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Asian Journal of Animal and Veterinary Advances

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OPEN ACCESS Asian Journal of Animal and Veterinary Advances

ISSN 1683-9919
DOI: 10.3923/ajava.2024.1.12

Review Article
Canthaxanthin and Vitamin D_3 in Commercial Poultry Breeders: A Review

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Abstract
The presence of large amounts of polyunsaturated fatty acids in the yolk, sperm and embryonic tissues predisposes them to lipid peroxidation, causing oxidative stress. Carotenoids such as canthaxanthin and the vitamin D metabolite, 25-hydroxycholecalciferol (25(OH)D_3), have been used to reduce these negative effects. This review addresses a description of the carotenoids and how they are absorbed and deposited in the egg yolk, the main aspects that indicate the use of 25(OH)D_3 in poultry production, as well as the main available papers in poultry production on the association between these two technologies. The main results support the hypothesis that the use of these substances is beneficial for the production of fertile eggs. In general, a reduction in liver and blood peroxidation, an increase in antioxidant activity and a possible direct effect on the vaginal mucosa were described by poultry breeders. Analyses of the antioxidant system in eggs and in breeders, embryos and progeny highlight significant improvements in antioxidant metabolic pathways with a reduction in lipid peroxidation and conservation of antioxidant capacity. In the progeny, effects on hatchability, chick quality, antioxidant capacity, immunity and performance are also described. Similarly, production and performance indicators such as egg and chick composition, hatchability, fertility and chick quality are positively improved in all species studied so far. Many aspects are not metabolically understood and deserve attention from researchers, but the recommendation to use these substances on farms producing fertile eggs is a reality for all poultry species studied.

Key words: Fertility, hatchability, embryo, oxidative stress, broiler, quail

Citation: dos Santos, T.C., L.C. Bittencourt, C.C. da Silva and C.A. Lozano-Poveda, 2024. Canthaxanthin and vitamin D_3 in commercial poultry breeders: A review. Asian J. Anim. Vet. Adv., 19: 1-12.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The pressure of genetic selection has been definitive for performance in broilers, but some undesirable effects have been manifested in breeders and progeny. Among the main problems reported in the poultry industry are the early decrease in fertility and consequent reduction in the number of chicks, as well as the quality of these chicks produced, since each low-quality chick housed generates a lower productive efficiency and a lower carcass yield in the slaughter plant.

In this way, the reduction in fertility and production of lower-quality chicks are considered key points in improving productive efficiency. Many products from various origins and sources are available as additives to be added to the diet of breeders, both male and female, to improve fertility as well as the health of the birds and their progeny. Although the effects of these additives are often not directly associated with reproduction, the end result is an increase in the number of viable chicks or breeders. A list of substances with this effect can be cited, among these, the most studied and used in the market today are organic microminerals such as selenium, vitamins such as E and D and different sources of carotenoids. All these substances mentioned have in common the ability to act in some way on the antioxidant defense mechanisms of the animal organism with positive effects and have been used in poultry production to improve the productive and reproductive performance of breeders, layers and broilers.

Egg yolk, sperm and embryonic tissues are rich in Polyunsaturated Fatty Acids (PUFAs) which perform functions that are essential to maintaining the integrity of phospholipid membranes. However, these fatty acids are more susceptible to lipid oxidation and free radicals ($^{-}O_2$) degrade lipids, proteins and DNA in these tissues, causing oxidative stress. When PUFAs are degraded, they cause a chain reaction that negatively affects performance and overall reproductive indices[1].

Oxidative stress causes greater losses in breeders than in breeder males because females are responsible for the main functions within the production chain, from the transfer of nutrients from the diet to the egg yolk, the formation of albumen and shell, the maintenance of sperm in the oviduct and fertilization of the oocyte, until the maintenance of early embryonic development. Thus, prevention against oxidative stress is essential, as several factors, such as egg storage, heat stress, the presence of mycotoxins in feed ingredients, the age of breeders, facilities and machinery, among others[2] can increase oxidative stress and worsen the quality of eggs, sperm and, consequently, the embryos and chicks produced.

In the process of oocyte fertilization, birds store spermatozoa for long periods after copulation in the sperm storage tubules, mainly at the uterus-vaginal junction[3]. For this storage, it is necessary to protect spermatozoa rich in PUFAs against lipid oxidation. In this protection, nutrients with antioxidant properties (carotenoids, vitamins C and E and selenium) are essential in the removal of free radicals. In general, these substances act in synergy with the enzymes superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px) and catalase (CA) in the female genitalia to maintain sperm characteristics and increase fertility[4,5].

Regarding the protection that the maternal organism provides to the embryos, their development is initiated after the fertilization of the oocyte forming the egg and the nutrients provided in the maternal diets directly interfere with embryonic development because fat-soluble vitamins (D, E, A and K), carotenoids and other nutrients are deposited in the egg yolk and subsequently transferred by the membrane of the yolk sac to the embryo[6]. Thus, increasing the egg content with antioxidant substances prevents metabolic processes harmful to the embryo and produces better-quality chicks. This is due to the intended positive correlation between maternal nutrition, egg composition and chick health.

