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Technical Bulletin

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Role of Vitamin D Metabolites in Animal Nutrition

The history of Vitamin D dates back to the early 20th century when a disease, now called rickets, used to be treated with cod liver oil. In 1924 Steenbock demonstrated that antirachitic activity could be generated by irradiation of foods with ultra-violet light. The antirachitic component was named Vitamin D by McCollum in 1925.

Further studies of Steenbock *et al*. and Bethke *et al*. showed the inability of fowl to utilize irradiated ergosterol from plant sources as effectively as cholecalciferol from fish oils. It was found that Vitamin D synthesis in plants is chemically different from its synthesis in animal bodies.

Introduction

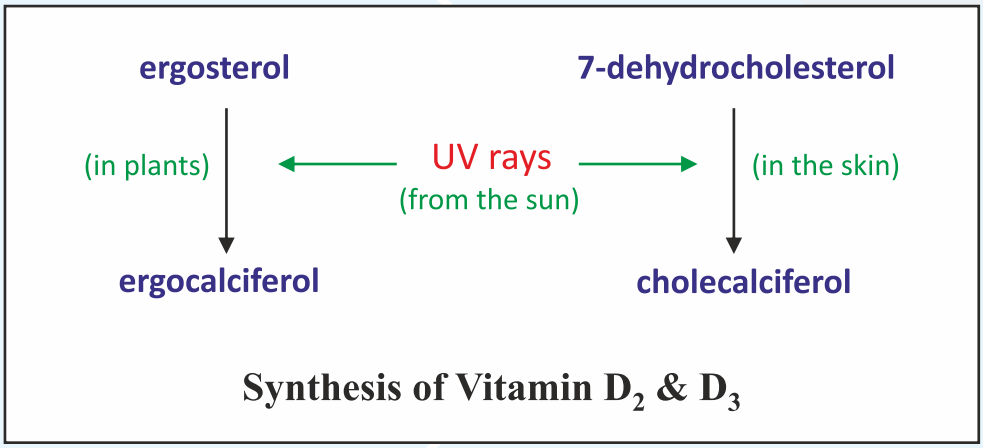
Vitamin D is also known as the "Sunshine Vitamin: as when skin is exposed to ample sunlight, Vitamin D is synthesized by different molecules. Cholecalciferol (Vitamin D_3), (occuring in animals) and Ergocalciferol (Vitamin D_2), (occurring in plants) are the two main natural sources of Vitamin D. As such Vitamin D would mean either Vitamin D_2 or Vitamin D_3 in the absence of a subscript.

Physiologically Vitamin D_3 metabolites 25 (OH) D_3, 1-α-(OH) D_3 and 1, 25 $(OH)_2$ D_3 are more effective than Cholecalciferol because all the Cholecalciferol entering the system is not converted into active metabolites. Active metabolites may directly reach the target tissue by bypassing hydroxylation in liver and kidney, hence have more efficacy than Vitamin D_3 (Goodgame *et al*., 2011).

When given through diet like all fat soluble vitamins, Vitamin D is absorbed from the digestive tract. Like the others, it requires the presence of bile salts for absorption (Braun, 1986), and is absorbed through chylomicron into the lymphatic system of animals along with other lipids.

Sunlight and Vitamin D

The 7-dehydrocholesterol present in the skin is converted into Vitamin D_3 by irradiation. Ultraviolet light of 230 to 320 nm wavelength affects the conversion by imparting a definite quantity of energy to the sterol molecule.



Synthesis of Vitamin D_2 & D_3

Chemical Nature

Vitamin D comprises a group of compounds with antirachitic activity that are closely related. It can be administered through the diet or through irradiation. There are around 10 pro-vitamins with variable antirachitic activity after irradiation. Ergocalciferol (Vitamin D_2) and cholecalciferol (Vitamin D_3) are the two most effective compounds of this group.

