Effects of mycotoxins on bone structure

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Various skeletal disorders affect most fast growing meat birds and also laying hens. A number of factors are known to influence normal bone development and these are discussed in relation to fast growth rate. The incidence of leg problems is generally higher in males than females.

Nutritional factors include protein and amino acids, vitamins, minerals and electrolyte balance as well as role of mycotoxins.

Genetics, sex of the bird and growth rate are also major factors affecting potential bone disorders. The anatomical difficulty in free movement and dominance of normal birds make the lame birds deprived of normal feeding and watering.

Furthermore, broilers with lameness are difficult to eviscerate properly with the automatic eviscerating machines, thereby, rendering them liable for discard, despite better body weights.

Most poultry are afflicted with varying degrees of skeletal disorders at some time during their productive life cycle. These problems are 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.

The leg bones are one of the fastest growing bones in the skeleton and coupled with their weight bearing characteristics it is not surprising that femur, tibiotarsus and tarso metatarsus bone problems are so prevalent.

In broilers and turkeys, leg problems occur early in life and lead eventually to morbidity with failure to eat and drink or in extreme cases, mortality.

Normal bone development

Bone is cartilaginous connective tissue with the unique property of being mineralised. To some extent, the limit of muscle growth is also 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.

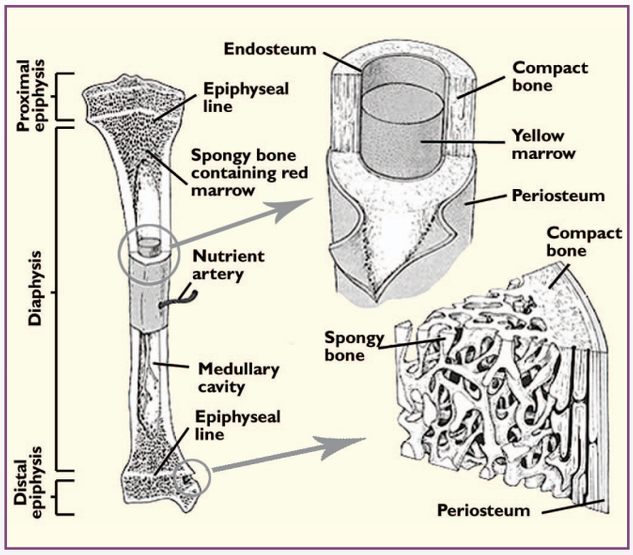


Fig. 1. The structure of bone.

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.

Both the longitudinal growth and bone thickness are controlled by the activity at the growth plate in the metaphysis region. Bone growth is accomplished by two basic processes.

First, there is formation of bone matrix (collagen and mucopolysaccharides) followed by calcification, mainly as calcium phosphate. Bone absorbing cells called osteoclasts break bone down and discard worn cells. After a few weeks the osteoclasts disappear and osteoblasts come to repair the bone. During the cycle calcium is deposited and withdrawn from the blood.

The periosteum, a fibrous membrane, covers the outside of bone. This membrane is rich with capillaries, which are responsible for nourishing bone. The outer layer of bone is called cortical bone. Skeletal bone mass is 80% cortical bone. Cancellous bone (also called trabecular bone) is an inner spongy structure that resembles honeycomb, which accounts for 20% of bone mass.

Potential contributors to abnormal bone development include:

- Body weight/growth rate.
- Nutrition.
- Protein and amino acids.
- Vitamins and minerals.
- Electrolyte balance.
- Mycotoxins (see Table 1).
- Dietary ingredients.

Various skeletal disorders found in poultry are known to have been influenced by mycotoxins as the etiological factor.

Tibial dyschondroplasia

Tibial dyschondroplasia (TD) is characterised by an abnormal cartilage mass in the proximal head of tibiotarsus. TD is seen most commonly in broiler chickens. Symptoms are first seen at 21-35 days.

Birds are reluctant to move and when forced to walk, do so with swaying motion or with stiff gait. TD relates to 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. A typical cartilage plug, therefore, develops and as bone grows there is lateral displacement of the growth plate causing characteristic bowing or bending of the legs.

Riddell, 1975b, suggests that between 21-24 days of age, the proximal tibiotarsus showed the greatest development of TD.