The association of canthaxanthin with the vitamin D metabolite 25-hydroxycholecalciferol (25(OH)D_3) has been indicated to improve production and reproductive indices in breeder farms. Therefore, the aim of this review was to describe the main aspects that involve these two active principles, going through the processes of yolk synthesis, the description of carotenoids and how they are absorbed and deposited in the egg yolk, the main aspects that indicate the use of 5(OH)D_3 in poultry production and the main studies available in poultry on the association between these two technologies.

VITELLOGENESIS IN POULTRY

The genital system of poultry has characteristics that place it in a unique location among production animals. The different compartments of the egg have distinctive synthesis rates. In chickens, the egg yolk in particular takes around 9 days to be completely synthesized and is produced in a process known as vitellogenesis[7]. Albumen and eggshell, on the other hand, are synthesized and excreted within a few hours, usually on the same day as the production of that egg. Vitellus, albumen and shell influence embryonic development differently, but all participate in the quality of the bird that will be produced.

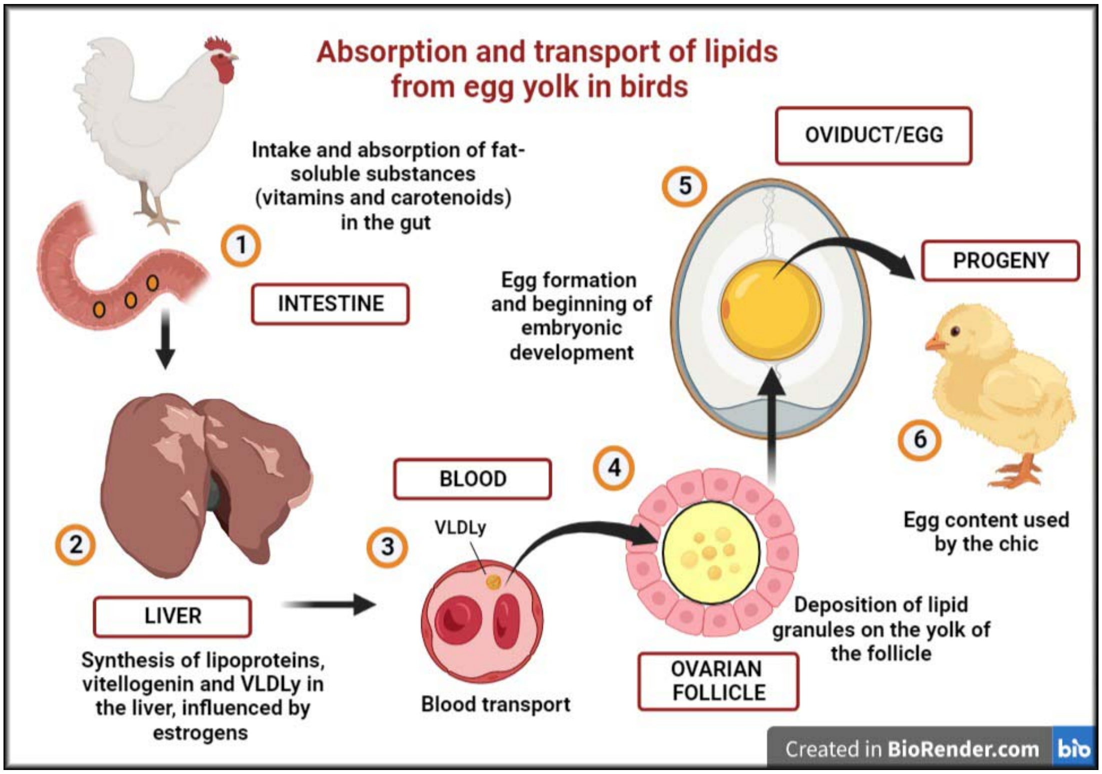

Fig. 1: Absorption and transport of lipids from egg yolk in birds

Illustration of the process of vitellogenesis from the intake of food containing fat-soluble substances, the lipoprotein synthesis in the liver and the transfer via the blood to the growing ovarian follicles and to the chick via the egg yolk

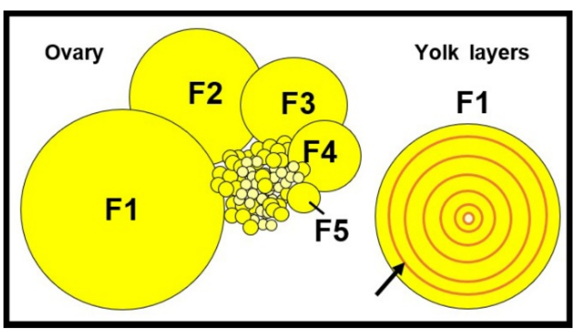

Fig. 2: Avian ovary during egg production

Diagram of an avian ovary showing the developing ovarian follicles and the hierarchy from F5 (smallest) to F1 (largest). The follicle is composed of consecutive yolk layers, identified by the lines from the center to the periphery (arrow) in the cross-section view of a F1 follicle