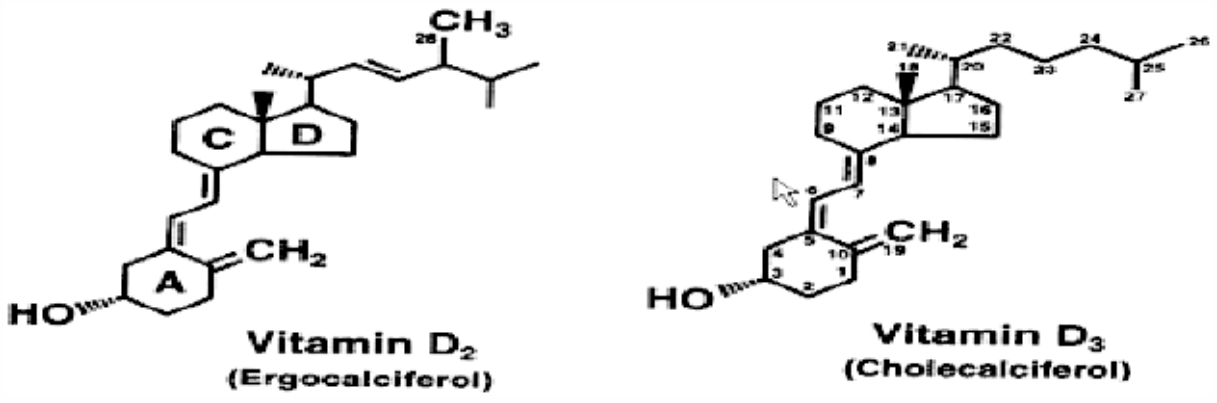
The term D_1 was originally suggested for activated sterol, which was later found to be impure and consisted primarily of ergocalciferol, which had already been termed as Vitamin D_2. The result of this confusion was that the term Vitamin D_1 was abolished in the D vitamin group. The D vitamins are insoluble in water but soluble in fats and fat solvents. Vitamin D_2 and Vitamin D_3 are both more oxidation-resistant than Vitamin A, whilst Vitamin D_3 is more stable than Vitamin D_2.

Metabolism

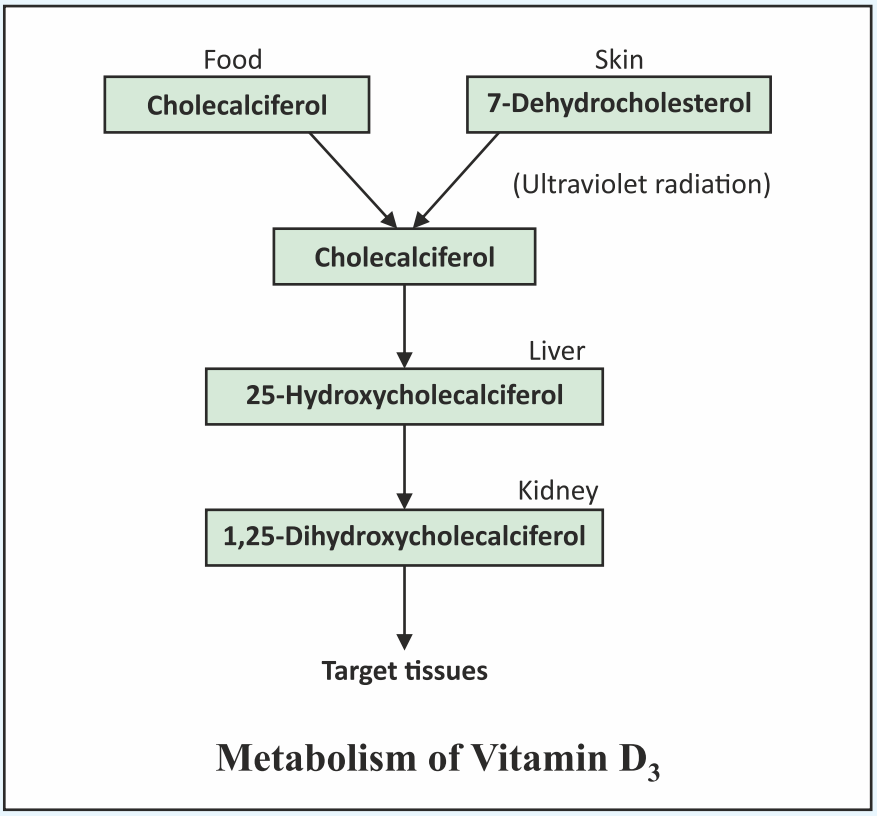
Dietary Vitamins D_2 (Ergocalciferol) and D_3 (Cholecalciferol) are absorbed from the small intestine and are transported by the blood to the liver, where they are converted into 25-hydroxycholecalciferol.