Among many causes like genetics, electrolyte balance, Ca, P leading to development of TD, mycotoxins does also play a vital role in its development. Mycotoxins produced by various Fusarium moulds are known to affect TD. Lee et al., 1985, isolated Fusarium roseum in oats and tested various fractions of the mycotoxins produced, as they affected TD.

Skeletal disorders	Mycotoxin involvement
Tibial dyschondroplasia	✓
Cage layer fatigue	✗
Rickets	✓
Chondrodystrophy	✗
Spondylolisthesis/kinky back	✗
Femoral head necrosis	✗
Foot pad dermatitis	✗
Articular gout	✓

Table 1. Some of the major skeletal disorders wherein involvement of mycotoxin has been implicated.

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 causing 100% TD when fed at 75ppm. TDP-1 has since been isolated as Fusarochromanone. Krough et al., 1989, reported what they claimed to be the first direct evidence of TD due to naturally occurring fusarochromanone.

The morphological characteristics of the cartilage of affected birds were classical to TD in that the typical cartilage was not penetrated by the meta physeal vascular system. Krough et al., 1989, showed that

Continued on page 12

Continued from page 11
while these changes were most pronounced in tibiotarsus, lesions also occurred in the humerus, femur and tarso metatarsus.

More recently Wu et al., 1993, indicated that moderately high levels (75ppm) of fusarochromanone caused 100% incidence of TD in broilers, and that the minimum dietary level of this toxin needed to produce leg problems was 20ppm.

Rickets

Rickets most commonly occurs in young meat birds, the main characteristic being inadequate bone mineralisation. Ca deficiency is the main problem, although this can be induced by feeding diets deficient in Ca, P, or vitamin D3. In most field outbreaks abnormal bird behaviour is seen between 7-10 days of age.

Characteristic weak bones and rib beading can be see at around 10-14 days of age, where 10-100% of the flock is affected. Their bones are rubbery and the rib cage is flattened and beaded at the attachment to the vertebrae.

In most of the cases of rickets, a deficiency of vitamin D3 is often suspected. This can be due to a simple dietary deficiency, inadequate potency of D3 supplement or other factors that reduce the absorption of vitamin D3. Rickets is often more problematic when diets contain mycotoxins and especially aflatoxins.

Normally, Vitamin D2 and D3 is converted to 25-hydroxycholecalciferol (25-OH), which is a circulatory form of Vitamin D3. This is then converted to 1,25 di hydroxycholecalciferol (1,25-OHD) or calcitrol, that is the most biological active form of the vitamin D. Aflatoxin reduces vitamin D absorption and liver damage prevents conversion to the active 25-OH form of vitamin D3 as shown in Fig. 2.

However, it is unclear if the mycotoxins create a specific metabolic deficiency of vitamin D3 and of other nutrients or if they simply affect the bird by reducing the feed intake. In mycotoxins contaminated diets especially those from Fusarium moulds, it is recommended to increase levels of vitamin D3.

Articular gout

Gout refers to the condition in which high plasma uric acid leads to precipitation of urate crystals either in synovial fluid and tendon sheaths of various joints especially the hock joint or on serous surface of various visceral organs when kidneys are dysfunctional. Sustained hyperurecaemia is most commonly caused by decreased renal clearance of urate.

Deposition of such urate crystals at joint is referred to as articular gout. Mycotoxins like aflatoxin, ochratoxin, and citrinin are all known for their kidney dysfunction mechanism.

Mollenhaur et al., 1989, fed aflatoxin at levels upto 5ppm of diet, and after 21 days, observed thickening of glomerular membranes of glomerular apparatus of chickens leading to articular gout.

Another mycotoxin namely oosporein (formed from mould, Chaetomium spp.) not only severely affects epithelium of proximal tubules of the nephron but also the basement membranes. These changes in kidney lead to hyperurecaemia and ultimately leading to gout.

Economic losses

Leg abnormalities probably cause more economic losses than any other single abnormality in the chicken house. It has been estimated that 2-6% of all broilers display some observable signs of skeletal problems, while many more will be affected in a less visible way.