Vitellogenesis starts in the liver (Fig. 1), where ovarian estrogen regulates the synthesis of precursor proteins rich in triacylglycerol like the Very Low-Density Lipoproteins of the yolk (VLDLy) and a large precursor protein, vitellogenin[7]. This metabolism is unique to females and is active during the laying phase. The characteristics of the VLDL in laying periods are unique, with a small size (30 nm) and only two apoproteins (apoB and apoVLDL-II). The chemical composition of VLDLs makes them sources of proteins, phospholipids and cholesterol, directly interfering with yolk composition and embryonic development[8].

After liver production, the VLDL and vitellogenin are transferred to the blood and then enter the yolk by endocitosis in the developing follicles of the ovary[9]. Therefore, the blood concentration of VLDL, vitellogenin and other yolk-specific proteins increases dramatically in female laying birds[10].

In the vitellogenesis process, there is an increase in size and a set of ovarian follicles is selected and grows in a hierarchy, from the smallest (F5) to the largest (F1) (Fig. 2). The yolk is deposited daily in layers in all growing follicles in the follicular hierarchy. As a consequence of this physiological process, the yolk content presents successive layers deposited over several days and its composition is influenced by the birds' diet during this period Fig. 3(a, b).

During embryonic development, the yolk components are transferred to the blood vessels in the yolk membranes and metabolized in the liver of the embryo. In this way, the fatty acid composition of the embryonic liver is totally different

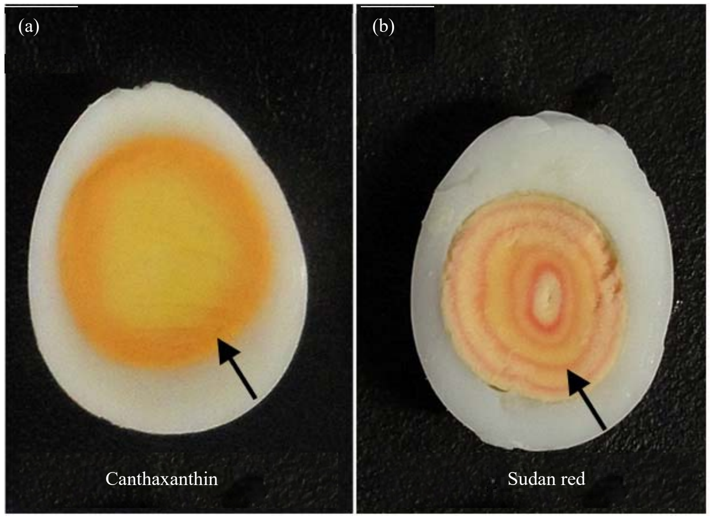

Fig. 3(a-b): A cross-section of quail eggs after cooking (a) In the left egg, the females received 6 mg of canthaxanthin for 4 days and the top layers of the yolk became more pigmented (arrow) and (b) In this egg, the female received a daily dose of Sudan red diluted in soybean oil for 5 consecutive days, marking the beginning of that day's yolk deposition (arrow)

from that of the yolk it originated from. Around 90% of the total energy requirement for embryonic development is provided by the beta-oxidation of fatty acids from the yolk, transferred to the organism of the embryo, mainly in the second half of incubation[10].

CAROTENOIDS

Carotenoids are fat-soluble organic compounds that, according to the way they absorb visible light, vary in color from pale yellow to dark red. They are produced by plants, microalgae, bacteria and fungi but not by animals. These substances are widely found in nature and more than 1,100 naturally occurring carotenoids are currently described[11,12], imparting color to fruits, flowers, vegetables and animals. In some animals, this carotenoid coloration is very evident, as in insects, canaries and flamingos, as well as in marine animals such as crustaceans and salmon[13].

Chemically, carotenoids can be classified into carotenes (hydrocarbons) and xanthophylls (oxygenated carotenes)[14]. In the first group, α-carotene, β-carotene, γ-carotene, δ-carotene and lycopene are present. The xanthophylls include canthaxanthin (Cx), lutein, zeaxanthin, β-cryptoxanthin and fucoxanthin[15]. A lot of functions are associated with carotenoids, including antioxidant activities, cell differentiation, anticarcinogens, immunomodulators, natural colorants, cell membrane stabilizers and action in fertility and embryonic development[8].

Corn is one of the main sources of energy in poultry diets in general and has in its composition carotenes and 11 to 0 mg kg^{-1} of xanthophylls (lutein, zeaxanthin and β-cryptoxanthin)[16,17]. The consumption of corn transfers carotenoids into the birds' bodies through their diet. Thus, the addition of natural or synthetic carotenoids increases their concentration in the body of birds, especially in the yolk, as carotenoids are the substances responsible for the yolk color[18].