The vitamin, whether intestinally absorbed or produced in the skin, is hydroxylated primarily in the liver by Vitamin D 25-hydroxylase to form 25-hydroxycholecalciferol (25-OH- D_3). If elevations in parathyroid hormone signal a need for increased circulating calcium levels, 25-OH-D_3-1 hydroxylase hydroxylates 25-OH-D_3 on carbon 1 to produce 1,25-dihydroxy Vitamin D_3 (1,25-$(OH)_2$ D_3).

Vitamin D_2 appears to be hydroxylated to 25-OH-D_2 and 1,25-$(OH)_2$-D_2 in a manner similar to Vitamin D_3 (Jones *et al.*, 1976). The dihydroxy



Chemical structures of ergocalciferol and cholecalciferol



Metabolism of Vitamin D_3

compound is then transported through the blood to the various target tissues - the intestine, the bones and the eggshell glands in birds.

Supplemental Forms of Vitamin D

Vitamin D is an essential nutrient with a major role in the regulation of a number of genes, many of which are involved in calcium absorption and transport along with cell development. Vitamin D is normally supplemented in animal diets as cholecalciferol. This form has to be hydroxylated first in the liver as 25-hydroxy-cholecalciferol and subsequently, in the kidney as 1,25-dihydroxy-cholecalciferol. This dihydroxy compound is the main metabolically active form of Vitamin D and binds to the Vitamin D receptor (VDR) which then binds to the Vitamin D response element in genes.

Vitamin D_3

The role of Vitamin D_3 in supporting animal production is well established. Vitamin D_3 is the most important molecule for calcium absorption in the intestine.

Vitamin D_3 (Cholecalciferol) is available to animals either by the conversion of 7-dehydrocholesterol to cholecalciferol in the skin or from dietary sources. In modern animal production where animals are kept in farms, the conversion of 7-dehydrocholesterol to cholecalciferol does not ensure enough cholecalciferol. Hence, Vitamin D_3 is regularly supplemented in animal diets through premixes.

25-Hydroxy Vitamin D_3

This metabolite is synthesized by hydroxylation of cholecalciferol in the liver.

Once in the liver, the first transformation occurs where a microsomal system hydroxylates the 25-position carbon in the side chain to produce 25-hydroxy-Vitamin D. This metabolite is the major circulating form of Vitamin D under normal conditions. The 25-(OH) D_3 is then transported to the kidney on the Vitamin D transport globulin. It is then converted in the proximal convoluted cells to a variety of compounds of which the most important is the 1,25 dihydroxy-Vitamin D_3.

1-α-Hydroxy Vitamin D_3

1-α-hydroxyvitamin D_3 (Alfacalcidiol) is a Vitamin D_3 metabolite in which the hydrogen at the 1-α position is replaced by a hydroxyl group.

Alfacalcidiol performs important functions in the regulation of the calcium balance and bone metabolism. Alfacalcidiol is Vitamin D-hormone analog which requires activation by the enzyme 25-hydroxylase in the liver. Alfacalcidiol is superior to Vitamin D (cholecalciferol) because it can bypass the renal 1-α-hydroxylase activation due to its pre-existing hydroxyl group at the 1st carbon, and can then undergo hydroxylation in the liver to yield 1,25 $(OH)_2$ D_3.

Alfacalcidiol has a weaker impact on calcium metabolism and parathyroid hormone levels than calcitriol.

1,25-Dihydroxy Vitamin D_3

Holick *et al*. (1971), Jones *et al*. (1975) pioneered the isolation and detection of 1,25$(OH)_2$ D_3 also known as Calcitriol. The 1-Hydroxylation of 25-OH-D_3 occurs in the kidney and was shown by the absence of 1-hydroxylation in a nephrectomized animal, contributing to the termination of the transport of intestinal calcium, mobilizing bone calcium and transporting intestinal phosphate. DeLuca (1974) concluded on the basis of many experiments with 1,25$(OH)_2D_3$ that 1,25$(OH)_2$ D_3 is a steroid hormone and is a biologically active Vitamin D metabolite. It is ten times more effective in ricket prevention and cure than Vitamin D_3.

