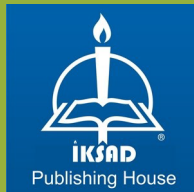


VITAMINS and MINERALS in POULTRY



Editor
Prof. Dr. Ergin Öztürk



VITAMINS and MINERALS in POULTRY

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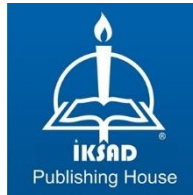
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PREFACE

Poultry is a very delicate and sensitive animal when it comes to its physiology and nutrition. Therefore, it's crucial to pay maximum attention to its diet. A deficiency of vitamins and trace minerals in their diet can lead to metabolic disorders, low productivity, and even death in poultry. When poultry are bred and pushed to produce higher yields, this case becomes more critical due to increase in their metabolic load. To tackle this and enhance their health and productivity, it's increasingly important to boost the levels of vitamins and minerals in their diets.

This book provides detailed information on the levels of macro and micro minerals and vitamins in poultry diets, factors affecting their needs, deficiency symptoms, and measures to address them based on current literature. For a long time, there has been a significant need for a comprehensive book on vitamins and minerals for both broilers and layers, and their breeding. *Vitamins and Minerals in Poultry* covers the fundamental chemical, metabolic, and functional roles of vitamins and minerals. It also addresses the requirements, deficiencies, and excess amounts of vitamins and minerals in poultry. This resource contains eight concise, up-to-date chapters on mineral and vitamin nutrition in poultry.

This book aims to serve as an authoritative reference for readers worldwide and be of considerable value to those in the poultry sector, including research and extension specialists, feed manufacturers, farmers, teachers, students, veterinarians, and the academic community.

Prof. Dr. Ergin ÖZTÜRK

CHAPTER 1

USE OF MICRO MINERALS IN LAYING HEN DIETS WITH CURRENT APPROACHES: A REVIEW

Şevket ÖZLÜ¹ Arif DARMAWAN ² and Ergin OZTURK³

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INTRODUCTION

Trace minerals are of great importance in the diet of laying hens because of their various roles in metabolism, directly or indirectly. The main ones are; immune system, repair of various tissues, reproductive parameters, hormonal mechanisms, growth, egg production, enzymatic reactions, and nervous system. Egg yield and egg quality are among the most important parameters in laying hens. In particular, eggshell quality is an important issue in terms of marketable eggs. Shell quality problems are observed in 10-15% of eggs produced worldwide, which causes economic losses (Stefanello et al., 2014). Most studies on eggshell quality focus on dietary calcium and phosphorus contents (Neijat et. al. 2011). However, the effects of trace minerals on shell quality are also a matter of study. They act as activators or constituents of enzymes involved in eggshell synthesis and directly interact with calcium crystals during eggshell formation, affecting the quality of the eggshell (Fernandez et al. 2008, Xiao et al. 2014).

In addition to minerals from feedstuffs, mineral additives are prepared for laying hen diets. Because the micro minerals in feedstuffs are insufficient to meet the needs of laying hens. For this reason, the mineral pre-mixes that are being prepared are used to meet the macro and micro mineral needs. Generally, micro minerals used in mineral pre-mixes are copper (Cu), iodine (I), iron (Fe), manganese (Mn), selenium (Se), zinc (Zn), magnesium (Mg), respectively. The form of the minerals used in the preparation of mineral additives is of great importance in terms of mineral bioavailability. Because not all forms of the mineral are equally useful in metabolism. For example, the utilization of iron (II) sulphate is high, while the utilization of iron (II) oxide is very low. Therefore, minerals are used in different inorganic forms such as phosphate, sulphate, oxide and carbonate. In addition, organic forms of minerals are also used as an alternative to their inorganic forms (Londero et. al. 2020). The usefulness of organic forms is higher than inorganic forms, but their higher cost than inorganic forms limits their use. The aim of this review is to bring together general information about trace minerals and current literature on the use of trace minerals in laying hen diets in recent years.

1. MANGANESE

Manganese is an essential trace element in poultry nutrition and feeding due to its role in eggshell and bone production, enzyme activity, and nutrient metabolism. It is found in all tissues and is essential for the normal digestion of amino acids, lipids, protein, and carbohydrates (Zhu and Richards, 2017; Tufarelli and Laudadio, 2017). Mn is important for the enzyme Mn-superoxide dismutase (SOD).

This enzyme is needed for additional defense against oxidative stress caused by inflammatory responses to certain infections. Manganese deficiency reduces SOD production and raises the peroxidative damage caused by elevated polyunsaturated fatty acid levels in the diet (Bortoluzzi et al 2020). Manganese is described as a trace mineral associated with better immunity, or to functions that support immunity where Junior et al. (2019) demonstrated that birds fed organic Mn had a more effective response against *Salmonella Enteritidis* vaccine than birds fed its inorganic minerals. In laying hens, Mn plays an important role in bone development, growth, optimal eggshell quality, and perosis prevention. Some researchers have recommended the use of organic Mn sources, which have been shown to have a significant impact on eggshell consistency and efficiency (Alagawany et al., 2021).

The lower absorption rate in poultry explains the higher requirement. Manganese absorption in the intestine is also inversely related to iron consumption. Mn is absorbed by mucosa cells in the small intestine, thus it is transported by α 2-macroglobulins or albumin to the liver. Manganese is secreted into bile in large amounts by the liver. Manganese is thus mainly excreted by feces. Manganese excretion in the urine is a small route of excretion that indicates endogenous homeostatic control. It was reported that Mn appears to be absorbed in the +2 valence and performs with I and Co for the same absorption sites (Galán and Drago, 2014). Developmental status, dietary constituents, and membrane factor translocation are three of the several determinants of Mn absorption and retention. Mn absorption has been associated with the high intake of calcium in the diet. The possible association between Ca, Mn, and bone health may be important in the prevalence of osteoporosis (Zhu and Richards, 2017; Tufarelli and Laudadio, 2017).

Due to the limited supply of Mn, it is widely agreed that maize–soybean meal–based poultry diets need to be supplemented with Mn (Liao et al., 2019). All Mn materials are supplemented to animal diets as a premixture. The use of three compounds namely; Manganous chloride, manganous sulphate (tetrahydrate), manganese chelate of glycine, hydrate (monohydrate) are used in drinking water by all animals species (EFSA, 2016). The detail requirement for animal species is presented in Table 2.

Excessive supplementation of Mn in the diets is able to result in cell dysfunctions such as neurotoxic effects (including neurobehavioral effects) as well as reproductive dysfunction (Adedara et al., 2017). According to some studies, when Mn is consumed in excess, it preferentially accumulates in the mitochondria and interferes with oxidative phosphorylation. As a result, it increases the production of reactive oxygen species, which can serve as mediators of oxidative damage to cell structures, proteins, lipids, and nucleic acids (Gunter et al., 2006; Jankowski et al., 2019).

Previous studies by Darvishi et al. (2020) also stated that organic Mn showed higher bioavailability compared with their inorganic sources when fed to laying hens, where the use of 50% (50 mg/kg Mn) organic and inorganic minerals affects positively laying performance and egg quality trait. Wang et al. (2015) found that dietary Mn deficiency-induced perosis in chicks by inhibiting chondrocyte proliferation and promoting chondrocyte apoptosis, which contributed to responsibility in bone mineralization. Cui et al. (2019) stated that Mn deficiency decreased egg production and shell quality of hens. Mn addition at a concentration of 120 mg Mn/kg either from Mn-sulphate or Mn-chelate of protein hydrolysate or Mn- chelate of glycine hydrate increased SOD in the blood of the hens, which is recognized to be one of the first components of the antioxidant defense system. The results of this experiment demonstrate that different sources of Mn used as dietary supplements significantly increase the biological antioxidant potential of laying hens, which could be used for preventing the formation of reactive oxygen species (ROS) (Piešová et al., 2019).

Further, Hy-Line Brown laying hens fed a corn-soybean meal basal diet (21.95 mg/kg, Mn) during 23 to 46 week, dietary level of 40 mg/kg from Mn-amino acid complexes could increase egg production, egg mass, feed efficiency and improve eggshell breaking strength of laying hens (Cui et al.,

2019). Related to this, Mn supplementation decreased follicular atresia while increasing preovulatory follicle development, which may explain the increased egg production. Mn has stimulated the development of reproductive organs and ovarian follicles, as well as egg formation, through modulating hormones of the hypothalamic-pituitary- gonadal axis (Zhang et al., 2020).

2. IRON

Iron is classified as a trace mineral that is needed in small amounts in poultry metabolism. Approximately 60% of the iron in metabolism is found in hemoglobin in the bloodstream. Although predictions about iron have focused on its relationship with hemoglobin for many years, it has been found that it has a wider physiological significance by detecting its presence in the cytochrome oxidase enzyme group (Suttle, 2010).

Hemoglobin: It is a complex of protoporphyrin, haem and globin. The Haem molecule contains 4 iron atoms, one in the center of each of the four linked porphyrin rings. Hemoglobin, in erythrocytes, transfers oxygen as oxyhemoglobin from the lungs to the tissues via arterial blood. In addition, it carries carbon dioxide as carboxyhemoglobin to the lungs via venous blood, allowing the tissues to "breathe" (Suttle, 2010). After the relationship of iron with various enzymes was discovered (Figure 1), other functions in metabolism were also clarified. Particularly, it participates in the structure of enzymes responsible for the Krebs cycle and activates them. It also ensures the removal of harmful substances in the reactions it is involved in.

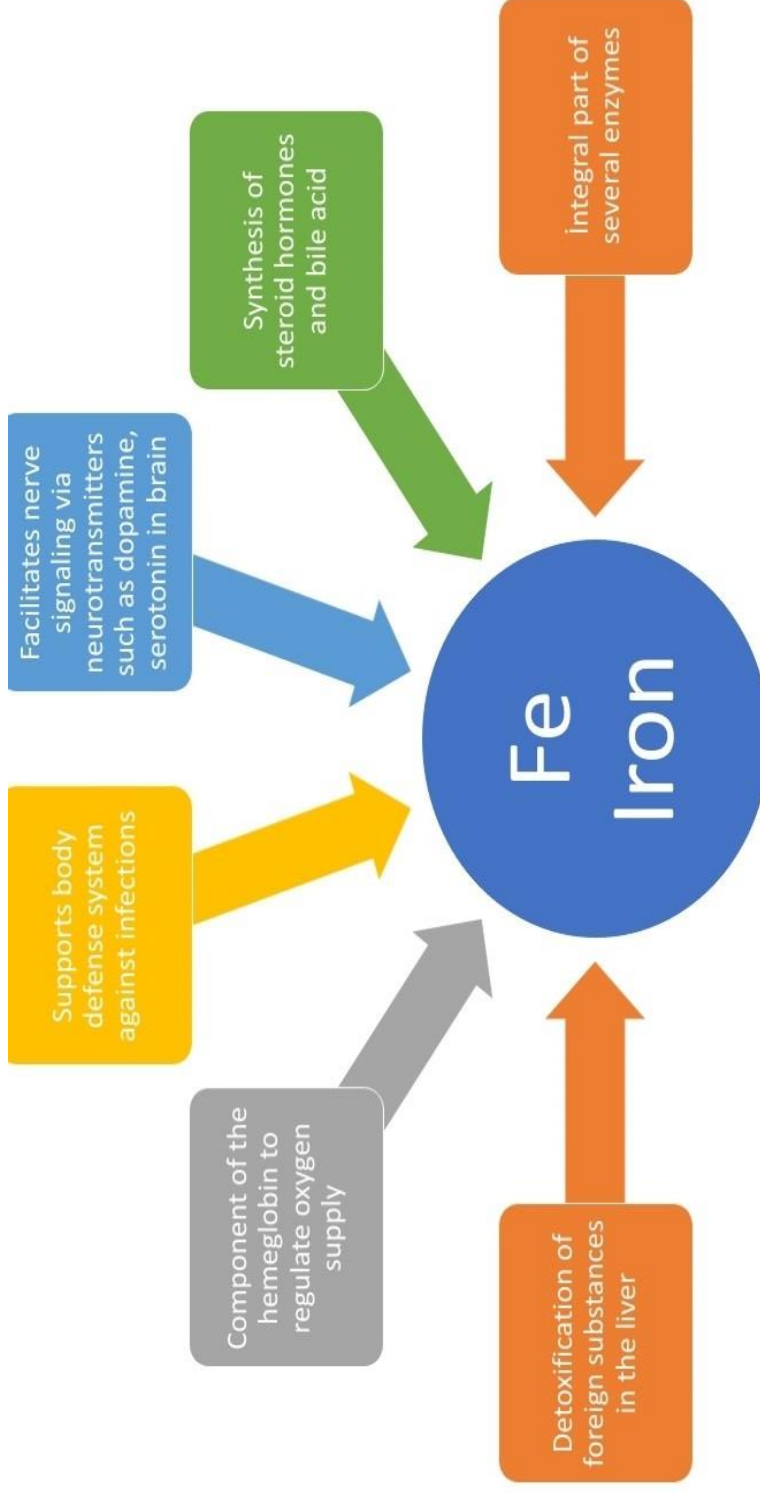


Figure 1. The functions of iron in the body

Iron is essentially absorbed in a 2-step manner. The first is mucosal absorption from the duodenum, the second is serosal transfer. Iron absorbed from haem and non-haem sources is oxidized to Fe^{3+} and bound to apotransferrin in a common mucosal pool before being given to serosal transfer. Subsequently, iron transported by serosal route is transported into the cell by endocytosis with transferrin receptors in cell membranes. Binding proteins that are sensitive to iron in the cell take iron from transferrin and carry it to the body for use or storage. Iron is stored mainly in the form of Haemosiderin in the intestine and liver (Suttle, 2010). The iron requirement in poultry is summarized in Table 2.

Iron deficiency is generally not seen in poultry. Unlike caged hens, even free-range hens are treated with soil to meet their iron needs. However, if there is not enough iron in the ration, anemia occurs. Anemia is generally seen as a decrease in the amount and size of hemoglobin and pale color (from red to white). Along with this, the skin color also becomes pale. In laying hens, an average of 1 mg of iron loss per day is observed during the laying period. Therefore, attention should be paid to the iron content in laying chicken diets. Iron is commonly added to the ration as ferrous sulfate, ferric chloride, ferric citrate, ferric ammonium citrate, and iron amino acid complexes. The availability of iron oxide for all poultry in the examinations is very few (Henry and Miller, 1995).

Olomola et al. (2019) reported that the addition of vitamin C and vitamin D to laying hen rations had no effect on egg iron accumulation. Qiu et al. (2020), found that the trace minerals (Fe, Cu, Mn, Zn) used in inorganic form (control) in laying hen rations at the end of the laying period, 1/3 less in mineral proteinate form (TRT) and inorganic form (ITM). The ITM group had lower egg production, eggshell resistance, serum estrogen, lutein, glycosaminoglycan concentration and carbonic anhydrase activity compared to the control group. Egg loss increased significantly in the ITM group compared to the control group. Also, the TRT group generally obtained similar results with the control group in all parameters. Therefore, they reported that the mineral form has important effects on laying hens.

Gou et al. (2020) investigated the effects of adding iron at different rates (50, 70, 90, 110, 130 and 150 mg/kg diet) to Chinese yellow broiler rations in 3 different feeding periods. Iron addition was added to the rations

in the form of $\text{FeSO}_4 + \text{H}_2\text{O}$. At the end of the study, body weight gain, feed intake and feed conversion ratio were not affected by Fe use at different rates. It has been reported that thymus, bursa and spleen weights and liver and kidney Fe contents are not affected by different Fe rates. Breast redness value of chickens in the group using 150 mg/kg Fe was found to be significantly higher than the group using 50 and 70 mg/kg iron. The drip loss rate decreased significantly in the group using 90 mg/kg Fe compared to the group using 150 mg/kg Fe. At the end of the research, it is reported that normal Fe supplementation (50mg / kg) is sufficient in Chinese yellow broiler starter and growing rations, but using 90 mg/kg Fe in the finishing period positively affects the meat quality.

Another study by Behroozlak et al. (2020), who investigated the effects of using Fe_2SO_4 in 3 different ratios (0, 40 and 80 mg / kg feed) in 3 different feeding periods (1-42 days (T), 11-42 days (GF) ve 25-42 days (F)). At the end of the study, it was reported that 80 mg/kg of Fe added to the ration increased the breast meat protein ratio. In addition, the use of 80 mg/kg Fe in the T period increased Fe accumulation in breast meat and was found to be higher than in other periods. Breast redness, the highest oxidative stability value of breast meat and it also decreased the malondialdehyde level by increasing Total Antioxidant Capacity (T-AOC) value was also reported to reach the highest level in the ration using 80 mg/kg Fe. As a result use of 80 mg/kg Fe_2SO_4 improved meat quality values and also 80 mg/kg Fe_2SO_4 is recommended level of one day broiler chickens.

In the EFSA (2020) report, the effects of the combined use of iron-lysine chelate, glutamic acid (Iron-LG) and Fe_2SO_4 on laying hens were investigated. In the study, they compared the effects of the ration without the addition of extra iron (negative control), the ration with the addition of 15, 30, 45, 60 and 75 mg/kg Iron- LG and the ration with the addition of 45 mg/kg Fe_2SO_4 (positive control). The highest egg production and egg weight was obtained in the group using 60 mg/kg Iron-LG. The highest egg yolk iron content was obtained in Iron-IG groups for all of the level of iron (45-60-75 mg/kg). In blood parameters results, it was reported that the use of 45-60-75 mg/kg Iron-IG significantly increased the amount of red blood cells and hemoglobin. However, there was no difference between the groups in terms of white blood cells, hematocrit and thrombocyte values. The use of 30-45-60-75

mg / kg Iron-LG significantly increased the total serum Fe amount compared to the other groups. Also use of Iron-LG has a positive effect on antioxidant parameters. As a result, it is reported that the use of 30 mg / kg Iron-LG is sufficient to meet the needs, but it is recommended to use at higher levels to achieve positive results in the above-mentioned parameters.

3. COPPER

Copper is an important trace mineral due to its presence in many enzymes in metabolism. These enzymes are also involved in the activity of many cofactors and reactive proteins. Because of these properties, copper plays a major role in reproduction and bone development. In addition, they are found in the structure of tyrosinase and lysyl oxidase enzymes that play a role in pigmentation and tissue development, and in case of deficiency, the performance of poultry is adversely affected. It plays a key role in hemoglobin synthesis, iron metabolism and erythrocyte formation. It also plays a role in the biosynthesis and cross- linking of elastin fibers and collagen and keratin and melanin synthesis (Suttle 2010). The enzymes with copper content in their structure and their functions are summarized in Figure 2.



Figure 2. The kinds of enzymes with copper content in their structure

A large amount of copper is stored in the liver. Copper concentration in the liver is higher in sheep, cattle and ducks (100-400 ppm) than in horses, pigs, chicks and turkeys (10- 50 ppm). Since the liver is the main storage organ for copper, it provides a more reliable indicator of the body's copper status than blood plasma. In most animal species, most of the foraged Cu is seen in the feces, and this is an unabsorbed mineral. The active excretion route of copper is in the bile. Apart from that, it is excreted with urine and feces. There is a reciprocal relationship between copper, molybdenum and sulfur. High levels of Cu intake reduces the amount of molybdenum stored in the liver. As the sulfur level increases, the amount of molybdenum excreted in urine increases, so the storage of the mineral is negatively affected (Suttle 2010). The amounts Cu required in poultry diets are summarized in the Table 2.

The general symptom of copper deficiency in poultry is anemia. Before severe anemia occurs, internal bleeding due to vascular defects can lead to death. Poultry fed with diets insufficient in terms of Cu causes lameness and easily fractured bones. Severe copper deficiency (0.7-0.9 ppm) in laying hens decreased egg yield, level of Cu in plasma and liver. Also, it can drop the incubation efficiency in breeding chickens to zero in 14 days. Anemia and regression of growth are seen in embryos from chickens fed with insufficient copper (Kutlu et al., 2005).

The copper requirement of animals is important, and the amount of available copper is more essential than the amount of copper in feed raw materials. The same is true for copper poisoning and the information on the useable copper content of feedstuffs is limited. However, various studies have shown the copper content in cereal by-products. Moreover, the usefulness of copper in feeds depends on the ration composition, especially the high amount of sulfur, molybdenum and iron. The copper content of some feedstuffs is presented in Table 3. The copper digestibility of poultry is generally lower than 30% and most of the copper absorption occurs in the proventriculus and duodenum. High levels of calcium, phosphorus, phytic acid, oxalate and tannins depending on the diet reduce copper absorption by forming insoluble complexes in the intestines. Copper homeostasis is maintained by predominantly control of the absorption rate. When the copper level is high in the diet, intestinal epithelial cells synthesize metallothionein, the cysteine-rich

protein binds tightly to copper and delays copper absorption. When zinc or cadmium levels are high in the diet, the transfer of zinc and cadmium between enterocytes stimulates metallothionein production, which reduces copper absorption (Suttle 2010). Copper is usually added to poultry rations in the forms of copper sulphate, copper chloride and copper oxide. In general, copper-cuprous- (Cu^+) compounds can be used more than copper-cupric- (Cu^{2+}) compounds (Baker and Ammerman, 1995). When there is a problem with poor bioavailability or interaction with other minerals, chelates or copper complexes with organic molecules are often used. Copper sulfate is sometimes added to rations at levels much higher than needed (100-250 mg kg^{-1}) because it has an antimicrobial and growth promoter effect (Marron et al., 2020).

A previous study by Nyungen et al. (2020) compared the effects of using copper hydroxychloride (CH) and copper sulphate (CS) in broiler feeds. It was reported that the use of CH improved the feed conversion ratio and higher body weight gain was obtained. However, it has been reported that the use of CH has positive effects on bone development. The use of CH on the amount of intestinal mucosa has positive effects and this result is positive in terms of performance and health parameters. Liao et al. (2020) reported that the use of high levels (220-330 mg / kg) of copper caused autophagy in the kidneys and had negative effects on energy metabolism. El-Katcha et al. (2020) investigated the effects of using 3 different Cu (inorganic, organic and copper nanoparticles) together with oxidized and normal oil on broilers. It was reported that the use of oxidized oil has a negative effect on body weight, feed intake and feed conversion ratio. The use of organic Cu negatively affected feed consumption, but the use of copper nanoparticles had a positive effect. The use of organic Cu at the rate of 50% of the requirement had a positive effect on feed conversion ratio. The addition of organic Cu or Cu nanoparticles up to 50% of the requirement contributed to phagocytosis, lysosomal and bactericidal activity. Incorporation of the oxidized oil with different Cu sources had no adverse effects on immune parameters. The addition of organic Cu or copper nanoparticles reduced the number of *E. coli* and lactobacilli in chicken secretion. Organic or nano-Cu supplementation in the diet containing oxidized fat reduce both hepatic vacuolar degeneration and inflammation. As a result of the research, it has been reported that the use of

nano or organic Cu at 50% of the requirement has positive effects on growth performance and immune system in broiler chickens. It has also been reported that the use of Cu nanoparticles reduces the negative effects caused by oxidized oils. Pereira et al. (2020) investigated the effects of using zinc, copper and manganese in 3 different forms (inorganic, amino acid metal chelate (AACM), inorganic + AACM) in laying hen diets. It was reported that 35% of the laying hens fed with AACM started laying 2 days earlier and their tibia weights were higher. In addition, higher oviduct weight, hematocrit, leukocyte, erythrocyte, mesophyll, monocyte, T4 and FSH ratio were detected in chickens fed with AACM. In line with these results, it has been reported that the use of the relevant minerals in the form of AACM gives more positive results on performance and health parameters. Zhou et al. (2020) investigated the effects of the amino acid Cu complex (Cu-Lys-Glu) in laying hens. Cu-Lys-Glu was added to feeds in 5 different rates (0, 15, 75, 150 and 300 mg / kg). It was reported that the use of high levels of Cu-Lys-Glu (300 mg / kg) negatively affected the ovulation rate. The use of 0, 15, 75 and 150 mg Cu / kg Cu-LysGlu does not cause a significant difference in hematological and serum biochemical parameters, organ indices and histopathological changes. However, the use of 300 mg Cu / kg Cu-LysGlu, mean corpuscular volume (MCV), albumin (ALB), total bilirubin (TBILI), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea nitrogen (UN) and creatinine (CRE) concentrations are significantly increased. It has also been reported to cause severe microscopic histopathological changes in the liver and kidney. It has been determined that the use of 150 mg / kg Cu-Lys-Glu is sufficient on performance and health parameters in laying hens.