CANTHAXANTHIN (Cx)

The same mechanism of lipid transport in general is used by other fat-soluble substances, such as vitamins and carotenoids, that become part of the vitellus. Dietary Cx is absorbed and transported and then fixed in the outer layer of lipid micelles because it is a polar substance with lipophilic characteristics. The transport mechanism of Cx in the bloodstream to the organs and other tissues is like that of other lipids, with Low-Density Lipoprotein (LDL) and high-density lipoprotein (HDL) being responsible for the transport[19].

Deposition of Cx in the egg yolk occurs through VLDLy, which deposits about 80% of the Cx ingested from the diet in the follicles of the ovary[20]. The plasma concentration of carotenoids is related to their concentration in the ingested food and in birds, carotenoids are deposited in the liver, yolk, thymus, cloacal pouch, adipose tissue and skin[21-23].

In diets supplemented with CX, this is transferred to the yolk content. The concentration of carotenoids in the egg yolk increased from 1.92 to 27.80 mg kg^{-1} from breeders supplemented with 6 mg kg^{-1} Cx[24]. A meta-analysis of 34 experiments concluded that the deposition of Cx in the yolk is 2.25 ppm of Cx in the diet[25]. In another study where Cx was microencapsulated in different forms, it was found that this increase in yolk coloration is maintained even after the egg is boiled or fried[26].

The Cx has antioxidant properties *in vivo* and *in vitro*[18]. Its antioxidant properties occur in part because of the high number of double bonds in its structure, which makes it, when compared to other carotenoids, more capable of removing free radicals and their reactive oxygen species (ROS), nitrogen species (RNS) and other metabolic toxins such as malonaldehyde[13,19,27].

The supplementation of Cx in the diet of layers and heavy breeders has been extensively studied. The antioxidant property of Cx is associated with reproductive performance (hatchability, fertile eggs and mortality), embryonic development and antioxidant and immune status in chicks. Studies describe that increasing the Cx concentration in the embryo's body tissue increases the ability to remove free radicals, peroxides and enhances the capacity to scavenge free radicals, peroxides and enhances the recycling of vitamins C and E, minimizing the oxidative stress of the metabolism during the incubation process and after hatching, thus improving the livability of the progeny[28].

The Cobb 500 breeders that received diets with 6 mg kg^{-1} of Cx had higher hatchability of fertile eggs and fertility and reduced embryonic mortality, resulting in a higher total hatching rate and lower embryonic mortality[29]. In another study with broiler breeders at different ages and corn or sorghum as an energy source, the use of 6 mg Cx improved egg laying and hatchability in older birds at 54 to 65 weeks[30]. The eggs had a high concentration of saturated (21.44%) and monounsaturated (24.96%) fatty acids and there was a reduction in progeny mortality, with a consequent improvement in livability, when the 64-week-old breeders were fed diets with corn and Cx[24].

Eggs that were stored before incubation had a reduction in the amount of malonaldehyde (mg kg^{-1}) in the yolk of breeders that received Cx. Similar effects were reported in Cherry Valley duck breeders supplemented with 6 mg kg^{-1} Cx+2700 UI 25(OH)D_3. Supplementation increased fertile egg hatchability and total hatchability and decreased mortality and the amount of malonaldehyde in the egg yolk and serum of male breeders. Supplementation improved shell quality by increasing the shell thickness and decreasing the rate of broken eggs, indicating that Cx improved the antioxidant activity of male's metabolism and the breeders livability[31-33].

Similar effects were also observed in another study with heavy breeders of the Chinese Tree-Yellow line from the 21st to the 47th week of age. In this, the mortality of incubated eggs was reduced (0 vs. 4%) with diets containing 6 mg kg^{-1} compared to the control diet and there was a higher level of antioxidant activity in egg yolk, breeder blood serum and tissues from day-old chicks[34].

Regarding the deposition of Cx in chick metabolism, the studies of Surai *et al.*[35] describe that the Cx concentrations in the liver of chicks from breeders supplemented with different levels (3, 6, 12 and 24 mg kg^{-1}) were highest at hatch (1d) and at 7d post-hatch. Meanwhile, MDA concentrations were lower in these tissues. The authors suggested that Cx decreased the susceptibility of PUFA-rich tissues to peroxidation. Supplementation of 6 mg kg^{-1} of Cx in broilers of the Ross 308 lineage from the 31st to the 59th week of life increased the antioxidant capacity in the plasma of one-day-old chicks and stimulated the immune system response due to the induction with *Escherichia coli*. These results suggested that Cx has effects on the immune system of birds with immunomodulatory characteristics[20].

Research was also undertaken to answer whether high doses of canthaxanthin in the diet could have adverse effects on poultry production in general. Tolerance to high Cx doses was tested in broilers (0, 25 and 250 mg kg^{-1}), layers (0, 8 and 80 mg kg^{-1}) and broiler breeders (0, 6 and 60 mg kg^{-1}). No adverse effects on serum biochemistry or mortality were recorded, however, the Cx overdose reduced egg fertility in broiler breeders[36].

