomegaly became

apparent due to lipid accumulation in the liver. Hematocrit and hemoglobin values were significantly decreased by 5.0 ppm aflatoxin at 12 d and by 2.5 ppm at 21 d of age. Serum levels of albumin and total protein were significantly reduced at 5.0 and 2.5 ppm aflatoxin by 3 and 6 d of age, respectively. Serum levels of uric acid, triglycerides and cholesterol were significantly decreased from control values from 12 through 21 d of age by 5.0 ppm aflatoxin and, to a lesser extent, by 2.5 ppm aflatoxin. The activity of serum lactate dehydrogenase was significantly decreased at all aflatoxin levels from 12 through 21 d of age. The authors concluded that the most sensitive indicators of aflatoxicosis in young chickens are the reduction in serum levels of albumin and protein.

7.1.   Clinical signs and effects on performance

The initial clinical signs reported during the outbreak of turkey "X" disease were anorexia and weight loss followed by depression, ataxia and recumbency. Affected birds died within a week or two and at the time of death frequently had opisthotonus characterized by arched neck, head drawn back, and legs extended backwards (Hamilton *et al*., 1972). Along with anorexia, weight loss, stunting, poor appearance, and paleness, nervous signs are frequently seen both in experimental trials and field outbreaks of aflatoxicosis in poultry. However, the most severe nervous signs (opisthotonus) are seen only in ducklings and turkey poults. Broiler chickens given a single oral dose of 4.0 mg AFB1/kg body weight showed dullness and decreased feed and water intake within 2-4 d while nervous signs included leg weakness and dropping of the wings (Rao and Joshi, 1993). Chickens exposed to dietary aflatoxin look paler than normal (Schaeffer *et al*., 1988a, 1988b). The poor pigmentation observed in chickens receiving aflatoxin-contaminated feed appears to be the result of decreased absorption, transport, and deposition of dietary carotenoids (Tyczkowski and Hamilton, 1987).

The adverse effects of aflatoxin on broiler performance are both dose- and time-dependent. Experimentally, levels of 0.5 ppm dietary AFB1 can cause a significant decrease in body weight and feed intake when administered for 4 wks whereas 1.0 ppm dietary aflatoxin for 1 or 2 wks does not affect performance. According to Osborne *et al*., (1982), the minimal dietary concentration of aflatoxin able to cause decreased growth in chickens appears to be 2.5 ppm. However, 0.5 ppm dietary AFB1 administered to 7 day old broilers for 4 wks caused a significant decrease in body weight and a significantly lower feed conversion compared with control chickens (DaFalla *et al*., 1987). The apparent discrepancy between these two studies may be related to the different sources of aflatoxin used. Huff *et al*. (1980) fed graded levels of dietary aflatoxin (0, 0.625, 1.25, 2.5, 5.0 and 10.0 ppm) to broiler chickens for 3 wks showing significantly decreased body weight and feed intake at levels of 2.5 ppm.

Adverse effects of aflatoxin on the productivity of commercial broilers have been reported. Doerr *et al*. (1983) evaluated the effect of low levels of dietary aflatoxin on performance and various processing parameters in broiler chickens exposed to the toxin for 7 wks. Aflatoxin at levels of 0.065-2.7 ppm significantly decreased live, and chilled eviscerated weight. Poor pigmentation and fatty livers were observed at processing. In another study, Jones *et al*. (1982) classified growers of broiler companies into good, or poor growers based on a productivity index. Aflatoxin concentration in feed of good growers was 6.1 ppb with an 18.0% frequency of contamination while the values for poor growers were 14.0 ppb and 31.3%, respectively.

Reddy *et al*. (1984) investigated the effect of dietary aflatoxin on performance parameters in broiler chickens. Dietary aflatoxin levels up to 1.0 ppm were fed to day-old broilers for up to 28 d.

Weight gain and feed consumption were depressed significantly by ≥ 0.75 ppm aflatoxin when given for ≥ 21 d. Administration of 1.0 ppm aflatoxin for 7 or 14 d had no effect on bird performance.

Giambrone *et al*. (1985) administered gelatin capsules containing pure AFB1 for 5 wks to 2-week-old broiler chickens at a dietary equivalent of up to 1.0 ppm AFB1. Pure AFB1 at levels of 0.5 and 1.0 ppm produced a significant decrease in 5-week weight gain and microscopic lesions of aflatoxicosis, although no mortality or effects on feed conversion were noted. Gross liver lesions indicative of aflatoxin toxicity occurred only at 1.0 ppm. Weight gain and feed conversion were not affected for broilers receiving pure AFB1 at doses below 0.5 ppm.

New Hampshire chickens fed a diet for 14 d containing 1.1 ppm AFB1, from naturally contaminated moldy wheat showed mortality rates of 58 to 73% (Rajion and Farrell, 1976). Postmortem examination showed enlarged livers while surviving chickens gained less weight and had a poorer feed conversion than did controls. The possibility of other mycotoxins occurring in the moldy wheat was not considered.

7.2.   *Gross and microscopic pathology*

Espada *et al*. (1992) described the lesions following experimental aflatoxicosis in chickens. Day old broilers were fed for 21 d with either 0.2 or 3 ppm AFB1, after which they were fed non-contaminated feed for an additional 10 d recovery period. Vacuolation of hepatocytes and lymphocytic depletion in the follicle medulla of the bursa of Fabricius were the first noticed lesions. These changes persisted during the recovery phase and followed a dose-response pattern. A significant reduction in the bodyweight and absolute weights of liver, bursa, spleen and thyroid were observed at 3 ppm AFB1. In another trial, petechial or ecchymotic hemorrhages on the medial surface of the thighs were observed in chickens exposed to 0.5 ppm dietary AFB1 for either 1, 2 or 3 wks. Liver hematomas and congestion of the spleen and kidneys were present in chickens receiving the toxin for 2 or 3 wks while chickens receiving 0.5 ppm dietary AFB1 for 4 wks had pale yellow livers, edema of the gall bladder wall and multifocal areas of congestion in the kidneys and perirenal edema. Histologic examination of the livers revealed congestion of the hepatic sinusoids, focal hemorrhages, centrolobular fatty cytoplasmic vacuolation and/or necrosis, biliary hyperplasia, and nodular lymphoid infiltration. In the kidneys, the epithelial cells of many convoluted tubules were vacuolated (DaFalla *et al*., 1987). In another study, histologic examination of day-old broilers fed a diet naturally contaminated with 2.65 ppm B1 showed massive necrosis of hepatocytes and renal hemorrhages. The tubular renal epithelium showed cloudy swelling, hydropic degeneration and necrosis (Asuzu and Shetty, 1986).

7.3.   *Biochemical changes and clinical pathology*

One of the most notable effects of continued low-level consumption of aflatoxin is an alteration of the serum protein profile. Total serum protein content is depressed due to reduced values for α- and β-globulins and albumin while γ-globulins are affected more variably (Pier, 1973). Tung *et al*. (1975a, 1975b) investigated the effects of graded levels of dietary aflatoxin up to 10 ppm on the concentration of different classes of serum proteins in chickens exposed for 3 wks to the toxic diets. Total serum proteins were significantly reduced by a dose of ≥ 1.25 ppm. The α-globulins and β-globulins were reduced at levels of 2.5 and 1.25 ppm, respectively. The IgG immunoglobulins were reduced at 2.5 ppm while IgM was not affected by any level of aflatoxin. The

most sensitive component was serum albumin that was decreased in a dose-response manner at even the lowest dose fed (Tung *et al*., 1975a). Jassar and Singh (1993) measured the weekly serum levels of total protein in broiler chickens receiving either 3.0 or 6.0 ppm dietary AFB1 for 42 d. Total serum protein decreased significantly in chickens fed 6.0 ppm AFB1 at all sampling times while at 3.0 ppm AFB1 a gradual decrease in total serum protein was observed up to day 21, followed by a slight increase.

The activity of serum or plasma enzymes has been extensively used as a measure of aflatoxin toxicity in chickens. In one study, broilers receiving 0.5 ppm dietary AFB1 for 4 wks had significantly higher activities of SDH and GDH (DaFalla *et al*., 1987). Increased activity of LDH, ALP, acid phosphatase, AST and ALT were reported in chickens receiving 3 or 6 ppm dietary AFB1 for 42 d. The increase in the levels of the serum enzymes measured was interpreted as a consequence of hepatocyte degeneration and subsequent leakage of enzymes. The serum enzyme levels were highest 21 d following exposure, after which the values declined indicating a recovery from the degenerative changes (DaFalla *et al*., 1987). In contrast with these results, broiler chickens receiving 2.5 or 5.0 ppm dietary AFB1 for 32 d did not show alterations in AST, ALT, LDH or GGT activities (Fernández *et al*., 1994). In a recent experiment, 96 h after the administration of a single oral dose of 4.0 mg/kg AFB1 to broiler chickens, serum AST levels were 2.5 times greater than those for control chickens (Rao and Joshi, 1993). Increased activity of hepatic ornithine decarboxylase was reported in chickens fed 2.5 ppm aflatoxin (Voight *et al*., 1987).

Several hematic parameters are affected by aflatoxin exposure. In experiments by Tung *et al*. (1975a, 1975b), chickens receiving graded levels of dietary AFB1 (0.625-10 ppm) showed a significant decrease in hemoglobin (Hb) content, hematocrit (PCV), and red blood cell (RBC) count. These parameters were significantly decreased to about the same extent by doses of 2.5 ppm AFB1 or more. However, Hb was lower at a dose of 1.25 ppm or higher, PCV was reduced by 2.5 ppm AFB1 or more, and RBC count was significantly reduced even by a dose of 0.625 ppm AFB1 (Tung *et al*., 1975b). Lanza *et al*. (1980) fed broiler chickens dietary aflatoxin at levels up to 50 ppm to 25 d of age. At 21 d a highly significant dose-related decrease in Hb, PCV, RBC, and mean corpuscular volume (MCV) were observed although mean corpuscular hemoglobin content (MCHC) was not affected. In another trial, broiler chickens fed dietary aflatoxin at levels of 0.75 or 1.0 ppm had significantly lower values for Hb and PCV than did control chickens (Reddy *et al*., 1984). Aflatoxins have also been shown to alter both the extrinsic and common clotting pathways in chickens. Broilers fed dietary aflatoxin at 2.5 ppm or more had reduced activities of clotting factors VII and V and fibrinogen. Factor X was affected at dietary levels of 5.0 and 10.0 ppm and the activity of prothrombin, the most sensitive factor, was reduced by doses of 0.625 ppm or greater (Doerr *et al*., 1976). Coagulopathy, as indicated by elevated prothrombin time, was observed in chickens receiving 2.5 ppm dietary aflatoxin for 3 wks (Huff *et al*., 1983) and 5.0 ppm for 6 wks (Chang and Hamilton, 1982a). The elevated prothrombin time was considered to be the result of impaired hepatic synthesis of clotting factors caused by the toxic action of aflatoxin on liver cells. In another trial, chickens injected I.P. with 58 μg/kg AFB1 had significantly higher blood clotting times than did controls, when measured 6 h after administration of the toxin (Bababunmi and Bassir, 1982).

The activity of some digestive enzymes, the absorption of carotenoid compounds from the GIT, and the metabolism of lipids can be altered by aflatoxin exposure. Dietary aflatoxin at 0.625 ppm or more produced a malabsorption syndrome characterized by steatorrhea, hypocarotenoidemia and decreased concentrations of bile salts and pancreatic lipase, trypsin, amylase, and RNAse (Osborne *et al*., 1982). In another experiment, the specific activities of

pancreatic chymotrypsin, amylase, and lipase, but not trypsin, were increased significantly by aflatoxin (Richardson and Hamilton, 1987). Plasma carotenoid levels were significantly decreased in broiler chickens fed rations containing 1.25 (Tung and Hamilton, 1973), 2.5 (Osborne *et al.*, 1982) or 2.7 ppm aflatoxin (Doerr *et al.*, 1983). Dietary aflatoxin administered at 5.0 ppm to 3-week old broilers significantly depressed absorption of dietary canthaxanthin into serum from 4 to 24 h after receiving the toxic diet (Tyczkowski *et al.*, 1991). Merkley *et al.* (1987) measured the hepatic content and fatty acid profile of broilers exposed to graded levels of dietary aflatoxin (0, 0.625, 1.25, 5.0 and 10.0 ppm). Total liver lipids increased significantly with increasing aflatoxin levels and this was almost entirely due to an increase in the neutral lipid fractions. In both neutral and polar lipid fractions a linear increase in monoenoic fatty acids [palmitic (16:0), stearic (18:0) and arachidonic (20:0)] was associated with increasing aflatoxin levels (Merkley *et al.*, 1987).

The effects of aflatoxin on the renal function of broiler chickens were reported by Glahn (1993). Aflatoxin-treated birds showed decreased fractional excretion of phosphate, decreased total plasma calcium concentration, decreased total plasma protein, decreased plasma 25-hydroxy vitamin D and decreased plasma 1,25-dihydroxy vitamin D. A sharp increase in urine hydrogen ion excretion was observed in aflatoxin-treated birds upon initiation of an intravenous 100 mM sodium phosphate infusion, suggesting increased Na^+/H^+ counterport. These effects were absent or significantly ameliorated 10 d after discontinuation of toxin administration although the glomerular filtration rate was decreased, indicating a possible reduction in functional renal mass and prolonged or permanent alteration in renal function.

7.4. *Natural outbreaks*

Archibald *et al.* (1962) reported one of the first cases of groundnut toxicosis. The groundnut meal had been included at the 5% level in the ration. Clinical signs appeared after 10-14 d of feeding the toxic diets, at which time mortality started and reached 8%. Clinical signs included depression, ataxia, and retarded growth. When the toxic meal was not included in the ration until after 4-5 wks of age, mortality was only 2-3%, compared with the higher mortality of 8% when the meal was included in the starter ration. This finding confirmed greater sensitivity of young chickens to the adverse effects of aflatoxin. On post mortem examination the most characteristic lesions appeared in the livers which were pale yellow to greyish brown and had a prominent reticular pattern. Petechial hemorrhages were observed on the surface of some livers. Gallbladders were enlarged and bile ducts distended. When the toxic meal was withdrawn from the ration, clinical signs disappeared and mortality ceased after 7-10 d.

Most of the reported natural outbreaks of aflatoxicosis in poultry are actually suspected cases where a variable number of feed samples are found positive to aflatoxins. However, the role of other primary etiologic agents (*e.g.* viral, bacterial or fungal diseases, parasitic infections, nutritional imbalances) on the clinical signs and adverse effects on performance shown by the affected birds is usually not taken into account. Decreased egg production and increased mortality rates in several poultry farms in India were associated with aflatoxin contamination of the feed; from 35 poultry feed samples analyzed, 23 were found positive for AFB1, with levels ranging from 0.07 to 0.914 ppm (Gupta *et al.*, 1985). Okoye *et al.* (1988) described the clinical signs and lesions of a suspected case of aflatoxicosis in broilers. The bird showed unusual signs including trembling, ataxia, paralysis of the legs and wings, and lameness. Histopathology revealed severe degeneration of hepatocytes, bile duct proliferation, and lymphocytic depletion in lymphoid organs. Aflatoxin B1 was detected in one sample of feed at a concentration of 2.4 ppm (Okoye *et al.*, 1988).

In another natural outbreak, over 1000 broilers died within two wks after being fed a diet contaminated with 2.65 ppm AFB1 (Asuzu and Shetty, 1986). Clinical signs included retarded growth, dullness, ruffled feathers, gasping, prostration and death. At necropsy, the livers were enlarged and pale, and there was blood in the intestinal lumen. Histologic examination of the livers showed vacuolation of hepatocytes, dilation of central veins and bile duct proliferation.

8.   AFLATOXICOSIS IN LAYING HENS

Reduced egg production and egg weight, increased liver fat, and alterations in some serum biochemical parameters are the most prominent manifestations of experimental aflatoxicosis in layers. Dietary levels of 1 ppm aflatoxin or more, adversely affect egg production when the toxin is given for 4 wks or more. It appears that aflatoxin affects egg production through an impairment of the normal mobilization of fat from the liver to the ovary. High mortality and a dramatic reduction of egg production were reported to occur during a natural outbreak.