Leg abnormalities result in mortality, reduced feed utilisation and growth rate, and down-grading in the processing plant. Unlike in

Table 2. Co-occurrence of aflatoxin B1, ochratoxin A and T2 toxin in feed ingredients and finished poultry feeds in India (Devegowda et al., 2005).

	Number of positive samples in respective range of mycotoxin concentrations					
	Aflatoxin B_1 (ppb)		Ochratoxin A (ppb)		T-2 toxin (ppb)	
	0-50	50-100	0-50	50-100	0-50	50-100
Cereals	88	44	68	44	72	44
Cereal byproducts	48	8	24	16	32	8
Oilseed meals	152	8	64	40	60	24
Finished feed	328	76	200	80	268	96

Ochratoxin A (ppb)	Aflatoxin B_1 (ppb) Negative	1-20	21-50	Total sample number
1-20	4	69	6	79
21-50	1	25	1	27
51-100	2	18	6	26
101-200	4	3	4	11
>200	3	0	0	3
Total sample number	14	115	17	146

Table 3. Co-occurrence of ochratoxin A with aflatoxin B1 in complete feed in India (Chandrasekaran et al., 2002).

North America, where efforts have been made to accurately monitor the losses incurred in the livestock industry due to mycotoxicoses, no such detailed information is available for the Asia-Pacific region.

Losses due to mycotoxicoses have been estimated at more than $1 billion in Canada and over $2.5 billion in the US during the 1990s.

To estimate production losses from skeletal problems, the chicken industry has been divided into two broad production systems – broiler flocks and breeder/layer flocks.

Broiler flocks

Broilers have a life span of approximately five to seven weeks after which they are slaughtered for meat. Therefore, the important production parameters that a disease may affect are weight gain, culling and mortality rates and condemnations at slaughter.

● **Disease effects:**
Weight gain/feed conversion. No published data were found on the effect of skeletal disorders on weight gain although they are said to have an impact.

● **Mortality and culling.**
In a UK trial quoted by Pattison (1992), 0.8% of broilers between 15 days of age and slaughter were culled for lameness. In a 1976 survey of broiler mortality on six farms in East Anglia, leg deformities were diagnosed in 2.06% of dead birds to give an overall mortality rate from leg deformities of around 0.06%. The suggested mortality/culling rates for skeletal problems are minimum 1%, mean 2% and maximum 3% of broilers placed per year.

● **Carcase condemnations.**
At a large poultry processing plant in 1992, 2.09% of broiler carcases were rejected in total and 1.57% of broiler carcases were rejected because of disease conditions. Joint lesions were responsible for 0.31% of condemnations in broilers from farms with rejection rates of 3% or more.

However, the author notes that the 19.5% of condemnations for emaciation were probably the result of leg weakness. If the assumption is true, around 20% of condemnations in broilers from farms with high condemnation rates were directly or indirectly attributable to leg weakness (skeletal problems).

If the relative importance of skeletal problems is the same in flocks with average condemnations rates, then around 0.3% (20% of 1.57%) of broilers slaughtered are likely to be condemned for skeletal problems.

The suggested carcase condemnation rates for skeletal problems are minimum 0.2%, mean 0.3% and maximum 0.5% of broilers slaughtered per year.

Egg laying flocks

Studies of losses due to disease in egg laying birds tend to be concentrated on birds in lay.

However, skeletal problems also cause losses during the rearing period in birds destined for breeder and commercial egg laying flocks.

The life span of egg laying birds has been divided into two periods: 0-19 weeks (growing period) and 20-70 weeks (laying period).

Mortality/culling is the most important production parameter affected by skeletal disorders in egg laying birds. All breeder and commercial egg laying flocks will contain birds with skeletal abnormalities.

● **Mortality and culling.**
Birds aged 0-19 weeks: Little data were found on mortality and culling rates for skeletal disorders in growing birds in breeder/ layer flocks.

In a survey of mortality in chicks from 0-70 days of age in nine breeder flocks, 1.3% of birds that died had deformed hocks to give an overall mortality rate from the condition of around 0.02%.

However, as the study only covers the early part of the birds' lives, it is an underestimate. The same rate applied proportionately over 19 weeks is 0.04%.The suggested mortality/culling rates for skeletal problems in birds during the growing period, is minimum 0.04% of birds aged 0-19 weeks.