Table 1 shows the main results observed in studies carried out with poultry breeders that used Cx in the diet and its effects on productive performance and on the progeny.

VITAMIN D

The process of eggshell mineralization in birds is known to be one of the fastest mineral deposition processes in nature. As modern poultry have been selected for high laying rates, the Ca and P requirements, as well as the control of their absorption and metabolization processes, are extremely important to ensure productivity indices. In this respect, the functions of vitamin D_3 in birds, in general, are well studied and known, especially in the skeletal system. The active metabolite of vitamin D is 1,25-dihydroxycholecalciferol and its main functions described involve the processes of Ca and P absorption in the intestine, the metabolic mechanisms of mineralization and demineralization of bone formation[37], effects related to improved hatchability and reduced embryonic mortality, positive participation in eggshell formation and quality[38], embryonic development in general[39] and in the immune system[40,41].

Table 1: Description of the main results reported in scientific articles that have used canthaxanthin (Cx) in poultry breeders

Authors	Animals and diets	Main results
Surai *et al.*[35]	Broiler breeders Cx 3, 6, 12 and 24 mg kg^{-1}	↑Cx and g-tocopherol in the yolk, ↑Cx in the liver of embryos and chicks 12 mg kg^{-1} Cx ↑ α-tocopherol in the liver of day-old chick and ↓lipid peroxidation in tissues 6 to 24 mg Cx delay in a-tocopherol depletion in the body of day-old chicks
Zhang *et al.*[34]	Chinese Tree-Yellow broiler breeders 0 or 6 mg kg^{-1} Cx	↑Total antioxidant capacity of hen's serum, egg yolk and day-old chick ↓MDA in yolk ↑Yolk color and shank skin pigmentation of chick ↓reduction of embryonic mortality
Rosa *et al.*[29]	Cobb 500 broiler breeders that were fed diets with 6 mg kg^{-1} Cx	↑Hatchability of fertile eggs and fertility ↓reduction of embryonic mortality ↓MDA in yolk of eggs stored preincubation
Weber *et al.*[36]	Ross 308 broiler breeders: Cx 0, 25 and 250 mg kg^{-1}) and Isa Brown layers: Cx 0, 80 and 80 mg kg^{-1}) Lohmann broiler breeders: Cx 0, 6 and 60 mg kg^{-1}	↑Mortality in overdose Cx overdose did not influence other analyzed variables
Rosa *et al.*[24]	Cobb 500 broiler breeders Corn or sorghum 0 or 6 mg kg^{-1} Cx	Chick weight and mortality were not affected Cx↓yolk lipid oxidation Corn-based diets: ↑Yolk monounsaturated and saturated FA↑n6:n3
Johnson-Dahl *et al.*[20]	Ross 308 broiler breeders Cx 0, 6 and 12 mg kg^{-1}	↑Cx in the egg and in chicks' liver No effect on performance and egg quality or progeny's performance ↑Plasma antioxidant capacity in chicks, ↑Bactericidal capacity against *E. coli*
Bonilla *et al.*[30]	Cobb 500 broiler breeders at 42 to 65 weeks without corn or sorghum 0 or 6 mg kg^{-1} Cx	↑Breeders laying and hatching at 54 to 65 weeks with Cx
Faruk *et al.*[25]	Meta analysis with 34 trials between 1997 and 2012, 21 to 65 weeks Up to 8 mg kg^{-1} Cx	Yolk 2.25 ppm deposition per ppm Cx in the diet ↑Egg mass, production Modulates immune and reproductive systems
Ismail *et al.*[64]	Indigenous Sinai chickens (male and females)	Spirulina and Cx, 6 and 8 mg kg^{-1} ↑body weight in females, egg production, sperm concentration

In general, vitamin D, or cholecalciferol, is a steroid hormone that is produced endogenously in the skin after exposure to sunlight. The UV rays convert 7-dehydrocholesterol into vitamin D_3 in the skin and the blood transports the metabolite to the liver. There, the 25-hydroxylase enzyme hydroxylates D_3 to 25-hydroxycholecalciferol (25(OH)D_3) or calcidiol. In the blood, 25(OH)D_3 conjugates with vitamin D transport proteins and reaches the kidneys. In the kidneys, 25(OH)D_3 is hydroxylated by the 1-alpha-hydroxylase enzyme to its active form, 1,25-dihydroxycholecalciferol (1,25(OH)$_2D_3$) or calcitriol[42-44], or can also be converted to 24,25-(OH)$_2$-D_3 by 24-hydroxylase, which is the first stage of vitamin D_3 elimination[45]. As 25(OH)D_3 can also be converted to its active form, 1,25(OH)$_2D_3$, in other organs and cells, producing local effects in tissues such as muscle, intestine, bursa, spleen and thymus[46].

The renal stage of metabolism is basically regulated by parathyroid hormone and responsive to Ca levels in the blood of birds[44]. Thus, liver and kidney health are essential for the complete metabolism of vitamin D to its active form. However, poultry in production has many challenges to overcome to maintain liver health due to general stress factors in production processes, the presence of mycotoxins in many feed ingredients even if in low doses and others.