4. IODINE

Iodine is the component of thyroid hormones that has a vital role in metabolism. These hormones are known as thyroxine (T4) and triiodothyronine (T3). Iodine deficiency is commonly referred to as goiter (enlarged thyroid) and is a discomfort seen all over the world. The metabolic efficiency and function of I are different from that of other trace elements. Its main known function is that it is a structural element of the thyroxine hormone. Thyroxine is the only hormone that contains an inorganic element.

Thyroxine is synthesized and stored in the thyroid gland. 80% of the I in the body is found in the thyroid gland. The thyroid gland makes up only 0.2% of body weight. Thyroxine hormone is transferred from the thyroid gland to various tissues and regulates and controls the energy metabolism events in these cells (Suttle, 2010). Like all anionic elements, I is very efficiently absorbed from the gastrointestinal tract. However, I secreted through various secretions is easily recycled in metabolism. Absorbed I is transported in the bloodstream by loosely binding to plasma proteins. A small amount of extra thyroid I circulates in a free ionic form such as chloride and accumulates in soft tissues such as muscle and liver when excess iodine is consumed. The recycling of thyroid I takes place through the iodide pool. Excess iodine is excreted predominantly in the urine as iodide (Suttle, 2010). The pathways followed by iodine in metabolism are summarized in Figure 3.

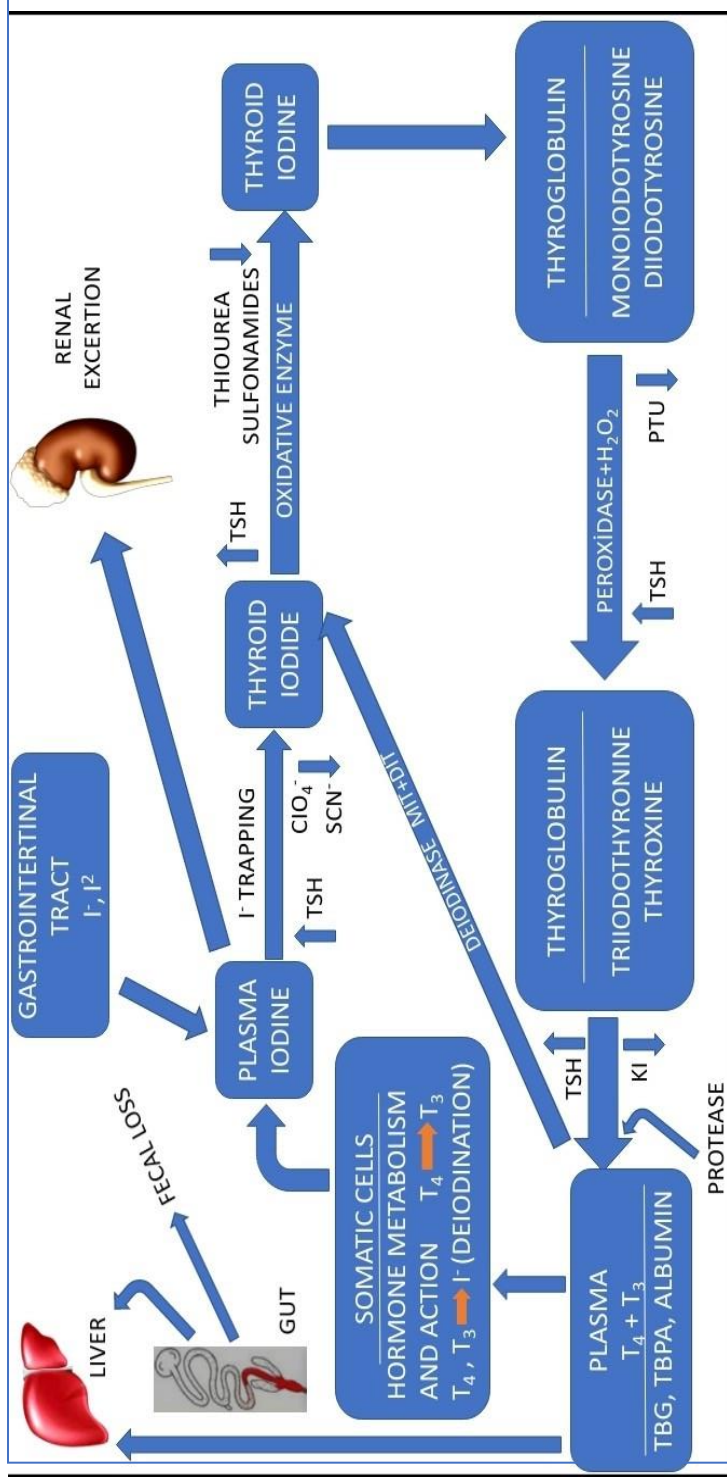


Figure 3. The pathways of iodine in metabolism

Iodine is commonly found in both organic and inorganic compounds in nature. I concentrations in feeds show intense changes. While oilseed meals contain approximately 0.11-0.20 ppm of I, cereal grains are found at levels of 0.04-0.10 ppm. Animal-based products have higher I content than plant-based products. For example, it varies between 0.3-3 ppm in fish. Also, amounts required in poultry diets are summarized in Table 2.

Goiter is a very rare disease in poultry. Insufficient thyroid hormones negatively affect growth, egg production and size. Especially in breeding chickens, I deficiency causes a decrease in I presence in the egg and a decrease in hatchability. Thyroid deficiency in laying hens causes weakening of feathers, small testicles and spermatozoa activity, and combs to shrink. The amount of I in poultry diets directly affects egg iodine accumulation and there is a linear relationship between them. Iraqi et al. (2020) reported that the use of 2 mg/kg organic I gave better results than inorganic form iodine in egg yield, feed conversion ratio, plasma IgM, HDL, HDL / LDL, T3 / T4, L%, H%, H / L values. Albdarani et al. (2020) reported that potassium I application by in ovo method had no effect on hatchability and embryonic death, and negatively affected hatching weight. Serum T3 value was negatively affected but had a positive effect on T4, and no effect on TSH was found. Only the amount of cholesterol and triglyceride in blood parameters was negatively affected by the administration. Sarlak et al. (2020) determined that performance and egg quality criteria were not affected by the use of 2 and 4 mg/kg organic or inorganic I. However, using 8 mg/kg of both iodine types negatively affected egg yield and feed intake. Especially the use of high doses of organic I reduced eggshell strength and Haugh units. It caused the increase of abnormal eggs in parallel with the disruption of blood serum and egg yolk lipids. It was reported that a linear increase was observed in the amount of egg shell and egg I with the amount of iodine added to the ration, and the use of organic iodine was higher than all the other groups. As a result of the research diet supplementation 2, 4, and 8 mg/kg iodine organic or inorganic sources improves I content of eggs. However, after 12-week period of supplementation does not any effect of I content of egg. Behroozlak et al. (2020) determined that feed consumption, body weight gain and feed conversion rate were not affected by iodine use (0, 2.5, 5 mg/kg). With the increase in iodine use, small intestine and jejunum weights were negatively

affected. However, the increase in iodine use increased the amount of iodine in breast meat.

5. SELENIUM

Oxidative stress is a serious damaging factor for cellular integrity through the continuous release of reactive oxygen species-mediated by various biotic (bacteria, viruses, fungi, etc.) and abiotic stressors. Trace elements such as Se with strong antioxidant potential have wide applicability as feed additives to reduce oxidative stress in living systems. The specific biochemical role of selenium was revealed by the discovery that glutathione peroxidase (GPX) is a selenoprotein (SeP) and correlations between GPX activity and selenium uptake in tissues emerged. Most SePs protect the tissue where they are formed from free radicals (ROS) and play important roles in cell signaling and transcription. If peroxidation gets out of control, it can initiate chain reactions of ROS formation and cause tissue damage. The task of terminating such reactions and protecting against peroxidation is shared by other tissue enzymes and non-enzyme compounds such as vitamin E. Biochemical and clinical abnormalities resulting from selenium deficiency, therefore, show various responses to vitamin E. Vitamin E functions as a fat-soluble antioxidant in cell membranes, while Se acts as water-soluble as GPX and mainly acts as an intracellular antioxidant (Suttle, 2010). Selenoproteins found in the body and their functions are shown in the Figure 4 and Table 1

Table 1. The kinds of enzyme with selenium content (Suttle, 2010)

Nomenclature	Selenoprotein	Principal location	Function
GPX1	Cytosolic glutathione peroxidases (GPX)	Tissue cytosol, red blood cells	Storage, antioxidant
GPX2	Phospholipid hydroperoxide GPX	Intracellular membranes, particularly testes	Intracellular antioxidant
GPX3	Plasma GPX	Plasma, kidney, lung	Extracellular antioxidant
GPX4	Gastrointestinal GPX	Intestinal mucosa	Mucosal antioxidant
GPX5	Epididymal GPX	Epididymis	Weak antioxidant
SPS-2	Selenophosphate synthetase 2	Ubiquitous	SeCys biosynthesis
ID1 ORD1	or Iodothyronine 5 α -deiodinase type I	Liver, kidney, muscle	Conversion of T4 to T3
ID2 ORD2	or Iodothyronine 5 α -deiodinase type II	BAT	Conversion of T4 to T3
ID3 ORD3	or Iodothyronine 5 α -deiodinase type III	Placenta	Conversion of T4 to T3
TR1 and 2	Thioredoxin reductase 1 and 2	Kidney, brain	Redox cycling
SePN	Selenoprotein N	Muscle	Cell proliferation
SePP	Selenoprotein P	Plasma	Transport, metal detoxifier
SePR	Selenoprotein R	Liver, kidney	Methionine sulfoxide reductase
SePW	Selenoprotein W	Muscle	Antioxidant, calcium-binding
MCSep	Mitochondrial capsular selenoprotein	Sperm capsule mitochondrial	Store for GPx

Selenium has a close relationship with vitamin E. Both nutrients protect biological membranes from oxidative degeneration. The lack of these nutrients causes tissue breakdown. Apart from that, they are found in the composition of proteins found in spermatozoa. It has a function in RNA as it can bind to purine and pyrimidine bases. It takes part in prostaglandin synthesis. It has various functions in the thyroid glands.

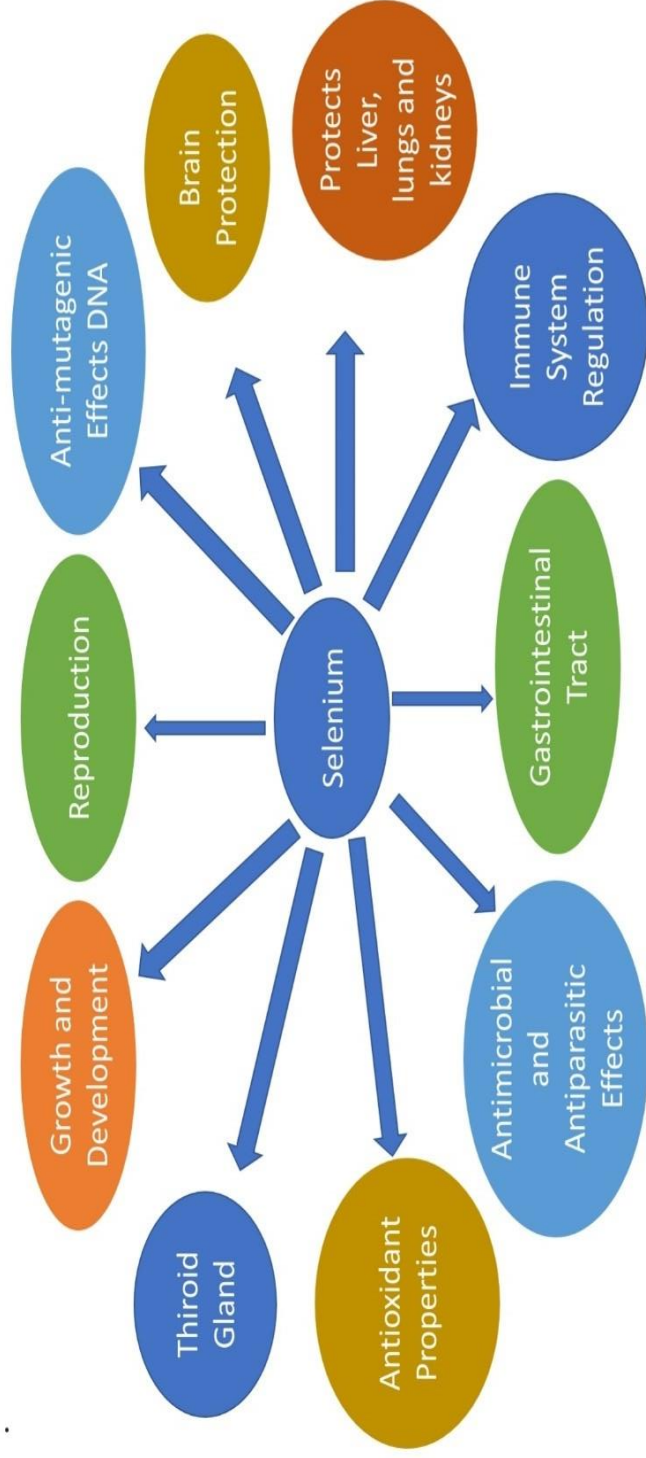


Figure 4. Selenium function in the body

Selenium is absorbed from the ileum, cecum and colon in poultry. Absorption does not occur in the stomach and duodenum. Absorption is higher in poultry than ruminants. Absorbed Se is transported to the tissues by plasma. The most storage takes place in the kidney. This is followed by tissues such as liver, spleen and pancreas. The Se content of nerve tissues is low. Its amount in feed should not exceed 4 ppm, which causes insufficiency at levels of lower than 0.1 ppm and toxic effects at levels higher than 4 ppm. Amounts of 0.5 ppm and above generally prevent deficiency from occurring (Filazi 2017). The needs of poultry are generally summarized in the Table 2.

Selenium deficiency can be seen in 3 different ways in poultry as exudative diathesis, pancreatic dystrophy and nutritional muscular dystrophy. The presence of sufficient Se in the diet for the first two diseases prevents the disease from occurring. However, in order to prevent muscle dystrophy, sulfur and vitamin E supplements should be present along with selenium. In white muscle disease (muscular dystrophy), as the name suggests, degenerations are seen in the muscles as white lines. Consequently, difficulties arise in animal movements. Exudative diathesis is seen in chicks fed with deficient rations of Se and vitamin E, which is characterized by fluid accumulation in the subcutaneous tissues in the breast area as a result of unprotected capillaries and oxidative damage. In severe Se deficiency, even if there is too much vitamin E in the diet, the chickens have atrophy of the pancreas and regression in growth. The target organ in Se deficiency is the pancreas. If vitamin E is sufficient, other organs are not affected by this deficiency. Pancreatic atrophy is associated with impaired lipid and vitamin E absorption. Selenium deficiency in all livestock results in impaired reproductive performance in males and females. Especially in poultry, it decreases egg yield and hatchability. Selenium level in plant-based feed varies depending on the plant species, the level of selenium in soil and water. The Se content of some plants is given in the Table 3. Additional selenium is commonly added to the ration as sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4), or selenomethionine. Selenium supplementation should be done carefully, as the amount of Se added to diets is very low and the margin between poultry requirement and toxic level is narrow.

Liu et al. (2020a) investigated the effect of using different Se sources (Sodium selenite, Se yeast) at different rates (0.3-0.5 mg / kg) in laying hens.

It was reported that different selenium sources had no effect on egg weight and feed conversion rate. Egg yield was positively affected by the use of 0.5 mg/kg sodium selenite. Egg selenium contents were also not affected by the amount and type of selenium. In addition, there was no difference between the groups in blood biochemistry values. Liu et al. (2020b) conducted similar research to Liu (2020a) and the use of different Se sources positively affected the Se content of eggs. However, it has been reported that it improves the antioxidant capacity in chickens and the use of 0.5 ppm Se yeast is particularly useful in obtaining eggs with high Se content. Sun et al. (2020) investigated the effects of using earthworm with increased selenium content in the diet of laying hens. As a result of the study, it was found that earthworm powder containing 1 mg/kg Se showed an increasing effect on total protein, albumin, glutathione peroxidase, superoxide dismutase, IgG and IL-2 values, but had a decreasing effect on triglyceride, total cholesterol, glucose and nitric oxide amounts. It was reported that these effects increase the antioxidant level in laying hens. Zhao et al. (2021) investigated the effects of using Se as selenized glucose in organic form on laying hens. It was reported that glutathione peroxidase and total antioxidant activity increased in the spleen and ovaries. It has been reported to have a preventive effect on oxidative stress parameters by increasing GSH-Px and T-AOC activities in the liver, although the hydrogen peroxide level decreases with the use of selenium. Zhou et al. (2020) reported that the use of glycine nano-selenium in laying hens contributes to antioxidant activity, positively affects intestinal parameters, and has no effect on egg performance and quality.

6. ZINC

Zinc is an essential trace mineral in metabolism. It exists as a cofactor in more than 300 metalloenzyme structures. It plays an important role in the metabolism of fat, carbohydrate, protein, nucleic acid and cell membranes. It is necessary for the continuity of feathering, growth, skeletal development, skin quality and reproductive parameters in poultry. However, it increases disease resistance by increasing immune system activity. It is involved in the carbonic anhydrase activity that ensures the improvement and continuity of the egg shell quality in laying hens (Suttle, 2010). The functions of zinc are summarized in Figure 5.

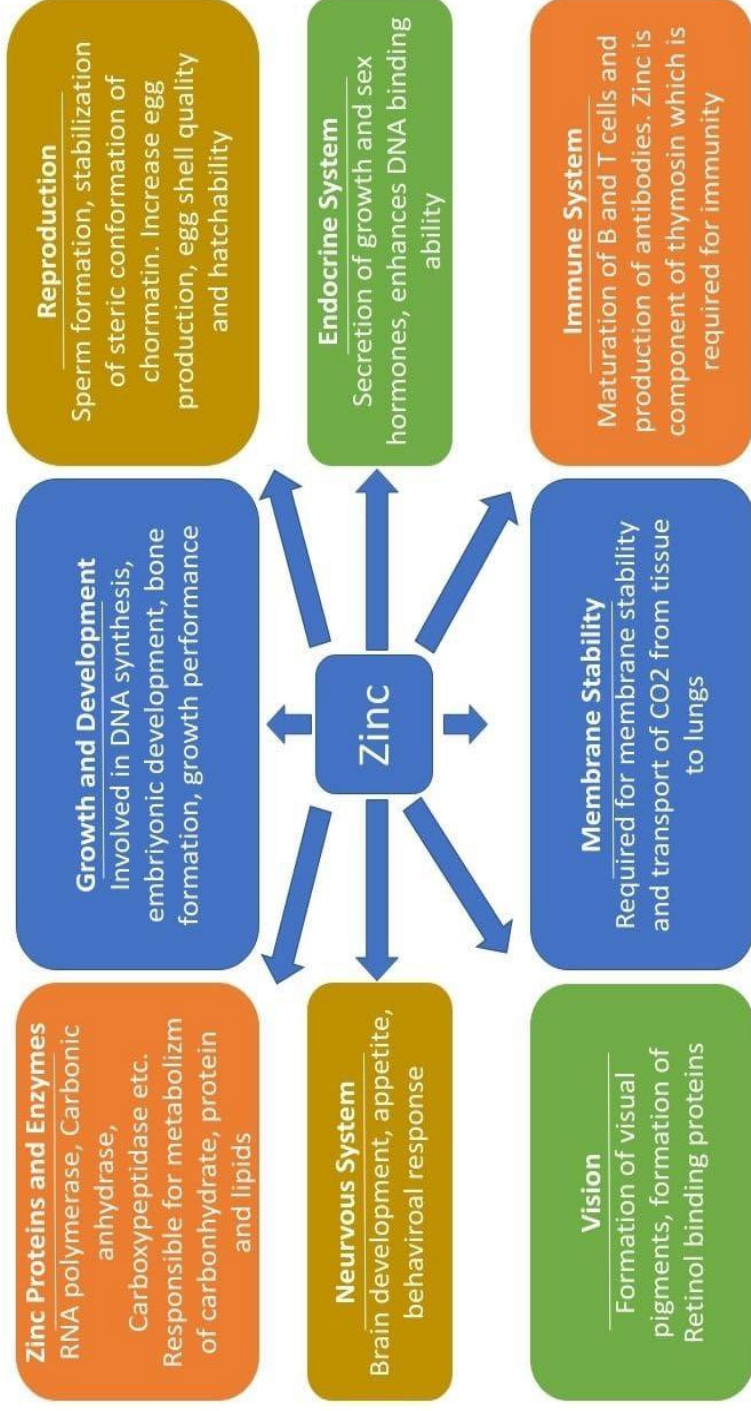


Figure 5. The functions of zinc mineral

Although zinc is mainly absorbed in the small intestine in poultry, very small amounts are also absorbed from the proventriculus. Many factors such as Ca, P, phytate, Cu, Kd, and Cr can reduce the absorption of zinc. In addition, raw materials such as casein, liver extract, soluble alcohol industry residues, corn oil and blood meal contribute to the absorption of zinc. EDTA and vitamin D also affect zinc absorption. The presence of zinc in the diet and its absorption in metabolism are inversely proportional (Suttle, 2010). Zinc is stored in soft tissues such as pancreas, liver, pituitary gland, kidneys and adrenals in metabolism. In addition, it is known that zinc content is high in male chickens, especially in the testicles and digestive tract secretions. Zinc concentration and bioavailability are highly variable in plant- based feeds. The amount of zinc in the vegetative parts of the plants is between 10 and 300 mg kg⁻¹ in dry matter, which depends on the species, the environment and the amount of zinc in the soil. However, the bioavailability of zinc is low due to phytic acid and oxalic acid in many seeds. Most feeds of animal origin are highly bioavailable and a very good source of zinc. Zinc can typically be added as zinc sulphate, zinc carbonate, zinc oxide, or zinc complex with amino acids. With galvanized pipe, it enriches the water supply and zinc so its deficiency can be prevented.

Poultry need more zinc, especially if fed vegetable rations containing soy and sesame pulp. An excess of calcium and phosphorus inhibits the use of zinc, whether in a simple diet or a mineral supplemented diet. Zinc deficiency is observed in poultry at certain periods. This deficiency may be caused by the age of the animal, availability of zinc in the diet, anti-nutritional factors and environmental factors. Zinc deficiency is mostly seen in young birds. Feed consumption and performance parameters are negatively affected in animals. However, especially the comb color is adversely affected and development is delayed. The age of first ovulation is also negatively affected by zinc deficiency. It is characterized by short and thick leg bones, widening of the heel joints, dandruff on the skin of the feet, very slow hair growth and reduced feed consumption.

Neto et al. (2020) reported that the use of zinc in combination with threonine has interaction effects on egg production and egg weight in laying hens. It has been reported that the use of zinc over 40mg/kg in layer diets negatively affects the egg quality. However, use of high levels of zinc has

been reported to negatively affect nutrient digestibility and energy utilization and adversely affect performance. Li et al. (2021) reported that the use of zinc methionine under in vitro conditions increases intracellular Ca^{2+} concentration and can make an extra contribution on growth in laying hens by improving mRNA metallothionein expressions. Yu et al. (2020) reported that the use of organic and inorganic zinc has no effect on feed consumption, average egg weight and egg quality, but an increase observed in antioxidant capacity in laying hens. It has been reported that different zinc sources cause an increase in the zinc content of eggs, but there is no difference between different zinc sources.

CONCLUSION

In fact, microminerals are often overlooked, although they play very important roles in animal nutrition science. The main reason for this is that researches are mostly focused on macro minerals. However, it is clear that trace minerals perform irreplaceable functions in metabolism, production parameters, protection of health and product quality. On the other hand, since the micro-mineral content of the feed materials that make up the compound feed is limited, it usually does not meet the needs of the animals. For this reason, micro mineral forms and amounts used in diets are constantly updated. In this way, it helps to obtain clearer information about micro minerals. Studies on the bioavailability of different mineral forms in metabolism aim to find answers to the question marks on this subject. Because different mineral forms are evaluated at different rates in metabolism. The usability of especially nano-form minerals in poultry feeds is being investigated. In this way, it is aimed both to prevent environmental pollution and to achieve a more effective performance by using less minerals. Current research is still not able to clearly reveal the needs of micro minerals in poultry nutrition. Therefore, future needs should be identified and needs updated and clarified through more effective and environmentalist research.