Nesheim and Ivy (1971) reported that liver fat in layers markedly increased in hens fed 8 ppm aflatoxin compared with controls (66 vs 47%). Small hemorrhages were observed in the liver of these hens although none died from the massive liver hemorrhages characteristic of fatty liver-hemorrhagic syndrome. In another study, hens receiving 1.25 - 20 ppm dietary aflatoxin for 3 wks showed a dose-related decrease in egg production and egg size although shell thickness was not affected. The lipid content of the liver was significantly increased by 5 ppm aflatoxin from 36% of dry matter in controls to 55% in aflatoxin-fed hens (Hamilton and Garlich, 1971). Hens receiving 1 ppm dietary aflatoxin for 4 wks showed a significant reduction in egg production whereas levels of 2 ppm affected feed conversion (Iqbal *et al*., 1983). In another study, Huff *et al*. (1975) investigated the effect of graded levels of dietary aflatoxin up to 10 ppm on layers. After 4 wks, liver size and liver lipid were increased by aflatoxin while egg production and egg size were decreased. Dry weight and lipid content of the yolk were not affected but yolk and plasma carotenoid concentrations were elevated.

Washburn *et al*. (1985) reported that dietary aflatoxin at 5 ppm fed for 3 wks had no detrimental effect on shell strength as assessed by various means although egg weight, was significantly lower. In another trial, laying hens showed a significant decrease in egg production at levels of $\geq$ 0.7 mg AFB1 per kg body weight per day. Using a dose of 5 mg/kg, production ceased after 3 d whereas at 0.7 or 1.0 mg/kg no effect on production was apparent until 4 to 5 wks after a 14-day dosing period (Exarchos and Gentry, 1982).

Laying hens receiving a diet containing up to 5 ppm aflatoxin have also been shown to have lower serum calcium and phosphorus levels (Fernandez *et al*., 1994). In a previous trial, 20 ppm dietary aflatoxin administered to hens in lay for 7 d did not adversely affect egg production but plasma calcium, protein, cholesterol and triglycerides were all decreased (Garlich *et al*., 1973). In this study a delayed adverse effect of aflatoxin on egg production was observed. At the end of the aflatoxin feeding period, the hens were returned to a control diet for recovery and egg production began to decline significantly on the first day of the recovery period. Egg production reached a minimum of 35% seven d later and returned to control rate 19 d after the withdrawal of the contaminated diet. This delayed effect on egg production emphasizes the severe epidemiological problem of mycotoxins. Under field conditions, the feed causing the problem can be totally

consumed before its adverse effects are noticed. Any therapeutic measure taken several d after observing of the adverse effect would appear to be solving the problem.

A natural outbreak of aflatoxicosis in layers was reported by Hamilton (1971). A flock of 1000 hens was fed moldy corn containing approximately 100 ppm aflatoxin for 2 d and then received clean corn. Almost 50% of the hens died within one week while egg production of the survivors declined to 5%.

9.   AFLATOXICOSIS IN OTHER AVIAN SPECIES

Comparative toxicological studies in avian species have shown that ducklings and turkey poults are the most sensitive species to aflatoxin. Goslings, quails and pheasants show intermediate sensitivity while chickens appear to be the most resistant. Muller *et al*. (1970) fed graded levels of dietary aflatoxins B1 and G1 (0.5, 1, 2, and 4 ppm) to ducklings, goslings, pheasants and turkey poults for 4 wks. Goslings and pheasants showed a dose-related increase in mortality and decrease in weight gain. All ducklings and turkey poults receiving $\geq$ 1 ppm aflatoxin died during the trial and therefore were the most severely affected species.

Giambrone *et al*. (1985a) investigated the effect of dietary aflatoxin on turkeys. Levels $\geq$ 0.4 ppm were highly toxic and caused significant decrease in weight gain and feed conversion when administered for 5 wks. Microscopic lesions indicative of aflatoxicosis were evident at 0.1 ppm and significant decreases in cell-mediated immunity (CMI) were noted at 0.2 ppm. In a separate trial, two-week old turkeys receiving an equivalent dietary concentration of 0.5 or 1.0 ppm pure AFB1 for 5 wks showed 100% morbidity, mortality and gross and microscopic lesions. Levels of 0.2 ppm AFB1 caused none of these changes but caused a significant depression in feed conversion and CMI without affecting weight gain (Giambrone *et al*., 1985c). In contrast, Richard *et al*. (1987) fed turkeys a diet containing 0.05 or 0.15 ppm aflatoxin for 13 wks and no mortality or adverse effects on weight gain, feed conversion, or histopathology of selected organs were detected.

Hetzel *et al*. (1984) reported the mortality and post-mortem findings from flocks of Alabio, Bali, Tegal and Khaki Campbell ducks fed aflatoxin-contaminated diets (25-50 ppb) for 27 months. The average mortality for the 4 breeds was 37% and liver lesions were observed in 39% of these birds. Laying ducks were significantly more susceptible than were drakes. Some ducks had distended abdomens due to liver tumors and secondary ascites. Alabio ducks had a higher frequency of bile duct hyperplasia and bile duct carcinoma while the occurrence of hepatocellular carcinomas in Kakhi Campbell ducks was significantly higher than for other breeds. A decrease in egg production was observed in all flocks after 12 months lay. In experiments by Ostrowski-Meissner (1983), diets contaminated with *A. flavus* and containing $\geq$ 50 ppb B1 significantly reduced body weight gain and utilization of dietary protein in growing Alabio ducks. Those diets that caused liver lesions in the ducks had no effect on White Leghorn chickens receiving the same dietary levels of AFB1.

Chang and Hamilton (1982a) fed Japanese quail up to 20 ppm aflatoxin to 4 wks of age. Growth inhibition occurred at $\geq$ 5 ppm and 50% mortality was observed at 20 ppm. The acute oral LD$_{50}$ was determined to be 19.5 $\pm$ 4.8 mg/kg. The most sensitive indicators of aflatoxicosis were depressed serum proteins and serum carotenoids and enlarged liver and pancreas, all of which occurred at the smallest dose administered (1.25 ppm). In a previous experiment, laying Japanese

quail receiving 2, 4 or 6 ppm dietary aflatoxin had decreased feed conversion, egg production, egg weight, hatchability, and egg quality (Sawhney *et al*., 1973a). Aflatoxin at a dietary level of 5 ppm caused delayed reproductive development in juvenile Japanese quail (Doerr and Ottinger, 1980). Livers from 6 to 8-week-old Japanese quail intubated with 0.3 mg per kg body weight with mixed aflatoxins (B1, B2, G1, and G2) showed bile duct proliferation, cellular necrosis, vacuolation, congestion, fatty changes and mild hepatitis (Dashek *et al*., 1983). Ruff *et al*. (1992b) fed bobwhite and Japanese quail diets containing 1.25, 2.5 or 5 ppm aflatoxin for 3 wks. Body weights were significantly decreased by levels of 2.5 ppm or higher. No effect on feed conversion was observed but a dose-dependent mortality was observed in these bobwhite quails. Johri *et al*. (1990) investigated the effects of low levels dietary aflatoxin (≤0.75 ppm) in Japanese quail fed toxic diets for 100 d. Feed consumption and hatchability of fertile eggs were adversely affected by 0.3 ppm, whereas egg production, protein utilization and body weight were adversely affected by 0.5 and 0.75 ppm. At 0.75 ppm, fertility and serum total protein decreased, and serum AST and ALT activities increased. Egg weight, hemoglobin content, PCV, blood glucose and serum uric acid remained unaffected. Young male Japanese quail receiving a diet containing 10 ppm aflatoxin showed a delay in physiological and behavioral sexual maturation (Ottinger and Doerr, 1980).

Severe mortality was also observed in ringneck pheasants fed diets containing 2.5 or 5 ppm aflatoxin for 3 wks. Body weight gain and feed conversion of these pheasants were significantly affected by doses of ≥ 1.25 ppm aflatoxin (Huff *et al*., 1992).

10.   EFFECTS OF AFLATOXIN ON REPRODUCTION

In layers, dietary levels of 5 and 10 ppm aflatoxin caused decreased hatchability but did not affect fertility (Howarth and Wyatt, 1976). Levels of 0.6 and 1.8 ppm AFB1 caused decreased hatchability due to 0-6 d embryo mortality (Cottier *et al*., 1969). Hafez *et al*. (1982) fed laying hens and mature cocks diets containing 8.1 ppm AFB1 or 1.6 ppm AFG1 for 3 wks. The layers ceased egg production during the experiment and histological examination of the ovaries showed follicular atresia although no testicular lesions were seen in the males.

Aflatoxin does not appear to affect the semen characteristics of broiler breeder males although White Leghorn males appear to be more sensitive to aflatoxin. In one study, 20 ppm dietary aflatoxin administered for 4 wks to mature broiler breeder males did not alter spermatozoa counts, semen volume, or semen DNA, RNA or protein content (Briggs *et al*., 1974). In contrast, the same level of aflatoxin administered to mature White Leghorn males for 5 wks resulted in decreased semen volume and testes weight and a disruption of the germinal epithelium. A significant decrease in feed intake and body weight preceded the decline in semen volume. However, there was no effect on percent fertile eggs or percent hatch of fertile eggs from hens artificially inseminated with spermatozoa from treated males (Sharlin *et al*., 1980, 1981). In another trial, White Leghorn breeder males were fed diets containing 0, 10 or 20 ppm AFB1 for 8 wks. Body weight and feed intake were progressively reduced and the semen quality was adversely affected from week 3 onwards in the males receiving 20 ppm AFB1. Only 50% of these birds continued to produce semen in contrast to 90% of the males receiving 10 ppm AFB1. Histologically, the testes of the birds fed 20 ppm AFB1 had marked disruption of the germinal epithelium, debris-filled lumen, and spermatogenesis was absent (Jagadeesh *et al*., 1986). Histological evidence of adverse effects of aflatoxin on the germinal epithelium of the testes was reported in immature chickens dosed with 200 μg of aflatoxin/day/chick for 35 d (Mohiuddin, 1982). Clarke and Ottinger (1987) reported that

the luteinizing hormone secretory capacity of the anterior pituitary was not affected by aflatoxin in maturing male chickens, however, the testicular response to exogenous luteinizing hormone-releasing hormone was altered during aflatoxicosis.

11.   EFFECTS OF AFLATOXIN ON THE IMMUNE SYSTEM

Immunosuppression caused by AFB1 has been demonstrated in chickens and turkeys as well as in laboratory animals (Sharma, 1993). The exact mechanism of aflatoxin-induced immunosuppression is unknown. The adverse effects of aflatoxin on complement, interferon and serum proteins are presumably the result of liver injury and inhibition of protein synthesis. Pier and McLoughlin (1985) summarized the effects of aflatoxins on the animal immune system as follows: aflatoxins impair immunogenesis without suppressing antibody formation; aflatoxins suppress formation of non-specific humoral substances related to resistance and immunity (complement and interferon) and suppress phagocytosis by macrophages; aflatoxins cause thymic aplasia and suppress CMI, notably delayed cutaneous hypersensitivity; lymphoblastogenesis and leucocyte migration are also variably suppressed. In addition to the thymic aplasia, a reduction of 25-38% in the weight of the bursa of Fabricius has been observed in chickens receiving dietary aflatoxin (Virdi *et al*. 1989).

Aflatoxin decreases complement activity in chickens (Campbell *et al*., 1983; Stewart *et al*., 1985) and turkeys (Corrier, 1991). Antibody responses to *Pasteurella multocida*, *Salmonella pullorum* and Newcastle disease virus are normal in chickens and turkeys exposed to low levels of dietary aflatoxin (0.2-0.5 ppm) but higher levels (0.6-10 ppm) can suppress immunoglobulin (Ig) IgG or IgA and antibody response to *Salmonella* and sheep RBC's (Corrier, 1991). Edds *et al*. (1973) reported that chickens whether vaccinated or not against Marek's disease (MD) receiving a diet containing 0.2 ppm AFB1 were more susceptible to challenge inoculation with MD virus than were controls. Similarly, chickens receiving 0.5 ppm dietary AFB1 and vaccinated against MD showed a significantly higher frequency of gross and microscopical lesions of MD than did chickens receiving aflatoxin-free diets (Batra *et al*., 1991). The presence of low levels of AFB1 in the feed appears to decrease vaccinal immunity and may therefore lead to the occurrence of disease even in properly vaccinated flocks.

Cell-mediated immune responses and effector cell function are also affected during aflatoxicosis. T lymphocyte-mediated delayed hypersensitivity and graft versus host responses are suppressed in chickens exposed to aflatoxins. Impaired chemotaxis, phagocytosis and intracellular killing by heterophils and monocytes are also seen in chickens during aflatoxicosis. Cell-mediated responses appear to be affected by low levels of aflatoxin whereas high levels affect immunoglobulin production and antibody responses (Corrier, 1991). *In vitro* studies suggest that turkey macrophages are resistant to the direct exposure to AFB1 and that AFB1-induced alterations in macrophage effector functions occur after metabolic activation of AFB1 by microsomal enzymes (Neldon-Ortiz and Qureshi, 1991). The rate of clearance of colloidal carbon from the circulation (a measurement of the function of the reticuloendothelial system) was significantly decreased in chickens by 1.25 ppm dietary aflatoxin (Michael *et al*., 1973). Using a similar methodology, Mohiuddin *et al*. (1986) observed a depressed activity of heterophils and Kupffer cells in chickens receiving 20 ppm dietary aflatoxin. However, dietary levels of 2.5 ppm aflatoxin had no effect on the phagocytic activity of heterophils (Campbell *et al*., 1983). In contrast to the situation in mammals, the avian thrombocyte has been shown to be phagocytic in the blood and phagocytizes more

bacteria than the heterophil and monocyte combined. Although thrombocytic counts are depressed by dietary aflatoxin (Mohiuddin *et al*., 1986), their phagocytic activity is not affected by aflatoxin (Chang and Hamilton (1979b). However, other phagocytic cells (heterophils, macrophages and monocytes) were affected by low (0.625 ppm) dietary levels in an experiment by Chang and Hamilton (1979a). *In vitro* experiments have shown increased sensitivity to staphylococcal ß-hemolysins of erythrocytes from growing chickens exposed to 10 ppm dietary aflatoxin (Doerr *et al*. 1987) and impairment of macrophage-mediated functions in chicken embryos injected with AFB1 (Neldon-Ortiz and Qureshi, 1992). Chickens receiving aflatoxin-contaminated diets showed higher susceptibility to duodenal (*Eimeria acervulina*) (Southern *et al*., 1984) and cecal (*E. tenella*) coccidiosis (Edds *et al*., 1973) than chickens receiving aflatoxin-free diets. Similarly, turkey poults were more susceptible to cecal coccidiosis (*E. adenoeides*) when they received dietary aflatoxin (Witlock *et al*., 1982). The adverse effect of AFB1 on cell-mediated immunity could partially explain the high susceptibility and mortality from cecal coccidiosis that is observed in birds fed aflatoxin-contaminated diets.

12.   __INTERACTIONS__

Under field conditions many different factors can interact deleteriously with aflatoxin. Some of these factors include presence of other mycotoxins in the feed, level and sources of dietary protein and lipids, disease status and vitamin deficiencies. The dose-response relationships of all these factors need to be studied before rational and justifiable statements can be made about safe levels (Richardson *et al*., 1987). These authors observed that the apparent minimum effective dose of dietary aflatoxin associated with acceptable growth rate in chickens can vary from 1.21 to 8 ppm depending on dietary protein level. It appears that the only way to gain a practical understanding of aflatoxicosis is by duplicating in the laboratory the many factors interacting in field conditions.

12.1.   *Interactions with other mycotoxins*

A toxic synergism between aflatoxin and ochratoxin A (Huff and Doerr, 1981; Huff et al., 1984), and aflatoxin and T-2 toxin (Huff *et al*., 1986) has been reported to occur in chickens exposed to a combination of toxins as compared with those exposed to a single toxin. Additive toxic interaction was reported to occur between aflatoxin and deoxynivalenol (Huff *et al*., 1986) and aflatoxin and cyclopiazonic acid (Smith *et al*., 1992). In contrast, when the individual and combined toxicity of kojic acid and aflatoxin was investigated in broilers, no additive or synergistic interactions were observed (Giroir *et al*., 1991).

