

# Invited Speakers

## S3.2

### Vitamin Requirements and Economic Responses

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NRC (1994) recommends dietary vitamin supplementation levels, which prevent clinical deficiencies. These levels may not support optimum health, performance and welfare of modern poultry. In order to entirely exploit the genetic growth potential of recent genotypes, to strengthen the resistance against various infectious diseases and to improve welfare as well as to optimize meat and egg quality, the vitamin nutrition of poultry must be adapted to today's husbandry and management conditions.

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### Introduction

Vitamins are essential micronutrients, occupying a central role in the metabolism. They are required for optimum health and normal physiological functions such as growth, development, maintenance or reproduction. As most vitamins cannot be synthesised by poultry, they must be obtained from the feed. If vitamins are absent from the diet or improperly absorbed or utilised, specific diseases or deficiency disorders are occurring.

Classically, vitamins have been divided into two groups, based on their solubility in lipids or in water. The fat-soluble group includes the vitamins A, D<sub>3</sub>, E and K, while the vitamins of the B complex (B<sub>1</sub>, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub>, niacin, pantothenic acid, folic acid and biotin) as well as vitamin C are classified as water-soluble. The fat-soluble vitamins are absorbed by mechanisms similar to those involved in fat absorption. Uptake of water-soluble vitamins occurs usually via simple diffusion. Fat-soluble vitamins may be deposited in the animal body, while water-soluble vitamins are not stored and excesses are rapidly excreted. A regular uptake of water-soluble vitamins is therefore essential for securing adequate vitamin availability. As the poultry breeds of today are very productive, their vitamin nutrition needs particular attention in order to allow the birds to perform up to their genetic potential. While the need for added vitamins in animal nutrition is not disputed on principle, there are many questions related to the economic supplementation levels, necessary to achieve optimum health and performance under production conditions.

### Vitamin Deficiencies

In general, vitamin deficiencies generate loss of appetite, growth retardation or inhibition and impaired feed conversion, which results in bad performance. Meat-type poultry grow slowly, laying birds suffer from a decline in egg production as well as insufficient shell quality, while in breeder hens both fertility and hatchability might be impaired. Insufficient supply of certain key vitamins can induce serious disorders, which may lead to death of the bird. The clinical appearance of such drastic deficiency symptoms is specific for each vitamin and thus can be used as diagnostic tool:

- Vitamin A (Retinol): dry and scaly skin, rough plumage, keratinisation of epithelia, poor absorption, reduced immune response and increased susceptibility to infection.
- Vitamin D<sub>3</sub> (Cholecalciferol): lameness and muscular weakness, stiff and hesitant gait, soft and pliant beak of chicks, deformed and brittle bones, enlargement of the epiphyses and occurrence of thin-shelled eggs.
- Vitamin E: muscular myopathy, exudative diathesis, encephalomalacia, frequent health problems and diminished fertility.
- Vitamin K: increased blood-clotting time, hemorrhagic diseases, weakness, rough plumage, paleness of the comb and the wattles.
- Vitamin B<sub>1</sub> (Thiamin): fatty degeneration and necrosis of heart fibers, bradycardia, heart failure, mucosal inflammation, ulcers and haemorrhages, skin oedema and cyanosis, progressive paralysis and atrophied ovaries.
- Vitamin B<sub>2</sub> (Riboflavin): inflamed mouth, nasal mucous membranes and skin, muscular debility, trembling, spasms and paralysis, vomiting, resorption disorders and diarrhoea.
- Vitamin B<sub>6</sub> (Pyridoxine): dermatitis, rough and deficient plumage, inflamed oedema of the eyelids, anaemia and ascites, muscular convulsions, followed by paralysis.
- Vitamin B<sub>12</sub> (Cyanocobalamin): defective feathering, leg weakness, perosis and gizzard erosion.
- Niacin (Nicotinic acid): lameness in young birds, inflammation of the mouth, diarrhoea and reduced feathering.
- Pantothenic acid: rough feathering and depigmentation, crusts at the corner of the beak, exudate on eye-lids and fatty degeneration of the liver.
- Folic acid (Pteroylglutamic acid): rough plumage, feather depigmentation, cervical paralysis, leg weakness and white watery diarrhoea.