Table 2. Micro Mineral Requirements for Poultry* (Dale, 1994; Anonymous, 2015)

White-Egg-Laying Strains						Brown-Egg-Laying Strains			
Nutrient	Unit	0-6 Weeks	6-12 Weeks	12-18 Weeks	18 Weeks to First egg	0-6 Weeks	6-12 Weeks	12-18 Weeks	18 Weeks to First egg
Manganese	mg	60.0 (100.0)	30.0 (100.0)	30.0 (100.0)	30.0 (60.0)	56.0 (100.0)	28.0 (100.0)	28.0 (100.0)	28.0 (60.0)
Zinc	mg	40.0 (70.0)	35.0 (70.0)	35.0 (70.0)	35.0 (40.0)	38.0 (70.0)	33.0 (70.0)	33.0 (70.0)	33.0 (40.0)
Iron	mg	80.0 (40.0)	60.0 (40.0)	60.0 (40.0)	60.0 (40.0)	75.0 (40.0)	56.0 (40.0)	56.0 (40.0)	56.0 (40.0)
Copper	mg	5.0 (7.0)	4.0 (7.0)	4.0 (7.0)	4.0 (7.0)	5.0 (7.0)	4.0 (7.0)	4.0 (7.0)	4.0 (7.0)
Iodine	mg	0.35 (1.0)	0.35 (1.0)	0.35 (1.0)	0.35 (1.0)	0.33 (1.0)	0.33 (1.0)	0.33 (1.0)	0.33 (1.0)
Selenium	mg	0.15 (0.2)	0.10 (0.2)	0.10 (0.2)	0.10 (0.2)	0.14 (0.2)	0.10 (0.2)	0.10 (0.2)	0.10 (0.2)

*Parameters outside parentheses are NRC data, and data in parentheses are based on current data.

Table 3. Some Feedstuffs Micro Mineral Ingredients mg/kg Dry Matter (Anonymous, 2021)

Feedstuff	Manganese	Zinc	Iron	Copper	Selenium
Alfa alfa meal	32.0	32.0	180.0	10.0	0.15-0.5
Barley	19.0	15.0	50.0	7.5	0.075-0.15
Maize	5.0	10.0	35.0	4.5	0.075-0.15
Dried corn distillate	-	-	500.0	80.0	0.15-0.5
Oat	43.0	140.0	70.0	8.0	0.5-1.0
Wheat	40.0	14.0	50.0	7.8	0.5-1.0
Pulp from soybean meal	-	27.0	500.0	20.0	0.075-0.15
Soybean meal	44.0	27.0	150.0	20.0	0.075-0.15
Rice bran	44.0	-	190.0	13.0	
Wheat bran	114.0	-	150.0	12.0	0.5-1.0
Dried skim milk		40.0	30.0	3.0	0.075-0.15
Fish meal	10.0	110.0	270.0	20.0	-

Table 3. Continue

Feedstuff	Manganese	Zinc	Iron	Copper	Selenium
Bone meal	-	90.0	500.0	12.0	-
Blood meal	1.0	-	2000.0	40.0	-
Cottonseed Meal	23.0	44.0	170.0	117.0	-
Sunflower Meal	38.0	96.0	271.0	32.0	-

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CHAPTER 2

THE STRATEGY OF MACROMINERALS APPLICATION IN IMPROVING THE PERFORMANCES AND EGG QUALITY OF LAYING HENS

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INTRODUCTION

Minerals are catalysts or components of several enzymes and hormones, therefore, they are necessary for normal growth and many metabolic processes in living organisms. Minerals can be classified into macro and microminerals. Macrominerals are expressed in concentrations higher than 100 ppm, while microminerals are expressed in concentrations less than 100 ppm and can even be expressed in ppb values (Lukić et al, 2009). Currently, it is necessary to supply adequate minerals in modern laying hens to maintain their genetic improvement and prevent deficiency diseases in birds. As a result, the macrominerals recommended by the National Research Council (NRC 1994) may not be appropriate due to genetic growth potential differences and the use of ingredients that interfere with the utilization of macrominerals. Moreover, under certain conditions such as hot stress conditions, laying hens may need to be added more than the minimum requirement. In addition, balanced relationships among minerals such as Mg, Ca, and P, as well as Na, K, and Cl, and mineral interactions and antagonisms are necessary for growth, production, bone development, health, egg quality, and litter quality. Both deficiency and excess of minerals in the poultry diets cause problems, mainly affecting the performance and egg quality causing serious economic loss. For example, Mg, Ca, P, and sulfur deficiency in laying hens lowers egg production, feed consumption, body and egg weight, eggshell thickness, eggshell weight, and these minerals concentrations in the blood, bone, and eggshell (Belkameh 2020). Meanwhile, excessive NaCl increases water consumption and leads to wet excreta. Thus, it causes a decrease in egg fertility and poor quality of the eggshell (Balos et al., 2016). Therefore, this paper aims to review and discuss the findings strategies from previous experiments in supplying the macrominerals for laying hens in which the effects, including the performance and egg quality, as well as and metabolic status of laying hens, requirement, bioavailability, retention, and excretion.

1. CALCIUM AND PHOSPHORUS

Calcium (Ca) and phosphorus (P) are important nutrients in the poultry diet, especially for bone and eggshell formation, and activators of enzymes and some hormone secretions. Ca is the major structural element in eggshells

which is highly mineralized in the form of Ca carbonate making up more than 90% of the shell (Li et al 2016). Most laying-hen lines have very few eggshell weaknesses during the first 35 weeks of life. Meanwhile, egg production and shell thickness gradually decrease due to a decrease of Ca absorption in the intestinal up to 50% on week 40 compared to the first period of egg production and the metabolic demand for Ca can contribute to osteoporosis when the chickens are 38 weeks or older. Therefore, Ca must be present in sufficient amounts and a well-balanced ratio (Carrillo et al., 2020). In addition, based on the laying phase, Rodrigues et al. (2013) also reported that a 0.8% level in the pre-laying phase was enough to ensure good performance and eggshell quality during the laying phase. In the case of the laying phase, the best results were obtained with a 3.5% Ca level. Otherwise, based on different Ca sources, Ganjigohari et al. (2017) found that administration of the nano calcium carbonate (0.126–2.015%) could replace calcium carbonate at a lower inclusion level without negative effect on egg production performance and egg quality in laying hens. The application of nanotechnology can increase the bioavailability of minerals particularly Ca by enhancing their surface area which could increase their absorption and utilization (Vijayakumar and Balakrishnan, 2014).

P has an important role in nerve function, a component of phospholipids and nucleic acids, and a key mediator of energy metabolism through ATP (Li et al., 2017). P is the second most abundant mineral in the animal body, and about 80% is found in the bones. Also, P is involved in gluconeogenesis, fatty acid transport, amino acid, and protein synthesis (Suttle, 2010). P is mostly found in plant-based diets as phytic acid (dihydrogen phosphate) and its salts (phytate). Nonruminant animals lack endogenous phytase and phosphatase to hydrolyze phytic acid to myoinositol phosphates and myoinositol, and finally, it becomes the phytate-P biologically available. Therefore, nonruminant animals' diets are usually supplemented with inorganic P, exogenous phytase, or both to meet their phosphorus requirements (Jing et al., 2018). By meta-analyzed, Ahmadi and Rodehutscord (2012) concluded that an optimal dietary non-phytate phosphorus level for laying performance and found that 0.14% non-phytate phosphorus is adequate for layers fed with 400 FTU/kg phytase. Another result by Kim et al (2017) that a super dosing level of 20,000 FTU/kg phytase

in Hy-Line Brown laying hens diets at 42 weeks to 47 weeks of age has a positive effect on egg production rate, but no beneficial effect on egg quality in laying hens. They explained that the beneficial effects of super-dosing phytase have been associated with more available P from phytate-P, which can decrease its anti-nutritional effect and generate myoinositol. Also increasing the utilization of phytate-P may further improve the utilization of energy and other nutrients such as amino acids and minerals in diets.

Calcium and Phosphorus Absorption

As the eggshell is mostly composed of CaCO_3 , it is commonly considered that Ca is an important mineral for eggshell quality. However, phosphorus and Vitamin D3, as well as some trace elements, are included. Ca absorption and Vitamin D3 activity are influenced by gut health and kidney function (Figure 1). The interactions of Ca and P with vitamin D3 are important because these interrelationships determine Ca and P homeostasis (Lie et al., 2017). Vitamin D is important for Ca homeostasis and responsiveness to parathyroid hormone in chickens. In the case of a low Ca concentration for an extended time, the parathyroid hormone stimulates the conversion of vitamin D3 to $1,25(\text{OH})_2\text{D}_3$. Thus, In the skeleton, $1,25(\text{OH})_2\text{D}_3$ together with parathyroid hormone promotes the mobilization of Ca from bone to maintain a constant blood Ca concentration and improves intestinal Ca absorption. In intestinal Ca can be transported through active and passive transport. Active transport including transcellular and metabolically driven transport involves 3 steps: 1) entry across the cell wall, 2) diffusion through the cytoplasm, and 3) exit at the basolateral cell membrane. Conversely, passive transport is characterized by ion movement from the intestinal lumen to the circulation along the chemical gradient through spaces between cells. The passive transport of Ca dominates when Ca intake is high or at adequate levels due to the inhibition of active calcium transport by high plasma Ca concentrations (Weglarz and Angel, 2013).

The concentration of P in the body is tightly regulated by renal excretion in which hormones and metabolic factors are involved in maintaining P homeostasis. P can be absorbed effectively even when it is in excess of requirement because there is limited control of P absorption from the gastrointestinal tract compared to Ca. A large portion of dietary P that is

available will be absorbed but may be eliminated through the urine which is regulated by parathyroid hormone. Unlike broilers in which absorption of P was most efficient from the duodenum to the upper jejunum, layers absorb P throughout the whole intestine, but the rate of absorption declines in the lower tract (Li et al., 2016)

According to Rodrigues et al. (2013), there are some factors directly related to Ca and P absorption in the intestine, mainly in the duodenum and jejunum such as protein levels, chelating agents and mineral interactions, dietary concentration and sources, physical and chemical forms of these minerals, intestinal pH (acidity promotes absorption), phosphate (high Ca/P enhances the formation of calcium phosphate), free fatty acids and vitamin D. P absorption is optimal at pH 5.5-6.0. Excess-free fatty acids in the diet can cause a decrease in pH in the gastrointestinal tract and thereby, interfere with Ca and P absorption.

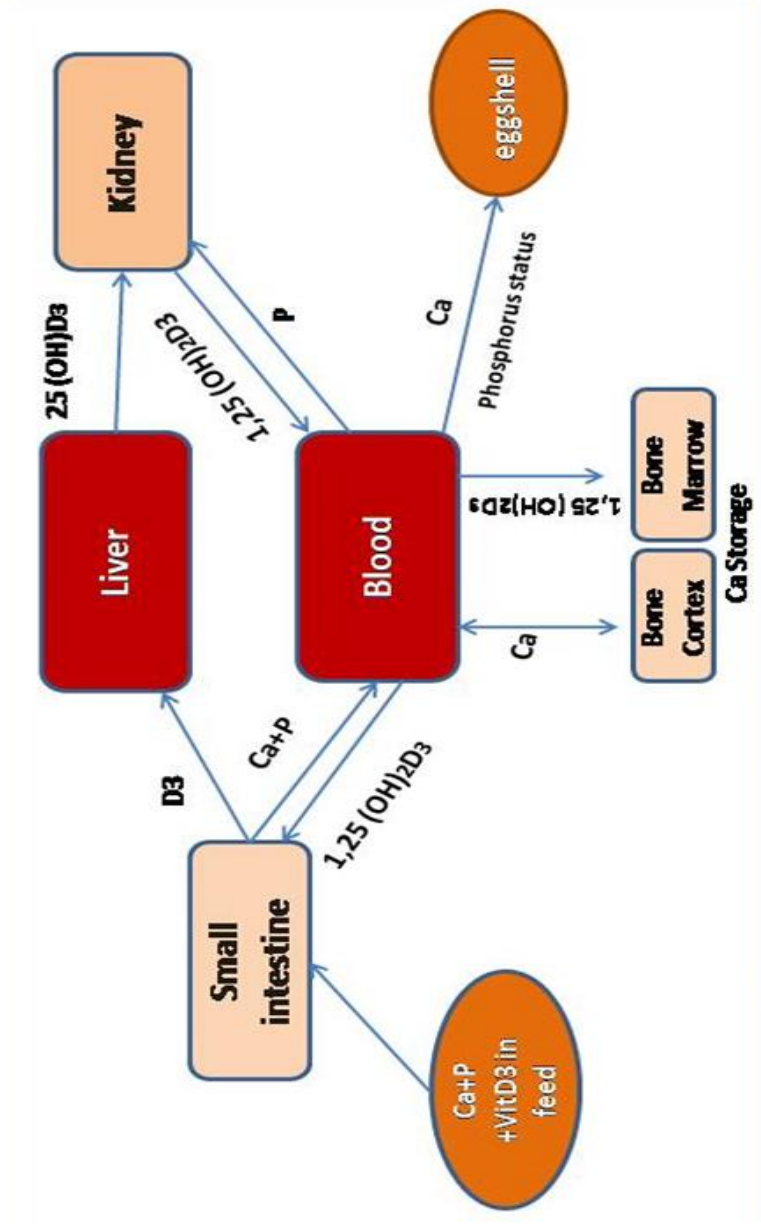


Figure 1. Biochemical pathway of calcium and phosphorus metabolism in laying hens

Calcium and Phosphorus Imbalance

Ca and P are critical for optimal egg production and eggshell quality. The imbalance between dietary Ca and P damages growth performance and bone development in poultry. Usually, the Ca to P ratio is used to evaluate the balance between dietary Ca and P. High levels of dietary Ca and low levels of P had detrimental effects on laying hens. Elevating dietary Ca levels increase pH in the gut and as a result, P absorption and retention will decrease. Therefore, the correct dietary Ca to P ratio is the most important for optimum egg production and eggshell quality in laying hens (Han et al., 2016). Walk et al.(2012) stated that high dietary Ca significantly increased gastrointestinal pH, which may decrease pepsin activity in the proventriculus and gizzard and reduce apparent ileal crude protein digestibility. The NRC (1994) recommended the ratio Ca and on-phytate phosphorus (Ca/ non-phytate phosphorus) is 2.22 for 1 to 21 day-old birds, and 2.57 for 22 to 42 day-old broilers. While Ca requirement of a laying hen is 4 -6 times higher than that of a non-laying hen due to the high requirement for bone formation and eggshell formation. Ca and P requirements of Leghorn laying hens are presented in Table 2.

A deficiency of either Ca or P results in a lack of normal skeletal calcification. Rickets is seen mainly in growing birds, whereas Ca deficiency in laying hens results in reduced shell quality and subsequently osteoporosis. When calcium is mobilized from bone to overcome a dietary deficiency, the cortical bone weakens and is unable to support the weight of the hen (Leeson, 2020). Laying hens that are not properly supplemented are at the highest risk for calcium deficiency disorders. Acute hypocalcemia in laying hens is thought to result in partial paresis (weakness). Under hypocalcemia, a Ca-deficient condition, hens use the Ca reserved in bones to fulfill the Ca demand during eggshell formation, and this could result in the recovery of shell thickness. Under long-term Ca deficiency, hens use the structural bone to replace the Ca reserved in the medullary bone. This remodeling process of the skeletal system results in a net loss of structural bone, leading to osteomalacia and eventually osteoporosis with a condition of paralysis known as cage layer fatigue (Hu, 2013).

2. SODIUM, POTASSIUM, AND CHLORIDE

All classes of poultry have specific requirements for sodium (Na), potassium (K), and chloride (Cl) in the correct amounts or ratio for physiological functions, particularly acid-base homeostasis. These minerals play an important role in the maintenance of cellular osmotic pressure and the metabolism of water in tissues (Melo et al., 2020). Moreover, according to Pohl et al. (2013), these electrolytes' functions include maintaining osmotic pressure and water distribution in various body fluid compartments, maintaining proper pH, regulating the proper function of the heart and other muscles, participating in oxidation-reduction (electron transport) reactions, and catalysis as cofactors for enzymes.

Na ions are the major cations of extracellular fluid and K ions are the major cations of the intracellular fluid. To maintain internal fluid and electrolyte balance, water, Na, and K are constantly moving between the intracellular and extracellular. Both deficiency and excess of Na and potassium in the poultry diets cause problems for the birds, mainly affecting their performance (Lima et al., 2015). Diets deficient in Na lead to adrenal malfunction, and this results in increased uric acid levels, which may cause physiological shock and death in the chicks. Chickens can maintain Na, K, and Cl homeostasis in their blood and tissues by excreting excess of these minerals (Mushtaq and Pasha, 2013).

Cl is the most common and essential anion in intracellular fluid for poultry, and it plays a role in a variety of body functions such as osmotic and acid-base balancing, muscular and nervous function, and the transport of water and solutes within fluid compartments. Cl is also a necessary part of hydrochloric acid. In poultry, this mineral is used as a component of an activator of hormones and enzymes, as well as for the development and replacement of the skeleton and eggshell. (Ravindran, 2013). The small amount of Cl also can be added in organic form (metal ion + amino acid ligand, chelated amino acids, proteinases) as organic forms are better assimilated by poultry than mineral salts. Organic mineral sources in poultry nutrition can prevent minerals from forming indigestible complexes with certain dietary components, as well as similar mineral antagonisms in the intestine, which could reduce mineral absorption rates (Swiatkiewicz et al., 2014; Algawany et al., 2021).

Sodium, Potassium, Chloride Absorption

Minerals in the diet must be absorbed across the epithelial cells that line the gastrointestinal tract to enter the blood and be used by the tissues. Minerals can be absorbed from any part of the digestive tract. However, most minerals are absorbed primarily in the small intestine. Both the small and large intestines are lined by a single layer of epithelial cells joined together by proteins such as occludins, claudins, and e-cadherins that form a tight junction between adjacent cells (Goff, 2017). Furthermore, at the basolateral membrane, an ion gradient created by the ATP-driven movement of Na^+ , K^+ , and Cl^- through sodium-potassium ATPase aids the passive diffusion of Na^+ , K^+ , and Cl^- via the Na^+ - K^+ - Cl^- cotransporter NKCC. Cotransporter NKCC1 is a protein that aids in the secondary active transport of Na, K, and Cl into cells. For Na, absorption occurs at the apical membrane by three main mechanisms. The first mechanism involves the sodium glucose-linked transporter (SGLT-1), which is activated by the presence of glucose in the lumen. The movement of Na and glucose creates a gradient that induces the movement of Na and water through the paracellular space, a phenomenon known as "sodium drag." The second mechanism Na absorption is related to the sodium hydrogen exchanger isoform-3 (NHE3), which is found throughout the small intestine and exchanges one Na ion for every proton. The last mechanism is the electrogenic sodium channels (ENaC) which are important in the large intestine and it is the key targets of the renin-angiotensin-aldosterone system, which regulates blood pressure and fluid balance (Nighot and Nighot., 2018).

Potassium is absorbed in the small intestines and eliminated in the urine. K^+ is quickly absorbed by active uptake in the mucosal lining of the intestine after ingestion. This rapid uptake could lead to severe K^+ imbalance if it was not for the rapid absorption of K^+ into cells. A total of 98% of K^+ absorbed through the gastrointestinal tract is contained in cells, with just 2% occurring extracellularly (Pohl et al., 2013). Cl is absorbed in the small intestines and transferred by the intestinal mucosa throughout the small and large intestines. In excess, Cl is excreted in the urine, which is normally associated with high Na and K levels (Bolos, et al 2016). Proximally, Cl is taken up either actively (via exchange mechanisms such as Cl/HCO_3 , Cl/OH) or passively (via electrochemical or concentration gradients). Electroneutral

sodium chloride absorption is blocked when the intestinal mucosa is activated by agents that increase intracellular second messengers. Electroneutral sodium chloride absorption is impaired when the intestinal mucosa is activated by substances that enhance intracellular second messengers. Thus, transport proteins in the intestinal mucosa (the most important being the CFTR channel) and basolateral membranes ($\text{Na}^+/\text{Cl}^-/\text{K}^+$ cotransporter, K^+ channels, Na^+/K^+ -ATPase) stimulate sodium chloride and potassium chloride secretion (Kato and Romero, 2011; Turck et al., 2019).

Dietary Electrolyte Balance

Dietary electrolyte balance plays an important role in the body's water management and the acid-base ratio regulation of blood. The electrolyte balance is most commonly expressed in mEq/kg, with a value of 250 mEq/kg considered ideal for normal physiological function. However, electrolyte imbalance is uncommon because the body's buffering system maintains normal physiological pH levels. Three major factors influence the maintenance of this value: electrolyte balance and ratio in feed, endogenous acid content, and renal activity level (Balos et al., 2016). The amount of electrolytes expressed as g/kg for each ingredient is used to calculate the dietary electrolyte balance, as follows: Dietary electrolyte balance = $\text{Na} + \text{K} - \text{Cl}$. (mEq/kg). When sodium (Na^+) and potassium (K^+) ion levels in the diet are too high, too many H^+ ions are pumped out of the blood. Conversely, the addition of Cl^- lowers blood HCO_3^- levels, resulting in acidosis, thereby lowering blood buffering capacity (Figure 2). The metabolic acid load will rise as a result of the imbalance, triggering a regulatory mechanism in the bones, lungs, or kidneys. This condition is costly in terms of energy since it requires nutrients that can otherwise be used for growth and other purposes (Mercier et al., 2017).

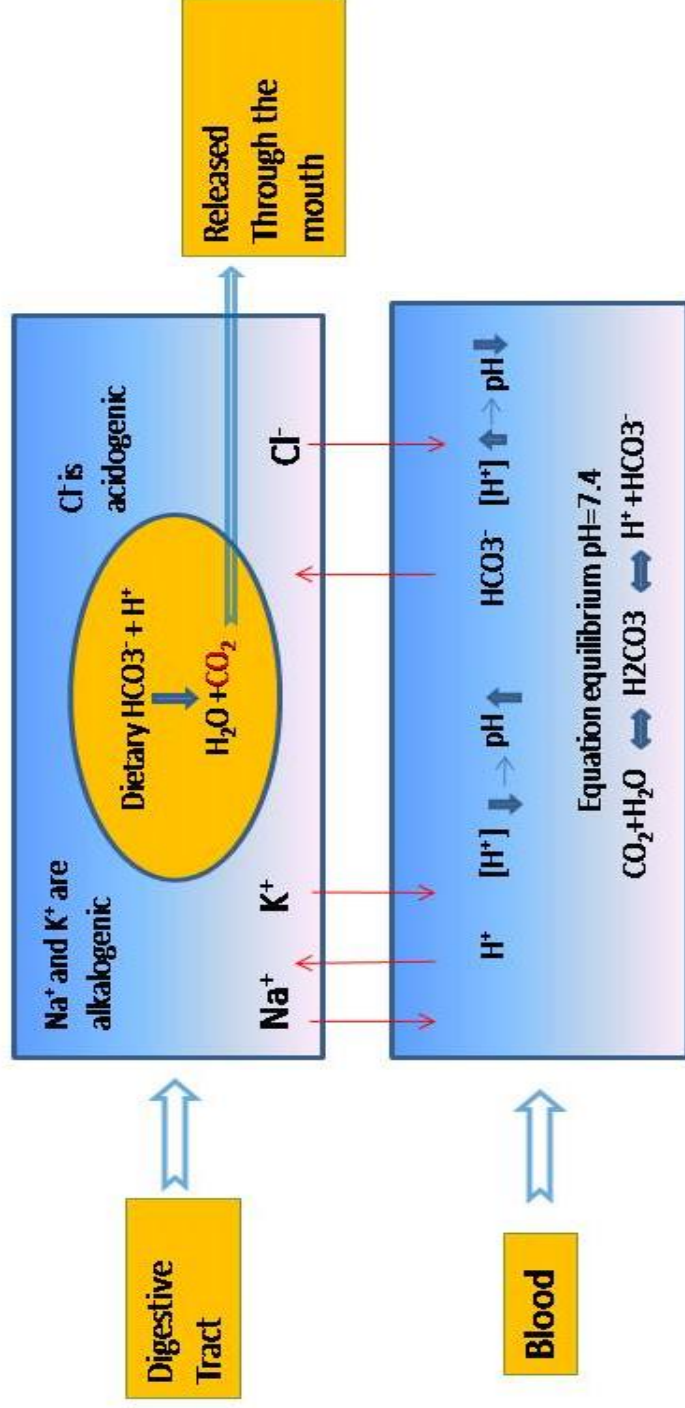


Figure 2. Blood buffering capacity mechanism

Sodium Requirements

Common salt is included in all diets as a source of sodium and an appetite stimulant. Salt is added in poultry diets at levels of 0.2% to 0.4% whereas sodium content in poultry feed at levels above 0.5% is considered toxic (Ravindran, 2013). Sodium deficiency decreases osmotic pressure and consequent disturbance of acid-base balance. Heart failure, decreased blood pressure, increased hematocrit, and decreased elasticity of subcutaneous tissue are all symptoms of severe sodium deficiency. Sodium deficiency also impairs adrenal gland function, resulting in elevated uric acid levels in the blood and finally, shock and death. Excessive salt increases water consumption and leads to wet excreta. Thus, it causes a decrease in egg fertility and poor quality of the eggshell (Balos et al., 2016). As a result, the NRC recommends a minimum sodium requirement of 0.13-0.19% for the layer period to reduce this risk. The sodium requirement of Leghorn laying hens is presented in Table 2 and sodium application for laying hens by different authors is presented in Table 3. Meanwhile, in the case of postmolt-laying hens, sodium administration of up to 0.15% provided better performance and egg quality, especially in the eggshell (Melo et al., 2020). However, under hot climates, supplemental salt with 0.2 to 0.3 % sodium bicarbonate is recommended (Ravindran, 2013). Moreover, Abbas et al. (2019) proved that 1% supplementation of sodium bicarbonate in layers' diet had a beneficial impact in terms of immunity, increased diet digestibility, and reduced or at least ameliorated the harmful effects of heat stress on immune response against ND virus during summer conditions.