12.2.   *Interactions with fungal infections*

Concomitant occurrence of aflatoxicosis and pulmonary aspergillosis has been reported in ducklings (Rao *et al*., 1985) and chickens (Shoyinka and Onyekweodiri, 1987). A flock of 400 Kakhi Campbell ducklings receiving a diet containing 280 ppb aflatoxin developed aspergillosis and 50% of the birds died (Rao *et al*., 1985). Similarly, pullets receiving aflatoxin-contaminated feed developed clinical signs and histopathological lesions of aflatoxicosis and were simultaneously affected by aspergillosis, with a mortality of more than 50% (Shoyinka and Onyekweodiri, 1987). In both outbreaks, aflatoxin-induced immunosuppression was considered to be a predisposing factor

in the presentation of aspergillosis. At this point it is important to emphasize that, although *A. flavus* is able to cause pulmonary aspergillosis in poultry (Richard, 1991), the fungus does not seem able to synthesize aflatoxin when it is growing in the birds tissues. Richard *et al*. (1981) exposed turkey poults to aerosols containing spores of aflatoxigenic strains of *A. flavus*. The fungus caused pulmonary aspergillosis but no aflatoxins were detected in tissues and no lesions of aflatoxicosis were seen in sections from the livers. It is believed that the potential of aflatoxigenic fungi to produce aflatoxins during aspergillosis is remote because the molds are incapable of aflatoxin production at 42°C, the normal body temperature in birds (Kahn *et al*., 1978).

12.3.   *Interactions with dietary nutrients*

The interactions of several nutrients (protein, fat, vitamins, selenium) with aflatoxin in experimental animals have been reviewed by Newberne (1987). In rats and Rhesus monkeys the effect of aflatoxin is enhanced by feeding severely protein-deficient diets as measured by incidence of liver tumors and mortality rate. In male rats, the carcinogenicity of AFB1 is enhanced by a diet marginal in methionine or choline, deficient in folate or high in fat. The source of dietary fat also influences the response of rat liver to aflatoxin. Corn oil resulted in a significant enhancement of liver tumors and microsomal enzyme activity when compared with feeding tallow. Severe liver damage was observed in vitamin A-deficient, AFB1-treated rats, while minimal liver lesions were seen in rats given adequate levels of vitamin A. Further, a single oral dose of AFB1 given to rats at 7 mg/kg body weight was less toxic in animals fed a high selenium diet than in animals fed diets adequate or marginal in selenium (Newberne, 1987).

The source and level of dietary protein affects the response of birds to aflatoxin. Ducks fed isonitrogenous diets containing soybean meal or peanut meal were more affected by the same concentration of dietary aflatoxin than those fed diets with fish meal (Ostrowski-Meissner, 1983). In another study, body weight, proportional liver weight, and liver lipids were more adversely affected by aflatoxin in chickens receiving a 10% protein diet than in chickens fed a 12.75% protein diet (Richardson *et al*., 1987). Laying hen diets supplemented with the amino acids cystine (Diaz *et al*., 1994) or tryptophan (Rogers *et al*., 1990) exhibited a significant reduction in total liver lipids; however, when tryptophan was supplemented to a diet containing 10 ppm aflatoxin, an increase in the severity of the lesions associated with aflatoxicosis was observed.

The effect of aflatoxin in poultry is greater in birds fed a low fat diet, but the reason for this response is unknown. In turkey poults the effect of 1.0 ppm dietary aflatoxin on growth rate was similar when feeding diets containing 2, 6 or 18% fat; however, a diet with 18% fat ameliorated the lethal effect of aflatoxin and restored the relative bursal weight to normal (Hamilton *et al*., 1972). In another experiment, liver lipid and bursal weight of growing chickens were more adversely affected by aflatoxin when the diet contained 2% fat compared with a diet containing 4% fat (Richardson *et al*., 1987).

The interaction of aflatoxin with vitamins has not been clearly established in poultry. Feeding trials showed that supplementing a diet with 4 times the NRC recommendations for vitamins afforded no protection against the adverse effect of aflatoxin on chicken growth (Hamilton *et al*., 1974). However, when the combined effect of vitamin deficiencies and aflatoxin were investigated, various interesting responses were observed. Diets deficient in riboflavin or cholecalciferol made chickens sensitive to levels of aflatoxin normally too small to affect their growth rate while using vitamin E and K_3 deficient-diets had no effect on aflatoxicosis as measured by growth rate. A

thiamine deficiency on the other hand had a protective effect against the growth inhibitory effect of dietary aflatoxin. This protective effect was thought to be the result of the stimulation of fatty acid oxidation caused by the thiamine deficiency since aflatoxin inhibits the transport of fat from the liver (Hamilton et al., 1974).

13.   TREATMENT, PREVENTION AND DETOXIFICATION

13.1.   Dietary treatments for aflatoxicosis

Aflatoxin contamination of feeds and feedstuffs is virtually inevitable, particularly in tropical areas of the world where environmental temperature and humidity favor the development of *Aspergillus* fungi and their production of mycotoxins. For this reason, several strategies have been developed in order to minimize the adverse effects of aflatoxins on livestock and to prevent human exposure. Strategies developed to control aflatoxicosis include the use of dietary supplements, prevention through genetic selection, and detoxification of aflatoxin-contaminated feeds and feedstuffs by either physical or chemical methods.

Dietary supplementation with natural and synthetic antioxidants, lipid and water soluble vitamins, selenium, sulfur amino acids, the tripeptide glutathione (γ-L-glutamyl-L-cysteinyl-glycine), microsomal enzyme inducers, and antibiotics has been used to counteract the adverse effects of aflatoxin in poultry. Increasing the dietary level of crude protein is also considered to have a protective effect against aflatoxin. Raising crude protein by 3% and supplementation of additional levels of riboflavin, pyridoxine, folic acid and choline protected laying Japanese quails from the performance-depressing effects of 0.75 ppm aflatoxin (Johri *et al*., 1990). Although some of these treatments have shown positive results in controlled experiments, the economic impact under field conditions needs careful study.

The addition of 3 or 8 times the normal level of the synthetic antioxidant butylated hydroxytoluene (BHT), to broiler diets containing 1.0 or 3.0 ppm aflatoxin gave significant protection against the adverse effect of the toxin on chicken growth (Larsen *et al*., 1985; Ehrich *et al*., 1986). However another synthetic antioxidant, did not show any protection against aflatoxin (Ehrich *et al*., 1986). Vitamin C (ascorbic acid), supplemented at dietary levels of 150 or 300 ppm, failed to prevent the adverse effects caused by 1.25 to 5.0 ppm aflatoxin in the diet of broilers (Huff *et al*., 1987). Studies *in vitro* with mammalian hepatocytes showed that vitamin A inhibits the formation of AFB1 adducts in a dose-dependent manner. Ascorbyl palmitate, a synthetic lipophilic derivative of ascorbic acid, reduced adduct levels at low AFB1 concentrations but had no effect at high levels. Interestingly, β-carotene and vitamin E, which are known liposoluble free radical scavengers, enhanced covalent binding of AFB1 to DNA (Yu *et al*., 1994).

Phenobarbital, a known cytochrome P-450 enzyme inducer, has been administered to aflatoxin-exposed animals in order to accelerate the catabolism and excretion of the toxin and thus alter the duration and intensity of any toxic effects. In experiments with Rhesus monkeys, phenobarbital pretreatment reduced plasma levels of total aflatoxins (B1, M1, and water soluble metabolites) and enhanced the circulation and elimination of less toxic, less mutagenic, and more soluble urinary products (Wong *et al*., 1981). Broiler chickens administered phenobarbital intermittently in the drinking water showed a substantial increase in cytochrome P-450 content and

in the activity of benzphetamine N-demethylase, enhancing the metabolism and elimination of absorbed AFB1. An improvement in feed consumption, weight gain, and microsomal enzyme activity and a decrease in AST values, reflecting on normal liver functions, were noted in chickens receiving phenobarbital (0.05%) and graded levels of AFB1 (0, 2.5, 5 or 10 ppm) for 8 wks (Dalvi and McGowan, 1984).

The tripeptide glutathione (GSH) plays a key role in the cellular defense against injuries by oxidants and free radicals and also in the detoxification of electrophilic xenobiotics. However, when GSH or its precursor amino acid, cystine, are supplemented in the diet at 500 ppm, mild protection against AFB1-induced growth depression was observed in broilers receiving a diet containing 10 ppm AFB1 (Dalvi and Ademoyero, 1984). The lack of response to GSH or cystine supplementation suggests that GSH-dependent detoxification is not important in aflatoxicosis. Studies *in vitro* using rat hepatocytes showed that GSH-dependent detoxification mechanisms do not play a major role in removing necrogenic metabolites of AFB1 and that prelethal responses of AFB1-injured hepatocytes are not affected by GSH-dependent cytoprotective mechanisms (Hayes *et al*., 1986).

Dietary supplementation with selenium has protected against the adverse effect of dietary aflatoxin on plasma protein levels and liver weight in turkey poults (Burguera *et al*., 1983). In another trial, turkey poults receiving diets containing 500 ppb AFB1 were supplemented with up to 4 ppm Se as selenite (Gregory and Edds, 1984). The proportion of liver aflatoxins in conjugated forms increased and the ratio of free aflatoxin B1/M1 decreased with increasing dietary selenium concentrations. The results indicated that selenium enhances aflatoxin detoxification processes *in vivo*.

Picroliv, a standardized iridoid glycoside fraction of *Picrorhiza kurroa*, has been shown to protect against the hepatotoxic action of a single oral dose of 7 mg AFB1/kg in rats (Dwivedi *et al*., 1993). Picroliv was administered at a dose of 25 mg/kg for 7 d, where most biochemical and histological liver parameters were essentially unchanged. Dimethylsulfoxide at dietary levels of 0.5-2.0 ppm had no protective action against the decreased growth, feed conversion and livability of young broilers exposed to 10 ppm dietary aflatoxin (Yen and Hamilton, 1982).

13.2. *Prevention through genetic selection*

The development of genetic strains resistant to aflatoxin was proposed by Smith and Hamilton (1970) after observing that six inbred strains of chickens had marked differences in their susceptibility to aflatoxin. Gumbmann *et al*. (1970) investigated the comparative aflatoxin susceptibility of 18 different strains, crosses, or breeds of chickens, turkeys and quail. Chicks were fed a diet containing 800 ppb AFB1 for periods of 2 to 6 wks and were evaluated for sensitive indicators of aflatoxicosis such as plasma albumin levels, and liver succinic dehydrogenase and DNA levels. New Hampshire chickens and turkey poults were the most severely affected by AFB1. When New Hampshire hens were crossed with Leghorn males or vice versa, the sensitivity of the chicks to aflatoxin was no longer detectable, suggesting that the susceptibility relates to genetic makeup. Birds showing considerable resistance to B1 included Barred Rock and Austrolop chickens and also guinea fowl (Gumbmann *et al*., 1970).

Selection for genetic resistance has been carried out by breeding survivors from a population that had received a single oral dose of aflatoxin that resulted in high mortality (Marks and Wyatt, 1979; Wyatt *et al*., 1987) and by selection of chickens for high and low plasma protein

concentrations in response to AFB1 (Scott *et al*., 1991). In chickens selected by resistance to mortality after a single oral dose, LD_{50} values of 9.42 and 17.05 mg AFB1/kg were determined after five generations for nonselected and selected lines, respectively (Wyatt *et al*., 1987). However, the progress for resistance varied considerably among the different populations of birds tested. Pegram *et al*. (1985, 1986) evaluated the comparative responses of genetically resistant and nonselected Japanese quail to dietary aflatoxin. The selected lines showed significantly lower mortality and significantly higher growth rates than the nonselected line. In addition, resistant quails showed varying degrees of resistance to the following aflatoxin-induced effects: liver lipid accumulation and enlargement, hepatic protein depletion, elevated hepatic β-glucuronidase activity, bursal regression, and hypoproteinemia (Pegram *et al*., 1985). Such resistance was not related to differential intestinal absorption of the toxin but rather to rapid elimination of AFB1 *via* hepatobiliary excretion (Pegram *et al*., 1986).

13.3.   *Detoxification of aflatoxin-contaminated feeds and feedstuffs*

Decontamination procedures have focused on degrading, destroying, inactivating, or removing aflatoxin from commodities by physical, chemical or biological methods. A successful detoxification process must be economical, must be capable of eliminating all traces of toxin without leaving harmful residues and must not impair the nutritional quality of the commodity. The decontamination strategies for aflatoxin-contaminated feeds and ingredients have been reviewed by Ellis *et al*. (1991).

13.3.1. Detoxification by physical methods

Physical methods of decontamination include extraction with organic solvents, heat inactivation, irradiation, and adsorption. This later process has received considerable attention in the past few years.

Organic solvent extraction

Several solvents or mixtures of solvents can effectively extract aflatoxins from contaminated commodities with minimal effects on protein content or nutritional quality. These include 95% ethanol, 90% aqueous acetone, 80% isopropanol, hexane-ethanol, hexane-methanol and hexane-acetone-water. However, current extraction technology is still impractical and cost-prohibitive (Park and Liang, 1993). Johnson *et al*., (1986) studied the feasibility of using methylene chloride (CH_2Cl_2) to extract oil, aflatoxin and gossypol simultaneously from cottonseed flakes; aflatoxin content was reduced by 73-92% and no residual aflatoxin was detected in the oil after it was refined with alkali.

Heat inactivation

Aflatoxins are very resistant to thermal inactivation and therefore the procedures based on detoxification by heat (boiling water, autoclaving, roasting, baking) result in little change in aflatoxin levels (Park and Liang, 1993). However, Conway *et al*. (1978) reported that roasting significantly reduced the concentration of aflatoxin in peanuts and pecans and that levels of AFB1 have been reduced by 50-70%, depending on the initial levels and on the type and temperature of roasting.

Irradiation

This has been shown to result in a marked reduction in the concentration of aflatoxins in contaminated products. Peanut oil exposed to short-wave and long-wave ultraviolet (UV) light showed a significant reduction in aflatoxin levels, and a 14-hour exposure to sunlight destroyed about 50% of AFB1 present in naturally contaminated peanut flakes (Park and Liang, 1993). Destruction of aflatoxins in coconut and groundnut oils by solar irradiation constitutes a potential application by small scale oil press mills in rural areas of underdeveloped countries (Scott, 1989).

Adsorbents

These are compounds that are not absorbed from the GIT and have the ability to bind physically with chemical substances, precluding their absorption. Adsorption therapy has been one of the most important methods of preventing the absorption of ingested toxicants in the GIT, however, adsorbents will also bind concurrently administered drugs used for therapeutic purposes. The use of adsorbents such as activated charcoal and silicates have been extensively studied in livestock exposed to dietary aflatoxins.

Activated charcoal

Activated charcoal is the residue from destructive distillation of vegetable origin organic matter. It is porous, low in ash content, and high in surface area. The efficacy of adsorption is not affected by the pH of the toxicant and adsorbed material is usually retained through the entire GIT (Osweiller *et al*., 1985). Activated charcoal is usually administered at 20-120 mg/kg to domestic animals. The addition of 200 ppm of activated charcoal to a broiler diet containing 500 ppb AFB1 was found to be moderately effective in reducing the adverse effects of aflatoxin as assessed by growth response and various biochemical parameters. Activated charcoal reduced the adverse effect of AFB1 on body weight and feed intake and caused a significant improvement in serum levels of AST, AP, total proteins, calcium and phosphorus levels, although no significant improvement on cholesterol levels was observed (Jindal *et al*., 1994). Simultaneous administration of charcoal (0.5 g/kg as 5% slurry) with an oral dose of 4.0 mg/kg AFB1 given to broiler chickens had a protective effect against the toxin (Rao and Joshi, 1993). Chickens receiving the charcoal had significantly lower levels of serum AST and bilirubin than did control chickens, 96 h after the administration of the toxin. Administration of 0.1% activated charcoal to broiler diets containing 0, 2.5, 5.0 or 10.0 ppm AFB1 proved to be moderately effective in improving feed consumption and weight gain and in protecting birds from AFB1-induced liver injury manifested by decreased activity of microsomal enzymes and increased AST levels (Dalvi and McGowan, 1984). Other studies, however, have reported that charcoal has only minor benefits when added to aflatoxin-contaminated poultry diets (Dalvi and Ademoyero, 1984).