Birds aged 20 weeks (point of lay) to 70 weeks (slaughter): The suggested mortality/culling rates for skeletal problems in birds during lay are minimum 0.1%, mean-0.5% and maximum 1.0% of birds in lay.

Indian perspective

During 2004 and 2005, a survey was conducted to study the incidence of aflatoxin, ochratoxin and T-2 toxin in various feed ingredients and finished feeds collected from different states of the country.

Out of 984 samples analysed, 824 samples were found to be positive for the presence of aflatoxin, ochratoxin and T-2 toxin. Of these, 91, 94, 97 and 97% of cereals, cereal by-products, oilseed meals and finished feeds, respectively, tested positive for mycotoxins.

The authors reiterated that not only are aflatoxins a problem in the region; but also ochratoxins and T-2 toxin.

Over a five year period, Chandrasekaran et al., 2002, assayed 7,173 samples of oil cake, 3,842 samples of complete feed and 2,463 cereals for the presence of ochratoxin A (OTA), citrinin and aflatoxin. Ochratoxin was detected in all samples while aflatoxin was found in 90% of the samples (Table 3).

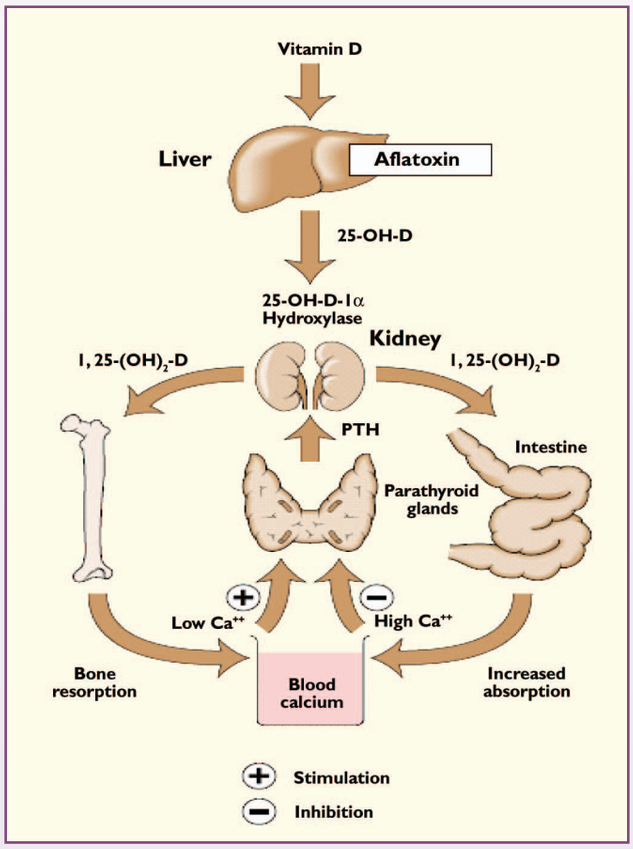
Nevertheless, mycotoxin surveys from around the world indicate that protein sources such as rapeseed meal, cottonseed meal, groundnut cake, sunflower cake, copra meal and palm kernel meal are more susceptible to mycotoxin contamination than conventional raw materials such as soybean meal.

Owing to high prices of conventional raw materials during certain years, feed manufacturers have been forced to opt for alternatives to soybean meal and this has increased the potential for mycotoxicoses for many livestock species.

Similarly, the cost of maize has forced a look at other energy sources, including byproducts such as rice bran, wheat bran and screenings. Many mycotoxins are concentrated in the outer covering of the seeds and therefore, the chances of mycotoxin related problems are increased when such materials are used in animal rations.

For example, during the milling process DON was found in the highest concentration in the bran and lowest in the flour. Mycotoxins from these byproducts in combination with mycotoxins from more traditional ingredients can result in toxicological interactions.

Fig. 2. Aflatoxin blocking vitamin D absorption.



Conclusion

It is quite evident that feed contaminated with mycotoxins play a role in inducing leg weakness.

Differential diagnosis will always allow us to determine what actions must be needed to control such incidences. Controlling all mycotoxin (polar and non polar) with available control strategy will undoubtedly bring down the leg weakness incidences. ■

References are available from the author on request
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