Poultry in production, especially female and male breeders, can obtain vitamin D_3 from feed, via supplementation by vitamin premix, or through endogenous production through UV radiation from light and animal by-products in the diet[37]. However, birds are confined to intensive production systems, which impair the endogenous pathway through radiation. On the other hand, the intake of products of animal origin may present a great variation in vitamin levels, besides the several processes in the industry that may affect and impair the stability of vitamin sources in the ingredients. Thus, supplementing the diets of female and male breeders is recommended.

The use of 25(OH)D_3 alone or together with vitamin D_3 in the diet of layers and breeders can influence calcium and phosphorus metabolism. The minerals are transferred to the egg yolk and their concentration is highly correlated with the amount supplied in the maternal diet[47]. In general, the absorption of vitamin D_3 and/or its metabolites occurs in the small intestine, where it possibly acts by modifying the morphology and development of the intestinal villi[48]. The metabolite $25(OH)D_3$, on the other hand, is absorbed twice as much as vitamin D_3 and has a longer half-life[49].

Due to the particularities of rapid intestinal absorption and a long half-life, the use of $25(OH)D_3$ in the diet of laying birds can lead to increased availability of P and Ca, improving the resistance and compaction of the eggshell[49]. These characteristics can reflect better incubation performance and embryonic development. Eggs with higher calcium and more compact and resistant shells have a better protection system for the embryo against infection, preventing water loss and increasing the amount of calcium for the embryo to use during bone development[50]. In this sense, research that analyzed heavy breeders fed diets with different levels of cholecalciferol and 2,720 IU kg^{-1} $25(OH)D_3$ showed better shell thickness and egg production, with higher 25-OH concentrations in the blood and egg yolk, evidencing that this metabolite has higher absorption and deposition compared to vitamin D_3[38].

Another known effect of vitamin D concerns its role in the circulatory system. Activation of the VDR receptor by D3 represses adipogenic genes, reducing the accumulation of adipose tissue and, consequently, improving the health of the cardiovascular system in general. Lin *et al.*[51] analyzed Cobb 500 breeders from 26 to 60 weeks of age with feeding programs with and without restriction and 2,760 IU kg^{-1} of $25(OH)D_3$ and described interesting results on the circulatory system when the metabolite was added. Heavy breeders fed *ad libitum* are predisposed to sudden death syndrome due to cardiac impairment[52] and thus the use of $25(OH)D_3$ in the feed improved the cardiorespiratory rates of the breeders, leading to a reduction in bird mortality. Regarding vitamin D concentrations in the egg and its transfer to the chicks, the avian vitamin D transporter protein has a high affinity mainly for cholecalciferol, releasing it into the ovarian follicles, where it is incorporated into the egg yolk. Both dietary D_3 and $25(OH)D_3$ levels have a high correlation with the amount found in the egg[47]. In developing chick embryos, the liver and kidney enzymes of the vitamin D metabolic pathway start to become active between the first and second week of incubation and the embryos start to convert the metabolites available in the egg to the active form of the vitamin, $1,25(OH)_2D$[53]. From this period on, the embryos can absorb the Ca and P of the yolk, regulate calcemic and absorb Ca from the eggshell via the chorioallantoic membrane to provide the Ca necessary for intense bone deposition that occurs within a few days[54].

In embryos, $1,25(OH)_2D_3$ takes part in the ossification processes and vitamin D deficiencies lead to embryonic death and reduced hatchability in general. Experiments with diets fed from 23 to 65 weeks of age containing 2,760 IU kg^{-1} $25(OH)D_3$ to Ross 308-line breeders reported increased hatchability and *in vitro* immune response of the progeny and reduced mortality[41]. This positive effect on hatchability with the use of $25(OH)D_3$ was also observed by Atencio *et al.*[37] and Coto *et al.*[38].

Older studies describe deficient or excessive vitamin D levels in the diet of breeders as reduced hatchability and increasing embryonic mortality[55]. Thus, both a deficit and an excess of vitamin D interfere with embryonic mortality, increasing this rate. When compared to diets with the recommended level of vitamin D, diets without vitamin D led to an increase in embryonic mortality[56,57]. Low D_3 levels are associated with reduced body and muscle weight and bone mineralization, leading chicks to fail in the process of changing their intra-egg positioning to initiate pulmonary ventilation in the egg air chamber. The chicks die of asphyxiation and become cyanotic, with edema and bleeding on the head[39].

In trials with diets that received other commercially available metabolites, such as $1,25(OH)D_3$ or $1\alpha,25(OH)D_3$, as the sole source of vitamin D, higher embryonic mortality was reported[57,58]. Only broiler breeder diets with the metabolite $25(OH)D_3$ showed the same embryonic mortality rates when compared to diets with vitamin D[59]. These studies indicate that the association between vitamin D and the metabolite $25(OH)D_3$ at optimal levels is essential for better embryonic mortality rates.