Other studies with different sources of sodium were reported by Zhang et al.(2020) where dietary 0.1 % sodium humate can enhance egg albumin quality and improve immunity in laying hens. Youssef et al. (2013) also reported that up to 0.2 % sodium formate significantly increased egg production and feed efficiency for aged laying hens (53-61 weeks) during the summer period. Supplementation of organic acid salt reduces the pH of diets and allows an increase in protein digestibility by enhancing digestive enzyme activity and reducing pathogenic bacteria activity. When the temperature of the environment is high, birds increase their respiration rate to increase the rate of evaporative cooling, resulting in excessive carbon dioxide loss and causing respiratory alkalosis. This may be reflected in a reduced growth rate

and a decline in eggshell quality, often seen in high-producing layers. (Ravindran, 2013; Youssef et al., 2013).

Potassium Requirements

The most common symptom of K deficiency is hypokalemia with symptoms of muscle weakness, decreased intestinal tone, cardiac insufficiency and respiratory insufficiency, and failure, which may occur as a result of extreme stress. Stress is associated with a rise in plasma proteins, which causes adrenalin-mediated renal excretion of K into the urine (Baloš et al., 2016). High dietary intakes of K may change the dietary electrolyte balance and increase water intake and excreta moisture (Koreleski et al., 2010). However, K toxicity is uncommon in animals due to the body's ability to excrete K quickly and control its absorption. The NRC (1994) recommends a minimum K requirement of 0.13-0.19% for laying hens to reduce this risk. The K requirement of Leghorn laying hens is presented in Table 2. However, under certain conditions such as hot stress conditions, K needs to be added more than the minimum requirement. K application for laying hens by different authors is presented in Table 3. Since, heat stress causes an increase in the concentrations of sodium and Cl ions in the blood (Abbas et al., 2012), whereas the concentrations of K ions and phosphate (PO_4^{++}) are reduced (Yosi et al., 2017). The addition of potassium chloride at 0.2–0.5% is helpful to maintain osmotic and acid-base balance and increase water consumption. Furthermore, as the temperature rises, the K can be added up to 0.6-0.7 percent (Saeed et al., 2019). Under heat stress, the addition of potassium chloride up to 0.4 % in drinking water can be a way to prevent the drop in egg production (Dai et al., 2009).

Chloride Requirements

In general, the concentration of Cl in the feed should be 10-15% higher than the sodium concentration. For broilers in the whole growth period, recommended Cl levels range from 0.16 to 0.23 % and 0.25% for turkey (Ramos and Brake, 2019). Meanwhile, the recommended requirement of laying hens for dietary Cl is 0.15% (NRC, 1994). The Cl requirement of Leghorn laying hens is presented in Table 2 and Cl application for laying hens by different authors is presented in Table 3. When low levels of salt are fed to monogastric, a Cl deficiency may occur. Cl deficiency is attributed to poor

growth and bone mineralization, feather picking, cannibalism limb weakness, high mortality rate, dehydration, and elevated Cl levels in the blood. Excess salt in drinking water has more severe toxic effects than excess salt in feed. Cl levels in the water that are considered tolerable range from 0.015 to 0.018 % or 0.25 to 0.30 % salt, whereas levels higher than 0.033 % or more than 0.54 percent salt were toxic (Bolos, et al., 2016).

In addition, the quality of eggshells is affected by Cl levels in diets and water because eggshell calcification is an ion transport process in which multiple ions transport, regulate, and collaborate to form calcium and bicarbonate ions. Thus it passes through the uterine apical membrane and into the uterine fluid to synthesize calcium carbonate (Jonchere et al., 2012). A study found that the breaking strength of laying hens fed diets containing 0.20 and 0.25% Cl significantly decreased compared with the 0.10 and 0.15% Cl levels during 47 to 54 weeks. The level of 0.2% and 0.25% levels were considered to reach the balance of Cl and HCO_3^- – and be able to meet the Cl and Na needs of the laying hens (Wang et al., 2020). Regarding the electrolyte balance among Na, K, and Cl, which were fundamental to the maintenance of osmotic pressure and the acid-base balance of body fluids, Silva et al. (2021) stated that the electrolyte balance values which gave the lowest possible feed conversion, improved yolk weight, and induced better uniformity were 1525, 1330 and 1250 in $\mu\text{eq} / \text{kg}$ of the feed respectively. Moreover, from the regression equations, the electrolyte balance value of 1390 $\mu\text{eq} / \text{kg}$ was identified as ideal, for laying hens 30 to 46 weeks of age. Another study by Dai et al.(2009) found that the supplementation of 0.2 % and 0.4% NaCl did not increase egg production during heat treatment, however, both treatments increased egg production after heat treatments. There was also a higher egg weight at 0.4 % NaCl supplementation on the fifth day of heat stress compared to 0.2 % NaCl. They reported that NaCl supplementation improved water intake during heat treatment as compared to the control group. An increase in water intake is normally followed by a decrease in feed intake and consequently, it can reduce egg production.

3. MAGNESIUM

Magnesium (Mg) is one of the most abundant cations in living cells, and it functions as a cofactor or activator for many important enzymes

involved in ATP-dependent reactions that power all major metabolic processes (Morii, 2007). Mg^{2+} is a cofactor in over 300 enzymatic reactions and is involved in many important biochemical pathways, including macronutrient degradation, oxidative phosphorylation, DNA and protein synthesis, neuromuscular excitability, and parathyroid hormone secretion regulation (Schuchardt and Hahn, 2017). Mg seems to play a central role in eggshell formation, although it is not clear whether there is a structural need or whether Mg simply gets deposited as a cofactor along with calcium (Leeson, 2020). Mg plays an important role in cellular metabolism and bone formation. Its activities are significantly related to Ca and P, so achieving the proper proportion of these elements in diets is critical in poultry feeding. Mg is involved in the metabolism of amino acids, fats, and sugars, as well as the metabolism of calcium and vitamin D in bones (Shastak and Rodehutscord, 2015). According to McDonald et al. (2011), Mg consumption increased Ca excretion in adult animals and inhibited Ca deposition in young animals. The addition of Mg to the chick rations also disrupted the Ca and P balance for normal bone formation during the first six weeks of age. Skřivan et al. (2016) stated that Mg, which is the Ca antagonist, improved the Ca, Mg, and P ratios and reduced the excess Ca. Furthermore, Matin et al. (2013) stated that the interrelationships between Mg and Ca and P suggest that hormones and enzymes involved with bone metabolism may be related to Mg metabolism. There can be a competition among ions for the active centers in enzyme systems as occurs with Mg and manganese in alkaline phosphatase.

Mg can pass through the intestinal epithelium using one of three mechanisms: passive diffusion, solvent drag, or active transport. Due to the chemical similarity of Ca and Mg, intestinal Mg absorption was assisted to some extent by vitamin D and its metabolites. In poultry, the main site of absorption of Mg was reported to lie between the lower duodenum and the lower jejunum. Mg is excreted endogenously in urine and feces, which is the main controller of Mg body homeostasis (Shastak and Rodehutscord, 2015)

Magnesium requirement

Considering the natural variation in Mg content in feedstuffs (Table 1), Mg deficiency is not to be expected under practical feeding in poultry. Thus, for supporting performance production, bone health, and litter quality, a more

balanced relationship between Mg, Ca, and P in rations is necessary. It has been suggested that the Mg requirements of laying hens should not exceed 0.5 g Mg/kg (NRC, 1994). The Mg requirement of Leghorn laying hens is presented in Table 2 and Mg application for laying hens by different authors is presented in Table 3. In laying hens, Mg deficiency lowers egg production, feed consumption, body and egg weight, eggshell thickness, eggshell weight, and Mg concentrations in the blood, bone, and eggshell (Belkameh, 2020). In growing laying hens, Mg deficiency is characterized by poor growth and feathering, decreased muscle tone, incoordination, tremors, convulsive, and death (Shastak and Rodehutsord, 2015).

The dietary either 300 or 600 mg/kg Mg significantly increased feed efficiency, egg mass, and eggshell thickness of laying hens in normal as well as heat stress conditions, but no significant effect was detected on egg production glucose, total cholesterol, and triglycerides (Gooya and Torki, 2018). In addition, according to Yang et al. (2012) Mg deficiency induces an increase in hydrogen peroxide production and decreases catalase activity in chick embryo hepatocytes in vitro and dietary MgSO₄ supplementation significantly prevented heat stress-induced oxidative damage and improved growth performance in broilers compared with that of control because of restoration of the activity of anti-oxidative enzymes. Gaál et al. (2015) reported that MgO supplementation increased the egg production of laying hens, as well as benefitting the quality of breeding eggs and improving hatching yield. Supplemented the diet to laying hens either with 0.4% Mg citrate or with 0.4% MgO produced 9.68% more eggs than did the non-supplemented hens.

Furthermore, Kim et al. (2013) reported that feeding 46-week-old laying hens with diets containing more than 4.2 g/kg Mg increased eggshell strength and shell thickness. Another study by Lilburn et al (2019) found that growing pullets and subsequently laying hens fed practical-type corn-soybean meal diets with and without 0.1% supplemental magnesium oxide (MgO) through 36 wk of age had a beneficial impact on body weight at 18 weeks and 21 weeks of age (beginning of egg production). The reason for these positive impact might be associated with increased concentration of Mg in eggshells because Mg is the mineral with the second-highest concentration in eggshells and It might also be attributed to Mg function as a cofactor in key metabolic

processes in the body including macronutrient degradation, oxidative phosphorylation, and protein synthesis (Schuchardt and Hahn, 2017). An antagonistic relationship also seems to exist between Ca and Mg concerning skeletal integrity and eggshell quality in laying hens. In one study, dietary laying hens with high dosing up to 1.56 g/kg Mg together with coarse-grained limestone at Ca: non-phytate phosphorus (12.8) ratio significantly increased Mg concentration in the eggshell and decreased Ca concentration. Excess of Mg in hens can reduce the activity of the parathyroid hormone, which reduces blood Ca and subsequently also egg production and shell quality (Skřivan et al 2016).

4. SULFUR

Sulfur (S) plays a variety of roles, including incorporation in amino acids, as well as enzymes and biomolecule metabolism. Elemental S is related to a variety of antioxidant metabolisms, including glutathione peroxidase and Reactive Oxygen Species (ROS) scavenging (Battin et al., 2009, Kim et al 2015). Poultry needs of S are also for the production of glutathione. In poultry, the supply of S can be achieved from various sources, such as synthetic amino acids (methionine, cysteine), sodium sulfate, ammonium sulfate, calcium sulfate, and elemental sulfur. However, the primary source of S absorbed in the digestive system is correlated with the intake of sulfur-containing amino acids. Only a small portion can be absorbed in the form of hydrogen sulfide and together with the S from the oxidation of methionine and cysteine contributes to the acid-base balance in the body. S is contained in amino acids or organic substances that are necessary nutrients for chickens that cannot be synthesized in the body (Alam and Anjum, 2003; Park and Park et al., 2017). Silva et al. (2014). S is included in the structure of biotin and thiamine, two B vitamins that are important actors in energy metabolism. Sulfur has a structural function in mucins (sulfomucins), which are made up of proteins, glycoproteins, and lipids that protect the intestinal tract from the contents of the lumen (Richter, 2011).

Sulfate S is absorbed up to 60% effective in the small intestine. S is transported across the apical membrane by a $2\text{ Na}^+/\text{SO}_4^{2-}$ cotransporter, and SO_4^{2-} is assumed to diffuse across the cell to the basolateral membrane. In return for 2 Cl^- or HCO_3^- anions, it is pushed over the basolateral membrane

into the extracellular fluids. Sulfate is a powerful anion that can affect acid-base balance and dietary S can be converted to sulfide, which can interfere with the absorption of other elements, particularly Cu and Se (Goff,2018). S is transformed into sulfate molecules and excreted in urine and feces (hydrogen sulfide) when there is an excess quantity. H₂S is mostly produced by anaerobic microbes decomposing S-containing amino acids, through bacterial sulfate reduction (BSR), or the chemical process by-products (Deng et al., 2018) (Figure 3) In addition, the case of toxic gas emissions from poultry operations, such as hydrogen sulfide (H₂S), potentially impacts the occurrence of various diseases. H₂S is rapidly absorbed into the bloodstream, where it dissociates, binds to haem molecules, and is partly metabolized to sulfate (SO₄) before being eliminated in the urine. As a result, excessive H₂S inhibits the enzyme cytochrome oxidase, which is required for mitochondrial respiration in cells. (Wang,2012; Fu et al., 2012) Therefore, the S supply in feed and water should be controlled to avoid excessive S content, especially if S-rich substances are used in the formulation.

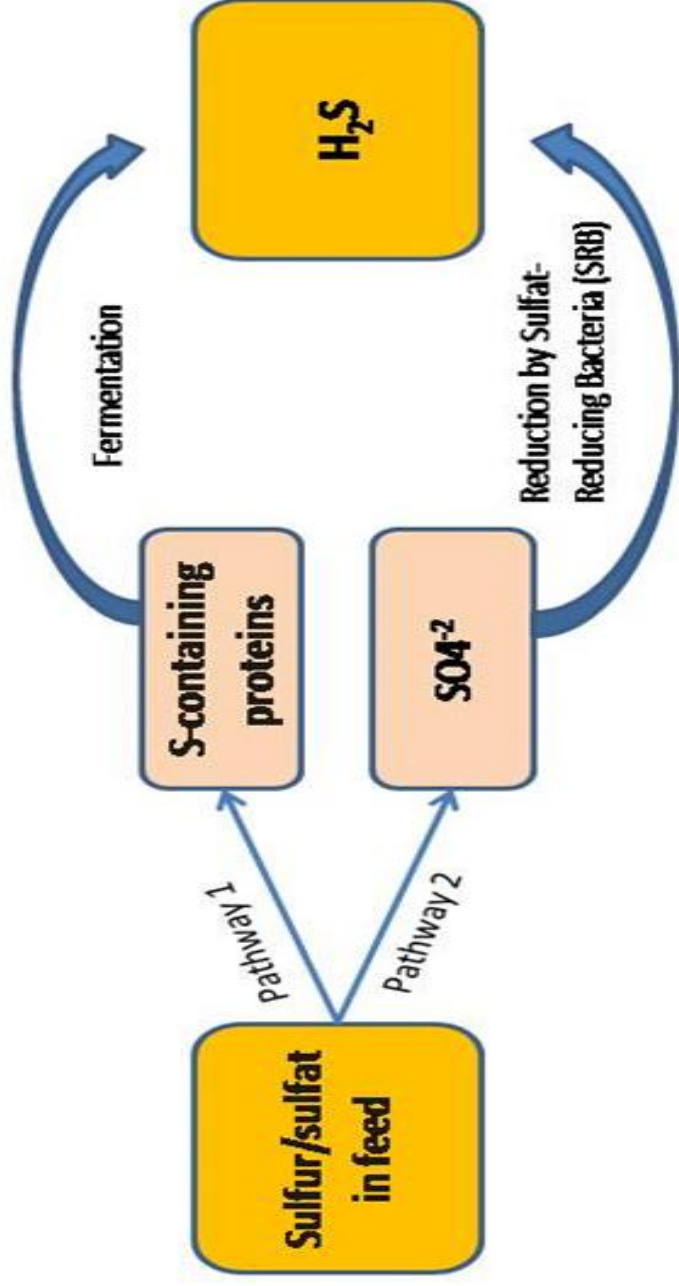


Figure 3. Production of H_2S pathways in the colon or caecum of monogastric

Sulfur Requirement

The type of feed raw material used influences the overall sulfur content of the feed as well as the quantity of S supplementation. For instance, synthetic sulfur amino acids need to be added during the formulation of diets to maintain the optimal growth and development of poultry due to the high use of soybean meal and corn. The high protein content in soybean makes it appropriate for animal diet formulation; nonetheless, one limiting characteristic of soybean protein is an apparent lack of sulfur amino acid. Corn is low in lysine but high in methionine, whereas soybeans have a high lysine content but a low S-containing amino acid content (Krishnan and Jez, 2018). However, S supplementation in the feed also needs to be considered, since acute S toxicity causes neurological changes, including blindness, coma, muscle twitches, and recumbency (Goff, 2018). Excess feeding of S destroys vitamin D, increases the production of feces, loss of membrane permeability, and fluid collection around the breast (Alam and Anjum, 2003). High levels of dietary S have been reported to complex intestinal calcium and lead to increased calcium excretion. Also when dietary canola meal with high levels of S is responsible for some of the leg problems and reduced feed intake. S content in corn meal is 1.1 g/kg whereas soybean meal is 4g/kg (Table 2). Broilers may tolerate dietary S levels up to 0.5 % without affecting performance while laying hens can tolerate even greater amounts (Leeson and Summers, 2005). However, the NRC (1994) states that S toxicity in chicks arises at 14,000 ppm (reduced growth) and in laying hens at 8,100 ppm (reduced egg production).

The previous study by Lim et al. (2018) reported that use the of organic S (0.0, 0.1, 0.2, and 0.4%) in the laying hen diet significantly increased albumen height, haugh unit, and egg production from 47 to 54 weeks of age. Organic S is necessary for the precursor of sulfur amino acid that supports egg production and albumen quality. Considering the dietary sulfur amino acid, Gomez and Angeles (2016) stated that the addition of 30 and 0.50% sulfur amino acid to laying hen from 68 to 83 weeks of age fed sorghum- and soybean meal-based produced the optimal egg production, egg mass, and feed efficiency. Conversely, Imik et al. (2006) demonstrated that the adverse effect of partially replacing corn with sorghum that containing low sulfur amino acid and high tannin with supplementation of 0.05%

Table 2. Nutrient Requirements of Immature Leghorn-Type Chickens as Percentages or Units per Kilogram of

Minerals	White-Egg-Laying Strains				Brown-Egg-Laying Strains			
	0-6 Weeks	6-12 Weeks	12-18 Weeks	18 Weeks to the 1st egg	0-6 Weeks	6-12 Weeks	12-18 Weeks	18 Weeks to the 1st egg
Calcium (%)	0.9 (1.0)	0.8 (0.95)	0.8 (0.92)	2 (2.25)	0.9 (1.00)	0.8 (0.95)	0.8 (0.90)	1.8 (2.25)
Nonphytate phosphorus (%)	0.4 (0.45)	0.35 (0.42)	0.3 (0.40)	0.32 (0.42)	0.4 (0.45)	0.35 (0.42)	0.3 (0.38)	0.35 (0.42)
Potassium (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Sodium (%)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)	0.15 (0.17)
Chlorine (%)	0.15	0.12	0.12	0.15	0.12	0.11	0.11	0.11
Magnesium (mg)	600	500	400	400	570	470	370	370

Note: The data in brackets is based on Leeson and Summers (2005)

Table 3. Macrominerals application for laying hens

No	Mineral Types and dosage	Strain and age	Effects	References
1.	Ca (0.8, 1.3, 1.8, 2.3%) for the pre-laying phase; Ca (2.5, 3.5%) for laying phase	Hisex Brown laying hens (6- weeks-old)	A good performance was obtained with a 0.8% calcium level in the pre-laying phase, whereas in the laying phase, the 3.5% calcium level provided a greater weight egg and better eggshell quality.	Rodrigues et al.(2013)
2.	Monocalcium phosphate (0, 0.05, 0.10, 0.15, 0.20, 0.25,and 0.30%)	Hy-Line Brown laying hens (26 weeks- old)	The supplementation of mono-dicalcium phosphate as a source of inorganic phosphorus had no effect on laying performance and egg quality in Hi-Line laying hens (from 29 to 40 weeks of age) fed with a corn-soybean meal-based diet containing 0.12% non-phytate phosphorus, 3.8% calcium, 2415 IU/kg vitamin and 2000 FTU/kg phytase.	Cheng et al. (2020)
3.	Ca (3.5, 3.8, 4.1, 4.4, and 4.7%)	Hy-Line Brown layers (70- weeks-old)	• The dietary Ca levels did not affect the total feed intake and laying performance in aged laying hens. • The eggshell quality can be improved by ingesting more Ca, up to 4.7% • It indicates that aged Brown layers require a relatively higher level of Ca to reduce cracked eggs and maximize eggshell qualities than required levels, 4.1% of the diet	An et al.(2016)
4.	Nanocalcium carbonate (2.015%, 1.01%, 0.252%,0.126%) Calcium	Laying hens (Bovans) (23 to 33 weeks of	• Replacing calcium carbonate with nano calcium carbonate at levels of 0.126–2.015% had no negative effect on egg production performance and egg quality in laying hens	Ganjigohari et al. (2017)

	carbonate (8.06%, 6.045%, 4.03%)	age)	• Extreme reduction of calcium concentration in diets (to 1.43% Ca in the T6 group) reduced production performance, egg quality characteristics, Tibia thickness and blood calcium of laying hens.	
5.	Di-calcium phosphate (2H ₂ O) (0, 0.05, 106 0.10, 0.15, 0.20, 0.25 and 0.30% inorganic phosphorus)	Hy-Line Brown laying hens (29-week-old)	Dietary supplementation of extra Di-calcium phosphate had no effects on laying performance, egg, quality and tibia quality parameters in Hy-Line Brown laying hens (from 29 to 40 weeks of age) fed with a corn-soybean meal-based commercial diet contained 0.12% non-phytate phosphorus, 3.8% calcium and 2000 FTU/phytase	Ren et al.(2020)
6.	Sodium (0.10, 0.15, 0.20, 0.25, and 0.30%)	Hisex White laying hens (84 weeks of age)	0.15% sodium in diets to postmolt laying hens provided better performance and egg quality, especially in the eggshell. Higher levels of sodium negatively affected the performance and egg quality.	Melo et al. (2020)
7.	1% NaHCO ₃	White Leghorn layers (24 weeks old)	1% NaHCO ₃ showed the best results. In general, results revealed that 1% supplementation of NaHCO ₃ in layers' diet has a beneficial impact in terms of immunity and diet digestibility.	Abbas et al. (2019)