Hydrated sodium calcium aluminosilicate (HSCAS)

Currently available as an anticaking agent for animal feeds, HSCAS, (a phyllosilicate clay) has been used to reduce the levels of bioavailable aflatoxins by selective chemisorption. HSCAS has been reported to remove aflatoxin from aqueous suspensions and to significantly reduce the uptake and distribution of aflatoxin in biological systems, prevent aflatoxicosis in domestic animals and reduce aflatoxin M1 residues in milk from lactating cows exposed to aflatoxin-contaminated diets (Park and Liang, 1993). Addition of HSCAS to the diets of growing broiler chicks (Kubena *et al*., 1990), turkey poults (Kubena *et al*., 1991), pigs (Lindemann *et al*., 1993), and lambs (Harvey *et al*., 1991) has resulted in a significant reduction in growth suppression normally associated with aflatoxicosis.

The addition of HSCAS at 0.5% to broiler diets contaminated with mycotoxins has protected from the toxic effects of aflatoxin but not from the toxicity of trichothecenes or ochratoxin A. The HSCAS, when added to the diet of Leghorn and broiler chickens significantly diminished the adverse effects of feeding 7.5 mg AFB1/kg of feed; the addition of HSCAS significantly decreased the growth inhibitory effect of the toxin and showed protective effect on gross hepatic changes produced by AFB1 (Phillips *et al*., 1988). The authors concluded that HSCAS can modulate the toxicity of AFB1 in chickens, perhaps via sequestration and reduced bioavailability *in vivo*. Kubena *et al*. (1990) investigated the effect of adding 0.5% HSCAS to broiler diets containing 3.5 ppm aflatoxin and/or 8.0 ppm T-2 toxin. The addition of HSCAS resulted in almost total protection against the effects of aflatoxin alone, limited protection against the combination, but no protection against T-2 toxin alone. In a similar trial, HSCAS at 0.5% was incorporated into diets containing 3.5 ppm aflatoxin and 5.0 ppm DAS, singly and in combination (Kubena *et al*., 1993). The addition of HSCAS diminished the toxicity of aflatoxin for many parameters but did not alter the toxicity of DAS. Addition of HSCAS to the aflatoxin plus DAS combination diet diminished some of the effects of the toxin combination. When HSCAS was tested for aflatoxin (3.5 ppm) and/or ochratoxin A (2 ppm) it reduced the toxicity of aflatoxin, but had little effect on either the toxicity of ochratoxin A alone or the toxicity resulting from the combination of aflatoxin and ochratoxin A (Huff *et al*., 1992). Kubena *et al*. (1991) evaluated the effects of adding 0.5% HSCAS to diets containing 0.5 or 1.0 ppm aflatoxin that were fed to turkey poults up to 3 wks of age. Mortality was 88% in poults receiving 1.0 ppm aflatoxin without HSCAS, while the addition of HSCAS reduced mortality to 28%. The HSCAS also diminished the adverse effects of aflatoxin on body weight, most relative organ weights, hematological values, serum biochemical values, and enzyme activities associated with 0.5 ppm aflatoxin but not with 1 ppm aflatoxin.

Other adsorbents

Silty clay loam soil added at 25% to a ration contaminated with 4.5 ppm aflatoxin reduced the residue concentrations of aflatoxins AFB1 and AFM1 in the livers of chicks fed the contaminated ration for 9 d (Madden and Stahr, 1992). Harvey *et al*. (1993) evaluated the ability of commercially available zeolitic ore compounds to reduce the deleterious effects of 3.5 ppm dietary aflatoxin on young growing broiler chickens. Weight-gain was improved by 29-41%. Sodium bentonite (clay) at 0.5% has been shown to be effective in reducing the toxicity of diets containing 0.8 ppm aflatoxin in growing swine, as evidence by the improvement in average daily gain, average daily feed intake, and serum clinical chemistry parameters usually affected by aflatoxin (Lindemann *et al*., 1993).

13.3.2. Detoxification by chemical methods

Ammonia or ammonia-related compounds appear to have the most practical application for the decontamination of aflatoxin in agricultural commodities. Ammoniation of aflatoxin-contaminated corn, cottonseed, cottonseed meal, and peanut meal has been shown to reduce the aflatoxin levels in these commodities by greater than 99% (Park, 1993b). Mexico has approved the procedure for corn and ammoniation of peanut meal is routinely used in France, South Africa, Senegal, Brazil and Sudan. Several members of the European Economic Community regularly import ammonia-treated peanut meal (Park, 1993b). Thiesen (1977) described a practical ammoniation method for groundnut meal to be used in compounded rations for ruminants where NPN (ammonia) is of value as a nutrient; a detoxification above 99% is obtained when groundnut meal is stored with 5% NH_3 and 20% water for 10 d in tight plastic bags. Currently, the first step in the ammoniation procedure consists of rehydration of the grain to 12-16% moisture. Either anhydrous ammonia (gas) or ammonium hydroxide (aqua-ammonia) can be used. Primarily, two procedures are used, namely, a high pressure and temperature process (HP/HT) used at feed mills or an atmospheric pressure and ambient temperature procedure (AP/AT) that can be used on farm (Park, 1993a). The HP/HT process lasts for 20-60 min and uses ammonia levels of 0.2-2%, pressures of 35-50 psi, and temperatures of 80-120°C. The AP/AT process uses higher levels of ammonia (1-5%) and must be applied for 2-3 wks (Park, 1993a). Because ammonia is highly volatile, corrosive, and less dense than air, the grain to be treated must be sealed in an air-tight container. During the ammoniation procedure, hydrated ammonia attacks the AFB1 lactone ring followed by decarboxylation to chemically inactivate the aflatoxin molecule (Cole, 1989). Dietary aflatoxin inactivated by ammoniation for layer-breeders had no detrimental effect on the immunological response elicited by Newcastle disease vaccination as measured by HI titers (Boulton *et al*., 1982).

Other chemical methods of detoxification of aflatoxin and substances capable of inhibiting aflatoxin production by *A. flavus* have been tested. Hydrogen peroxide (H_2O_2) is an oxidizing agent acceptable in foods and has the potential to destroy up to 97% of aflatoxins (Ellis *et al*., 1991). Peanut kernels submerged in 0.075, 0.150, or 0.25% hydrogen peroxide showed a reduction in aflatoxin content of 90% within 1 min, regardless of the initial aflatoxin content (Clavero *et al*., 1993). Treatment of maize with organic acids (isobutyric and propionic-acetic acid) has reduced the infection with *Aspergillus flavus* fungi and decreased aflatoxin production after 6 months of storage (Bothast et al., 1976). Rodriguez and Mahoney (1994) investigated the effect of 12 surfactants on aflatoxin production by *A. flavus*. Five nonionic surfactants (Triton X-100, Tergitol NP-7, Tergitol NP-10, polyoxyethylene 10 lauryl ether, and Latron AG-98) reduced aflatoxin production by 99% when added at a concentration of 1% (wt/vol) to the substrate.

Chapter 14

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CHAPTER 15.   FUMONISINS

Other names:	NONE
Species:	ALL POULTRY

1.   COMPENDIUM

Fumonisins are the most recently discovered group of mycotoxins. Fumonisins are produced by the fungus *Fusarium moniliforme* Sheldon, fumonisin B1 (FB1) being the most predominant molecular form produced by the fungus. Since their identification, fumonisins have been associated with previously known animal diseases such as equine leucoencephalomalacia (ELEM) and porcine pulmonary edema (PPE). The only commodities in which fumonisins have been detected so far are corn and corn-based animal feeds and human foods. The mechanism of action of fumonisins appears to be a disruption of the synthesis of sphingolipids. Exposure to toxic levels of FB1 results in an increase in the serum levels of sphinganine and sphingosine, while serum levels of complex sphingolipids are decreased. Even though FB1 is highly toxic for swine and equine species, it has very low toxicity for poultry. Recent research has shown that pure FB1 at dietary levels up to 80 ppm has no adverse effect on performance of growing broiler chickens.

2.   CHEMISTRY AND NATURAL OCCURRENCE

Six different fumonisins have so far been isolated and identified, namely fumonisins A1, A2, B1, B2, B3, and B4 (*Figure 15.1*). Fumonisins of the A series are amides, while those of the B series have a free amine. Differing hydroxyl substitutions account for different fumonisins within each series. The most predominant molecular form produced by *Fusarium moniliforme* strains is fumonisin B1 (FB1) (Norred, 1993). The structures of FB1, FB2, FA1 and FA2 were first reported by Bezuidenhout *et al*. (1988). Recently, two other fumonisins, B3 and B4, were isolated from *F. moniliforme* cultures and their structures were elucidated (Cawood *et al*., 1991; Plattner *et al*., 1992). Apart from reports of FB1 and FB2 in "black oats" from Brazil and in New Zealand forage grass, the only commodities in which fumonisins have been detected so far are corn and corn-based animal feeds and human foods (Scott, 1993). Fumonisins were first isolated from cultures of the fungus *F. moniliforme*; however, other *Fusarium spp* have been found to be producers of fumonisins including *F. proliferatum* (Ross *et al*., 1990), *F. nygamai* (Thiel *et al*., 1992), *F. anthophilum, F. dlamini*, and *F. napiforme* (Nelson, 1992). Recently, a fungus of the *Alternaria spp* was also shown to produce FB1 (Chen *et al*., 1992).

Fumonisins are strongly polar compounds. They are soluble in water, more soluble in acetonitrile-water or methanol and insoluble in organic solvents. Hydrolysis of fumonisins by heating with 6M hydrochloric acid or 0.05-2M potassium hydroxide produces tricarballylic acid (1,2,3-propanetricarboxylic acid) and the corresponding aminopolyol (Scott, 1993).

Although isolation and structural elucidation of six fumonisins has been reported, only fumonisins B1, B2, and B3 have been detected in naturally contaminated corn (Henry and Wyatt, 1993). Corn-based food and feeds from different countries have been shown to contain FB1 at levels ranging from 0.055 to 5.0 μg/g (ppm), and usually less than one third of the samples tested are positive for the presence of fumonisins. Levels of FB2 and FB3 are much lower than those of FB1. A recent survey of occurrence of FB1 and FB2 in corn-based food and feed products purchased in Switzerland (Pittet *et al*., 1992) showed that 36.7% of the 120 samples analyzed contained detectable levels of FB1 (0.055-0.79 μg/g) while only 12.5% were found positive for FB2 (0.05-0.16 μg/g). Levels of fumonisins ranging between 1.6 and 10 μg/g were found in 17 samples of Argentinean field-trial corn (Sydenham *et al*., 1993). Using a thin layer chromatography (TLC) method, levels of 0.1-5.0 μg/g FB1 were found in 15% of 193 samples of corn in the USA (Rottinghaus *et al*., 1992). In another study, samples of USA corn were found to contain levels of 0.38-2.8 μg/g FB1 with an average value of 1.22 μg/g (Holcomb *et al*., 1993). Average fumonisin content of Iowa, Wisconsin and Illinois corn from the 1988-1991 crop years was found to be 2.9, 0.8 and 0.3 μg/g for FB1, FB2 and FB3, respectively (Murphy *et al*., 1993). In another survey, (Bauer and Binder, 1993) FB1 was detected in 7 of 50 samples of German maize at a mean concentration of 0.37 μg/g (range 0.28-0.64 μg/g). In contrast with these figures, the fumonisin levels in feeds associated with episodes of equine leucoencephalomalacia (ELEM) and porcine pulmonary edema (PPE) have been found to be about two orders of magnitude higher. For example, feeds associated with ELEM contained FB1 levels ranging from 1 μg/g to 126 μg/g while those associated with toxicosis in swine ranged from 1 μg/g to 330 μg/g (Ross *et al*., 1991). Wilson *et al*. (1990) found FB1 levels of 37-122 μg/g in samples of corn screenings associated with ELEM that resulted in very high mortality. Ross *et al*. (1992) suggested that concentrations $\geq$10 μg/g FB1 in horse feed were likely to be involved in ELEM. In 40 of 45 confirmed cases of ELEM, FB1 levels were $\geq$10 μg/g while non-problem feeds contained <10 μg/g FB1.

3.   <u>MECHANISM OF ACTION OF FUMONISINS</u>

Wang *et al*. (1991) proposed a possible mechanism of action for the biological effects of fumonisins. The authors theorized that FB1 might interfere in some way with the biosynthesis of sphingolipids or sphingosine turnover because of the similarity of the polyhydric alcohol moiety of FB1 to the complex amino alcohol sphingosine, which is the backbone of the sphingolipids (*Figure 15.2*). Sphingomyelins (phosphosphingolipids) contain a fatty acid, phosphate, choline, and sphingosine. Glycosphingolipids are glycolipids that contain the sphingosine-fatty acid combination "ceramide", combined with one or more sugar residues. Sphingolipids (phosphosphingolipids and glycosphingolipids), are important for cell membrane integrity, intercellular communication and contact, and physiological activity. Sphingolipids are found in large quantities in brain and nerve tissue. For instance, the glycosphingolipid galactosyl-ceramide is the major lipid constituent of myelin, which is a membrane constituent of oligodendrocytes and Schwann cells, in the central and peripheral nervous system, respectively. Sphingosine is synthesized in the endoplasmic reticulum. The combination of palmitoyl-CoA and the amino acid serine forms 3-ketosphinganine, which is then reduced to sphinganine, and further reduced to form sphingosine. Ceramide is formed by a

combination of either a free fatty acid or an acyl-CoA and sphingosine. Sphingomyelin is formed when ceramide reacts with either CDP-choline or phosphatidylcholine (Mayes, 1988).

To test the hypothesis that fumonisins act by alteration of sphingolipid biosynthesis, Wang *et al*. (1991) examined the effect of FB1 on the ability of rat hepatocytes to convert [^{14}C]serine to labelled sphingolipids. Incorporation of the label was inhibited by FB1, with an IC_{50} of 0.1 μM. A similar degree of inhibition was obtained with FB2. Therefore, fumonisins appeared to have a potent action against sphingolipid biosynthesis at concentrations that would conceivably be reached or exceeded by the consumption of naturally contaminated corn. The specific site of action of FB1 appears to be the enzymes sphinganine- and sphingosine-*N*-acyltransferases (*Figure 15.3*). The structural basis for this inhibition is unknown. It has been postulated that the similarities between fumonisins and long-chain (sphingoid) bases allow them to be recognized as substrate (transition state, or product analogs) of sphingosine- or sphinganine-*N*-acetyltransferase. Disruption of this pathway could explain at least some of the pathological effects of fumonisins. The degeneration of neuronal cells seen in ELEM may be due to inhibition of sphingolipid biosynthesis because of their high concentration in the brain. On the other hand, accumulation of sphinganine in cells exposed to fumonisins may lead to cell death (since long-chain bases are highly cytotoxic), or to cell proliferation, since these compounds are mitogenic for some cell types (Wang *et al*., 1991).

The alteration in sphingolipid metabolism caused by fumonisins can be monitored by measuring serum levels of sphingosine, sphinganine, and complex sphingolipids. Wang *et al*. (1992) measured these parameters in ponies fed a diet containing 44 ppm FB1. Results indicated that both sphinganine and sphingosine, and especially their ratio, increased after fumonisin exposure, while serum levels of complex sphingolipids decreased. Because alteration in the ratio sphinganine:sphingosine was observed before the elevation in serum liver enzymes, the authors proposed the use of this ratio as an early indicator of fumonisin toxicity.