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- Biotin: rough and brittle feathers, dry skin, dermatitis of foot pads and deformation of the beak, fatty liver and kidney syndrome as well as perosis.
- Vitamin C (Ascorbic acid): classical vitamin C deficiency does not occur, since poultry are able to synthesise ascorbic acid in the kidneys.

Although rare, clinical vitamin deficiencies can still occur in poultry under production conditions. Encephalomalacia has occasionally been reported from broiler flocks, particularly in stressful conditions, when vitamin E demand is high. Furthermore, bouts of digestive disorders, resulting in malabsorption, lead to an insufficient uptake of fat-soluble vitamins. Recently a serious vitamin A deficiency was diagnosed in a turkey flock, which was due to complete absence of supplemental vitamin A in the feed. Affected birds had stunted growth and ruffled feathers, showed signs of incoordination and were depressed. Mortality was almost doubled (Cortes *et al.*, 2006).

In order to avoid vitamin deficiencies, nutritional recommendations for the dietary supply of these micronutrients have been developed. The most prominent compilation is found in the nutrient requirements of the National Research Council (NRC, 1994), which is based on respective scientific publications. The NRC recommendations, however, indicate just the minimum vitamin levels, necessary to avoid clinical deficiency symptoms. Furthermore, many of the references are 20 to 40 years old and therefore outdated. In order to give nutritionists a more practical guidance, breeding companies and vitamin suppliers (DSM, 2006) have published own vitamin supplementation guidelines, which are based on experience from industry practice as well. These supplementation levels generally exceed the minimum requirements of governmental institutions, since they aim at achieving optimum health and performance of domestic animals in the most cost-effective way.

## Genetic Development of Poultry Breeds

A considerable part of the classical vitamin requirement studies, which have been taken as a basis for the recommendations of the NRC (1994), date back to the years 1960-1980. For two reasons, the information from this database might not be directly relevant for today's poultry production. On the one hand side, many studies were carried out under perfect husbandry conditions, i.e. individual housing of birds (no struggle for feed and water), climate controlled ventilation (no temperature stress or accumulation of ammonia) and clean cages with wire floors (no infectious pressure from diseases). It is known that vitamin requirements are lower, when the stressors, occurring under commercial conditions, are absent. On the other hand side, the poultry breeds, both meat-type and laying, which were used decades ago have nothing in common with current modern genotypes. Havenstein *et al.* (2003) reported that the Ross 308 broilers reached 1,815 g BW at 32 d of age with a FC of 1.47, whereas a breed from 1957 would not have reached that BW until 101 d of age with a FC of 4.42.

The growth rate of meat-type poultry continues to increase year by year and feed conversion tends to decrease, i.e. less feed is required for the same development of biomass. Likewise, performance of laying hens has positively developed, both in terms of egg productivity as of feed efficiency. Since the dietary inclusion levels of vitamins are expressed in units of feed, the absolute uptake of vitamins for an equivalent muscle or egg production has steadily decreased. Whether a constant adjustment is necessary for the growing genetic potential of modern breeds has been addressed in several studies. The NRC (1994) requirement of broilers for vitamin B<sub>1</sub> of 1.8 mg/kg feed, based on research from Thornton and Shutze (1960), was challenged with a classical requirement study for broiler chicks under more recent conditions (Olkowski and Classen, 1996). The results showed that a vitamin B<sub>1</sub> supplementation level above 2 mg/kg did not result in better performance of chicks, indicating that the basic needs of thiamin for maximizing performance have not increased over time. However, a minimum of 8 mg/kg was necessary to avoid blood thiamin concentrations to decline. This finding was taken as an indication that a higher supplementation of vitamin B<sub>1</sub> was needed to sustain thiamin supply for high-yielding poultry breeds. For vitamin D<sub>3</sub> it was recently demonstrated that a dietary supplementation with 10,000 IU/kg feed significantly improved growth, enhanced tibia breaking strength and reduced the incidence of tibial dyschondroplasia in comparison to 5,000 IU/kg, which represents the currently approved maximum level of vitamin D<sub>3</sub> supply for meat-type chickens in Europe (Whitehead *et al.*, 2004). Accordingly the authors asked the question, whether the maximum legal limit for vitamin D<sub>3</sub> in broiler diets should not be increased.