8.	Sodium humate (0.1, 0.3 or 0.5%)	Laying hens, (24 weeks old)	• Sodium humate had no significant effect on egg production or egg and shell quality, but all supplemental levels significantly improved Haugh unit values and egg yolk color. • Supplementation with all levels of sodium humate significantly increased serum immunoglobulin (Ig) levels compared to the hens fed the control diet. • Feeding either 0.1 or 0.5% sodium humate significantly increased serum IgM levels	Zhang et al.(2020)
9.	Sodium format (0.1,0.2, and 0.3%)	Laying hens (53-61 weeks)	0.1 % and 0.2 % sodium format improved egg production, HU, and shell weight (53-61 weeks) during the hot summer period	Youssef et al. (2013)
10.	Sodium butyrate (500 ppm doses)	Bovans laying hens (63 weeks of age)	Sodium butyrate increased the laying percentage, egg mass and conversion index. A decrease in the percentage of broken eggs was also present, as well as in shell fissure and increment of nutrient absorption area in the intestinal villi	Herrera et al. (2009)
11.	Sodium butyrate (300, 600, and 1200 mg/kg)	72 laying hens at the age of 60 weeks	The addition of sodium butyrate did not have a statistically significant effect on the eggshell-breaking strength, eggshell ratio, shell thickness, egg shape index, albumen-yolk index, damaged egg ratio, and egg yolk color criteria	Sevim et al. (2020)

12.	KCl (0, 0.2 and 0.4%) in drinking water	48 Hisex hens (76 weeks old)	• There was a tendency towards lower body temperature and thicker eggshells with 0.4% KCl solution. • Feed intake, egg deformation, yolk color, and HU were not significantly affected by KCl supplementation. • KCl supplementation through drinking water may be a means to maintain egg production and egg quality which usually occurs when the temperature in the layer house increases.	Dai et al. (2009)
13.	Cl inclusion (0.06, 0.10, 0.15, 0.20, and 0.25%)	Jing Brown laying hens at 43 weeks of age	• Better eggshell quality could be obtained when NaCl was partly replaced by Na₂SO₄ in laying hen diets maintaining Cl level at 0.10 or 0.15%. • Better eggshell quality could be obtained when NaCl was partly replaced by Na₂SO₄ in laying hen diets maintaining Cl level at 0.15%.	Wang et al. (2020)
14.	Total electrolyte balance (BET)(1000, 1250, 1500, 1750 and 2000 µeq / kg)	Hy-Line Brown laying hens, of 30 to 46 weeks of age	• The electrolyte balance values showing minimum feed conversion, higher yolk weight, and better uniformity were, respectively, BET = 1400, 1330, and 1250 in µeq / kg of loads at different temperatures. • From the regression equations, the value indicated was BET=1390 for the 30- to 46-week-old laying hens	Silva et al. (2021)
15.	MgO (2, 4, or 6 g/kg) to provide Mg (4.03, 4.87, or 5.71 g/kg)	Bovan white layers at the age of 35 weeks	Supplementation of Mg up to 5.71 g/kg diet improved eggshell quality and laying hen's performance.	Belkameh (2020)
16.	Mg (0, 300 and 600 mg)	Lohmann LSL	Feed conversation ratio and egg mass improved in the birds	Gooya and Torki

	/kg from Mg oxide)	Lite laying hens (30 wk age,	fed either 300 or 600 mg /kg of Mg	(2018)
17.	2.3, 2.6, or 3.0 g/kg Mg in diets by adding 1.0, 1.5, or 2.0 g of MgO to the basal diet	Hy-Line Brown laying hens of 72 weeks of age	Feeding aged laying hens with diets containing increasing concentrations of Mg up to 3.0 g/kg improves eggshell strength, but has no detrimental effects on laying performance.	Kim et al. (2013)
18.	0.1% MgO	Hy-Line W-36 pullets	Supplemental MgO had a positive effect on selected aspects of pullet skeletal development during the early phases of growth and body weight near photostimulation and the onset of egg production.	Lilburn et al. (2019)
19.	Mg (1.56 and 4.0 g/kg)	laying hens at 50 and 52 weeks of age	• The addition of Mg to the mixed feed, together with coarse-grained limestone, decreased yolk percentage, increased eggshell percentage, increased eggshell thickness, and eggshell breaking strength. • Higher dietary Mg, together with a wider Ca : non-phytate phosphorus ratio (12.8 and 18) increased egg production and egg weight, but it did not influence eggshell-breaking strength	Skrivan et al. (2016)
20.	Organic sulfur (0.0, 0.1, 0.2, and 0.4%)	Lohmann brown laying hens at 31 weeks of age	• Egg production tended to increase with 0.4% organic sulfur in diet after 39 weeks of age and, there was a significant effect from 47 to 54 weeks of age. • Egg quality traits of albumen height and haugh unit increased significantly	Lim et al (2018)

CONCLUSION

Macrominerals studies in modern laying hens are gaining attention. Numerous approaches were conducted not only supporting adequate mineral, optimum performances and egg quality but also considering laying hens' health, animal welfare, and environmental protection. Therefore, the macromineral strategies that can be implemented are: 1) providing adequate and balanced minerals to maximize the genetic potential of laying hens. Since both deficiency and excess of minerals in the poultry diets cause problems, mainly affecting the performance and egg quality, 2) applying nanotechnology or organic form (metal ion + amino acid ligand, chelated amino acids, proteinases). The application of nanotechnology can increase the bioavailability of minerals by enhancing the mineral's surface area. Meanwhile, the organic mineral form can prevent the minerals from forming indigestible complexes with certain dietary components, as well as mineral antagonisms in the intestine, which can reduce mineral absorption rates, 3) using enzymes such as phytase to improve mineral bioavailabilities. The beneficial effects of phytase have been related to the increased availability of P which may also promote the use of energy and other nutrients in diets such as amino acids and other minerals.

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CHAPTER 3

THE STRATEGIC APPLICATION OF VITAMIN TO INCREASE LAYING HENS' PERFORMANCE AND EGG QUALITY: A REVIEW

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INTRODUCTION

Vitamins are organic substances that are not a group of carbohydrates, proteins, or lipids, and vitamins are also required in very small amounts (mg or μg) and they perform several functions in a reduction in the risk of disease, maintenance of good health and are essential for growth and metabolism (Akram et al. 2020). In the birds, not all vitamins can be synthesized in sufficient quantities to meet their requirements in all physiological phases and certain condition such as heat stress condition, therefore dietary supplementation is required due to inadequate vitamins can produce numerous health problems and lead to dysfunction of the immune system, which can result in increased rates of infection or inflammation and ultimately decreased growth and death in some cases. The vitamins are of two types, one is fat-soluble (vitamin A, D, E, and K) and the other is water-soluble (vitamin B complex group). Fat-soluble vitamins (A, D, E, and K) are digested and absorbed in the same way as fats. In the body, they are stored in fatty tissues, mainly the liver and adipose tissue, which acts as a reserve for the animal, but in excess is excreted in bile through the feces, but it can reach toxic levels especially vitamins A and D (Reddy and Jialal, 2020).

For laying hens, vitamins have a positive effect on the growth, development of reproductive organs, egg production and egg quality. Vitamin A and D can impact the gut microbiota by protecting intestinal barrier integrity and immune status, thus guarding the host from certain diseases (Riccio and Rossano, 2018). Vitamin D also plays a role in Ca and P absorption, regulation of parathyroid hormone, bone mineralization, and for the laying hen, Ca one of the key nutrients required for optimal eggshell quality in laying hens (Hester, 2017). Vitamin E is necessary for the cellular defense system that plays an important role in scavenging free radicals, preventing lipids peroxidation, and protecting animals from the adverse effects caused by oxidative stress (Attia et al., 2018) and it is also essential to maintain fertility and hatchability in parent stocks (Shojadoost et al., 2021). Meanwhile, vitamin K has a vital role in the secretion of male sex hormones such as testosterone and calcium metabolism through activating the calcium-binding proteins that assistance to build and maintain calcium for bone as well as an eggshell (Katarzyna, 2015; Elbossat, 2018).

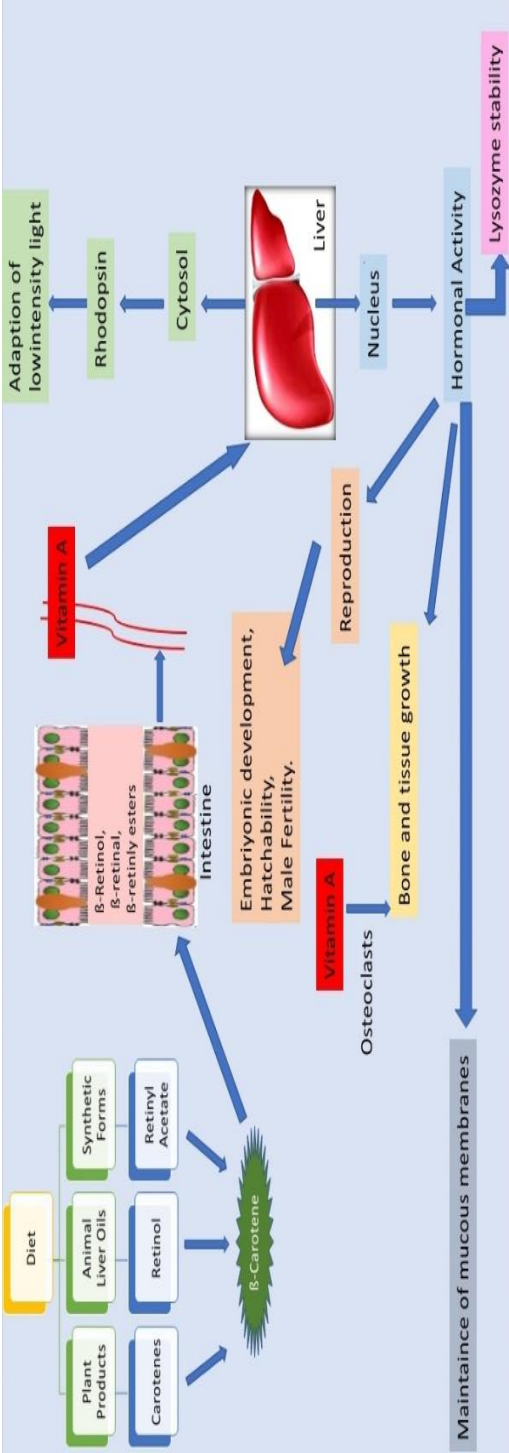
Choline, folic acid and inositol are considered a member of the B complex vitamin group. In laying hen diets, choline is needed for the formation of phosphatidylcholine that is the most abundant phospholipid in the body which is not only a major component of cellular membranes and but also needed for cell division and growth (Arias et al., 2020). Also, folic acid is important for methionine metabolism and plays a critical role in breeder performance and folic acid deficient embryos preserve methionine, rather than catabolize it to cysteine (Lu et al., 2021). Meanwhile, myo-inositol is needed to be involved in energy and lipid metabolism, bone and muscle formation, reproduction, general metabolic performance and glucose metabolism (Gonzalez-Uarquin et al., 2021). PABA is a cofactor and precursor in the synthesis of folic acid, purines and thymine in most species of bacteria, algae and higher plants (Krátký et al., 2019).

This review is divided into sections on the different vitamins with a description of its role in laying hens. Then, there is a discussion of various studies applying different vitamin levels effect on production parameters, egg quality, the immune system of the laying hen.

1. VITAMIN A

Vitamin A has importance in metabolic processes. Under conditions of adequate dietary vitamin A, the liver is the major site of vitamin A storage, with over 95% of the total neutral retinoid being present as retinyl esters, predominately retinyl palmitate, and stearate (Harrison, 2019). Deficiency of vitamin A is a decrease in sexual activity in males and failure of spermatogenesis, accompanied by a reduction in fertility and the number of hatched eggs. Other symptoms which can be found in deficient chicks are ataxia, xerophthalmia, and chronic purulent conjunctivitis (Barroeta et al., 2012). Figure 1 describes that vitamin A is a group of retinoid compounds that have properties similar to those of retinol, such as retinal, retinyl esters, and retinoic acid, and these are found naturally only in animals tissues. Meanwhile, the plant's tissues contain carotenoids that are classified into carotenes and xanthophylls. The most important of pro-vitamin A is β -carotene, β -cryptoxanthin, and α -carotene. Among these, β -carotene is had the greatest biological potency to be converted to retinol (García-de Blas et al., 2015; Lima and Souza, 2018). In the intestinal epithelium of the birds,

carotenes are converted into vitamin A. Vitamin A binds to proteins in the cytosol and converts dietary vitamin A (retinol) to 11-cis retinaldehyde, which serves for the visual pigment rhodopsin that is important for sight, especially in poultry adapting to low-intensity light in intensive production. In the nucleus, vitamin A binds to completely different retinoic acid-binding proteins (RBP). This new complex binds specific regions of chromatin and causes changes in the rate of transcription of specific genes. Vitamin A has important effects on bone growth and thus the development of young animals, the quantity and quality of semen produced, the growth and differentiation of epithelial tissues of the reproductive system, and the embryo, among others. Finally, vitamin A contributes to maintaining lysozyme stability inside the cells (Barroeta et al., 2012)



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Figure 1. The absorption of Vitamin A and the purpose of the metabolites and main functions

The recent studies on supplementation of vitamin A in laying hens diets under heat stress condition at the level of 0, 8000, and 16,000 IU/kg was conducted by El-Hack et al. (2016). They reported that dietary vitamin A up to 16,000 IU/kg diet significantly improved all productive traits without affected egg quality. This indicates that the supplementation of vitamin A might be advantageous for the laying performance of stressed hens. Vitamin A is a very effective antioxidant and plays a crucial role in membrane integrity restoration and normal development of reproductive organs in laying hens under heat stress (Kaya and Yildirim, 2011) that can slow down the lipid peroxidation process by breaking the chain reactions involved in this process and can check the membrane deterioration process induced by heat stress the birds increase the excretion of vitamin and mineral from tissues and thus may lead to increase its requirements. For this reason, the availability and activity of vitamins A related to the metabolism and antioxidant defense are of great importance. Elsherif (2017) founded that dietary vitamin A before and during early egg production of laying hen had a beneficial effect on egg production and egg mass laying hens. Egg production at peak period was higher with supplementation of vitamin A at levels from 30,000 to 50,000 IU than either 10,000 or 20,000 IU Vitamin A. The other studies on supplementation of vitamin A levels in broiler breeder at 46 to 56 weeks of age was conducted by Chen et al. (2015), whereas they compared different levels of supplementation vitamin A up to 21,600 IU/kg and focused on the effects on egg production parameters and reproductive performance. The results proved that the level of 5,400 IU/kg was improved egg production and egg mass. This indicates that supplementation of vitamin A can prevent a drop in egg production. This reason is supported by Yuan et al. (2014) that supplementation of vitamin A can increase ovarian stromal and white follicle weights along with oviduct weight and length that indicates the involvement of vitamin A in the function of chicken reproductive organs. Another result reported by Yuan et al. (2014), supplementation in a high level of vitamin A (45,000 IU/kg) decreased the laying performance and excessive vitamin A also increased the concentrations in the yolk and liver. Therefore, the authors indicated a linear relationship between dietary dosages used and vitamin A levels in the liver. This effect may be related

to vitamin absorption in the intestine and metabolism by the liver. In the body, vitamins are stored mainly in the liver and adipose tissue which acts as a reserve for the animal, but in excess is excreted in bile through the feces. However, it can reach toxic levels especially in the case of vitamins A and D (Reddy and Jialal, 2020). Therefore, the decrease of laying performance due to excessive vitamin A might be attributed to the affected liver function (hepatotoxic effect) (Yuan et al., 2014).

Vitamin A also improves the immunity in laying hens. According to Yuan et al. (2014) supplementation of vitamin A from 5,000 IU/kg to 20,000 IU/kg appears to increase the Newcastle disease virus (NDV) antibody titer, but supplementation from 20,000 to 35,000 IU/kg decreased the NDV antibody titer. Supplemental levels of vitamin A above the tolerable dose could impair T-cell immunity. These findings suggest that excessive vitamin A was detrimental to humoral immunity and a comprehensive experiment with different doses is needed to determine optimum dietary vitamin doses. The supplementation of vitamin A in the laying diet can strengthen the immune system against parasites, viruses, and bacteria as it exerts a protective effect on mucous membranes and adrenal glands. Besides, retinoic acid increased the response of T-lymphocytes (El-Hack et al., 2016; Lima and Souza, 2018).

Vitamin A and Retinoic acid as active metabolites of vitamin A have been shown to produce anti-inflammatory effects in different species through different mechanisms. Vitamin A induces dendritic cells to exhibit anti-inflammatory effects (Klebanoff et al., 2013). Dendritic cells are the immune system's guardians that sense, process, and present antigens to T lymphocytes (Sesti-Costa et al., 2020). Also, vitamin A binds to its nuclear receptor (Retinoid X receptor) which modulates a specific immune system. Thus, Vitamin A activates the innate immune cells (macrophages and dendritic cells). In mucosal sites such as the lungs and gut, treatment leads to an increase in mucin and secretory IgA antibody production. The activity of this vitamin is highly dose-dependent, as low and high doses induce inflammatory and anti-inflammatory responses respectively (Shojadoost et al., 2021).

2. VITAMIN D

Vitamin D is a group of ester compounds whose main function is to regulate the functions of genes essential for calcium absorption and phosphorus metabolism, bone mineralization and egg formation. Ergocalciferol (D2) is a compound of plant origin and is synthesized from ergosterol (plant steroid), whereas cholecalciferol (D3) is synthesized from precursor 7-dehydroxycholesterol, which is present exclusively in animal tissues. The synthesis of vitamin D3 from its precursor occurs in the skin under the influence of UV irradiation. Hens reared in the closed house must have a supplementary of vitamin D due to insufficient endogenous synthesis (Swiatkiewicz et al., 2017). Cholecalciferol is stored in the liver and adipose tissues in an inactive form and it is activated by hydroxylation into 25-hydroxycholecalciferol. Thus, this metabolite is carried to the kidneys and converted to 1.25-hydroxycholecalciferol (calcitriol) as an active form in animals. Calcitriol regulates the absorption, transport, deposition, and mobilization of calcium together with parathyroid hormone. Calcitriol is transported to the intestine, to the bones, or to another part of the kidney where it participates in the metabolism of calcium and phosphorus. Increasing concentrations of vitamin D3 result in an increased concentration of ionized and total calcium and decreased concentration of sodium and phosphorus. In laying hens, the calcium for eggshell is from the ration and the mobilization of the calcium reserves stored in the medullary bone (Figure 2) (Barroeta et al., 2012)

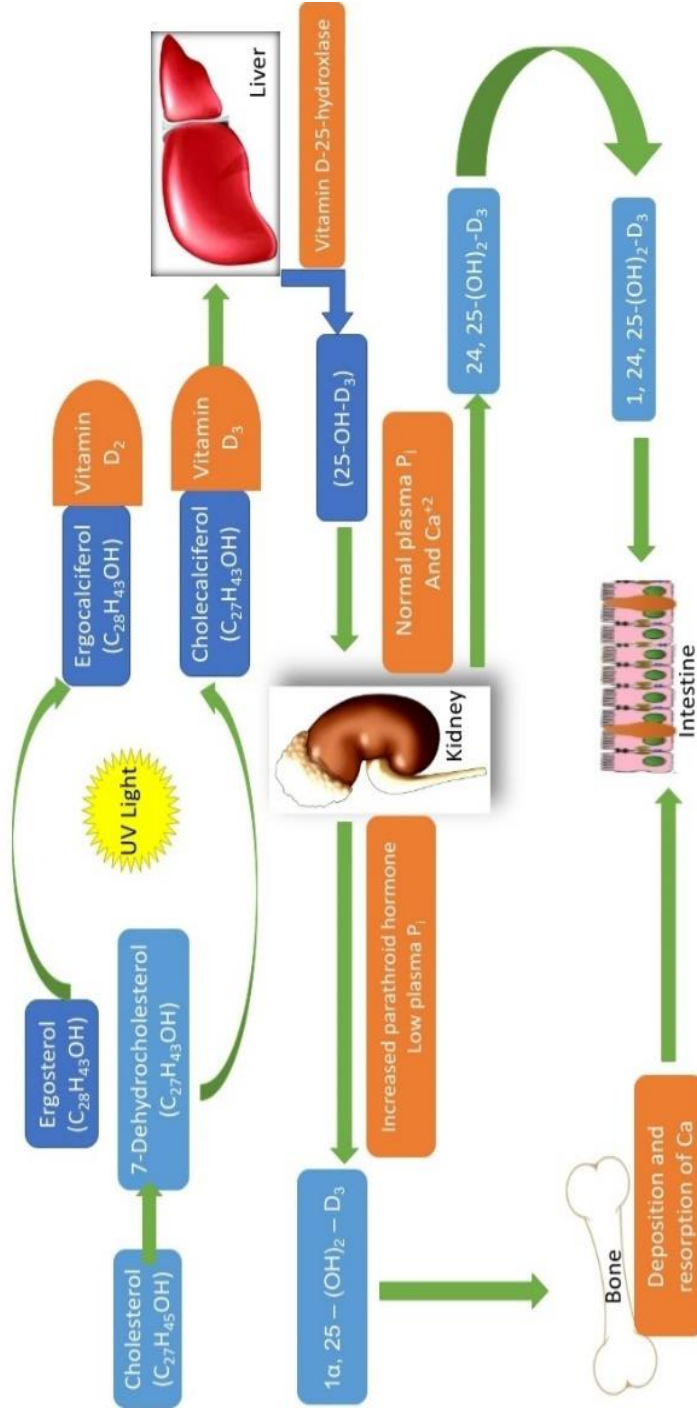


Figure 2 The absorption of Vitamin D and the purpose of the metabolites and main functions.

Vitamin D also plays a role in Ca and P absorption, regulation of parathyroid hormone, bone mineralization, and for the laying hen, Ca one of the key nutrients required for optimal eggshell quality in laying hens (Hester,2017). Numerous of study proved that supplementation of different forms and levels of vitamin D give a positive effect on a pullet, aged layer, and laying hen under high stocking density. Vitamin D3 is converted in the liver to become its primary circulating form of 25-hydroxycholecalciferol (25OHD) and dietary 69 µg/kg of 25OHD can increase in eggshell strengthen and lightness in high stocking density of laying hens compared to regular vitamin D supplementation (Wang et al., 2020). Supplementation of vitamin D2 in combination with vitamin D3 could result in an increase in Ca and P utilization, rate of laying, and egg mass in the late laying hen cycle (Adhikari et al.,2020; Atta et al., 2020). Browning and Cowieson (2015) found that a high dietary level of cholecalciferol (5,000 IU/kg) can increase egg weight, without giving a positive effect on laying performance, eggshell quality, and tibia mineralization. Moreover, Plaimast et al. (2015) stated that there was an improvement of eggshell quality in aged layers fed diets containing 6,000 IU/kg. Also, the dietary vitamin D3 at 2,760 IU/kg with 25OHD at 2,760 IU(69µg/kg) increased the growth rate and bone size of pullets and also increased bone mineral deposition and improvement of structural during the early laying period at 18 to 60 weeks of age (Chen et al.,2020) which early bone development is ver important before sexual maturity, and its prolonged effects on bone health during laying periods (Casey- Trott et al., 2017).

Unfortunately, dietary 69 µg/kg of 25OHD did not affect egg-laying rate and egg weight (Wang et al 2020). Adhikari et al (2020), the addition of different isoforms of vitamin D (vitamin D2, vitamin D3, or 25-OHD) did not improve egg quality parameters. Akbari Moghaddam Kakhki et al. (2018) also reported that additional 25-OHD supplementation (69 or 138µg/kg) on the basal diet containing 3,300 IU of vitamin D3/kg did not show any egg production improvement of Lohmann at 72 to81 weeks old. Similarly, the Nascimento et al. (2014) study did not show any positive effect of cholecalciferol substitution by 25-OH-D3 in the diet for older laying hens on egg production, eggshell quality, or bone breaking strength. The reason behind no effect of different forms and levels of vitamin D on egg quality

parameters may be due to the inclusion of adequate levels of Ca, available P, and vitamin D3 in the diet. It has been indicated that only when vitamin D3 was deficient in the diet, the exogenous addition of vitamin D3 or its analog can exert its improving effect on the performance of laying hens (Wang et al., 2020; Geng et al., 2018)

In birds, the role of vitamin D3 in calcium and phosphorus metabolism is crucial for its well-documented involvement in bone development and eggshell formation in laying hens. Also, some studies reported that vitamin D plays a role in the immunomodulatory, anti-inflammatory, and anti-coccidia roles in chickens. A previous study reported by Geng *et al.* (2018) that vitamin D₃ supplementation at 500, 1500, and 3000 IU/ kg decreased IgG and IgM in the vitamin D₃-sufficient hens compared with the non supplemented hens under *Escherichia coli* lipopolysaccharide (LPS) challenge. Thus, they found that vitamin D3 supplementation could be beneficial to protect layer hens in preventing immunological stress chickens by induced splenic immunological stress through suppressing the transcription of NF- κ B genes. Likewise, Shojadoost et al. (2015) observed that treatment of LPS stimulated chicken macrophages with 1,25(OH)₂D₃ can downregulation of IL-1 β expression. Morris et al. (2015) found that a high dietary level of 25-OH-D₃ (100 μ g/kg) had a positive influence on immune response in layer chickens after the *coccidia* challenge. The highest dose of vitamin D (2,760 IU/kg) of vitamin D supplementation of the low Ca and P diet resulted in higher Th1 cytokines in the spleen and bursa of Fabricius (Rodriguez-Lecompte et al., 2016).