4. FUMONISIN TOXICOSIS IN POULTRY

Studies on the toxic effect of fumonisins for avian species have been conducted primarily using *F. moniliforme* culture material (FCM) as the source of fumonisin. Usually, a noncontaminated poultry ration is mixed with FCM containing known amounts of fumonisins in order to achieve the desired dietary mycotoxin concentration. Preparation of FCM starts with the incubation of a fumonisin-producing *F. moniliforme* fungal strain in sterile corn for 5 wk (Weibking *et al*., 1993ab). After this period, the substrate is blended with an organic solvent mixture (acetone:chloroform, 75:25), filtered, and the solvent discarded. The solid residue is re-extracted with an identical solvent mixture, refiltered, dried, and ground to a fine powder. This culture material is then analyzed for fumonisins and incorporated into the experimental diets. The average concentration of FB1 in FCM is usually very low, comprising only about 2 mg/g (0.2%) or less of the total FCM. Therefore, in order to achieve the required toxic dietary concentrations, significant amounts of FCM need to be added, and in most cases FCM

constitutes more than 10% of the experimental diet (Weibking *et al.*, 1993a). Because the concentration of FB1 in FCM is usually around 0.2%, there is a large proportion of the culture material (≥99.8%) which may be the source of unknown *Fusarium* metabolites that may be responsible for confounding effects. Norred *et al*. (1991) reported the presence of yet unidentified cytotoxic water soluble metabolites in *F. moniliforme* cultures. These compounds may be playing an important role in the wide variety of clinical signs and lesions observed in birds fed diets containing FCM. Furthermore, studies on the toxic effects of fumonisin in poultry have been done using relatively high dietary levels (20-525 mg/kg). As the levels of FB1 in corn normally range from 0 to 5 ppm (Pittet *et al*., 1992; Rottinghaus *et al*., 1992; Holcomb *et al*., 1993a; Murphy *et al*., 1993) and corn is usually added as 50% of the poultry ration, levels of 2.5 mg/kg FB1 can be expected in poultry feeds. In one study, only 6 of 22 samples of poultry feed were positive to FB1 and only 2 of 22 had detectable FB2 levels. Values ranged from 0 to 0.48 ppm FB1, with an average content of 0.235 ppm FB1 in positive samples (Pittet *et al*., 1992).

In an early trial, FCM was incorporated into corn-based diets at 12.5, 25, or 50% (Engelhardt *et al*., 1989) and fed to day-old chicks, ducklings, and turkey poults for two wk. All experimental diets caused a high mortality that ranged from 80 to 100%. The few survivors had an average body weight 50-75% lower than their respective controls. Gross lesions were similar in all three species and included ascites, hydropericardium, and myocardial pallor. Oral ulcerations were present in some turkey poults. Histologically, myofibrillar degeneration and necrosis were seen in the heart while the liver showed multifocal vacuolation and swelling of hepatocytes with foci of hepatocellular necrosis. It is important to note that the FCM used in this trial was not extracted with organic solvents before drying and grinding. This FCM was only tested for moniliformin, because no methods for fumonisin analysis had been reported at that time. Moniliformin was present at a concentration of 1.15 mg/g. Since *F. moniliforme* is not only able to synthesize fumonisins but also moniliformin, fusarins, fusaric acid, and a number of unknown cytotoxic metabolites (Marasas, 1991; Norred, 1991) the results from this experiment are difficult to interpret.

Ledoux *et al*. (1992) fed day-old chicks diets containing FCM that supplied 0, 100, 200, 300, or 400 ppm FB1, for 21 d. Body weights and average daily gain decreased with increasing dietary FB1, and liver, proventriculus and gizzard weights increased. Histopathological lesions in chicks receiving FB1 included thymic cortical atrophy, multifocal hepatic necrosis, biliary hyperplasia and widening of the proliferating cartilage zone in proximal tibiotarsal physes from some chickens receiving 200 or 300 ppm FB1. Serum calcium, cholesterol, and AST activity all increased at higher fumonisin dietary levels. The authors concluded that FB1 from FCM is toxic to growing chickens. In a similar trial, Brown *et al*. (1992) fed day-old broiler chicks diets that contained 0 or 300 ppm FB1 from FCM, for 2 wk. Chicks fed the fumonisin contaminated diet showed 19% reduction in average body weight and 30% increase in relative liver weight. Histologically, the livers of these chicks had acute multifocal randomly oriented hepatic necrosis with moderate biliary hyperplasia and kupffer-cell hypertrophy. Other lesions included mild villus atrophy and goblet-cell hyperplasia in the lower small intestine, small foci of acute myocardial and skeletal muscle necrosis, and widening of both proliferating and hypertrophic zones in proximal tibiotarsal physes. In the same trial, the addition of 0.5% aluminosilicate did not prevent the adverse effects of a diet containing 300 ppm FB1 from FCM given another group of day-old chicks for 14 d.

Weibking *et al*. (1993a) fed day-old broilers diets containing up to 525 ppm FB1 supplied by FCM, for 3 wk. Chickens receiving 450 and 525 ppm FB1 had significantly decreased body weight gain and feed intake, increased liver and kidney weights and increased mean cell hemoglobin concentrations. Histopathological lesions were only seen in the livers of chickens receiving 225

ppm FB1 or more and consisted of isolated foci of hepatic necrosis, moderate diffuse hepatocellular hyperplasia and mild biliary hyperplasia. Compared with controls, all chicks fed FB1 had significantly higher serum free sphinganine levels and elevated sphinganine:sphingosine ratios. Because inhibition of sphingolipid biosynthesis has been hypothesized as the mechanism of action of fumonisins, the authors suggested that diets containing 75 ppm FB1 from FCM may be toxic to young broiler chickens.

Few trials have been conducted using purified fumonisin as the source of toxin, Javed *et al*. (1993) fed chickens diets containing 125 or 274 ppm purified FB1 for 14 d. The toxin induced dose-responsive clinical signs, reduced weight gains and mortality. In the same experiment, day-old chickens were more sensitive to the adverse effects of FB1 and FB2 from *F. proliferatum* culture material than were 7 or 21 day old chickens. According to Javed *et al*. (1993), acute deaths similar to those described for "spiking mortality syndrome" were observed in groups of chickens receiving diets containing a *F. proliferatum* culture material that contained FB1, FB2, and moniliformin. Henry and Wyatt (1994) studied the toxicity of purified FB1 in growing broilers. Purified FB1 was incorporated into the diets of day-old broilers at 0, 20, 40, or 80 ppm and the diets were fed to the chicks for 21 days. Dietary levels up to 80 ppm FB1 did not adversely affect body weight, feed efficiency, or water consumption. No difference in liver, spleen, kidney, proventriculus, or bursal weights were seen between control and FB1-treated chicks. However, liver lipids of chicks receiving FB1 were significantly lower than those of controls, while liver sphinganine and spinganine/sphingosine ratio were significantly higher in treated groups. The authors concluded that dietary FB1 at 80 ppm does not affect performance of broiler chickens.

Turkey poults have also been shown to be susceptible to the adverse effects of FB1 from FCM. Weibking *et al*. (1993b) fed day-old poults diets containing up to 200 ppm FB1 for 3 wk. Body weight gain and feed conversion decreased linearly with increasing dietary fumonisin. Liver, kidney and pancreas weights increased linearly with dietary FB1, and spleen and heart weights decreased. Serum AST levels increased with increasing FB1 concentration while serum cholesterol, alkaline phosphatase, mean cell volume, and mean cell hemoglobin all decreased. Histopathological lesions in the poults receiving FB1 included biliary hyperplasia, hypertrophy of kupffer cells, thymic cortical atrophy, and moderate widening of the proliferating and degenerating hypertrophied zones of proximal tibiotarsal physes.

Few studies on the interaction of FB1 with other mycotoxins in poultry have been conducted. Weibking *et al*. (1992) evaluated the individual and combined effects of FB1 and aflatoxin in turkey poults and found no additive or synergistic effects between these two mycotoxins. However, additive effects were observed when pure FB1 and moniliformin were administered together to growing chickens (Javed *et al*., 1993). In a recent trial, turkey poults were fed diets containing 300 ppm FB1 (from FCM) and 5 ppm T-2 toxin, either singly or in combination; additive toxic interactions for reduced body weight gain and other parameters were observed (Kubena *et al.* 1994).

From the results of the fumonisin trials in poultry it appears that this toxin does not represent a threat to the health and/or productivity of chickens and turkeys. The minimum effective dose of 75 ppm dietary FB1 (from FCM) proposed by Weibking *et al*. (1993a) is about 150 times higher than the highest level of contamination with FB1 in poultry feeds reported by Pittet *et al*. (1992). Further, Henry and Wyatt (1994) showed that 80 ppm purified FB1 has no effect in broiler performance. However, further studies to determine the potential effects of fumonisin on laying hens and other avian species (e.g. ducks, quails) would be useful. The potential toxic interaction of fumonisin and other mycotoxins also warrants investigation.

Chapter 15

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CHAPTER 16   OTHER MYCOTOXINS

Other names:	NONE
Species:	ALL POULTRY

1.   COMPENDIUM

An overview of the role of cyclopiazonic acid, oosporein, citrinin, ergot alkaloids, fusarochromanone, fusaric acid, moniliformin, and zearalenone on poultry health and performance is given in this Chapter. The major toxic effects caused by these mycotoxins in chickens as well as the major fungal species responsible for their production are summarized in *(Table 16.1.)*.

2.   CYCLOPIAZONIC ACID

Chemically, cyclopiazonic acid (CPA) is an indole-tetramic acid *(Fig. 16.1)*. This mycotoxin was first isolated from a culture of *Penicillium cyclopium Westling* (Holzapfel, 1968), but subsequent studies revealed producers of CPA in other *Penicillium* species including *P. camemberti, P. commune, P. chrysogenum, P. crustosum, P. griseofulvum, P. hirsutum*, and *P. viridicatum* (Pitt and Leistner, 1991). Fungi of the *Aspergillus* genus are also capable of producing CPA, including the aflatoxigenic fungus *A. flavus*, as well as *A. tamarii* and *A. versicolor* (Smith and Ross, 1991). In the past, CPA was regarded as being commonly produced by *P. cyclopium* (now known as *P. aurantiogriseum*). However, isolates producing CPA and previously assigned to *P. cyclopium* or *P. puberulum* are now identified as *P. commune*, which, apart from *A. flavus*, appears to be the most common natural source of CPA (Pitt and Leistner, 1991). Cyclopiazonic acid tends to co-occur with aflatoxin in several substrates. Gallagher *et al*. (1978) found that 14 of 54 isolates of *A. flavus* grown on various agricultural commodities produced both aflatoxins and CPA. The first report on the natural occurrence of CPA was made in 1978 in samples of *A. flavus* contaminated corn that also contained aflatoxin (Gallagher *et al*., 1978). Since then, CPA has been reported in meats (*eg.* ham, sausage, frankfurters), cheese, cereal grain based mixed feeds, and nuts (Dorner *et al*., 1983). Levels found in cheese ranged from 0.05 to 0.4 ppm (Le Bars, 1979) while those in peanuts were over the range of 0.032-0.065 ppm in sound mature kernel fractions and 0.13-1.088 ppm in loose-shell kernel fractions (Lansden and Davidson, 1983). In this later study, almost all the CPA-contaminated peanuts also had detectable levels of aflatoxin. Urano *et al*. (1992) found 0.82 ppm CPA in corn naturally contaminated with aflatoxin B1. Toxicological studies in several animal species have shown that the major target organs of CPA are the liver, kidney, and gastrointestinal tract, with liver involvement occurring primarily in rats (Cole, 1986). Due to the effects on the central nervous system observed in experimental animals exposed to CPA (*i.e*., ataxia, extensor spasms), this mycotoxin has been classified as a neurotoxin (Wannemacher *et al*., 1991).

TABLE 16.1 Main toxic effects of selected mycotoxins affecting chickens

Mycotoxin	Major producers	Main effects in chickens
Cyclopiazonic acid	*Penicillium commune* *Aspergillus flavus*	Inflammation and ulceration of the mucosal lining of the GIT. Nervous signs (opisthotonus).
Oosporein	*Chaetomium trilaterale*	Primary renal tubular damage with secondary visceral gout.
Citrinin	*Penicillium citrinum*	Reversible renal damage causing increased diuresis, increased water consumption, and watery diarrhea.
Ergot alkaloids (e.g. ergotamine, ergocristine)	*Claviceps purpurea*	Necrosis of peripheral tissues (eg. toes, beak, wattles, comb). Nervous signs (ataxia).
Fusarochromanone	*Fusarium equiseti*	Dyschondroplastic lesions in the proximal physis of long bones, particularly tibiotarsi.
Fusaric acid	*Fusarium moniliforme*	Decreased cell-mediated immunity.
Moniliformin	*Fusarium moniliforme*	Acute death without gross lesions at post mortem examination.
Zearalenone	*Fusarium graminearum*	Hypertrophy of the oviduct at extremely high dietary levels (800 ppm).

Experimentally, high dietary levels of CPA (50-100 ppm) can cause mucosal epithelial inflammation and necrosis of the crop, proventriculus and gizzard, proventricular mucosal hyperplasia, and multifocal necrosis of the liver, spleen and myocardium of broilers. Dorner *et al*. (1983) fed broiler chickens diets containing 10, 50, or 100 ppm CPA (purified from cultures of *A. flavus*) to day-old broilers for 7 weeks. Feed containing 10 or 50 ppm CPA produced no change in mortality or weight gain compared with controls; however, 30% mortality and a dramatic reduction in mean body weight (496 g vs 1,728 g in controls) were observed in chickens fed 100 ppm CPA. Gross post-mortem lesions in birds given 100 ppm CPA were present in the proventriculus, liver, spleen, and bursa of Fabricius. The proventriculi of these birds were dilated, had excessive mucus overlaying the mucosal surface, and showed areas of hyperemia, erosion, and ulceration of the mucosa. The livers were moderately pale and had multiple, irregular, and variable-sized yellow foci (histologically, areas of coagulative necrosis). These yellow foci were also present in the spleen. Small bursae of Fabricius were also observed in this group of chickens. Birds given a diet

containing 50 ppm CPA showed only dilation of the proventriculus and a thick proventricular mucosal surface. Gross lesions were not seen in birds receiving 10 ppm CPA or in controls. Histological lesions, characterized by focal to diffuse areas of necrosis, were seen in the mucosa of the gastrointestinal tract (crop, proventriculus, and gizzard), and in several organs (liver, spleen, and heart) of chickens receiving ≥50 ppm CPA. Lesions were milder in birds on 50 ppm CPA than in those fed 100 ppm CPA. Lymphoid depletion in the follicular cortex and medulla of the bursa of Fabricius was observed in chickens receiving 100 ppm CPA (Dorner *et al*., 1983). Sukspath *et al*. (1990) investigated the toxicity of CPA in mature male chickens. Roosters tolerated daily oral administration of 2 mg CPA/kg body weight for 4 weeks. However, at 4 mg/kg CPA caused decreased semen volume, reduced concentration of spermatozoa and increased number of abnormal spermatozoa. Within 6 d of the start of the treatment, 4 out of 6 roosters died.

In experiments reported by Cole (1986), day-old chicks and turkey poults acutely intoxicated with CPA showed nervous signs (opisthotonus prior to death) similar to those described by Blount (1961) during the outbreak of turkey "X" disease which occurred in England in 1960 (*see Chapter 14*). Cole (1986) postulated that CPA may also have been involved in the original turkey "X" disease syndrome. Recently, Bradburn *et al*. (1994) found CPA at a level of 31 ppm in a 1960s sample of groundnut cake which had been implicated in the first record of turkey "X" disease. According to the authors, the presence of CPA would explain the catarrhal and hemorrhagic enteritis and opisthotonus originally recorded after the groundnut cake had been ingested by turkeys.