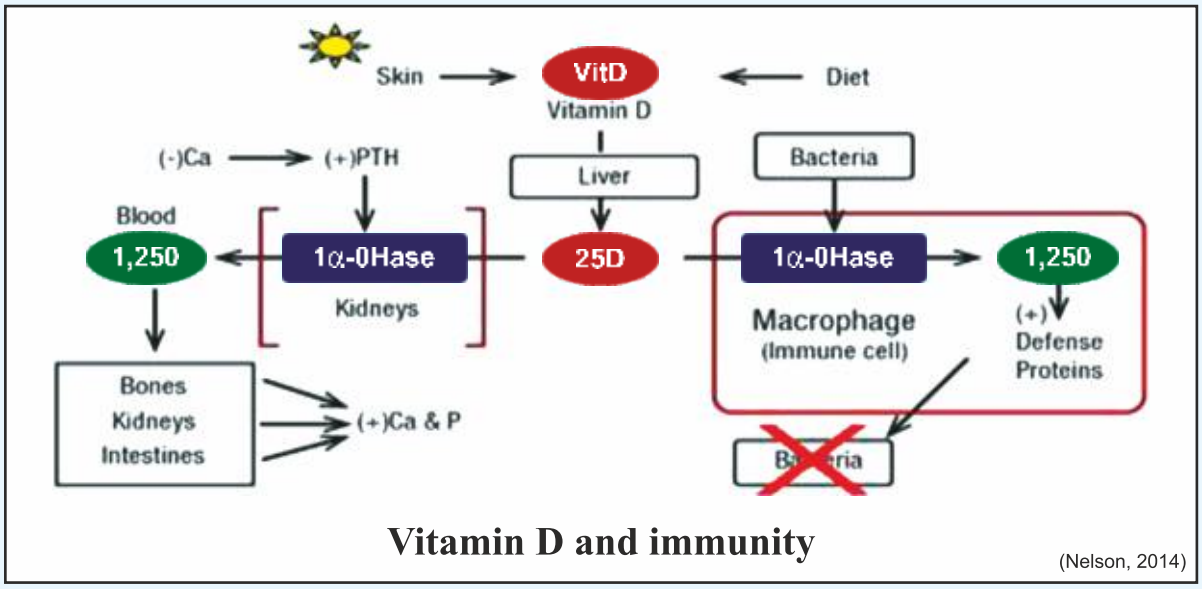
1,25$(OH)_2$ D_3 acts directly on the intestine by stimulating the intestinal calcium, phosphorus absorption and parathyroid hormone thereby causing a large phosphate diuresis while negating the phosphataemic effect of 1,25$(OH)_2D_3$. This results in a net increase in serum calcium with no change in serum inorganic phosphate.

Glycoside form of 1,25-dihydroxy Vitamin D_3

1,25-dihydroxy Vitamin D_3 glycoside is of plant origin and is derived from the waxy-leaf nightshade *(Solanum glaucophyllum*). This plant naturally contains 1,25 $(OH)_2$ D_3, the metabolically active form of Vitamin D_3, in a glycosidic form. Several studies with different animal species have proved its role in correcting problems associated with Vitamin D deficiency. The heat stability of the glycoside form is preferred over other metabolic forms of Vitamin D_3 hence making it ideal for on top supplementation.

Comparison of commercially available variants of Vitamin D_3

Various Forms	Activation Status	Metabolic Loss
Vitamin D_3	Metabolized in liver & kidney and then gets activated	High
25 (OH) D_3	Metabolized in kidney and then gets activated	High
1-α-(OH) D_3	Metabolized in liver and then gets activated	High
1, 25 $(OH)_2$ D_3	Already in active form	Low



Vitamin D and immunity

(Nelson, 2014)