## Immune Responses and Health

Due to intensive husbandry of poultry in commercial conditions, infectious diseases can spread quickly throughout a broiler or a turkey flock. The efficacy of the immune system, which is the primary line of defence, is dependent on an adequate nutrient supply for the development of its key organs, for the rapid expansion of effector cells and for the subsequent synthesis of antibodies. Among the micronutrients, the vitamins A, D, and E were demonstrated to have a direct modulating activity on the immune system (Klasing, 1998).

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## *Vitamin A*

As early as 1977, Leutskaya and Fais have demonstrated that the antibody content in chickens depended on the dose of vitamin A in the diet. In birds fed a high dose of dietary vitamin A, the antibody content in the serum was found to be up to 5 times as high as in control chickens. In a further investigation growth of bursa and thymus was found to be impaired in chicks, fed a vitamin A-free diet. A partial deficiency of vitamin A adversely affected relative bursa weight, but not that of the thymus (Davis and Sell, 1983). Also T-lymphocyte proliferative responses were decreased at low vitamin A intakes and enhanced at the high vitamin A intake (Sklan *et al.*, 1989). Vitamin A deficiency, but also long-term excess caused impairment of the immune response as demonstrated by antibody production and T-lymphocyte proliferation *in vitro*. In contrast to the effects of long-term vitamin excess, a large vitamin A bolus did not cause immune response impairment and even restored normal immune functions in previously vitamin A-depleted chicks (Friedman and Sklan, 1989). Excess or insufficient vitamin A led to increased susceptibility of chicks to *E. coli* infection, which was accompanied by depressed immune responses. Chicks receiving excess vitamin A were also more sensitive to *E. coli* than vitamin A-depleted chicks. In contrast to chicks receiving excess vitamin A, T lymphocyte responses of vitamin A-depleted chicks achieved levels similar to those of vitamin A-sufficient birds with a certain lag period, indicating a delayed immune response (Friedman *et al.*, 1991). In chicks with no added dietary vitamin A, antibody production and proliferative response against *Mycobacterium tuberculosis* were depressed in comparison with chicks receiving vitamin A. The immune responses were maximized with dietary vitamin A until the diet contained 6,660 micrograms/kg, above which the responses decreased (Sklan *et al.*, 1994). Using Newcastle disease virus (NDV) in broiler chickens it was demonstrated that both humoral and cellular immune responses were modulated by dietary vitamin A, suggesting that vitamin A-deficient chickens developed a T helper (Th)2 immune response, whereas the chickens fed highly enriched vitamin A diet showed a Th1 immune response (Lessard *et al.*, 1997). In turkeys, the effect of dietary vitamin A on antibody production and T-cell proliferative response was determined after immunisation with NDV and turkey pox vaccines. Poults receiving the diet with no added dietary vitamin A died by 22 days and had very low concentrations of plasma and liver vitamin A. Increasing dietary concentrations of vitamin A enhanced the proliferative response as well as the antibody titres to NDV and turkey pox until the diet contained 6.0 micrograms/g, above which the response began to decrease (Sklan *et al.*, 1995). In laying hens Coşkun *et al.* (1998) investigated dietary levels of up to 24,000 IU vitamin A per kg over one year, but could not record any beneficial effects on the various parameters of the immune response. In contrast, a high level of dietary vitamin A (12,000 IU/kg) increased the antibody titer against NDV of heat-stressed hens (Lin *et al.*, 2002).

## *Vitamin D<sub>3</sub>*

Plasmid DNA expressing the major outer membrane protein of an avian *Chlamydophila psittaci* have been tested for the ability to elicit an immune response and induce protection in turkeys against challenge with the same serovar. The vitamin D<sub>3</sub>-metabolite 1alpha,25-dihydroxycholecalciferol augmented serum and mucosal antibody titres. However, higher antibody titres were not related to better protection and even had a negative effect on bacterial excretion (Verminnen *et al.*, 2005).