According to Shojadoost et al. (2021), vitamin D3 binds to its nuclear receptor (Vitamin D Receptor) where it has a broad modulatory activity on immune system cells. Treatment with vitamin D3 increases the host cell's ability to express Toll-like receptor -2 (TLR-2) and Toll-like receptor -4 (TLR-4) leading to an increase in antimicrobial peptide expression (cathelicidins). Furthermore, it exerts anti-inflammatory effects by downregulating macrophage and dendritic cell activity (decrease interleukin -8 (IL-8) and interleukin (IL)-1 β expression) as well as T cell activation (increased interleukin-10 (IL-10) and decrease in IFN- γ , interleukin-2 (IL-2), and interleukin-17 (IL-17) expression) and an increase in Treg cells

(regulatory activity).

3. VITAMIN E

Vitamin E activity is fat-soluble compounds consisting of α , β , γ , and δ -forms of tocopherols and tocotrienols. α -tocopherol is most abundant in animal tissues that have the highest activity (Yang et al., 2020). Vitamin E is necessary for the cellular defense system in the face of oxidation. As a natural antioxidant, it plays an important role in scavenging free radicals, preventing lipids peroxidation, and protecting animals from the adverse effects caused by oxidative stress. Tocopherols remove the peroxy radical by donating a hydrogen atom and converting it to peroxide (Attia et al., 2018). In poultry, vitamin E also has a crucial role in the prevention of nutritional encephalopathy and myopathies in chickens and turkeys (Klasing and Korver, 2020). Also, it is essential to maintain fertility and hatchability in parent stocks (Shojadoost et al., 2021). Most vitamin E deficiency effects are caused by oxidation of polyunsaturated fatty acids (PUFA), which causes cell membrane disorders, therefore, vitamin E supplementation may affect the response of dietary PUFA (Raederstorff et al., 2015). Moreover, keeping a balance with other antioxidant nutrients including vitamin C, β -carotene, and selenium is important to act in protecting against free radicals. Furthermore, it has been observed that vitamin E promotes the activity of the immune and reproductive systems such as egg production, egg fertility, hatchability, and sperm motility (Fouad et al., 2020). Figure 3 describes that vitamin E treatment leads to the proliferation of T cells and it decreases the cytokines such as IL-4, IL-10, and TGF- β . Vitamin E also leads to B cell activation and subsequent increases in IgM, IgY, and IgA antibody secretion to help induce immunity against infections.

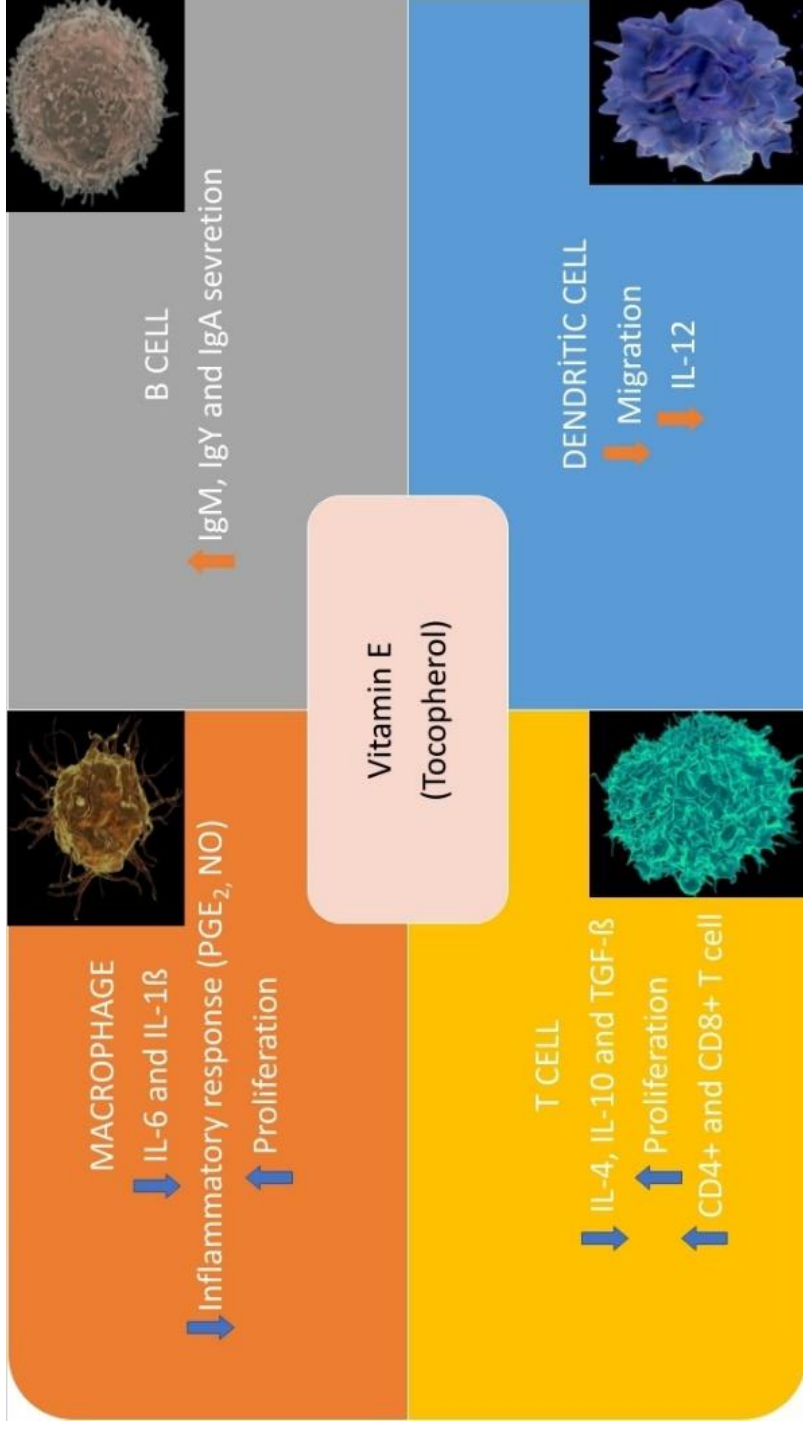


Figure 3. Mechanism of vitamin E on the immune system

Numerous studies have been aimed at establishing the relationship between vitamin E consumed and the prevention of oxidation. In a recent study, the addition of 30 IU/kg vitamin E to the layer hen diet improved serum superoxide dismutase and glutathione peroxidase as antioxidant enzymes (Liu et al., 2019). Ding et al. (2021) also reported that the addition of 100 IU/kg vitamin E prevented aged corn-induced lipid peroxidation in laying hens, possibly via a direct increase in antioxidant enzyme activities and enhancing the relative expressing of antioxidant genes (SOD1mRNA) in the ovaries. Supplementation of 250 mg/kg vitamin E in single or combination with selenium decreased malondialdehyde (MDA) concentration and increased the enzyme activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) in liver, heart, breast and thigh muscle of laying hens, whereas these enzymes are antioxidant enzyme and MDA is one of the final products of polyunsaturated fatty acid peroxidation (Çelebi, 2019). The protection of the liver and other organs from oxidative damage can support a good metabolism and nutrients utilization as well as hen's performances, where egg yield and feed efficiency increased by dietary 500 mg vitamin E/kg (El-Hack et al., 2017). The addition of vitamin E 60 IU/kg feed increased egg production yolk weight, albumen weight and vitelline membrane strength (Parolini et al., 2015) and similar result founded by Karadas et al. (2017) that the supplementation of vitamin E at 125 to 300mg/kg improved egg production and eggshell density. Further, the improvement in egg production is due to the beneficial effects of vitamin E by facilitating the release of vitellogenin from the liver and by increasing its concentration in the blood (El-Hack et al., 2017). Additionally, vitamin E improves laying hens performance by reducing the negative effects of corticosterone hormone induced by heat (Li et al., 2020) Corticosterone is a common hormone that is released in many stress situations by the hypothalamus-pituitary-adrenal axis that was potentially harmful to the health of animals (Bodegom et al., 2017). Indeed, vitamin E protects male poultry reproductive systems against oxidative damage (Fouad et al., 2020). Dietary 200 mg of Vit E/kg diet increased semen quality and fertility rate (Zanussi et al., 2019). Vitamin E also reduced lipid peroxidation

of the sperm membrane and enhanced the functions of sperm mitochondria (Asl et al., 2018).

4. VITAMIN K

Vitamin K has important physiological functions which relate to blood coagulation, bone turnover and strength, inhibition of arterial calcification, and anti-inflammatory (O'Sullivan et al., 2020). Vitamin K derived from plants is phylloquinone (K_1), vitamin K obtained from bacterial fermentation is menaquinone (K_2) and vitamin K_3 or menadione is produced through chemical synthesis and is the form normally used for feeding animals (Walther and Chollet, 2017). Vitamin K is absorbed from the small intestine and transported via chylomicrons in the circulation by triacylglycerol-rich lipoproteins. Thus, it is used in the activation of calcium-binding proteins after that vitamin K transport through the bloodstream into the liver. Vitamin K plays important role in the activation of calcium-bound protein which responsible for transfer calcium into bones. Also, it increases the secretion of male sex hormones such as testosterone (Elbossat, 2018).

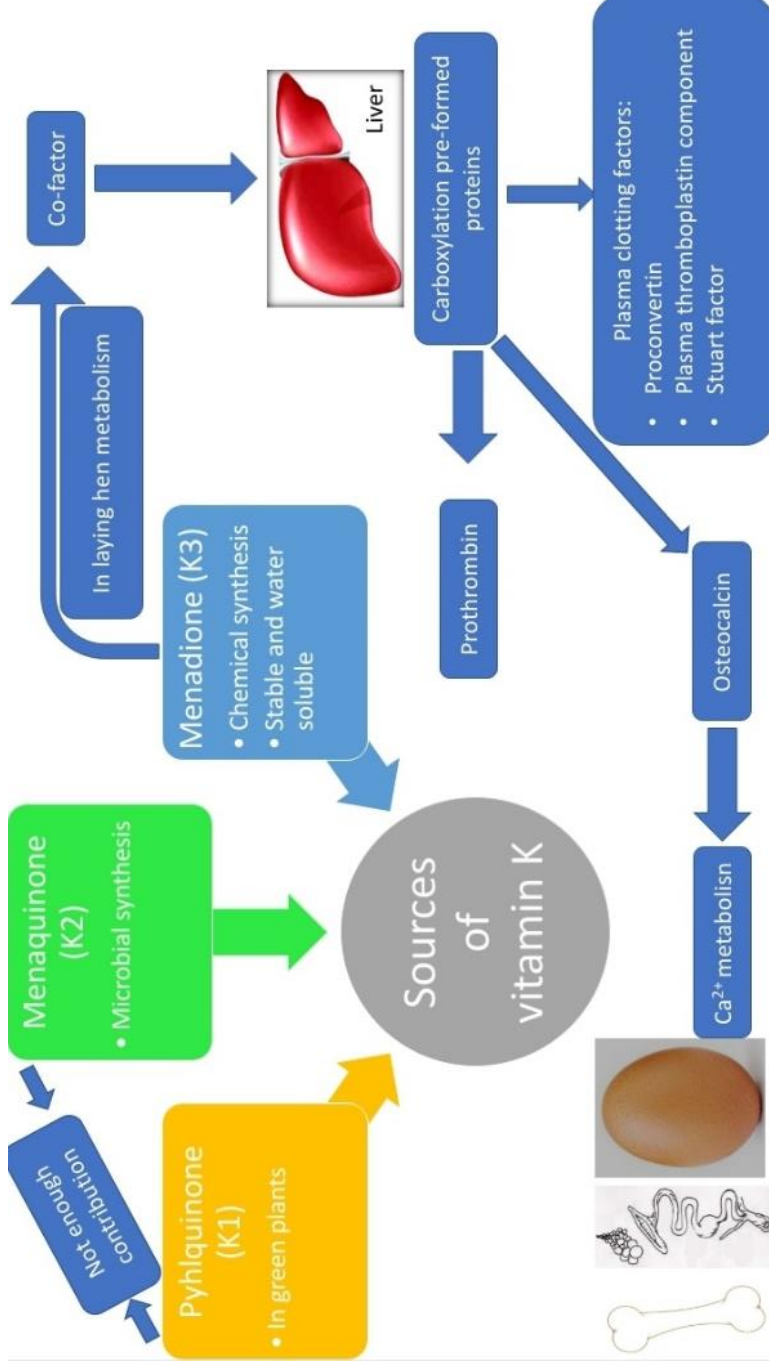


Figure 4. Metabolism of vitamin K

In laying hens, Fares et al. (2018) reported that the supplementation of up to 19 mg/kg vitamin K₃ increased egg production, egg mass, egg weight, feed efficiency, eggshell weight percentage and shell thickness, but it significantly decreased embryonic mortalities during 15-21 days of incubation. They stated that the significant improvement of feed efficiency with supplementation of vitamin K₃ could be related to the role of vitamin K on digestive enzymes. Meanwhile, vitamin K also plays a vital role in calcium metabolism through activating the calcium-binding proteins such as Matrix gla protein and osteocalcin (Katarzyna, 2015) which assistance to build and maintain calcium for bone as well as an eggshell. The other result reported by Souza et al. (2017) that supplementation of 17.86 mg/kg vitamin K₃ and 1.4% Ca in the pullets phase presented greater levels of total serum Ca and it indicates that there is an increase in Ca binding proteins, which can lead to greater Ca absorption with a consequent increase in serum levels. They explained that vitamin K acts as an enzymatic cofactor of a post-transduction reaction by participating in the carboxylation of osteocalcin, which consequently indirectly increases osteoblastic activity and gives benefits to bone formation. These results suggest that these hens had better bone reserves for labile calcium and the formation of eggshells. When the supplementation levels in the breeder rations are inadequate, eggs are produced with low vitamin K content, accompanied by high levels of embryonic mortality due to hemorrhagic processes during the final period of incubation.

5. CHOLINE

Choline is considered a member of the B complex vitamin group and in laying hen diets, it is needed for the formation of phosphatidylcholine. Both choline and phosphatidylcholine are required for very-low-density lipoprotein secretion and lipid transport from the liver to blood and peripheral tissues (Aziza et al., 2019). Moreover, Phosphatidylcholine is the most abundant phospholipid in the body, which is not only a major component of cellular membranes and needed for cell division and growth (Arias et al., 2020).

Choline metabolism can be divided into four main pathways, which are involved in the synthesis of acetylcholine, betaine, phospholipids, and trimethylamine. Choline is catalyzed by choline acyltransferase into

acetylcholine, which is key in cholinergic neurotransmission and it can be oxidized to obtain betaine a methyl donor implicated in the epigenetic regulation of DNA, and a requirement in the synthesis of phosphatidylcholine. Choline is taken from the diet and gut microbiota trimethylamine lyases transform it into trimethylamine. Trimethylamine is absorbed by the intestine and delivered to the liver, where trimethylamine is metabolized into trimethylamine N-oxide by host hepatic monooxygenases and is distributed to organs. (Wiedeman et al., 2018; Arias et al., 2020)

In poultry, choline can be synthesized in the liver from serine and methyl groups that have three essential metabolic roles including a constituent of phospholipids, preventing fatty liver and a precursor for acetylcholine synthesis (Igwe et al., 2015). Laying hens fed with a diet containing 0–2500 mg choline/kg for 6 weeks (Janist et al., 2019) and 1,000 mg/kg choline for 4 weeks (Wang et al., 2019) did not show any effects on production performance and egg quality. Also, feeding laying hens diets with microalgae and 2,460 mg/kg choline for 16 weeks led to a significant increase in egg production performance and egg quality (Yonke and Cherian, 2019). This difference may be caused by the difference in the dosage of supplemental choline or the experimental period. A recent study showed that choline supplementation enhanced antioxidant status while reducing lipid peroxidation in hens fed flaxseed rich in omega-3 polyunsaturated fatty acids (Aziza et al., 2019). The addition of 3400 mg/kg choline increases significantly the total lipid in egg yolk and leads to a reduction in liver total lipids. This condition can be explained that the lipids in the form of very-low-density lipoprotein, triglycerides, or cholesteryl esters and phospholipids are chiefly exported from the liver to tissues including egg yolk due to the elevation of phosphatidylcholine concentration (Dong et al., 2019).

Additionally, dietary choline supplementation elevated the GSH-Px activity and T-AOC and tended to reduce MDA levels in liver in laying hens (Yonke and Cherian, 2019). Choline is the major source of methyl groups in the diet that participates in the methylation of homocysteine to form methionine. Thus it can improve the methionine biosynthesis which is an essential amino acid that plays a key role in protein synthesis and synthesis of adenosylmethionine and GSH (Dong et al., 2019).

6. FOLIC ACID

Folic acid is part of the vitamin B complex that has essential in the transfer of monocarbonate units in metabolic processes and affects the synthesis of purines and pyrimidines. It is also involved in the metabolism of serine, glycine, histidine, methionine, choline and thiamine (Barroeta et al., 2012). Interaction of folic acid with pyridoxine is important for methionine metabolism and plays a critical role in breeder performance and folic acid deficient embryos preserve methionine, rather than catabolize it to cysteine (Lu et al., 2021).

In laying hens, the study by Janist *et al.* (2019) found that supplementation up to 8 mg/kg folic acid did not result in any beneficial effects on production performance and egg quality. However, egg number and shell thickness also increased significantly by dietary 20 mg/kg folic acid in the summer season (Nofal et al., 2018). Another study reported that there was a different response of dietary folic acid in young and older laying hen where dietary 4 mg/kg folic acid increased production performance, egg weight, and egg mass in the young laying hen but no significant differences were found in any performance measurements in the older laying hen. This difference may be associated with the inference that the capacity of enhancing folate status via dietary folic acid supplementation was relatively high in the young laying hens, especially the increased egg weight or egg mass is likely related to protein metabolism (Jing et al., 2014).

Increased egg production with folic acid supplementation may be related to its role in methionine metabolism (Figure 4). According to Thaler (2014), folic acid is a synthetic compound with a monoglutamate residue at the end of the carboxyl group and it is the most stable form of folate. The importance of folate is because of its role as a precursor to 5-methyltetrahydrofolate, which acts as a methyl group donor for the re-methylation of homocysteine to methionine. Folic acid and folates are precursors to 5 methyltetrahydrofolate, which

in turn acts as a methyl group donor for the re-methylation of homocysteine to methionine. Folate deficiency results in lower concentrations of S-adenosyl methionine, an important methyl group donor for epigenetic processes (gene methylation) and basic processes of cell metabolism including DNA synthesis and protein synthesis.

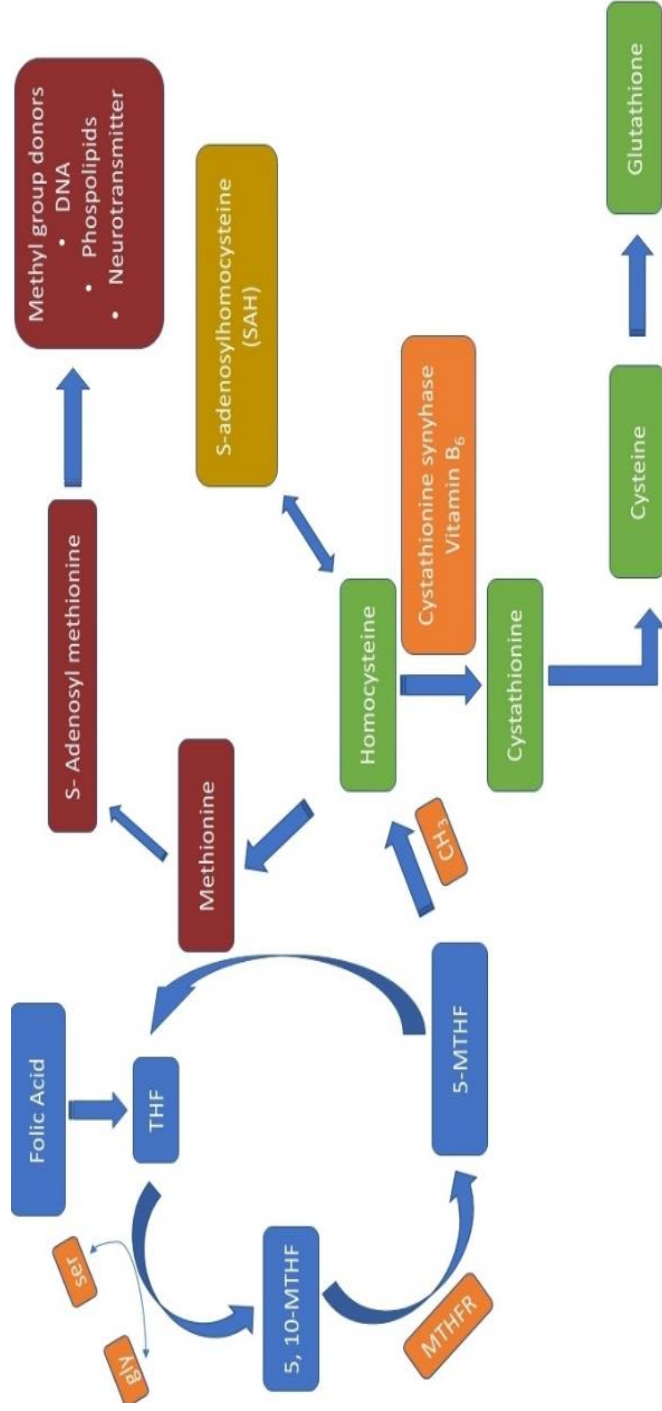


Figure 5. Mechanism of folic acid as a methyl group donor for the re-methylation of homocysteine to methionine

7. INOSITOL

Inositol is a cyclical isomer of glucose, which is involved in many physiological processes, such as insulin sensitivity (inositol pyrophosphate), lipid and cell metabolism (various inositol forms), transduction of brain signals (inositol triphosphate), and modulation of circadian rhythms (Wei et al., 2016; Herwig et al., 2019). Inositol has 9 possible stereoisomeric forms including myo-inositol, scyllo-inositol, mucoinositol, D-chiro-inositol, L-chiro-inositol, neo-inositol, allo-inositol, epi-inositol, and cis-inositol. Among these forms, Myo-inositol is the predominant form occurring in nature (Gonzalez-Uarquin et al., 2020) and it can be found in a variety of tissues, such as the kidney, brain, pancreas, heart, skeletal muscle, lung, and digestive tract. In addition to endogenous synthesis, inositol can be derived from dietary sources, such as the action of exogenous phytase on dietary phytate (Herwig et al., 2019). Myo-inositol is synthesized endogenously from D-glucose or inositol phosphates dephosphorylation by the action of inositol monophosphatase 1. Furthermore, Myo-inositol appeared to be involved in brain osmolarity regulation, energy and lipid metabolism, bone and muscle formation, reproduction, and general metabolic performance. Myo-inositol also modulates insulin secretion from pancreatic beta-cells, thereby influencing glucose metabolism (Gonzalez-Uarquin et al., 2021).