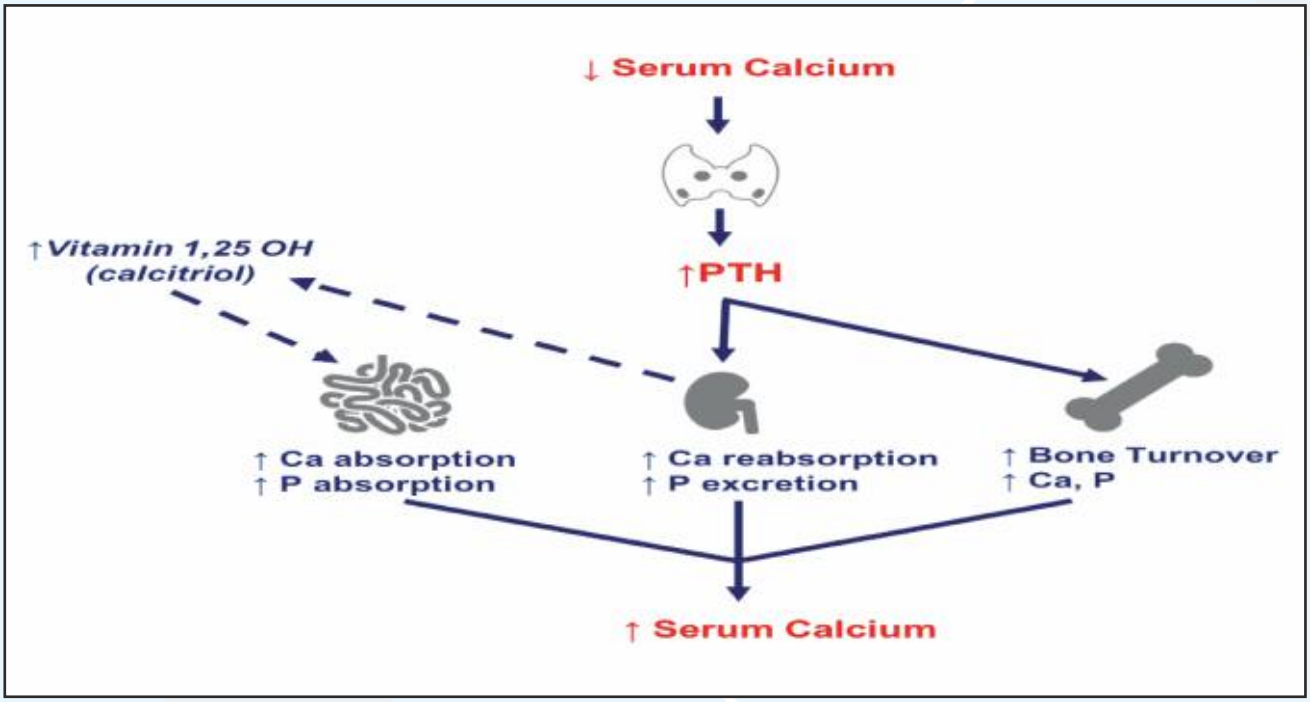
Role of Vitamin D in supporting immunity

When pathogens invade the body, the immune system first activates the innate and then the acquired host defense systems. Antimicrobial defences of the macrophages depend on the 1α-hydroxylase activity in the macrophages and the availability of Vitamin D. If Vitamin D is insufficient, the immune system is compromised, and the animal is at greater risk of infectious diseases.

1α-hydroxylase activity in immune cells produces 1,25 dihydroxycholecalciferol which triggers innate defenses of the immune system by stimulating production of defense proteins that kill pathogens. When there is a deficiency of Vitamin D, immune system function may be impaired even if the animal does not exhibit symptoms of conventional Vitamin D deficiency.

Role of Vitamin D in Regulation of Ca & P Absorption

The amount of 1,25-dihydroxycholecalciferol produced by the kidney is controlled by the parathyroid hormone. When the level of calcium



in the blood is low (hypocalcemia), the parathyroid gland is stimulated to secrete more parathyroid hormones, which induce the kidney to produce more 1,25-dihydroxycholecalciferol. This in turn enhances the intestinal absorption of calcium. In addition to increasing intestinal absorption of calcium, 1,25-dihydroxycholecalciferol increases the absorption of phosphorus from the intestine and enhances calcium and phosphorus reabsorption from the kidney and bone.

Deficiency Symptoms

In young animals, a deficiency of Vitamin D results in rickets, a growing bone disease in which the deposition of calcium and phosphorus is disturbed, the bones become fragile and the legs can bow. Symptoms of swollen knees and hocks and arching of the back can occur in young cattle. In swine swollen joints, broken bones, joint stiffness and sometimes paralysis is observed.

Vitamin D deficiency causes Osteomalacia in older animals, in which bone reabsorption has already developed. Osteomalacia arising out of Vitamin D deficiency is not normal in farm animals, although pregnant and lactating animals that need increased amounts of calcium and phosphorus may experience a similar condition. Rickets and Osteomalacia are not particular disorders that are exclusively caused by Vitamin D deficiency; they may also be caused by a lack of or an imbalance of calcium and phosphorus.