## *Vitamin E*

Tengerdy *et al.* (1972) were the first group, reporting a significantly increased immune response as measured by the antibody plaque-forming cell test or by haemagglutination in chicks and hens, supplemented with elevated dietary levels of vitamin E. Interestingly the effect was much greater on hypoxic birds. Chicks and turkeys, infected with *E. coli* and fed supplemental vitamin E, were found to have reduced mortality and increased HA titers (Nockels, 1979). Vitamin E was also demonstrated to induce a higher production of circulating antibodies against NDV and *Pasteurella anatipestifer*, following vaccination of broiler chicks against these antigens (Franchini *et al.*, 1986). In broilers, immunised against NDV, the highest vitamin E level (300 ppm) in combination with various selenium levels resulted in elevated body weight gain, favourable feed conversion ratio, significantly higher antibody titres and improved cellular immune responses (Swain *et al.*, 2000). In cockerels, fed diets containing either 10 or 300 mg/kg of vitamin E, the ratio of heterophils to lymphocytes increased and cutaneous basophil hypersensitivity response was depressed by feeding the higher vitamin E diet. The enhanced heterophil:lymphocyte ratio from feeding vitamin E suggested an improved phagocytic ability of the immune system (Boa-Amponsem *et al.*, 2000). A dose-dependent increase in antibody production in response to attenuated infectious bursal disease virus (IBDV) between 0 and 25 IU/kg of supplemented vitamin E was found, but no further increase at higher levels. Antibody levels to sheep red blood cells were higher in birds supplemented with 50 IU/kg vitamin E compared to those supplemented with 0 or 200 IU/kg. The conclusion was that moderate levels of vitamin E supplementation were most immunomodulatory and that high levels were less effective (Leshchinsky and Klasing, 2001). Only one study reported a negative effect of vitamin E on the immune response. Antibody production in turkeys to naturally occurring *E. coli*, to NDV and turkey pox vaccines were significantly higher, when the dietary supply of chicks with vitamin E was limited. Concentrations of circulating antibodies to NDV and to turkey pox were highest in the group, receiving no added vitamin E, whereas titers in groups on 150 mg/kg were significantly lower. Responses of groups receiving 50 mg/kg added vitamin E were slightly lower than the control, though not significantly (Friedman *et al.*, 1998).

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In a study on the effect of selenium (Se) or vitamin E on the immune response of broilers to coccidiosis immunized chickens, supplemented with elevated Se or vitamin E, had increased body weight gain and a tendency for lower feed conversion ratio than chickens, fed the unsupplemented diet after an *Eimeria tenella* challenge. Furthermore, dietary supplementation with Se or vitamin E reduced mortality and increased body weight gain of nonimmunised chickens infected with *E. tenella* (Colnago *et al.*, 1984). In contrast a later investigation found vitamin E not to counteract the negative impact of coccidiosis. Plasma alpha-tocopherol was consistently depressed during the acute phase of infection in chicks, consuming either high or low dietary vitamin E. Neither weight gain depression was reversed, nor lesion scores or amount of oocysts shed were consistently influenced. The likely reason for the ineffectiveness of vitamin E might be malabsorption during *E. maxima* infection, making vitamin E less biologically available to the infected tissues (Allen and Fetterer, 2002).

Supplemental vitamin E was also demonstrated to improve maternal immunity. When breeding hens on elevated vitamin E were immunized with *Brucella abortus*, the passive immunity of their offspring was improved. Furthermore vitamin E, fed to nonimmunised hens, significantly increased the primary immune response of their chicks following immunization (Nockels, 1979). Chicks hatched from eggs of breeders, supplemented with high vitamin E and vaccinated against NDV showed accelerated proliferation of splenic lymphocytes and had significantly higher antibody titers than chicks from the control group, indicating that vitamin E supplementation of breeder birds increased the immune response of their progeny (Haq *et al.*, 1996).

## Vitamin C

Birds fed diets, supplemented with vitamin C, experienced significant increases in serum levels of ascorbic acid. The antibody response to *Brucella abortus* was lower in neonates and higher in adults in the presence of increased levels of vitamin C (McCorkle *et al.*, 1980). Chickens, either fed a diet supplemented with 1,000 ppm ascorbic acid or not and vaccinated against IBDV, were subsequently challenged with this pathogenic virus. The number of CD8+ in spleen and IgM+ cells in bursa were significantly higher in the ascorbic acid supplemented group. Furthermore production of IL-2 by splenocytes and the number of anti-IBDV antibody secreting cells in spleen were significantly higher with addition of ascorbic acid. It was concluded that dietary supplementation of vitamin C ameliorates the immunosuppression caused by IBDV vaccination and improves humoral and cellular immune responses (Wu *et al.*, 2000). In laying hens a synergistic effect of vitamin C for their health was demonstrated (Puthongsiriporn *et al.*, 2001). Ascorbic acid, combined with vitamin E, during heat stress generated the highest lymphocyte proliferative responses to concanavalin A and *Salmonella typhimurium* lipopolysaccharide in comparison to treatments with lower vitamin supplementation.