3.   OOSPOREIN

Oosporein (chaetomidin, isooosporein), a red crystalline dibenzoquinone (3,3',6,6'-tetrahydroxy-5,5'-dimethyl-2,2'-bi-*p*-benzoquinone, *Fig. 16.2*), was first isolated from the mycelium of *Oospora colorans*, hence the name (Kögl and Van Wessem, 1944). Even though oosporein had been reported to be produced by several fungi since the 1940s, the first study on the toxic effects of oosporein in animals and plants was reported in 1974 by Cole and co-workers. They purified the toxin from culture broth of a strain of *Chaetomium trilaterale* isolated from moldy peanuts and investigated the lethal effects of the toxin on day-old chicks. The median lethal dose (LD_{50}) of oosporein was found to be 6.12 mg/kg, whereas the LD_{100} was approximately 10 mg/kg or 400 μg/chick. Therefore, in terms of LD_{50}, oosporein is comparable in toxicity to aflatoxin, which has an LD_{50} value of 6.8 mg/kg in day-old chicks. *Chaetomium trilaterale* and other fungi capable of producing oosporein are common contaminants of cereal grains and other substrates (Cole *et al*., 1974); however, few studies to investigate the natural occurrence of oosporein in feeds and feedstuffs have been conducted. Manning and Wyatt (1984) reported the occurrence of 300 ppm oosporein in moldy corn contaminated with *Chaetomium trilaterale*.

Laboratory studies conducted to investigate the effect of purified dietary oosporein in broiler chickens and turkey poults have shown that the toxin causes a primary renal tubular lesion (Brown *et al*., 1987) that results in visceral gout (Pegram and Wyatt, 1979, 1981; Pegram *et al*., 1982). However, the dietary levels required to induce toxicity are several orders of magnitude higher than those required for other mycotoxins. Gout is a metabolic disorder resulting from the deposition of crystals of monosodium urate (MSU), from supersaturated hyperurecemic body fluids in soft tissues and joints (*See Chapter 7*). Sustained hyperurecemia is most commonly caused by decreased renal clearance of urate, especially in primary renal diseases that decrease glomerular filtration rate (GFR). Plasma is saturated with uric acid above 7.0 mg/100 ml at 37°C; however, the solubility of urate at 30°C is only 4 mg/100 ml. For this reason, during sustained hyperurecemia needle-shaped MSU crystals (also known as tophi) are deposited in avascular (*eg.* cartilage) or relatively avascular (*eg.* tendons, ligaments) tissues, serous surfaces of internal organs, and in cooler areas of the body such as the feet. In birds, gout occurs as two different syndromes, namely articular and visceral gout. Articular gout is a relatively rare condition that affects only the musculoskeletal system and appears to be multifactorial in origin but with a strong hereditary basis (Duff, 1990). Visceral gout (visceral urate deposition) is caused by renal failure and is characterized by precipitation of urates in the kidneys and on serous surfaces of the heart, liver, mesenteries, air sacs, and peritoneum. In severe cases, surfaces of muscles and synovial sheaths of tendons and joints may be involved, and precipitation of urates may occur within the liver and spleen (Duff, 1990; Riddell, 1991).

Pegram and Wyatt (1979, 1981) investigated the effect of dietary oosporein in broilers. Purified mycotoxin was added to attain graded dietary levels from 0 to 600 ppm and the toxic feed was given to day-old broilers for three weeks. Dietary levels ≤100 ppm oosporein had no detrimental effect on broiler performance; however, levels ≥200 ppm caused a dose-related increase in mortality, with decreased feed consumption and body weight gain. Increased water consumption was observed at levels of 400 and 600 ppm. Plasma concentration of uric acid was significantly higher in chickens receiving 400 ppm oosporein (8.84 mg/100 ml) than in controls (5.99 mg/100 ml). Pegram *et al*. (1982) fed day-old turkey poults graded levels of oosporein (0, 500, 1000, and 1500 ppm) for three weeks. Feed consumption and body weight decreased proportional to dose, whereas water intake increased. Visceral gout and mortality rates of 24 and 52% were observed in poults receiving 1000 and 1500 ppm oosporein, respectively. However, gout and mortality were absent at 500 ppm oosporein. Manning and Wyatt (1984) investigated the toxicity of *Chaetomium trilaterale* contaminated corn and various chemical forms of oosporein in broiler chicks. Feeding a diet containing 60% contaminated corn (300 ppm oosporein) to day-old broilers caused 100% mortality during the first week. Post mortem examination revealed extensive visceral

urate deposition, enlarged pale kidneys, dehydration, proventricular enlargement with mucosal necrosis, and a dark-green discoloration of the gizzard lining. Based on mortality from 1 to 10 days of age, the LD_{50} values for oosporein acid, oosporein Na salt, and oosporein K salt were 5.77, 5.00, and 4.56 mg/kg, respectively. The K and Na salts of oosporein caused significantly higher mortality than did the organic acid form. The K salt caused the most severe lesions while the organic acid caused the least severe lesions.

In addition to visceral gout (and secondary articular gout in severe cases), gross pathological findings reported in experimental oosporein-toxicosis in chickens and poults include dehydration, swollen and pale kidneys, proventricular enlargement with mucosal necrosis, bile staining of the gizzard koilin layer, and increased relative weights of the liver, kidney, and proventriculus (Pegram and Wyatt, 1979, 1981; Pegram *et al*., 1982). Microscopically, the most severe effect of oosporein occurs in the epithelium of the proximal tubules (PT) of the nephron. Kidneys from broilers fed oosporein (300 ppm) from 0 to 21 days had nephrosis of initial proximal tubular segments (with periodic-acid-Schiff positive granules in the macula densa), interstitial granulomas around tophi, interstitial fibrosis, hyperplasia of remaining tubular epithelial cells and dilation of centrolobular distal tubules (Brown, 1986; Brown *et al*., 1987). Oosporein damages not only the epithelial tubular cells but also the PT basement membranes, preventing regeneration of affected tubules and reducing functional nephron numbers. Damage to initial and subsequent PT segments may be responsible for the hyperurecemia and visceral gout seen during oosporein toxicosis (Brown *et al*., 1987).

4.   CITRININ

Citrinin, a yellow crystalline bicyclic phenol derivative (*Fig. 16.3*), was first isolated in the 1930s from *Penicillium citrinum* (Hetherington and Raistrick, 1931). Further studies revealed that citrinin was also produced by other species of *Penicillium* (*e.g. P. expansum, P. lanosum, P. verrucosum*) and by species of *Aspergillus* (*e.g. A. candidus, A. terreus*). Initially, citrinin attracted much attention due to its antibacterial activity; however, toxicity studies in animals showed that the compound was too toxic for use as an antibiotic. Citrinin has been shown to produce severe renal damage in pigs, rats, and dogs, and is also responsible for the "yellowed rice syndrome", an animal mycotoxicosis reported in Japan (Reiss, 1977). Citrinin often coexists in cereals along with ochratoxin A. Its occurrence has been reported in cereal grains such as corn, wheat, barley, oats, rye, and rice, and the levels of contamination range from 0.001 to 80 ppm (Roberts and Mora, 1978; Yoshisawa, 1991).

In broiler chickens, the clinical signs observed in citrinin toxicosis are either non-specific or are reflective of kidney damage. The major toxic effects are increased water consumption and urine excretion, resulting in watery diarrhea, with a minimum dietary toxic level of approximately 130 ppm. In terms of LD_{50}, citrinin is less toxic than other nephrotoxic mycotoxins such as ochratoxin A and oosporein. The single-oral LD_{50} value for citrinin in 7 d old male broiler chickens was reported to be 95 mg/kg (Mehdi *et al*., 1981), which is about 45 and 15 times higher than the corresponding values for ochratoxin A (2.1 mg/kg Huff *et al*., 1974) and oosporein (6.1 mg/kg, Cole *et al*., 1974). The toxicity of citrinin to chick embryos has also been investigated. Citrinin was found to be teratogenic, and had an LD_{50} value of 80.5 μg/egg (Ciegler *et al*., 1977); 67% of the chick embryos inoculated with 0.5 μg/egg died before hatching and showed microscopic degenerative changes in the kidney and liver (Lalithakunjamma and Nair, 1991).

The effect of citrinin on broiler performance was investigated by Ames *et al*. (1976), who fed broiler chicks diets containing up to 500 ppm citrinin for three weeks. Body weight was decreased by the 500 ppm level whereas all levels of citrinin resulted in enlarged kidneys and an improvement in feed conversion when compared with controls. Levels ≥250 ppm citrinin caused a dose-related increase in water consumption accompanied by diarrhea. Using colostomized broiler chickens, Gustavson *et al*. (1981) found that citrinin significantly increases both water consumption and urine excretion without affecting fecal moisture. Therefore, the observation of watery diarrhea during citrinin toxicosis is the result of increased urine excretion and not due to a toxic effect on the gastrointestinal tract. In another trial, broiler chickens receiving graded levels of dietary citrinin (0, 33, 65, 130, and 260 ppm) showed increased water intake and diarrhea at levels of 130 and 260 ppm (Roberts and Mora, 1978). Nelson *et al*. (1981) investigated the effects of citrinin on growing chicks by feeding day-old broilers diets containing up to 440 ppm citrinin for three weeks. Body weight was significantly decreased only by levels ≥330 ppm whereas feed utilization decreased only at 440 ppm. In this trial, analysis of thigh muscle, kidney, liver, and blood for citrinin revealed detectable levels in the liver and blood of chicks fed 440 ppm.

Gross lesions observed in chickens receiving 130 or 260 ppm dietary citrinin included hemorrhagic jejunums, mottled livers, and enlarged kidneys (Roberts and Mora, 1978). Histopathology of broiler chicks given lethal doses of citrinin by crop gavage revealed renal degeneration and necrosis of the tubular epithelial cells of both the proximal and distal tubules. Hepatic lesions included multiple foci of necrosis and hemorrhage, and lymphoid necrosis and depletion were found in the bursa of Fabricius, thymus, cecal tonsil and spleen (Mehdi *et al*., 1981). In the same trial, when chickens were fed diets containing 100, 250 or 500 ppm citrinin for three weeks they showed clinical signs of citrinin toxicosis and enlargement of the kidneys although no histologic lesions were found. Therefore, lesions in extra-renal tissues are unlikely to be found in chickens exposed to the dietary levels of citrinin present in naturally contaminated feedstuffs (usually <80 ppm). Campbell *et al*. (1981) investigated the effects of dietary citrinin on broiler chicken immunity and found no adverse effects on either humoral or cell-mediated immune functions.

In laying hens, dietary levels up to 250 ppm citrinin had no adverse effect on layer performance. Ames *et al*. (1976) fed layers diets containing 0, 50 or 250 ppm citrinin for three weeks and no effect on body weight, feed consumption, egg production, egg weight, or egg shell quality were observed. A moderate diarrhea that subsided once the birds returned to a non-toxic diet was observed about three days after feeding the 250 ppm level. Mehdi *et al*. (1984) investigated the effect of graded levels of dietary citrinin (100, 250, or 500 ppm) in White Pekin ducklings. Body weight gain was significantly decreased with 500 ppm and nephropathy was observed at levels ≥250 ppm. Microscopically, the nephropathy was characterized by degeneration, necrosis, mineralization, and regeneration of tubular epithelial cells of both the cortical and medullary regions. Interstitial fibrosis was found in the medullary regions of the 500 ppm group only.

Studies on the effect of citrinin on chicken renal function showed that the toxin alters several tubular transport processes and that the effect is reversible. Intravenous infusion of citrinin in SCWL pullets had no acute direct effect on GFR, renal plasma flow rate, urine pH, or fractional calcium or magnesium excretion. However, citrinin caused rapid increases in urine flow rate, free water clearance, and in fractional sodium, potassium, and inorganic phosphate excretion. Citrinin-induced diuresis recovered to normal values in 30-40 minutes upon removal of the toxin from the infusion solution (Hnatow and Wideman, 1985).

5.   ERGOT ALKALOIDS

Ergotism is the disease caused by the ingestion of alkaloids contained in the sclerotia (ergots) of *Claviceps* species. The pharmacological use of ergots in obstetrics was known in China 5000 years ago while the Romans and Egyptians were aware of their toxicity by 79 B.C. and 1000 A.D., respectively. The great epidemic of ergotism, so well documented in Europe during the latter Middle Ages, resulted in the death of thousands of people. The toxicosis, also known as "St. Antony's Fire", caused ischemia, post-ischemic inflammation, necrosis, and gangrene of the limbs. Nowadays, ergotism has almost disappeared as a human disease but it is still observed in animals consuming ergot contaminated grains. The word ergot derives from the French, and means rooster's spur. Ergots are formed when ascospores of the fungus *Claviceps purpurea* (or other *Claviceps* species) infect the open inflorescence of graminaceous plants such as rye, wheat, triticale, barley, oats, sorghum, corn, rice, and several grass species (Lorenz, 1979). Initially, the ascospores replace the ovary with a white-yellow mycelial mass that produces a nectarlike material

known as "honeydew". This conidial stage (*Sphacelia)* can spread as asexual conidia to other inflorescence and eventually enlarges, darkens, and hardens into a sclerotium, the ergot. The sclerotia resemble the seeds which they replace but are usually larger and more angular, similar to a rooster's spur. However, the external characteristics of the ergots produced by the various *Claviceps* species vary considerably. Differences in length (3 mm to 8 cm), weight (8.2-24.5 g/100 sclerotia), shape (curved, straight, or round), and color (white, brown, yellow, green, black, or purplish brown) have been recorded (Lorenz, 1979). Sclerotia that fall to the ground or are sown with seed germinate in spring in temperate climates to produce stalked stromata bearing perithecia which produce ascospores to complete the life cycle (Lacey, 1991). *Claviceps* sclerotia contain a number of different chemical substances. Toxicologically significant are the alkaloids derived from lysergic acid and the clavine alkaloids, derived from dimethylergoline. Ergocornine, ergocristine, ergokryptine, and ergotamine (*Fig. 16.4*) are among the most pharmacologically active peptides and are the main alkaloids of *C. purpurea* (Lacey, 1991).

The amount of ergot in feeds is usually expressed as the percentage of ergot sclerotia by weight present in a given grain. Ergot tolerances have been set in several countries including Canada, the USA, and the EEC. Depending on the country and specific commodity, grain samples containing 0.1-0.33% ergot are graded "ergoty" (Lorenz, 1979). The ergot alkaloid content present in sclerotia from cereal grains ranges from 0 to 1.0%, and, when present, the proportions of different alkaloids vary considerably (Lacey, 1991).

In surveys quoted by Yoshisawa (1991), ergots found in samples of winter wheat contained 0.185-0.379% total alkaloids (expressed as ergotoxine); ergotamine comprised 36-64% of the total alkaloids while ergometrine and ergokryptine content ranged from 3 to 8%. Samples of commercial wheat and rye flour contained ergocristine (2.7-62.2 ppb), ergometrine (0.27-10.4 ppb), ergosine ($\leq$10.8 ppb), ergotamine (1.7-36.9 ppb), ergocornine (traces to 7.9 ppb), and α-ergocryptine (traces to 10.3 ppb) (Yoshisawa, 1991). Scott *et al*. (1992) conducted a survey to determine the prevalence and concentrations of ergonovine (ergometrine), ergosine, ergotamine, ergocornine, α-ergokryptine, and ergocristine in over 400 samples of grain foods sold in Canada. The predominant alkaloids found were ergotamine and ergocristine. Rye flour was the most contaminated food, with a mean total alkaloid concentration of 239 ppb and a range of 70-414 ppb. Wheat flour total alkaloid concentrations were much lower than those of rye flour, and had a mean of 31 ppb and a range of 15-68 ppb.

Two forms of ergotism have been described in mammals: a gangrenous form with necrosis and sloughing of the extremities and a convulsive form characterized by staggers, ataxia, tremors and convulsions (Osweiller *et al*., 1985). Natural ergot alkaloids (especially ergotamine) cause arterial and venous vasoconstriction, increased blood pressure, decreased blood flow to the extremities, and may also damage the capillary endothelium. The combined effect of vasoconstriction and endothelial damage results in vascular stasis, thrombosis and eventually gangrene (Osweiller *et al*., 1985).