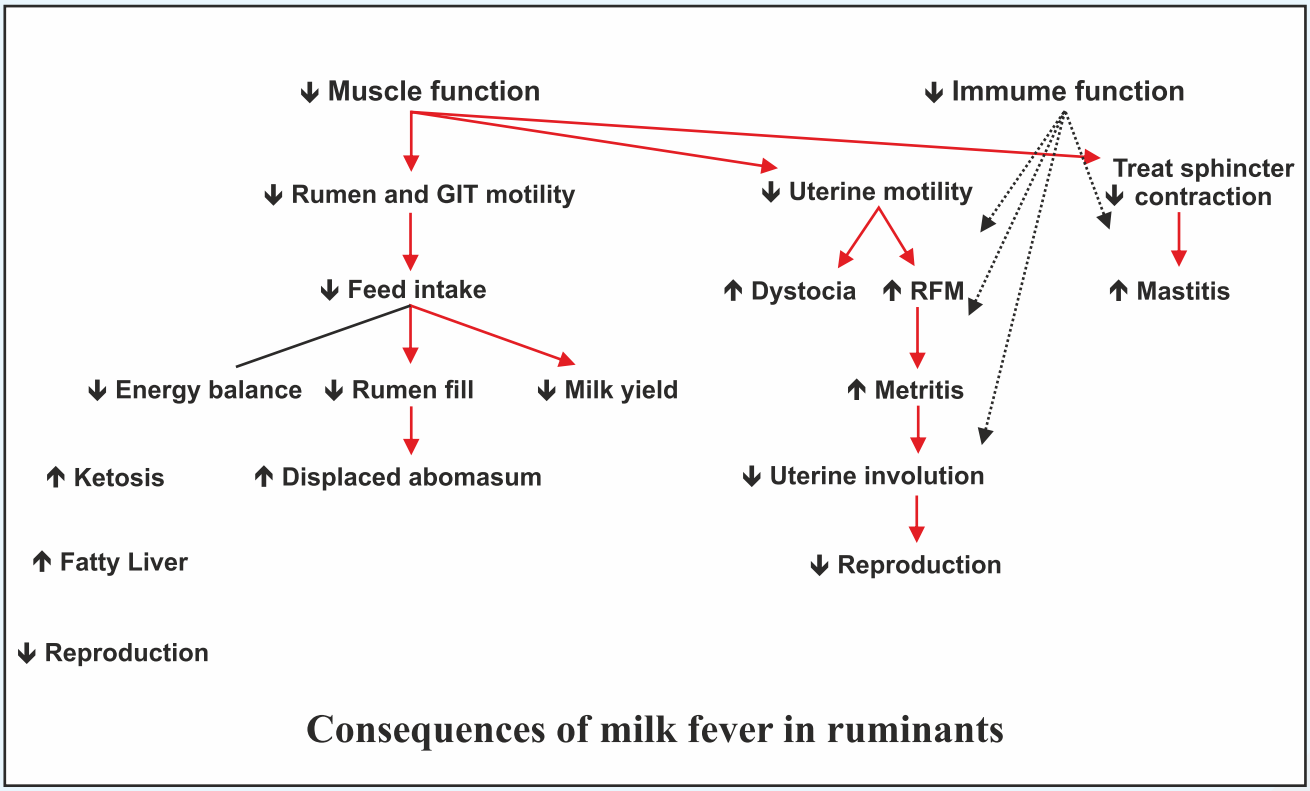
In Poultry

In poultry, a deficiency of Vitamin D causes the bones and the beak to become soft and rubbery. It also is the cause for retarded growth and leg weaknesses. Egg production can reduce and eggshell quality may deteriorate. Poultry feeds with the exception of fishmeal, contain little or no Vitamin D. This vitamin is generally supplied in the form of fish-liver oils or synthetic preparations.

The first sign of Vitamin D deficiency in chicks is rickets, which is characterized by severe weakness of the legs. During Vitamin D deficiency, growing birds develop hypocalcemia, which in turn stunts skeletal development through widened cartilage at epiphyses of long bones and weakened shafts. Young growing chickens and turkeys tend to rest frequently in a squatting position, are disinclined to walk, and have lame stiff-legged gait. These are distinguished from the clinical signs of Vitamin A deficiency as Vitamin D-deficient birds are alert rather than droopy and walk with a lame rather than a staggering gait. The beaks and claws become soft and pliable, usually between the 2nd and 3rd week. The most characteristic internal sign of Vitamin D deficiency in chicks is the beading of ribs at the juncture of the spinal column.