## Welfare of Poultry

Tibial dyschondroplasia (TD) is a disorder, occurring in the growth plates of long bones, which results in a deformation of the bones and subsequent lameness and heavy pain. Although vitamin D<sub>3</sub> was described not to be effective, several vitamin D<sub>3</sub> metabolites were found to prevent the development of TD in broilers (Edwards, 1990). The efficacy of the first D<sub>3</sub>-metabolite 25-hydroxycholecalciferol to reduce the incidence of TD in young broilers was found to be better when the dietary calcium level was below optimum (Ledwaba and Roberson, 2003). Recently, a dietary supplementation of 10,000 IU vitamin D<sub>3</sub> per kg of feed was demonstrated to minimise TD incidence. This result indicates that the vitamin D<sub>3</sub> requirement of broilers for prevention of TD is higher than earlier estimates and may be related to increased calcium requirements of modern broiler genotypes (Whitehead *et al.*, 2004).

## Product Quality

Poultry meat has a relatively short shelf life, since oxidation of lipids give rise to the typical off-odours and off-flavours of spoiled meat. Vitamin E is nature's most powerful lipid-soluble antioxidant, being able to break the free radical induced chain reaction of lipid oxidation. Numerous studies with meat from poultry, fed on diets supplemented with elevated levels of vitamin E, have shown that the oxidative stability of lipids was improved and that the development of rancid deterioration of the meat was delayed. Therefore supplementation of poultry with extra vitamin E is the most promising approach to achieve and maintain an optimum quality of poultry meat (Weber, 2001).

For most people meat is an important part of their daily nutrition, which represents a reliable source of vitamins, particularly of the B-group. Supplementation of meat-type poultry with elevated levels of vitamins will therefore improve the nutritional value of the end product. Furthermore, eggs are a particularly nutritious food and represent a perfect vehicle for the transfer of high-quality nutrients, such as vitamins to human subjects. When alpha-tocopherol and beta-carotene, two antioxidants which are associated with health benefits for humans, were supplemented to laying hens at supranutritional dietary levels, their concentration in egg yolks could be significantly improved (Jiang *et al.*, 1994). Likewise, very high doses of vitamin E (up to 20,000 mg/kg) for laying hens increased the vitamin E content to 51 mg per egg (Sünder *et al.*, 1999). Multiple-enriched eggs have been obtained by feeding laying hens with linseed, minerals, vitamins and lutein. These eggs had greater nutritional value than standard eggs, containing several times more of the omega-3 fatty acid ALA and DHA, more vitamin D, folic acid and

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vitamin E, more lutein and zeaxanthine as well as more iodine and selenium. Furthermore these eggs were rich in vitamin B<sub>12</sub> and vitamin A, plus vitamin B<sub>2</sub>, pantothenic acid and phosphorus (Bourre and Galea, 2006).

## Conclusions

It is widely accepted by the poultry industry that the minimum dietary vitamin levels, required to prevent clinical deficiencies may not support optimum health, performance and welfare of poultry. The predominant reason for that is the rapid progress in genetic development of the breeds, which tends to increase the demand for vitamins. Furthermore, certain vitamins have been demonstrated to exert additional effects, when supplied at elevated supplementation levels. The vitamins A, E, D<sub>3</sub> and C modulate the immune response and thereby improve the resistance of poultry against various infectious diseases, which ultimately contributes to the welfare of farmed birds. Vitamin E has the ability to protect meat from oxidative deterioration through its powerful antioxidant potential. Finally, dietary vitamins are deposited in meat and eggs, which increases the nutritional value of these important food products. In summary, the vitamin nutrition of poultry deserves special attention in order to entirely exploit the productivity potential of modern birds under today's husbandry conditions.

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