The NRC's[60] minimum vitamin D intake recommendations for layers are 7.5 μg kg^{-1} of feed (300 IU kg^{-1}), representing 1 μg/bird/day, while more recent recommendations indicate higher intakes. Research in adult birds testing high doses of vitamin D_3 metabolites shows that they have a wide safety margin. In Hy-line layers, doses of up to 68,348 IU D_3 kg^{-1} in birds up to 68 weeks of age were analyzed and the results demonstrated that dietary D_3 supplementation of up to 35,014 IU D_3 kg^{-1} did not affect performance negatively and improved skeletal quality in pullets and layers, as well as the D_3 content of yolk and eggshell quality[61].

In Table 2 the references about vitamin D in broiler breeders are organized with the main effects described in poultry breeders, eggs, incubation and progeny.

ASSOCIATION OF CANTHAXANTHIN AND 25-HYDROXYCHOLECALCIFEROL

The association of Cx with $25(OH)D_3$ has been used commercially for some time and is indicated for

Table 2: Description of the main results reported in scientific articles that have used 25(OH)D_3 in poultry breeders and their results on breeders and progeny

Authors	Animals and diets	Main results
Torres *et al.*[65]	Cobb 500 broiler breeders 1,400 and 2,760 IU kg^{-1} 25(OH)D_3 32 to 67 wk	25(OH)D_3 ↑eggshell quality (60 wk), ↓embryo mortality (67 wk)
Coto *et al.*[38]	Cobb 500 broiler breeders Factorial 2×5 (0 and 2,720 IU kg^{-1} 25(OH)D_3×0, 300, 600, 1,200 and 2,400 IU kg^{-1} cholecalciferol)	↑25-OH plasma and yolk ↑Shell thickness, egg production, egg mass
Peng *et al.*[66]	Cobb broiler breeders with 2,760 IU kg^{-1} 25(OH)D_3 Progeny with or without 25(OH)D_3	No effect on reproductive performance and breeders' incubation ↑Ca in day-old chick's tibia No effect on MDA in serum of day-old chick or weight ↑growth immunity and bone quality ↓body weight variation among individuals when in the progeny feed
Saunders-Blades and Korver[41]	ROSS 308 broiler breeders, 23 to 65 weeks of age 2,760 UI vitamin D and 2,760 IU kg^{-1} 25(OH)D_3	↑Increased hatchability in older breeders ↑Innate immunity against *E. coli* in phagocytosis tests
Lin *et al.* [51]	Cobb 500 broiler breeders at 26 to 60 weeks, with or without feed restriction, 2,760 IU kg^{-1} 25(OH)D_3	↑Breeders' survival ↑Cardiorespiratory rate

Table 3: Description of the main results reported in scientific articles that have used the canthaxanthin and 25(OH)D_3 association in poultry breeders and progeny

Authors	Animals and diets	Main results
Ren *et al.*[31-33]	Cherry Valley ducks and progeny, 6 mg kg^{-1} Cx+2,700 IU kg^{-1} 25(OH)D_3 Progeny control feed	↓Broken eggs, ↑Thickness of eggshell, ↑yolk pigmentation ↑Hatchability and fertility, ↓MDA in yolk and breeders' serum and carbonylated protein in the liver of males and females ↑Weight gain (1-14 d) and feed intake (15 -35 d), ↑Feet pigmentation, ↑SOD in day-old chick liver and total antioxidant capacity, ↓ MDA in serum and carbonylated protein in the yolk (14d)
Araujo *et al.* [67]	Cobb 500 breeders and progeny, 6 mg kg^{-1} Cx+2,760 IU kg^{-1} 25(OH)D_3 Progeny with and without 6 ppm Cx+2,760 IU kg^{-1} 25(OH)D_3	↑Egg production, hatchability of fertile eggs, fertility ↓Reduction of embryonic mortality Broilers with 6 ppm Cx+2,760 IU kg^{-1} 25(OH)D_3 from the breeder and diet had better feed conversion, carcass and breast
Bonagurio *et al.*[62,63]	European quail breeders Cx (0, 3, 6, 9 and 12 mg kg^{-1}) and 25(OH)D_3 (1,380, 2,760, 4,140 and 5,520 IU kg^{-1})	No effect on production performance and egg quality ↑Yolk color ↑DPPH in liver and serum, ↓MDA in liver up to 4.8 mg Cx ↑*SOD* up to 6 mg Cx and *GP*× *7* gene in the vaginal mucosa ↓Ashes and Ca of bones of breeders Maintained fertility and high sperm survival in the oviduct of older females ↑Hatchability of fertile eggs and fertility, ↓Embryonic mortality up to 5.7 mg Cx, ↑length and Pasgar© Score up to levels around 6 mg Cx

broiler breeders with positive effects on hatchery performance on indices such as hatchability, fertility, mortality and infertility and chick quality. Studies with European quail breeders testing Cx levels (0, 3, 6, 9 and 12 mg kg^{-1}) associated with increasing levels of 25(OH)D_3 (1,380, 2,760, 4,140 and 5,520 IU kg^{-1}) demonstrated that Cx+ association affects serum biochemistry and bone quality by modifying the breeder's Ca and P metabolism. The antioxidant analysis of serum and liver showed an increase in systemic and tissue antioxidant capacity in birds as the product level increased, but at higher levels, it proved to be pro-oxidant, with 6 mg already used in the industry being recommended for other birds[62].