In the laying hens, myo-inositol concentration jejunum and ileum was lower when fed by high calcium in the diet, where calcium might be had a diminishing effect on endogenous phosphatase promoting the degradation of phytic acid to myo-inositol (Sommerfeld et al., 2020). Sahin et al.(2018) founded that supplementation of arginine-silicate-inositol complex up to 1000mg/kg for 90 days increased egg production, feed efficiency, and eggshell quality. The increase of performance might be related to the improvement myo-inositol, calcium and vitamin D concentrations and alkaline phosphatase levels in serum and ileum that indicators of bone formation and calcium mobilization in eggshell (Sahin et al., 2018; Herwig et al., 2021)

The addition of phytase in the diet as the hydrolyzing phytate increases the release of both inositol and nutrients for absorption in the chicken digestive tract. For example, Taylor et al. (2018) stated that the addition of

1500 FTU/kg phytase to layer diets increased myo-inositol in the proventriculus, gizzard and the ileum with improvement of performance, such as FCR. The increase of inositol concentrations implies that the phytase releases the inositol from dietary phytate in the gut where it is absorbed and enters circulation and it is expected to result in increased uptake into various tissues. Also, inositol can induce glucose as the energy supply for bird production (Lee and Bedford. 2016) including growth and egg production. Besides that, myo-inositol is essential for an optimal ovary performance being involved in gonadotropin pathways promoting ovulation, thus, the myo-inositol needs of the ovary increased strongly at the start of egg-laying or before week 24 (Lagana et al.,2018; Gonzalez-Uarquin et al., 2021)

On the contrary, reported by Zyla et al. (2012) that the supplemental 0.1% of myo-inositol in the corn-soybean meal-based diets has lower performance compared to the wheat soybean-based diet. The supplemental 0.1% myo-inositol in the corn-soybean diets suppressed feed intakes, reduced egg production, had no effect on eggshell quality and reduced the deposition of eicosanoid fatty acids in yolks. They stated that this phenomenon may be explained by the possible involvement of different inositols in the hormonal control of hen reproductive physiology and the hens fed the wheat soybean meal diet, high concentrations of different inositol phosphates must have been released by the wheat endogenous phytase in the intestine to accompany the supplemental myo-inositol. Furthermore, according to Gonzalez-Uarquin et al (2020), the myo-inositol efficiency depended on animal-related factors (species, age of animals, genetic background and microbial phosphatases), dietary-related factors (myo-inositol content, type of substrates, intrinsic phytases or phosphatases, total Ca, P levels, and Ca:P ratio), and myo-inositol related factors (doses, and source).

8. PARA-AMINOBENZOIC ACID

Para-aminobenzoic acid (PABA), being a B vitamin, is a cofactor and precursor in the synthesis of folic acid, purines and thymine in most species of bacteria, algae and higher plants. However, PABA is neither essential nor biosynthesized in mammals and it is not essential to human health, therefore not officially classified as a vitamin (Krátký et al., 2019). This reason may be the cause of the lack of information on the use of PABA in poultry. In cattle,

the use of PABA reported by Smolentsev et al. (2021) that the use of PABA at a dose of 0.5 mg per 1 kg of live weight had a positive effect on the growth and development of young cattle.

PABA also showed antibacterial activity against gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and gram-negative (*Pseudomonas aeruginosa*, *Salmonella enterica* and It has also exhibited antifungal activity against *Candida albicans* with the highest zone of inhibition (Kapoor and Dahiya, 2016). PABA administered in the adult rat as the Aktipol preparation acts as an epigenetic regulator of regenerative processes in renewable cell populations. The protective and therapeutic use of Aktipol allows for optimization of the state of eye surface tissues under deleterious hypoxic conditions and maintains the state of the eye at a physiologically normal level (Markitantova et al.,2018). According to Horozić et al. (2019) PABA has shown a stronger antimicrobial activity at lower pH values. It is supposed that this acid reacts in at least two mechanisms of antibacterial activity, one mechanism in common with other organic acids and the other mechanism by interfering with the synthesis of the peptidoglycan layer by an action on the dihydrofolate reductase enzyme.

9. PYRIDOXINE

There are three different forms of pyridoxine or vitamin B6: pyridoxine, pyridoxal and pyridoxamine. Pyridoxal is the active form and the most stable form (Barroeta et al. 2012). Pyridoxine participates in phosphorylation reactions in the liver with niacin and riboflavin (Barroeta et al. 2012).

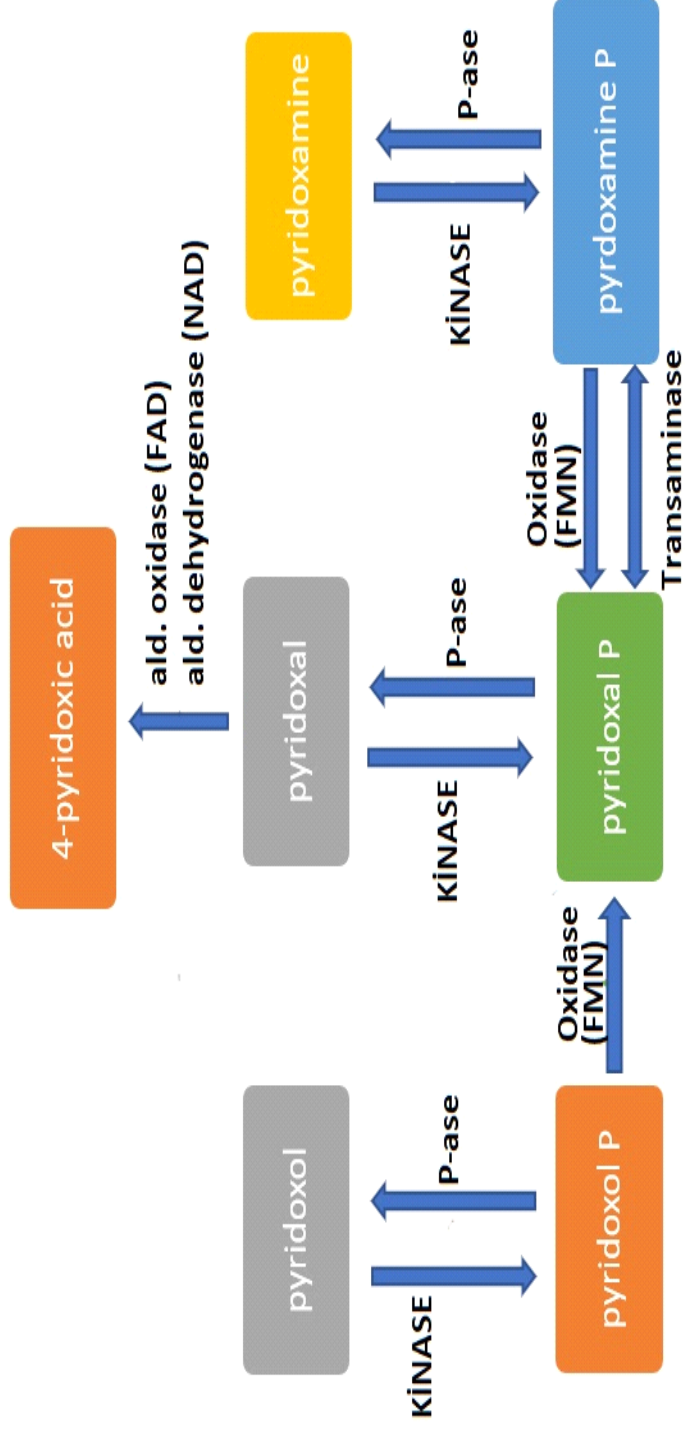


Figure 6. Metabolism of Pyridoxine

Pyridoxine is found in high amounts in cereals and fish. The table 1 below contains the pyridoxine content of some feedstuffs. There are various opinions regarding the amount of pyridoxine that should be added to laying hen diets (Combs and McClung, 2016). In an 88% dry matter diet, NRC (1994) at least 2.5 mg / kg feed, Larbier and Leclercq (1994), PAN (2005) and Leeson and Summers (2005) at least 1-3 mg / kg feed, Whitehead (1988), AWT (2001) and DSM (2006) at least 0-6 mg / kg feed is recommended to add pyridoxine. Commercial laying hens generally report their need for pyridoxine as 0-5 mg / kg feed (Leeson and Summers, 2009). Generally, in normal laying hen diets, the need for pyridoxine is sufficient. Pyridoxine is in the structure of enzymes that catalyze reactions involving many amino acids. In addition, it contributes to energy production by participating in fatty acid and carbohydrate metabolism. Table 2 shows the enzymes containing pyridoxine and in which reactions they take part.

Table 1. Pyridoxine content of some feedstuffs (Combs and McClung, 2016).

Feed	Vitamin B6 (mg/kg)
Alfalfa meal, dehydrated	10,0
Alfalfa meal, sun-cured	9,0
Barley	2,9
Corn	7,0
Corn Gluten Meal	8,0
Poultry by-product meal	NV
Soybean meal	8,0
Soybean meal, dehulled	8,0
Soybeans, full-fat, processed	11,0
Sunflower Seed Meal	16,0
Wheat	4,0

Table 2 Pyridoxine containing enzymes and reactions involving enzymes (Combs and McClung, 2016).

Type of Reaction	Enzyme
Decarboxylation	Aspartate 1-decarboxylase
	Glutamate decarboxylase
	Ornithine decarboxylase
	Aromatic amino acid decarboxylase
	Histidine decarboxylase
R-group interconversion	Serine hydroxymethyltransferase
	δ -Aminolevulinic acid synthase
Transamination	Aspartate aminotransferase
	Alanine aminotransferase
	γ -Aminobutyrate aminotransferase
	Cysteine aminotransferase
	Tyrosine aminotransferase
	Leucine aminotransferase
	Ornithine aminotransferase
	Glutamine aminotransferase
	Branched-chain amino acid aminotransferase
	Serine–pyruvate aminotransferase
Racemization	Aromatic amino acid transferase
	Histidine aminotransferase
	Cystathionine β -synthase
α,β -Elimination	Serine dehydratase
γ -Elimination	Cystathionine γ -lyase
	Kynureninase

Although the symptoms of pyridoxine deficiency in laying hens have not been clearly explained, it has been reported that they occur in the form of

decreased body weight, body fat reserves, egg production and anorexia (Weiss ve Scott, 1979). Pyridoxine is stable in acidic environment. However, it loses its stability in an alkaline environment or under the influence of heat and light (Combs and McClung, 2016). The pyridoxine loss caused by heat treatment of feeds has a high variation (%0-70). This loss is few in plant-based materials, whereas there is a high amount of loss in animal-based materials. The loss of pyridoxine during the storage of feed varies between 25 and 50% per year. The bioavailability of pyridoxine varies according to the form in feeds. Generally, the bioavailability of pyridoxine from plant-derived sources is higher than animal-based sources (Combs and McClung, 2016). The effect of pyridoxine together with flaxseed meal on egg parameters was investigated by Khan (2019). As a result of the research, egg shell thickness and egg weight increased with the use of pyridoxine. The highest egg yolk / albumen ratio was obtained by using pyridoxine. The amount of egg yolk cholesterol also decreased in the use of pyridoxine. Also, addition of pyridoxine to the diet, reduce the omega-6 level in egg and hens have lower blood triglyceride levels ($p = 0.016$). Bealish et al. (2019) aimed to compare the effects of add pyridoxine in laying hens diet and pyridoxine injection into the hatching egg. At the end of the study, the addition of pyridoxine in diet improved hatchability and chick weight, but injection method had a statistically positive effect. In general, the addition of pyridoxine by the in-ovo method improves the incubation parameters and decreasing embryonic deaths. Silaban et al. (2020) investigated the effects of using pyridoxine in three different ways (drinking water, diet and intravenous injection) on blood albumin and globulin levels in laying hens. At the end of the study, it was reported that the addition of pyridoxine by intravenous injection had positive effects on blood albumin level but not on globulin level.

10. PANTOTHENIC ACID

Besides being the building block of coenzyme-A, it also has very important functions in fatty acid metabolism. Pantothenic acid also serves as the main precursor of the acyl carrier protein. In this way, it also plays an important role in amino acid and carbohydrate metabolism. Especially it plays a role in the acylation of proteins in protein metabolism (Combs and

McClung, 2016). Pantothenic acid is found in very few amounts in grains. The table 3 below shows the pyridoxine content of some feedstuffs.

Table 3. Pantothenic Acid content of some feedstuffs (Combs and McClung, 2016)

Feedstuff	Pantothenic (mg/kg)	Acid
Alfalfa meal, dehydrated	33.0	
Alfalfa meal, sun-cured	20.0	
Barley	6.6	
Corn	5.7	
Corn Gluten Meal	10.0	
Poultry by-product meal	8.8	
Soybean meal	14.5	
Soybean meal, dehulled	14.5	
Soybeans, full-fat, processed	15.0	
Sunflower Seed Meal	10.0	
Wheat	13.0	

Pantothenic acid maintains its stability under storage conditions. However, heat treatments, acid or alkaline environments affect its stability negatively. Studies report that 37-78% loss occurs when feedstuffs are exposed to heat treatment (Combs and McClung, 2016). In an 88% dry matter ration, NRC (1994) at least 2 mg / kg feed, Larbier and Leclercq (1994) and PAN (2005), at least 5-10 mg / kg feed, Leeson and Summers (2005), AWT (2001) and DSM (2006) at least 0-6 mg / kg feed is recommended to add pantothenic acid. Pantothenic acid deficiency in laying hens causes problems such as nervous system problems, dermatitis and Addison's disease (Barroeta et al. 2012). However, it has been reported that it has no effect on egg production, and may have negative effects on hatchability. There has been no

specific study on laying hens in the last 30 years regarding pantothenic acid. In general, Beer et al. (1963) reported that the diet was at least 1.9 ppm, 4 ppm for optimum egg production and 8 ppm for maximum hatching efficiency. Gonzalez (1987) reported that 0-15 mg / kg should be added to laying hen diets.

11. BIOTIN

Biotin is abundant in nature, plant and animal products. Bioavailability varies due to the fact that biotin found in nature is usually bonded with lysine or other amino acids. In general, biotin bioavailability is reported to be at higher levels in protein-based feedstuffs than grain group feedstuffs (Baker, 1995). Table 4 shows the existence and bioavailability value of biotin in various feedstuffs (Combs and McClung, 2016).

Table 4 The existence and bioavailability value of biotin in various feedstuffs (Combs and McClung, 2016)

Feed	Total Biotin (µg/100 g)	Available Biotin (µg/100 g)	Bioavailability (%)
Barley	10.9	1.2	11.0
Corn	5.0	6.5	133.0
Wheat	8.4	0.4	5.0
Rapeseed Meal	93.0	57.4	62.0
Sunflower Seed Meal	119.0	41.5	35.0
Soybean Meal	25.8	27.8	108.0

The requirement of biotin in laying hens varies according to the type of carbohydrates and fats in the diet and the synthesis capacity of the microorganisms in the metabolism. If the synthesis is not sufficient, it must be added to the diet. Biotin in feedstuffs is absorbed in the small intestine. Biotin

deficiency generally causes negative effects on yield parameters (Barroeta et. al. 2012). Biotin can be destroyed by heat treatment. In addition, technological processes applied to feeds also significantly damage the presence of biotin. These losses can be reduced by various antioxidants (Vitamin C, Vitamin E) (Combs and McClung, 2016). Biotin participates in carboxylation, gluconeogenesis and protein synthesis reactions in the body. For this reason, biotin is of great importance in terms of ensuring the continuity of life and continuity of yield parameters (Barroeta et. al. 2012).

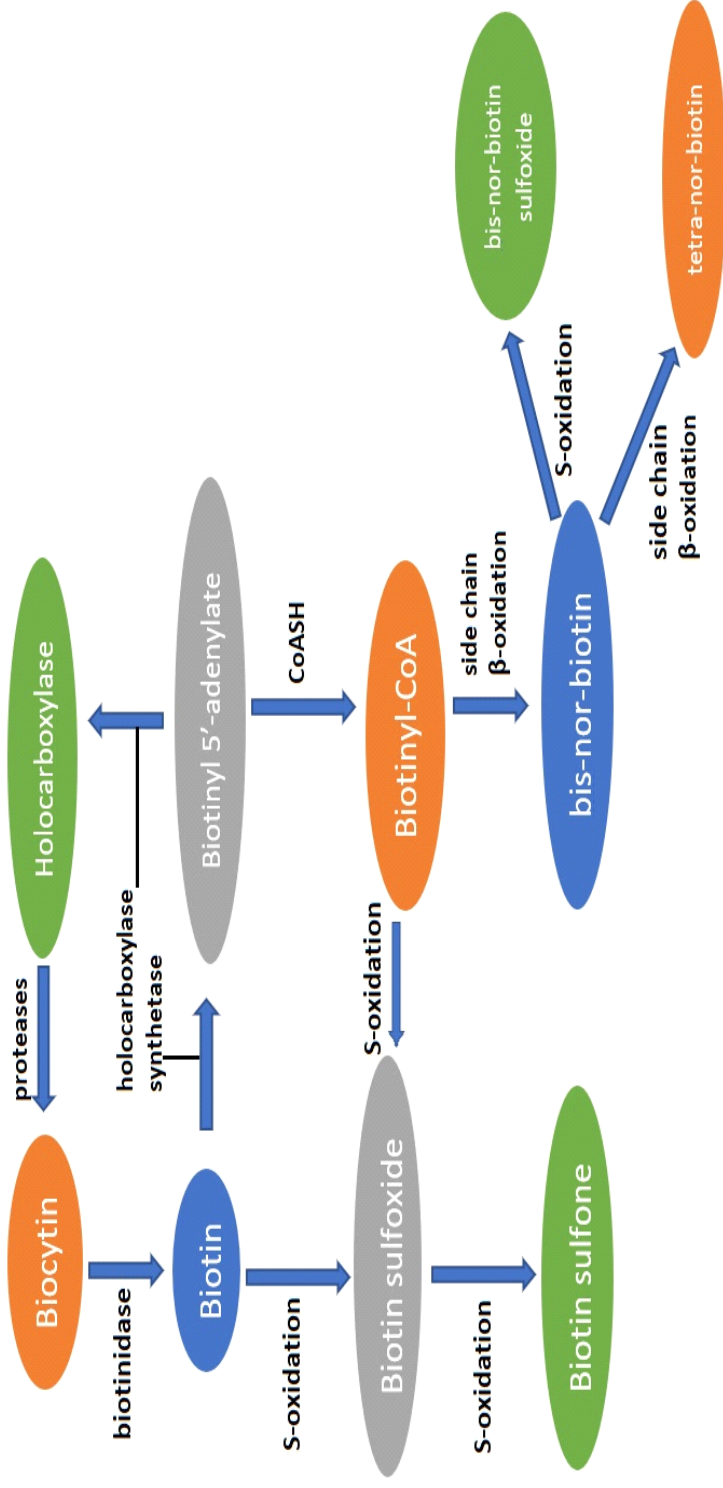


Figure 7. Metabolism of Biotin.

NRC (1994), Larbier and Leclercq (1994), PAN (2005) and Leeson and Summers (2005) at least 0.1 mg / kg feed, Whitehead (1988), AWT (2001) and DSM (2006) at least 0-0.3 mg / kg feed is recommended to add biotin to laying hen diets. El-Katcha et al. (2019) examined the effects of using biotin alone or in combination with bile acid in laying hens. It was reported that while an improvement in feed conversion ratio was achieved, feed intake was negatively affected and it had no effect on other performance parameters. In addition, fat digestibility was not affected by the use of biotin with bile acid. In terms of, only egg yolk weight was positively affected by the use of biotin with bile acid, and no difference was found between the control group and the group using only biotin. The use of biotin in either way did not show a significant effect on blood parameters. Huang et al. (2020) reported that the use of biotin reduces the effects of fatty liver syndrome in laying hens. It has also been reported that regular use of biotin can prevent this disease.

12. COBALAMIN (VITAMIN B12)

Cobalamin or vitamin B12 is the last vitamin to be discovered, but it is the most effective vitamin that can supply of animals in few quantities. Vitamin B12 can only be synthesized naturally by microorganisms in the intestines, so plant-based feeds are poor in cobalamin (Barroeta et. al. 2012). Vitamin B12 contains cobalt in its structure. With this feature, it is the only vitamin that contains elements in its structure. Cobalamin stability is high in aqueous solution and crystalline form. The combination with protein factors (hapcorcorrin and intrinsic factor (IF)) is necessary for the absorption of vitamin B12 in the intestine. The cobalamin-IF complex is absorbed in the ileum through a receptor-mediated process. The possibility of this active transport mechanism limits the absorption rate of cyanocobalamin. In addition, about 1% of the dose is absorbed by passive diffusion. Cobalamin is converted into an active form of methyl or adenosylcobalamin in intestinal cells, and is transported in the blood through a specific binding protein after release. Unlike other B vitamins, cobalamin is not used immediately after being absorbed by the body. Vitamin B12 is mainly stored in the liver, and a small amount is stored in the kidneys, muscles, bones and skin. The main way of elimination is through bile. The excrement in the urine is the least. Cobalamin synthesizes nucleic acid with folic acid in metabolism. In addition,

it is also involved in the cholesterol metabolism and the methionine synthesis. It can also prevent anemia by synthesizing erythrocytes. However, according to the NRC recommendation laying hen diets contain approximately 0.004 mg / kg cobalamin.

Cobalamin is related to choline and methionine, so methionine or choline deficiency can cause cobalamin deficiency (Barroeta et. al. 2012). In general, no evidence of cobalamin deficiency. A sufficient amount of cobalamin can be stored in the body's reserves for a long time. Johnson and Korver (2008), added 16 and 116 μg / kg cobalamin to the laying hen diet for 6 weeks, an accumulation of 0.72 and 0.92 μg cobalamin was reported in the egg. Similarly, Leeson and Caston (2003), added 10 and 100 μg cobalamin in laying hen diet for three months experiment, an accumulation of 0.87 and 3.35 μg cobalamin was reported in the egg. Although the addition of cobalamin to laying hen rations increases the accumulation of cobalamin in eggs, the results of the available studies are highly variable. Overall, the data show that the extra use of B12 in laying hen diets has a significant effect on the vitamin B12 content of eggs. Bunchasak et al. (2009) investigated the accumulation of different amounts of Vitamin B12 supplementation (0, 0.01, 0.08) on the liver, blood serum and egg yolk of laying hen diets. It has been reported that the extra addition of vitamin B12 to the diet is not significant ($P > 0.05$). As a mean value, an accumulation of 743.14 ng / g, 20.39 ng / ml, 45.13 ng / g was observed in liver, blood serum and egg yolk, respectively. Although vitamin B12 deficiency has no effect on egg production in general, it has been reported to have negative effects on egg weight (Skinner et al. 1951).

13. VITAMIN C

In poultry, Vitamin C or ascorbic acid is synthesized in the kidney from glucose (Barroeta et. al. 2012). Although it is not generally found to be deficient, supplements are needed if stress factors increase. In addition, there is no evidence of microbial synthesis of ascorbic acid (VITAMIN KİTAP 269-270). Vitamin C is present in feedstuffs of approximately 80-90% as ascorbic acid or dehydroascorbic acid. Vitamin C supplemented in the diet reduces the negative effects of stress, provides immunological support and thus helps the birds to protect their health. Vitamin C is very sensitive to oxidation. Therefore, it is adversely affected by the long-term storage and heat treatment

of feeds. Bioavailability and active derivates of vitamin C are shown in table 5(Combs and McClung, 2016).

Table 5. Bioavailability and active derivates of vitamin C (Combs and McClung, 2016)

Strongly Activity)	Biopotent (> %50	Weakly Activity)	Biopotent (< %50
Ascorbic acid 2-O- α -glucoside		l-ascorbyl palmitate	
6-Bromo-6-deoxyl-ascorbic acid		l-ascorbyl-2-sulfate	
l-ascorbate 2-phosphate		l-ascorbate-O-methyl ether	
l-ascorbate 2-triphosphate			

Vitamin C is involved in more than one metabolic event in the body. It plays a role in calcitriol biosynthesis, which has the role of regulating calcium homeostasis in collagen and aldosterone in the body. Aldosterone plays a role in maintaining and regulating electrolyte balance in tissues (Barroeta et. al. 2012).Vitamin C has a protective effect on macrophages during phagocytosis, so it can improve the cellular immune response and ensure the continuity of corticosterone production under environmental and immune stress (Sahin and Onderci, 2002). When vitamin C is used under heat stress conditions, the stressor has been shown to reduce and contribute to egg production and body weight (Abd-Ellah, 1995; Andrews et al., 1987; Dzhambulatov et al., 1996). In case of stress factors, ascorbic acid should be added to laying hen diets.