Tibial dyschondroplasia (TD) is one of the most common skeletal abnormalities observed due to Vitamin D deficiency. TD represents deformed bones and lameness in birds. It is a disease common in rapidly growing birds, especially broilers and turkeys. Comparisons between fast-growing and slow-growing strains have revealed less mineralization and more porous cortical bone in fast growing birds.

Signs of Vitamin D deficiency start occuring in laying birds in confinement within one to two months of having been deprived of Vitamin D. When laying chickens are fed a diet deficient in Vitamin D, the first sign of the deficiency is a thinning of the egg shells. Commercial layers may continue to lay eggs with reduced shell strength for weeks. If the diet is completely devoid of Vitamin D_3, egg production may decrease rapidly, and eggs with a very thin shell or no shell can be produced. In layers, eggshell strength tends to decrease as the birds age. The decline in shell strength may be due to a decrease in the bird's ability to synthesize 1,25-$(OH)_2 D_3$. A study of the effect of dietary 1,25- $(OH)_2$ D_3 on eggshell strength in older birds found that within 3 weeks, the percentage of cracked or broken eggs were lower for birds supplemented with 1,25-$(OH)_2 D_3$ (Tsang, 1992).



Consequences of milk fever in ruminants

Vitamin D supplementation in breeder birds impacts its content in egg yolks. Deficiency leads to marked reduction in hatchability and frequent mortality of embryos. These embryos show a short upper mandible or incomplete formation at the base of the beak.

In Ruminants

Clinical signs of Vitamin D deficiency in ruminants are decreased appetite, decreased growth rate, digestive disturbances, stiffness in gait, labored breathing, irritability, weakness, and occasionally tetany and convulsions. There is an enlargement of joints, slight arching of the back, and bowing of legs, with an erosion of joint surfaces causing difficulty in locomotion. Sometimes, young ruminants may be born weak, deformed or dead.

Milk fever (parturient paresis) is a paralyzing metabolic disease caused by hypocalcemia near parturition and initiation of lactation in high milk-producing dairy cows. Milk fever is an impaired metabolic condition that is related to Ca status, historical Ca intake, and malfunction of the hormone form of Vitamin D [1,25-$(OH)_2D_3$] and the Parathyroid hormone PTH. Animals that develop milk fever are unable to meet the sudden demand for Ca that is brought about by the initiation of lactation.

Milk fever usually occurs within 72 hours after parturition and is manifested by circulatory collapse, generalized paresis, and depression of consciousness. The most noticeable and commonly occurring abnormality is acute hypocalcemia, in which serum Ca decreases from a normal range of 8 to 10 mg/dL to a range of 3 to 7 mg/dL. In the early stages of onset, the cow may exhibit some unsteadiness during walking. More frequently, the cow lies on her sternum with the head displaced to one side, causing a kink in the neck, or turned into the flank. The eyes are dull and staring and the pupils dilated. If treatment is delayed, the dullness gives way to coma, which may lead to death.

In Swine

In swine, Vitamin D deficiency causes poor growth, stiffness, lameness, stilted gait, posterior paralysis, fractures, softness of bones, bone deformities, beading of the ribs and enlargement of joints. Bones may get deformed by the weight of the animal and the pull of body muscles.

Conclusion

Fast growing and high performing birds and animals frequently experience bone and health challenges due to poor absorption or imbalance or deficiency of Calcium and Phosphorus, all of which lead to reduced performance. In the modern animal farming industry, where the birds and animals are mostly raised indoors, adequate availability of Vitamin D is essential to ensure proper turnover of calcium and phosphorus in the body.

In practice, deficiency of Vitamin D is quite common and leads to several performance related issues. While regular supplementation of Vitamin D_3 through premixes is common, there are more bioactive forms of this vitamin which do not require liver and kidney level conversions. Often, due to disease conditions, the liver and kidney do not work efficiently which adversely affects the conversion of Vitamin D into its active form. To meet such challenges, a biologically active form of Vitamin D represents an answer in managing the gap between Vitamin D requirement and availability.

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