In the same model, incubation and reproductive performance in European quail breeders were also analyzed. In this study, estimation of 5.90 mg kg^{-1} Cx+2714 IU of 25(OH)D_3 had better results for hatchability (total and in fertile eggs) and fertility and mortality and infertility were reduced. These results were associated with improvements in fertility in old breeders, until the 40th week and progeny showed improvements in chick length and Pasgar©Score, which resulted in better chick quality[63].

The following Table 3 shows other articles and those previously mentioned and their main observations in poultry when using canthaxanthin, 25(OH)D_3, or their associations. Thus, a scheme is presented below summarizing the main

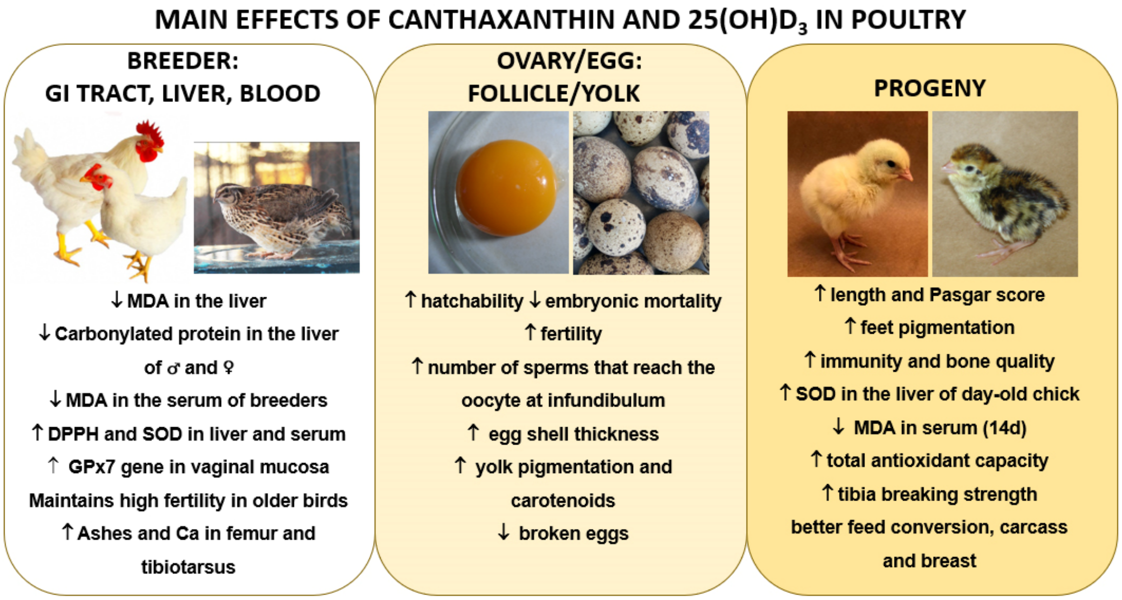

Fig. 4: Synthesis of the main effects of canthaxanthin and 25(OH)D_3 in poultry

benefits of the Cx and 25(OH)D_3 association, that have been researched and described so far in different breeders, both females and males and in their progeny (Fig. 4).

CONCLUSION

Research has shown positive results using Cx and 25(OH)D_3 in poultry diets. This review, focus was on aspects related to female and male breeders. The main results found over the years by different research groups support the hypothesis that the use of these substances is beneficial to produce fertile eggs. Many aspects are not yet fully known or metabolically understood and some effects still deserve attention from researchers. However, analyses of the antioxidant system in eggs and the breeder's body, embryos and progeny highlight significant improvements in antioxidant metabolic pathways, with reduced lipid peroxidation and preservation of other metabolic pathways such as those using vitamin E. Similarly, production and performance indicators such as egg and chick composition, hatchability, fertility and chick quality are positively improved in all species studied to date. Therefore, it is possible to state that the recommendation for the use of these substances in fertile egg production farms is a reality for poultry.

SIGNIFICANCE STATEMENT

This review focused on those scientific articles that provided Cx and 25-hydroxycholecalciferol alone or in combination for breeders in different species of poultry and described their effects both on the breeders and on the progeny. Many articles focus on the use of Cx for laying hens to color the yolk or on D_3 in general for broilers or layers and breeders for its known role in the bone system. This review sought to demonstrate what is known as the effects of these products when used in the diet of breeders. The positive results of this association are similar when using both substances separately. In this way, this review substantiates the indication of the use of both Cx and 25(OH)D_3 to produce fertile eggs in all production birds.

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