Both gangrenous-like lesions and nervous signs have been reported in chickens exposed to dietary ergot alkaloids. Safe dietary levels of ergot for chickens appear to range between 0.3 and 0.8% by weight. However, the wide biological variation in both the quantity and quality of the alkaloids present in ergot sclerotia makes it very difficult to establish safe levels. Perek (1958) administered SCWL layers and cockerels ergot powder containing 1% alkaloids twice daily by crop gavage. Lesions described as vesicular dermatitis of the comb and wattles, as well as necrotic lesions in the feet were observed; some hens developed purple combs, had decreased feed intake, and ceased laying. In another trial, the inclusion of ≥ 0.3% ergot in wheat-based chick diets resulted in growth depression and mortality (O'Neil and Rae, 1965). However, Bragg *et al*. (1970) found that broilers could tolerate up to 0.8% triticale ergot added to wheat diets without adverse effect. This apparent discrepancy could be due to differences in the type and amount of alkaloids present in the sclerotia added to the experimental diets. In the experiment reported by Bragg *et al*. (1970) graded levels of dietary triticale ergot up to 12.8% were fed to day-old broiler chicks for either 4 or 8 weeks. Dietary ergot levels ≤0.8% were tolerated without adverse effect, but levels ≥1.6% significantly depressed growth and increased the feed/gain ratio. Ergot levels ≥3.2% caused necrosis of the toe, nails, beak, and caused high mortality. Ergotism was characterized by an initial depression in growth, poor feathering, nervousness, loss of coordination, and finally, inability to stand. In experiments by Misir and Marquardt (1978), ergot infestation of rye at levels of 0.11% did not adversely affect chick performance. Rotter *et al*. (1985a) investigated the effect of graded levels of dietary wheat ergot (1-8%) on the performance of growing Leghorn and broiler chicks. A dose-dependent adverse effect on performance was observed for both strains of birds; however, broilers were slightly more sensitive than were Leghorns. After 3 weeks of exposure, birds receiving 1% dietary ergot had about 10% lower body weight gain than controls whereas those receiving 8% ergot were 80% smaller. Mortality was low on diets containing up to 3% dietary ergot but above this level there was a dramatic dose-dependent increase in mortality. The effect of different sources of ergot on chick performance was investigated by Rotter *et al*. (1985b) who fed 7 day old Leghorn chicks diets containing ground ergot from rye, wheat, or triticale for 7 days. The ground ergot was added to the diets in order to achieve total alkaloid concentrations of either 45 or 90 ppm. The relative body weight gain varied considerably; values of 49-65% and 18-42% of controls were recorded for birds fed 45 and 90 ppm total alkaloids, respectively. The authors concluded that even though the total alkaloid content can be used to predict the effect of ergot on chick performance, the prediction is not always accurate due to the variable content and variable effects of individual alkaloids present in a given sample.

Experiments have shown that chickens receiving dietary ergot can recover from its adverse effect once they are changed to an ergot-free diet. Rotter *et al*. (1985c) observed that chickens receiving up to 2% dietary ergot (0.31% total alkaloids) from day of age for 32 d showed a recovery when the birds were given an ergot-free diet. The effect of adding purified ergotamine tartrate in the diet of growing chickens was evaluated by Young and Marquardt (1982). In short-term studies (7-10 days), dietary levels of 30 - 40 ppm ergotamine tartrate did not alter feed intake or weight gain of broiler or Leghorn chicks. Levels ≥250 ppm caused toe necrosis and levels of 800 ppm had only a

slight effect on the feed:gain ratio and failed to produce gross lesions in brain or muscle. A case of suspected ergotism in Muscovy ducks that consumed wheat containing 1.17% ergot sclerotia was described by Swarbrick and Swarbrick (1968). Heavy mortality occurred within 48 h in ducklings aged between two to three months, but older birds were unaffected. Mortality was preceded by lethargy and diarrhea, and death occurred within 24-48 h after the onset of signs.

6.   FUSAROCHROMANONE

Fusarochromanone (FC) is a *Fusarium equiseti*-produced mycotoxin that has been shown to increase the incidence of dyschondroplasia in broilers. The minimum dietary level causing increased incidence of dyschondroplasia in growing broilers has been shown to be 20 ppm. However, no surveys to determine the natural occurrence of FC in feeds and feedstuffs have been conducted and the role of this mycotoxin on the occurrence of long bone dyschondroplasia under field conditions is uncertain.

Physeal osteochondrosis (dyschondroplasia) is an abnormality of growth plate cartilage of growing broilers characterized by a mass of avascular opaque cartilage in the proximal metaphysis of long bones (*Chapter 11*). Dyschondroplastic lesions are commonly present in proximal and distal tibiotarsi and proximal femora and tarsometatarsi but can be found anywhere in the skeleton. The etiology of tibial dyschondroplasia (TD) is uncertain but nutritional, genetic, and environmental factors have been shown to play a role (Duff, 1990; Leach and Lilburn, 1992). Walser *et al*. (1982) found that broilers fed diets containing *Fusarium roseum*-contaminated corn had an extremely high incidence (85-90%) of osteochondrosis in proximal tibiosarsi. The specific compound responsible for the high incidence of TD lesions was later purified from an isolate of *F. roseum* "Graminearum" and named TDP-1 (Lee *et al*., 1985); when chickens were fed a diet containing 75 ppm pure TDP-1, a 100% incidence of TD was observed, thus establishing a cause and effect relationship between the mycotoxin and dyschondroplasia. Interestingly, the fungal isolate initially identified as *F. roseum* "Graminearum" was in fact a degenerate strain of *F. equiseti* (Thrane, 1989). When 62 *Fusarium* isolates representing nine species were screened for FC production only three strains of *F. equiseti* were capable of FC biosynthesis (Wu *et al*., 1990). The chemical structure of TDP-1 was elucidated by Pathre *et al*. (1986) who gave the toxin the name fusarochromanone to reflect its *Fusarium* origin and its chromanone ring structure (*Fig. 16.5*).

Recently, Wu *et al*. (1993) investigated the effect of purified FC in broiler chickens. At a dietary level of 75 ppm, FC caused a 33% reduction in body weight, a 100% incidence of TD and decreased the humoral response to sheep erythrocytes by 34-50%. The minimum dietary concentration of FC capable of inducing a significant increase in the incidence of TD was found to be 20 ppm. Fusarochromanone did not induce TD in Leghorn chicks. Haynes and Walser (1986) investigated the ultrastructure of FC-induced TD in broiler chickens fed twice daily purified FC by crop gavage for 14 days. Chickens examined after 2, 4, or 6 days of treatment had either no gross lesions (2 days) or mild gross lesions (4 and 6 days) and did not show ultrastructural lesions. However, after 8 or more days of exposure, moderate to severe gross lesions of TD and ultrastructural lesions including intracellular lipid accumulation and necrosis of chondrocytes were seen. The authors concluded that the cellular changes in TD develop only after the cartilage accumulates. In layers, pure FC has been shown to reduce the hatchability of fertile eggs (Pathre *et al*., 1986).

7.   **FUSARIC ACID**

Fusaric acid (5-butylpicolinic acid; 5-butyl pyridine-2-carboxylic acid, (*Fig. 16.6*) is a phytotoxin with weak antibacterial activity produced mainly by *F. moniliforme* (Smith and Sousadias, 1993). Other *Fusarium* species, including *F. oxysporum, F. sacchari, F. solani*, and *F. verticillioides*, can also produce this metabolite (Thrane, 1989). A recent Canadian survey of swine feedstuffs (whole swine feeds, dry corn, high-moisture corn, wheat, and barley) showed average concentrations of fusaric acid ranging from 11.6 to 35.76 ppm; the highest level found was 135.6 ppm in high-moisture corn (Smith and Sousadias, 1993). Fusaric acid inhibits dopamine-ß-hydroxylase, one of the enzymes involved in the biosynthesis of norepinephrine, and has potent hypotensive activity in rabbits, rats, cats and dogs (Hidaka *et al*., 1969).

The effect of fusaric acid on broilers was investigated by Chu *et al*. (1993) who fed female broiler chicks on diets containing graded levels of fusaric acid (0, 35, 75, and 150 ppm) for three weeks. Fusaric acid did not increase the incidence of TD, leg shape deformities, or rickets, and no effect on body weight gain or feed intake was observed. However, fusaric acid decreased cell-mediated immune response to phytohemagglutinin-P challenge at all dietary levels (Chu *et al*., 1993). In a more recent trial, Ogunbo *et al*. (1994) fed day-old turkey poults and broilers up to 400 ppm fusaric acid for 21 d. Fusaric acid had no effect on feed intake or weight gain and it was concluded that fusaric acid is not toxic to young broilers or turkeys at these dietary concentrations. Although fusaric acid may not cause overt toxicosis in poultry, possible toxic interactions between this and other *Fusarium* toxins (*e.g.* moniliformin, fusarochromanone, fumonisin, trichothecenes) should be investigated.

8.   MONILIFORMIN

Moniliformin (1-hydroxy-cyclobut-1-ene-3,4-dione, *Fig. 16.7*) is a plant growth regulator and phytotoxin, produced mainly by strains of *Fusarium moniliforme* (Vesonder and Golinski, 1989). Moniliformin was discovered while screening for toxic products of an isolate of *F. moniliforme* cultured on corn (Cole *et al*., 1973). Later, other *Fusarium* fungi were shown to produce moniliformin including *F. acuminatum, F. avenaceum, F. concolor, F. equiseti, F. oxysporum, F.*

proliferatum and *F. semitectum* (Rabie *et al*., 1982). In terms of lethality, moniliformin is highly toxic for chickens; however, the mechanism of action for this lethality is unknown.

The single oral LD_{50} value for 1 day old chicks was found to be 5.4 mg/kg, whereas the LD_{50} value for chick embryos was 2.8 μg/egg (Burmeister *et al*., 1979). Acute death without gross lesions has been observed in broilers fed diets containing fumonisins and moniliformin from *F. proliferatum* culture material (Javed *et al*., 1993). For this reason, these and possibly other yet unidentified *Fusarium* toxins have been proposed to play a role in the so-called "spiking mortality syndrome". The minimum dietary level of moniliformin causing mortality in growing broilers appears to be in the range of 16-27 ppm. In trials using purified moniliformin no mortality was seen at 16 ppm (Allen *et al*., 1981c) but 40% mortality was reported when a dietary level of 27 ppm was fed (Javed *et al*., 1993).

Allen *et al*., (1981b) fed moniliformin, either purified (0, 8, and 16 ppm) or from culture of *F. moniliforme* on corn grits (8, 16, and 64 ppm) to day-old broiler chicks for 21 d. Up to 16 ppm dietary moniliformin from either source was without effect on weight gain, feed consumption, and mortality. However, chickens receiving 64 ppm dietary moniliformin from culture material had reduced weight gain and feed consumption. Three of 10 chicks fed 64 ppm moniliformin died

without gross lesions. These birds were growing normally and had feed in their crops and digestive tracts at time of death. *F. moniliforme* is capable of producing not only moniliformin but also other toxins such as fumonisin B1 (FB1) and fusaric acid. Therefore, the possibility exists of having feedstuffs contaminated with moniliformin and FB1 and/or fusaric acid under field conditions. Recently, Javed *et al*. (1993) investigated the individual and combined effects of purified moniliformin and FB1 in broiler chicks. Six experimental diets including one control, two levels of FB1 (125 and 274 ppm), two levels of moniliformin (27 and 154 ppm), and one combination treatment (137 ppm FB1 + 77 ppm moniliformin) were fed to six groups of day-old chicks for 14 days. Mortality was 100% in the combination treatment, 70% in chicks receiving 154 ppm moniliformin, 50% in chicks fed 274 ppm FB1, 40% in chicks fed 27 ppm moniliformin, and 20% in chicks fed 125 ppm FB1. Weight gains were significantly depressed in all treatment groups compared with controls; moniliformin at 27 and 154 ppm reduced the 14-day body weight by 62.7 and 72.8%, respectively, whereas FB1 caused a reduction of 51.9 and 60.9% at dietary levels of 125 and 274 ppm, respectively. Clinical signs including depression, weakness, ataxia, dyspnea and gasping terminating in flaccidity and death were observed in birds receiving the combination treatment, 48 h after the administration of the toxic diet. Similar but milder clinical signs were observed in the other treatment groups after 5-7 days of exposure to the toxins. Javed *et al*. (1993) concluded that moniliformin may be more toxic to young broilers than is FB1 and that their toxicities appear to be additive.

9.   ZEARALENONE

Zearalenone (ZEA) is a phenolic resorcyclic acid lactone with potent estrogenic properties, produced primarily by *Fusarium roseum*. ZEA can induce signs of estrus in ovariectomized sows or in prepubertal gilts and dietary concentrations as low as 1-5 ppm can cause vulvovaginitis in young female swine (Osweiller *et al*., 1985).

Zearalenone concentrations of 2.9, 5, and 35.9 ppm were reported in US animal feed, US corn, and Yugoslavian corn, respectively (Pier *et al*., 1980). However, almost all findings of ZEA surveys in Egypt, Denmark, France, Kenya, Taiwan, and the United States in corn, corn products, rice, and animal feeds showed <1 ppm in grains and foods with higher levels in feeds (Jelinek et al., 1989). A survey conducted in the Netherlands revealed that from 89 randomly selected samples of feedstuffs, 31% were contaminated with ZEA. The mean ZEA content of all positive samples was 0.062 ppm and the highest level was 0.24 ppm in maize meal (Veldman *et al*., 1992). Results from a survey conducted in Ontario corn samples indicated that <50% of 98 samples contained ZEA, although the highest level measured was 0.7 ppm (Hunton, 1994).

Although the concentrations of ZEA normally found in contaminated feeds and feedstuffs represent a potential risk for swine, they appear to have no effect on poultry health and performance. Bacon and Marks (1976) fed broiler chickens a diet containing 30 ppm ZEA for 7 weeks and no adverse effect on performance parameters was observed. Similarly, when graded levels of dietary zearalenone from 10-800 ppm were fed to day-old broiler chicks for 21 days no effect on weight gain, feed consumption, or feed:gain ratio was observed. There were no prominent lesions at post mortem examination except for hypertrophy of the oviduct in some birds receiving 800 ppm ZEA (Chi *et al*., 1980). In another trial, older broiler chickens (6 to 9 weeks of age) receiving graded levels of dietary ZEA (50-800 ppm) showed no difference in performance parameters, relative organ weights, and selected blood parameters, when compared with controls

(Allen *et al*., 1981a). Studies conducted with mature male and female chickens indicated that dietary ZEA up to 800 ppm is without effect on reproductive performance of mature chickens. ZEA did not affect egg production, egg size, egg shell thickness, Haugh units, fertility, hatchability of fertile eggs, feed intake, body weight, or the relative weights of comb, oviduct, heart, liver and spleen. Post mortem and histological examination of tissues from these birds revealed no effects of ZEA feeding (Allen *et al*., 1981b).

Even though ZEA appears to be non toxic for poultry species, the detection of this mycotoxin in poultry feed has been suggested to be used as a "biomarker" for other yet unknown *Fusarium* toxins (Romer, 1990). The type B trichothecene deoxynivalenol (DON) is another example of a potential biomarker for poultry feed. The presence of biomarkers, whether toxic or not, indicates that the feed was exposed to conditions favorable for mold growth. This increases the possibility that the feed is contaminated with mycotoxins. Testing for the presence of biomarkers such as ZEA and DON is easily performed with currently available test kits and may be used to detect and control *Fusarium* field problems without actually identifying specific toxins (Romer, 1990).

Chapter 16

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CHAPTER 17   MINERAL AND VITAMIN TOXICITIES

| **Other names:** | NONE |
| **Species:** | ALL POULTRY |

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1.   COMPENDIUM

There has been relatively little recent work published on the toxic effects of minerals or vitamins. Toxicities of these nutrients can affect the bird directly or more commonly cause an antagonism of other nutrient systems. Toxic levels of most minerals are tabulated, while for vitamins we have listed general upper safe levels in relation to requirement. Detailed discussions on mineral toxicities centre around magnesium, copper and vanadium, because these can be found at toxic levels as contaminants to conventional feedstuffs, or as in the case of copper, added as a treatment/prevention of enteric disorders. Sodium toxicity is discussed in terms of electrolyte and water balance, while an excess intake of calcium and phosphorus is described relative to skeletal and eggshell integrity.

High intakes of fat soluble vitamins seem to be problematic, and especially for vitamin A which appears to increase the birds need for vitamin D_3. Toxic effects of vitamin A can be corrected by feeding high levels of vitamin D_3. Toxic levels of vitamin E also disrupt calcium and phosphorus metabolism, while moderately high levels of vitamin D_3 and especially metabolites such as $1\alpha(OH)D_3$ cause aberrations in circulating calcium concentrations.

2.   TRACE MINERAL TOXICITIES

There has been relatively little recent work published on the toxic effect of the various minerals. The summary shown in *Table 17.1* is adapted from that provided by the NRC (1994) in Nutrient Requirements of Poultry. Toxic levels shown in *Table 17.1* generally relate to the lowest inclusion level currently known to produce signs. In most instances these signs are a reduced growth rate in immature birds, and have been induced when feeding the appropriate levels of inorganic salts. In many instances, these toxicities can occur at much lower levels when the minerals are present as organic compounds. Toxicity will therefore vary with the form of mineral used, and likely be most severe for younger rather than older birds.

The bird response to trace mineral toxicities is not always easy to quantitate, because the interaction between minerals and other nutrients leads to the situation of a high level of one mineral perhaps inducing a "toxic effect" simply by precipitating a deficiency of another mineral or another nutrient. An example of such induced "toxicity" between sodium, potassium and chloride is now

well established, where balance as well as absolute levels of individual minerals is of prime concern (*Chapter 10*).

Table 17.1 Mineral toxicity	
Mineral	**Toxic level (ppm)**
Aluminium	500 - 2200
Arsenic	100
Barium	200
Bromine	5,000
Cadmium	20 - 40
Chloride	9 - 15,000
Chromium	300
Cobalt	100
Copper	250
Fluorine	400
Iodine	625
Iron	4,500
Lead	320
Magnesium	5700
Manganese	4,000
Mercury	250
Molybdenum	350
Nickel	400
Selenium	10
Silver	900
Sodium	8,900
Strontium	6,000
Tungston	500
Vanadium	5
Zinc	800

Adapted from NRC (1994)
References cited in Bibliography

For most trace minerals, toxicities are rare unless there is an error in formulation or the ingredients are contaminated with specific metals. Following is a discussion on some of the more common trace mineral toxicities and how these can occur under commercial conditions.

2.1.   *Magnesium*

High levels of magnesium will induce excessive drinking by birds as they attempt to clear the mineral through the kidney. Field cases of excessive water and feed consumption occurring when there is a reduced egg production or virtually no growth have been seen in birds drinking water obtained from wells adjacent to subterranean veins of gypsum. More commonly, magnesium

toxicity arises from the incorporation of dolomitic limestone into poultry diets. While normal limestone usually contains less than 1% magnesium, dolomitic limestone which is most commonly used in the steel industry, can contain as much as 10-13% magnesium. This high level of magnesium has the usual cathartic effect of most magnesium salts, although more importantly the magnesium competes with calcium for sites of absorption and transport. Eggshell thickness quickly declines in birds fed dolomitic limestone.

2.2. Iron

Excessive intake of iron may induce a deficiency of phosphorus through the formation of iron phosphate in the intestine. Apart from the potential for excess iron to induce rickets, the insoluble intestinal iron phosphate apparently produces a colloid that may adsorb vitamins and other trace minerals (Scott *et al.,* 1982). Corn distillers solubles are very high in iron content, although a toxicity may occur more frequently when iron is added to diets in order to detoxify gossypol. For treating cottonseed meal, iron is often added in a 1:1 ratio with expected gossypol content, and so under these conditions it may be important to critically review the vitamin and phosphorus levels of these diets.

2.3. Copper

High levels of copper are added to diets and especially for turkeys, as a growth promoter and/or to reduce excreta moisture content. This is usually accomplished by adding copper sulphate to the diet, and so a toxic level of 250 ppm (*Table 17.1*) is realised with about 1.3 kg $CuSO_4 5H_2O$/tonne feed, for chickens. Turkeys may be able to tolerate at least twice this amount of $CuSO_4$ in the diet. High levels of copper may interfere with sulfur metabolism, and as such, may increase the need for methionine and/or cystine.

2.4. Selenium

Selenium toxicity could only occur as a result of mixing error, because levels of 50-100 times the requirement are needed to cause a disruption of various enzyme systems. Affected birds will show a general loss of performance although there are few gross pathological abnormalities.

2.5. Vanadium

Vanadium toxicity has been reported in situations where birds are fed contaminated sources of phosphate. Growth rate in young chicks will be affected at 10-20 ppm vanadium, while 20-30 ppm vanadium fed to layers causes a significant reduction in albumen thickness. Layers fed vanadium-contaminated phosphates have also been shown to produce eggshells that have a characteristic smoothness and that break cleanly, like glass, when even gentle pressure is applied. While increasing levels of dietary chromium have been reported to partially alleviate vanadium toxicity in young birds, it has little beneficial effect for layers.

2.6.   *Sodium*

Sodium toxicity frequently occurs due to the incorporation of too much salt in the diet. Birds can tolerate up to 40-50 times normal intake, prior to there being serious problems of abnormal metabolism, although signs of "toxicity" occur with much lower margins of error in formulation. Toxicity *per se* is influenced by the birds ability to drink, and as long as sodium can be excreted, few derangements of metabolism occur. Even slight increases in sodium intake therefore result in increased water intake and urine output. Sodium toxicity therefore becomes problematic in terms of controlling excreta and litter moisture levels (*see Chapter 9*). Turkeys up to 8 wks of age seem most responsive to diet sodium in terms of water balance, although most young birds will start to drink more water when normal sodium intake is increased by only 20%. Doubling of sodium intake seems to have little effect on bird performance, other than an increase in the water flux. Such increased water intake can be advantageous during heat stress, and so therapy often involves feeding elevated levels of sodium chloride.

3.   **CALCIUM AND PHOSPHORUS TOXICITIES**

Problems of calcium and/or phosphorus toxicity must be considered together, because high levels of one mineral directly influences the metabolism of the other. Shafey (1993) presents an extensive current review of calcium toxicity of all ages of birds, and as one expects, there are implications for the metabolism of phosphorus and other microminerals. Shafey (1993) shows evidence for abnormal growth and development of broilers when the diet calcium level exceeds 1.2-1.5% of the diet, although variability in results will obviously relate to the associated levels of dietary phosphorus, vitamin D_3 and phytic acid. Excess calcium and/or phosphorus are solubilized by HCl in the stomach, although in the small intestine, because of an increase in pH, there can be a formation of flocculent precipitates. Shafey (1993) concludes that a major negative effect of a high calcium intake in young birds, may be a reduced bioavailability of other minerals in the intestine, and so when high calcium diets are necessarily used, then an increased supply of microminerals seems advisable. Excess calcium will also cause a phosphorus deficiency in birds and especially if diets are marginal in phosphorus. Again this is due to the formation of insoluble tricalcium phosphate. High levels of calcium may also cause increased soap formation in the small intestine, the consequence of which may be reduced fatty acid and energy uptake. There is also an indication of these soaps forming a calcium-fatty acid-phosphate complex in situations where both calcium and phosphorus are abundant.

Availability of both calcium and phosphorus is also influenced by phytate levels in the diet. Under normal conditions phytate complexes with calcium in a molar ratio of 1:5 (Nelson and Kirby, 1987). This situation is taken into account in formulation, and is partly responsible for the need to provide around 1% calcium in most poultry diets. If phytase enzymes are used, it is likely that more calcium (as well as phosphorus) will be available to the bird. While the increased phosphorus availability will be considered during formulation, there has apparently been little thought to the potential for calcium toxicity to arise when up to 0.15-0.2% more calcium is made available for absorption. Feeding too much calcium to immature pullets for a prolonged period of time can cause urolithiasis (*Chapter 7*). Calcium and phosphorus toxicity can also occur in layers, where effects on skeletal development and shell quality are seen. Because a deterioration in shell quality occurs quickly with excess of either calcium or phosphorus, then diet changes are usually made prior to the

onset of skeletal disorders such as osteopetrosis. Levels of available phosphorus much in excess of 0.45%, depending upon the feed intake, will likely cause a reduction in shell thickness related to a calcium deficiency induced either by reduced absorption, or reduced uptake by the shell gland due to a change in blood electrolyte balance. High levels of calcium (>4.5%) can also lead to excessive calcium deposits on the eggshell, most often referred to as "pimpling".

4.   <u>VITAMIN TOXICITIES</u>

A summary of toxic effects and toxic levels of vitamins where known, are shown in *Table 17.2*. Much of the data for the compilation of this table is taken from the excellent review of vitamin tolerance of animals prepared by NRC (1987), to which the reader is directed for extensive details of specific vitamin toxicities. Vitamin toxicities are not likely to occur under normal feeding conditions, although there is some evidence that even moderately high levels of certain vitamins can lead to a disruption in the metabolism of other vitamins. Also toxicities of some vitamins can occur when they are given indiscriminately during vitamin therapy. Potential toxicity and/or imbalance seems most prevalent with vitamins, A, D_3 and E.

4.1.   Vitamin A

Jensen *et al.* (1983) showed a growth depression in 6 wk old birds fed 12,000 IU vitamin A/kg diet, and this was associated with a reduced pigmentation of the body and impairment of skeletal integrity. The authors conclude that high levels of vitamin A are toxic to birds, and that feeding 4-10 times NRC requirement is a dated concept that was probably warranted some time ago when only unstable forms of vitamin A were available. In subsequent studies, Veltmann and Jensen (1986) found a strain-response to high levels of vitamin A. Dosing all birds with 300 IU vit A/g B.wt produced a growth depression within 16 d, although doubling the dose to 600 IU had an almost immediate effect. In both broiler and Leghorn chicks, excess vitamin A caused osteodystrophy in the long bones. However, while in broilers the toxicity was characterized by rachitic lesions, in the slower growing Leghorn there was only a thinning of the proliferative maturation zone and a thickening of the hypertrophic zone within the growth plate. The authors conclude that vitamin A toxicity in terms of deranged bone calcification was not due simply to reduced feed intake. Veltmann *et al.* (1987) in a subsequent study suggested that the effects of vitamin A toxicity may relate to the impaired uptake or metabolism of vitamin D_3. Giving chicks and poults 830 IU vit A/g B.wt resulted in a reduced growth, but this was corrected by giving additional vitamin D_3. It seems as though an excess of vitamin A inhibits the transport of fat soluble vitamins, and especially if a common fatty acid binding protein is used (Veltmann *et al.,* 1987). In these studies, many birds showed signs of rickets, although the authors conclude that TD may be a consequence of nutritional antagonism between excess vitamin A, and vitamin D_3. Data shown in *Table 17.3*, as developed by Metz *et al.* (1985) supports this idea of interrelation between vitamins A and D_3.

TABLE 17.2 Vitamin toxicity

Vitamin	Toxic Effects	Age	Vitamin Concentration	Comments	Refere
A	Depressed growth, reduced bone ash	1 d	650 - 2,600,000 IU/kg	Highest levels only depress growth	Combs Jensen et Veltman et
	Slight growth depression	14 - 56 d	52,800 IU/kg	Various forms Vit A tested	Castano et
General safe level 4 - 10 x requirement					
D	Weight loss, renal tubular calcification	1 d	10 - 12.5 mg/kg	Response dependent upon Ca and P levels of diet	Taylor et Morrissey e
General safe level 4 - 10 x requirement					
E	Weight-loss, reduced hematocrit, reduced plasma Ca, P	21 - 35 d	2 - 64,000 IU/kg	Depressed growth with lower inclusion levels	March et Nockels et Murphy et
General safe level 100 - 200 x requirement					
K	LD_{50} 804 mg/kg with oral dosing	Up to 7 d	> 100 mg/kg	Increased mortality with graded levels of menadione	Molitar & Rob Ansbacher e
General safe level 1,000 x requirement					
C	None	1 d	3 g/kg	Toxic levels not yet established	Schmeling & N
General safe level at least 1,000 x requirement					

TABLE 17.2   Vitamin toxicity cont.

Toxic Effects	Age	Vitamin concentration	Comments	F
Problems with nerve transmission in laboratory animals			Toxic levels not yet established	
General safe level at least 1,000 x requirement				
Depressed growth	8 - 16 d	5 - 20,000 mg/kg	Linear growth response to graded levels	Bak
General safe level 2,000 x requirement				
None			Toxic levels not yet established	
General safe level at least 1,000 x requirement				
Ataxia, muscle weakness and loss of balance with laboratory species			Toxic levels not yet established	
General safe level with laboratory species 1,000 x requirement				
None			Toxic levels not yet established	
General safe level with laboratory species 1,000 x requirement				

TABLE 17.2   Vitamin toxicity cont.

	Toxic Effects	Age	Vitamin Concentration	Comments	Re
cid	None			Toxic levels not yet established 10 g/kg B.wt had no effect with laboratory species	
General safe level with laboratory species 1,000 x requirements					
	None	1 d	Up to 0.5 mg/kg B.wt	Liver biotin levels elevated	Whi Rar Arend
General safe level 10 x requirement					
	None	1 d	30 μg/g	No effect on growth. No major response reported with other species	Schaef
General safe level at least 10 x requirement					
	Reduced body weight and feed efficiency	1 d	> 2,200 mg/kg	Pyridoxine deficiency noted in some studies after 6 weeks	Deeb March
	Fishy taint in eggs	Layer	3 - 5,000 mg/kg		
General safe level 2 - 5 x requirement					

Table 17.3 Effects of toxicities of vitamins A and D_3			
Diet treatment	25 d Body wt (g)	% Bone Ash	General observations
Control	641	44	No abnormalities
High vitamin A	313	29	Severe lameness, rickets
High vitamin D_3	527	43	Renal tubular mineralization
High vitamin A + D_3	596	48	No abnormalities
			Adapted from Metz et al. (1985)

It seems obvious that high levels of vitamin A can increase the requirement for vitamin D_3, and so diet and water therapy levels of vitamin A should be conservative.

4.2.   Vitamin E

Growth rate of young birds is not affected by feeding 1000 IU vitamin E/kg diet, although adverse effects are seen at 2200 IU vitamin E/kg (March *et al*., 1973). Toxic levels of vitamin E also depress thyroid function and bone calcification, and because of increased prothrombin time, there is an indication of impairment of vitamin K metabolism. Murphy *et al.* (1981) likewise showed 10,000 IU vitamin E/kg diet to be hazardous to young birds, with the major metabolic changes being reduced calcium and phosphorus levels in plasma together with reduced bone ash content. Murphy *et al.* (1981) conclude that excess vitamin E increases the birds requirement for vitamin D_3. Feeding a very wide range of vitamin E levels, Nockels *et al.* (1976) observed a linear decrease in the growth of 5 wk old birds. With levels exceeding 4,000 IU/kg diet there was reduced skin pigmentation, while at 8,000 IU/kg the most characteristic sign was development of so called "waxy" feathers.

4.3.   Vitamin D_3

High levels of vitamin D_3 can lead to calcium deposits in the kidney, and this is sometimes seen in turkey poults when the vitamin is topdressed on to feed as a treatment for rickets. Ameenuddin *et al.* (1986) found laying hens to be little affected by up to 5,000 µg vitamin D_3/kg feed. This apparently high level of D_3 had no effect on egg production or hatchability, although there was an indication of reduced egg weight, shell thickness and fertility, although it is not known if these effects were due simply to reduced feed intake (Ameenuddin *et al.,* 1986).

Over the last few years, there has been an interest in the use of various D_3 metabolites and how they influence calcium metabolism, especially for younger birds. Such metabolites are used at very low concentrations in the diet and so there is a potential for toxicity. Soares *et al.* (1982) studied the potential toxicity of some D_3 metabolites. Relatively high diet levels of the commonly used 25 (OH) D_3 were generally without effect, although at 10-15 µg/kg $1\alpha(OH)D_3$ caused extensive weight loss and reduction in shell thickness of layers. Soares *et al.* (1982) suggest that the pathological effects observed are related to the potent calcium homeostatic properties of these metabolites, and that toxic levels cause aberrations in circulating calcium concentrations.

Chapter 17

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