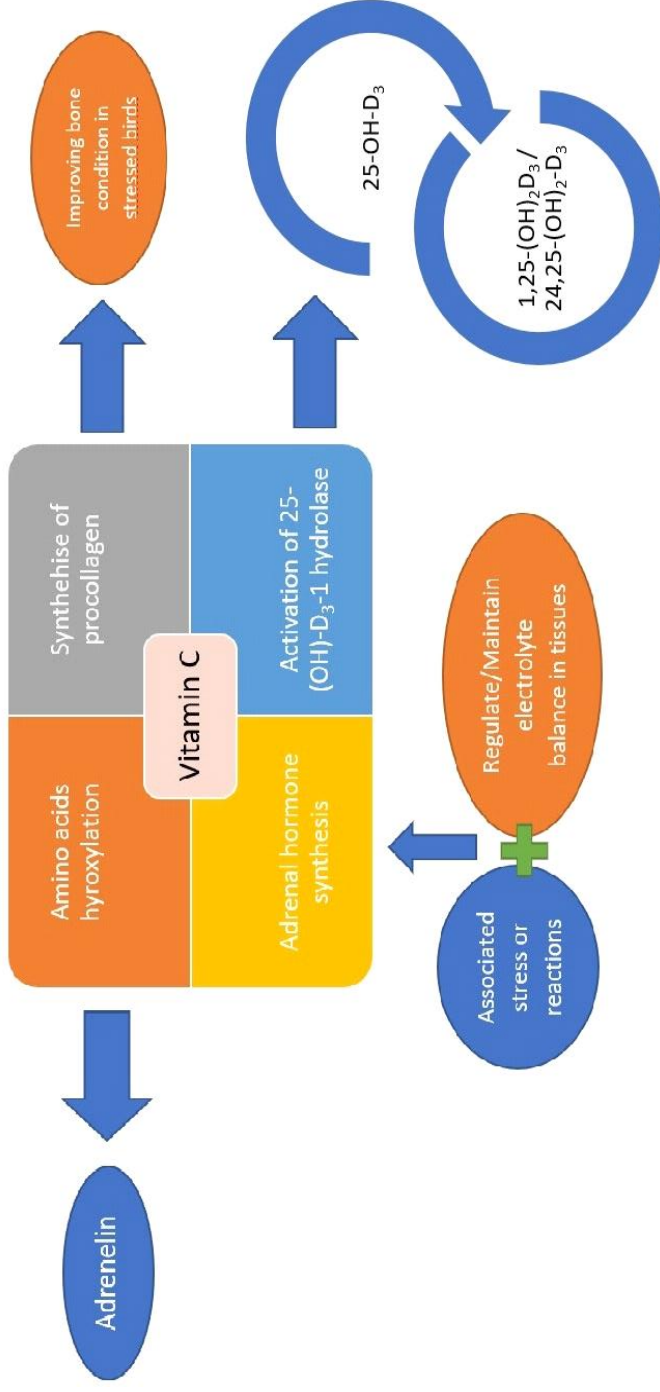


Figure 8. Functions in metabolism of Vitamin C.

Mirfendereski and Jahanian (2015) investigated the effects of using ascorbic acid and chromium together in laying hens housed at high stocking density. It was reported that the use of 500 ppm ascorbic acid improves feed conversion ratio, and the use of ascorbic acid together with chromium has a positive effect on egg production. In the same study, it was reported that the increase in the stocking density increases the amount of corticosterone in the blood, this situation can occur depending on the stress factor and the use of chromium with vitamin C decreases this parameter. It has been reported that the use of vitamin C has no effect on immune system parameters. Gerzilov et al. (2015) investigated the effects of using zinc and ascorbic acid in three different temperature environments (hot, cold and thermoneutral) in laying hens raised in the free range system. As a result of the research, it was reported that the lowest blood corticosterone level was detected with Zn and vitamin C supplements in laying hens raised in a thermoneutral environment. It has been reported that the use of Vitamin C together with Zn has antioxidant and antistress properties and can thus improve animal welfare by lowering the level of corticosterone in the blood. Zinc and vitamin C added to diets promote the reduction of serum corticosterone and cholesterol concentrations. In this way, it contributes to the reduction of the negative effect of stress due to environmental temperature. Also, the use of Zn and Vitamin C has a positive effect on the body weight parameters in all temperature conditions. It has been reported that the use of vitamin C and Zn has a positive effect on egg production under hot and cold stress conditions. Bozakova (2020) investigated the effects of Zn and vitamin C supplements on laying hen diet under cold stress. It was reported that the use of vitamin C together with zinc increased egg production. In addition, reduce blood corticosterone and Malondialdehyde (MDA) levels. In addition the oxidative stress index calculated from the related parameters and decreased significantly. Malondialdehyde (MDA) is one of the end products of polyunsaturated fatty acids peroxidation in cells. An increase in free radicals causes an overproduction of MDA. Malondialdehyde level is known as a marker of oxidative stress and antioxidant status. Therefore, it can be said that the use of Zn and vitamin C together has a positive effect on oxidative stress. Soltani et al. (2019) investigated the effects of vitamin C and ethanol extract of propolis on laying hen feed under normal conditions. As a result of the

research, vitamin C supplementation has no effect on performance and egg parameters, and reduces blood glucose levels. In the studies summarized above, it is concluded that vitamin C supplementation has a positive effect when laying hens are exposed to stress parameters. However, it is reported that it also reduces the negative effects of oxidative stress.

14. VITAMIN ANTAGONISMS

An anti-vitamin is a substance which makes the vitamin unavailable block the action of some vitamins within the body. Sometimes, antagonism can also indirectly increase the needs of target vitamins because of their excessive intake. Generally, the fat-soluble group can be considered antagonistic due to their competition for absorption and transport. Vitamin A and D are mutually antagonistic to each other where vitamin A reduces the toxic effects of vitamin D. High doses of vitamin A can also inhibit the absorption of vitamin K (Tripathy et al.,2017). The different effects on bone metabolism also indicate vitamin A and D antagonism. Excess vitamin A induces bone resorption and decalcification, while vitamin D improves calcium absorption and retention. Also, vitamin E may compete with vitamin A for absorption. Vitamin A is known to suppress vitamin E levels in immunoprotein tissue. The antagonistic effects are also indicated by the opposite effects of vitamins A and E on prostaglandin synthesis. Vitamin E suppresses prostaglandin E2 and enhances prostaglandin E1 synthesis, while vitamin A enhances prostaglandin E2 synthesis. High levels of vitamin E intake may also **interfere** with beta carotene absorption. Other vitamins having a potential to antagonize vitamin A include B1, B12, B6, and K, but their mechanisms of action are not completely understood (Figure 8)(Watts, 1991).

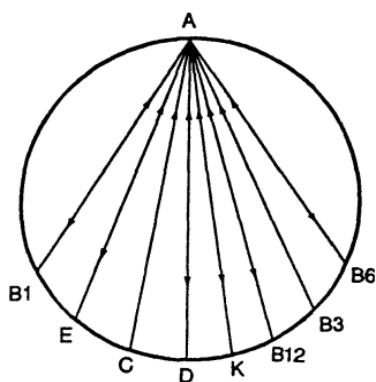


Figure 9. indicates the vitamins that are considered to be antagonistic to vitamin A (Watts,1991).

Other antagonism is between vitamin E and vitamin K which is high amount of vitamin E inhibit the vitamin K dependent carbboxylase activity and inhibit the activity of vitamin K in coagulation. The potential mechanisms for the vitamin E and K interactions include: 1) vitamin E competes for the enzyme that truncates the K1 side chain to form menadione; or 2) vitamin E increases xenobiotic pathways that increase the metabolism and excretion of all vitamin K forms from the liver; or 3) vitamin E competes with K1 for the cytochrome, thus preventing the β -oxidation of the tail to form menadione (Traber, 2008).

15. VITAMIN SYNERGISMS

Synergy is defined as the combined interaction of several system elements which produces an entirely different or greater effect compared to what they produce by their separate effects. For example, vitamins A and D help body to absorb zinc which in turn enables body to absorb fat soluble vitamins. Also, vitamins A, D, and K2 work together to build strong bones, promote growth, protect against calcification of the soft tissues, and support immune system. The interaction between folic acid and vitamin B12 can increase the effects of the anemia and lead to permanent neurological damage. For the conversion of folic to folinic acid, vitamin B12 is essential (Tripathy et al.,2017).

Moreover, the effects of vitamin C, vitamin E, and β -carotene have been demonstrated to have the greatest synergism effects as antioxidant to against the oxidation of lipid in chicken. Vitamin C has notable antioxidant properties because of its ability to donate electrons, and it protects the integrity of many cells, including lymphocytes, against damage from free radicals generated in response to infection or toxins (Nimse and Pal, 2015). Other result, vitamins D and K have potential synergy for bone mineralization. Vitamin K is needed for the carboxylation of vitamin K-dependent proteins such as osteocalcin and matrix Gla protein, while vitamin D promotes the production of vitamin K-dependent protein concentrations. These vitamin K-dependent proteins are needed for extrahepatic organs such as the bone. This will result in bone mineralization and will inhibit soft tissue calcification, which will ultimately lead to lower risks of fractures (Ballegooijen et al., 2017).

16. VITAMIN STABILITY

Several physical and chemical factors can affect the stability of vitamins. The major factors are temperature, humidity, presence of oxygen, light, pH, oxidizing and reducing agents, presence of metal ions and other ingredients in the matrix, or the combination of several of these factors (IADSA, 2014). The water molecules, oxygen in the air and temperature may influence the oxidation rate of fat-soluble vitamins; therefore, resulting in decreased vitamin stability. The individual vitamins vary in their susceptibility to degradation Table 6.

Table 6. Sensivityy of vitamins to factors affecting stability

Vitamin	Temperature	Humidity	Light	Oxygen	Acid Ph	Alkaline pH
Vitamin A	Very sensitive	Sensitive	Very sensitive	Very sensitive	Sensitive	Stable
Vitamin D	Sensitive	Sensitive	Sensitive	Very sensitive	Sensitive	Stable
Vitamin E	Stable	Stable	Sensitive	Sensitive	Sensitive	Sensitive
Vitamin K	Sensitive	Very sensitive	Stable	Sensitive	Very sensitive	Stable
Riboflavin	Stable	Sensitive	Sensitive	Stable	Stable	Stable
Niacin	Stable	Stable	Stable	Stable	Stable	Stable
Pantothenic acid	Sensitive	Sensitive	Stable	Stable	Stable	Stable
Vitamin B12	Very sensitive	Sensitive	Sensitive	Sensitive	Stable	Stable
Pyridoxine	Very sensitive	Sensitive	Sensitive	Stable	Sensitive	Stable
Biotin	Sensitive	Stable	Stable	Stable	Stable	Stable
Folic acid	Very sensitive	Sensitive	Very sensitive	Stable	Very sensitive	Stable

Vitamin A is the most light-sensitive micronutrient when subjected to light in unprotected bags . it suffers extensive photodegradation when exposed to wavelengths between 330 -350 nm. Vitamin E is degraded by oxygen in a reaction catalysed by light. Vitamin E is particularly sensitive to wavelengths between 285 nm and 305 nm. Meanwhile Phylloquinone (vitamin K1) has been reported that the concentration of phylloquinone can decrease by 50% following 3 hours in strong sunlight (Ferguson et al.,2014). According to Saensukjaroenphon et al (2020), the fat-soluble vitamins were stable when mixed with both vitamin and vitamin trace mineral premix and stored at 22°C with 28.4%RH. However, premixes were stored at 39.5°C with 78.8% RH, the vitamin A and D3 were stable up to 30 days while the vitamin E was stable up to 60 days. Also they found that the vitamin stability was greater than 90% after the premixes were heated at 60°C for approximately nine and a half hours.

The destruction of vitamins during manufacturing processes was explained by Riaz et al (2009) that the vitamins most sensitive to the extrusion process are A, E, C, B1, and folic acid, but the majority of B complex vitamins (B2,B6,B12, niacin, Ca-pantothenate, and biotin) have great stability in feed processing. The losses vitamin during pelleting and extrusion is presented in Table 7. Also, they found that microencapsulated vitamins was higher stability than that crystalline ones of vitamins, particularly ascorbic acid, menadione, pyridoxine, and folic acid.

Table 7. Typical losses of different vitamin during Pelleting and extrusion.

Vitamins	Mineral/ Vitamin premix	Pelleting (70°C)	Pelleting (90°C)	Extrusion (80°C)	Storage
Vitamin A	1% / month	10%	30–40%	30%	6–7% / month
Vitamin D	10% / month	15%	35%	25%	10% / month
Vitamin E	2% / 6 month as acetate	10%	15%	10%	–
Vitamin K	34–38% / month	20%	40%	50%	50% in compound feed
Vitamin B ₁	50% / 3 month	15%	50%	50%	5–20% / month
Vitamin B ₂	5–40%	10%	15%	20%	2–10% / month
Vitamin B ₆	20% / month	10%	30%	5–25%	2–5% / month
Niacin	2–4% / month	5%	10%	10–30%	1–2%
Pantothenic acid	1–8% / month	10%	20%	10–20%	0–5% / month
Choline	10% / 6 month	5%	5%	Low	3% / 6 month
Folic acid	10–40% p month	5–20%	45%	50%	10–50% / month
Biotin	5% / month	10%	35%	15–25%	1–2% / month
Vitamin C	1–2% / month	40%	85%	90%	2–5% / month

Source : Riaz et al. (2009)

CONCLUSION

For laying hens, vitamins have a positive effect on the growth, development of reproductive organs, egg production and egg quality. Moreover, vitamins A, D, E play critical roles in immune system development and antioxidant. Also, vitamin D and vitamin K have important role in calcium and phosphorus metabolism that its well-documented involvement in bone development and eggshell formation. Choline and folic acid are participates in improving the methionine biosynthesis which is an essential amino acid that plays a key role in egg production. Meanwhile inositol induces glucose as the energy supply for laying hens. PABA is neither essential nor biosynthesized in mammals and human. This reason may be the cause of the lack of information on the use of PABA in poultry. Nevertheless, some research has shown inconsistent results concerning dose–response relationships for some vitamins.

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CHAPTER 4

VITAMIN AND MINERAL REQUIREMENTS OF BREEDING LAYING HENS

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1. INTRODUCTION

Over the past century, advances in genetics, disease management, vaccination techniques, and general breeding procedures have all contributed to the advancements in layer breeder strains. These advancements have led to healthier, more fruitful flocks with improved reproductive and disease resistance, which in turn has improved the industry's sustainability and efficiency. Selection is used by layer breeding businesses to enhance more than 30 features crucial to the production of commercial eggs. Nevertheless, there aren't any globally or nationally recognized breeding objectives (Underwood et al., 2021). Layer breeding companies use a pyramidal structure, requiring a small number of layer breeders to produce laying hens, with each breeder producing approximately 115 hens (Peixoto et al., 2021). Nowadays, egg weight, body weight, feed conversion, laying rate, livability, albumen height, color, strength, and inclusions (such as blood and meat stains) in the eggs are the most important considerations to breeders (Thiruvankadan et al., 2010).

Specialized birds reared to lay fertilized eggs are known as layer breeders. In order to develop and improve layer breeds for effective egg production, layer breeding firms are essential. Hendrix poultry (Dekalb, Hisex, and Bovans), Hubbard-ISA (Shaver, ISA, Hubbard, and Babcock), and Erich Wesjohann (Hy-Line, Heisdorf & Nelson Farms Inc., and Lohmann Tierzucht), are the three main layer breeding businesses in the globe (Thiruvankadan et al., 2010).

Embryonic development is one of the critical factors that significantly affect the productivity and quality of birds for poultry breeding companies. For the transmission of necessary nutrients for embryonic development, the nutritional state of the male and female breeders, particularly with regard to minerals and vitamins, is crucial (Surai et al., 2016). Layer breeders' nutrient diets might change depending on the week, particular flock, and breeder habits during the last thirty years. For chickens to remain healthy, their immune systems to be supported, their production potential to be maximized, and the quality of their eggshells to be improved, their meals must contain sufficient amounts of minerals and vitamins (Smith & Akinbamijo, 2000; Yenice et al., 2015).

A variety of items from various sources can be added to breeders' diets, both male and female, in order to boost fertility and improve the health of the birds and their progeny (Santos et al., 2024). Many different chemicals have this effect at the moment, organic microminerals (including selenium), vitamin E, vitamin D, and different types of carotenoids are the most studied and commercially accessible ones (Bülbül et al., 2008; Puthongsiriporn et al., 2001; Wang et al., 2017). In poultry production, all of the feed supplements have been used to boost the productivity and reproductive capabilities of layers, broilers, and breeders. They are all capable of having a favorable impact on the animal organism's immune system defense and antioxidant levels. According to Gan et al. (2020), dietary vitamins play a crucial role in reducing free radical damage, boosting antioxidant levels, and enhancing immune system performance. Vitamins C and E, which function as antioxidants in the diet of laying hens, aid in preventing oxidative damage to other constituents such as cytosolic compounds and membrane phospholipids (Combs Jr & McClung, 2016). Layer breeders cannot generate fat-soluble vitamins A, D, E, and K internally, thus they, like other fowl, need to have supplements of these vital compounds in their diet. Fat-soluble vitamins serve essential roles in development, reproduction, immunity, and other metabolic processes by acting as catalysts. Unlike water soluble vitamins, which are rarely retained in the body for a long time, fat-soluble vitamins can be stored in adipose tissues and liver as a reserve against times of scarcity. To ensure good health and productivity among layer breeders, their diets must be balanced with respect to these considerations (Khan et al., 2023).

The two types of minerals found in layer breeder diets are macro and microelements. Macro elements are necessary nutrients, like calcium, phosphorus, sodium, potassium, magnesium, and chlorine, that chickens require in comparatively large amounts. In the diet of breeders, minerals are dietary accessible supplies in the forms of inorganic, organic, and nanoparticles (NP). During the preparation of chicken diets, the amount and source of mineral components are very important factors. They affect total economic results, bird health, product quality, and production efficiency (Attia et al., 2010). The ongoing investigation and enhancement of the vitamin and mineral sources in layer breeder diets has resulted in significant changes. Instead of depending exclusively on traditional inorganic sources,

essential trace minerals including copper, iron, iodine, manganese, selenium, and zinc are increasingly being given in organic forms, such as chelated compounds. These organic components are more bio-available, meaning that birds will be able to absorb and use them more efficiently (Araújo et al., 2019). However, shortages in these elements cause all major organ systems to develop abnormally, stunted growth, and even embryonic mortality in birds (Abd El-Ghany, 2022).

Micronutrients such as trace elements, organic acids, coccidiostats, and essential vitamins must be provided to parent stock feed for maximum hatching egg production and hatchability. By giving these micronutrients in the appropriate amounts, the parent stock's proper supply is safeguarded (Santos et al., 2024).

Important dietary guidelines for poultry, especially laying hens, were supplied by the National Research Council (NRC, 1994). It's important to understand that while the NRC tables have been revised on a regular basis, they still only represent the bare minimum of nutrient needs. According to recent studies, the NRC guidelines may need to be improved in several areas to better suit the demands of layer breeders today. Validation of suggested values for fat-soluble vitamins and trace elements bio-availability is crucial.

In this chapter, we investigated the impact of vitamins and minerals addition to diet on the fertility functions of female and male layer breeders based on data from several notable studies.

2. VITAMINS

2.1. Vitamin A

Even though vitamin A was one of the first vitamins to be discovered, scientists are still learning about the biological and physiological functions that it mediates, calling for a deeper comprehension (Shastak & Pelletier, 2023). It has been reported that vitamin A addition to ration of layer breeders has several significant effects. It can improve reproductive performance by increasing egg production and hatchability rates since the reproduction rate was significantly increased by adding vitamin A to layer breeders' diets (Kerti & Bárdos, 1997; Lin et al., 2002). Yuan et al. (2014) reported that adding vitamin A at 35000 IU/kg or above did not have a negative influence on production efficiency and improved accumulation of vitamin A in liver and

yolk. But at level of 45000 IU/kg and above vitamin A in diet of broiler breeders dramatically reduced egg quality and reproductive efficiency. Therefore, 35000 IU/kg dose of vitamin A reported as the highest safe limit for broiler breeders. Over this level has been found to harm liver function, reproductive health, and immune response. It was also reported that 10800 IU vitamin A supplement to 46-54 weeks of age Chinese yellow-feathered broiler diets increased production performance, liver accumulation of vitamin A, and regulation of ovarian hormone receptors expression and suppression of apoptosis gene transcriptional retinoic acid (Chen et al., 2016).

Vitamin A increases the strength of immune system against infectious diseases and inhibition of viral infections (Khan et al., 2023) and disease resistance therefore improving overall health. Villus height and crypt depth for better feed absorption and gastrointestinal health of layer breeders were observed by high vitamin A supplementation (El-Ratel et al., 2024). It controls the production of cytokines vital to immunity as well as influencing the number of peripheral T cells and antibody titers towards Newcastle disease virus. However, El-Ratel et al. (2024) observed that adding excess vitamin A above 6000 IU/kg negatively affected broiler immune system, which should be noted in relation to layer breeders. Wang et al. (2020) used different doses (0, 5400, 10800, and 21600 IU/kg) of vitamin A in diet of Chinese yellow-feathered breeder hens. Progeny hatched from these 4 treatments was fed with a basal chick diet added to different doses at (0 or 5000 IU/kg vitamin A) for 63 days. Results show that breeder and progeny diet supplementation to vitamin A had positive effects on meat quality and immune function of offspring. Even though this study was run with broiler breeders, it is crucial that layer breeder companies take these suggestions into account and modify their diets accordingly. Kucuk et al. (2003) studied on broilers under heat stress but supplemented with 15000 IU/kg of vitamin A had lower Malondialdehyde (MDA) in serum compared to their counterparts that were not given vitamin A. Yang et al. (2020), revealed a study where maternal geese got a diet supplemented with vitamin A from 0 to 16000 IU/kg feed, offspring received doses either at 0 or 9000 IU/kg. It was shown that providing maternal geese with 12000 IU/kg of vitamin A improved organ development and immunological function in goslings.

The recommended daily allowance for adults is given as being about 3000 I.U.s; this may range between different individuals based on age or sexual orientation; however it does not exceed this amount for any person whatsoever; hence it represents all individual's requirement (NRC, 1994). Turkey breeders must meet the 5000 IU per kg, duck breeder must meet the 2500IU per kg, quail breeders must meet the 3300IU per kg, and finally duck breeder must meet the 4000IU per kg diet (Khan et al., 2023). In earlier reports by Coskun et al. (1998) and Mendonça Jr. et al. (2002), a high dose of vitamin A did not show any discernible influence on the production rate with layer hens compared to NRC standard dose of vitamin A under commercial conditions. However, layer breeder companies (H&N MG and Lohman parent stock) recommended 10000 IU per kg of feed (Anonymous, 2020, 2022). Lima and Souza (2018) suggested that the dosage of vitamin A is roughly 9000 IU/kg in laying hens' feed. It is important that layer breeder farmers take these suggestions into account.

Most published literature have focused on layer, broiler, broiler breeder, and other poultry species compared to layer breeders on growth, performance, immunity, and development effects. Layer breeders seem to have received less attention than other poultry strains and species. Further investigations are needed in this area.

2.2. Vitamin D

Vitamin D is an essential vitamin that affects the eggshell quality of poultry. Calcium and phosphate intestinal absorption, mineralization, and demineralization of bone, as well as bone mobilization, is facilitated by vitamin D₃ or its active metabolite 1,25-dihydroxycholecalciferol (Newman & Leeson, 1999; Shivazad et al., 2005). According to the NRC (1994), the vitamin D₃ requirement for white-egg layer has been recommended at 300 IU of vitamin D₃/kg with a daily intake of 100 g of feed per chicken. Brown egg layers are estimated to have a daily intake of 110 g of feed per chicken, requiring 33 IU of vitamin D₃ per daily chicken. To observe how changing vitamin D levels in nutrition affect the hatchability, growth performance and general health is crucial to choosing the best nutritional formulation for layer breeders (El-Din et al., 2004; Fatemi et al., 2020). By increasing mammillary knobs and palisade layer thickness, it might be possible to strengthen

eggshells through raising the density of eggs by heightening amounts of vitamin D₃ or its metabolites (Jing et al., 2022).

For broiler breeders, Atencio et al. (2006) reported that using varying levels of vitamin D (0, 125, 250, 500, 1000, and 2000 D₃/kg for 27 to 36 wk of age) indicates a requirement of approximately 1400 IU D₃/kg. Between 36-66 weeks of age, treatments provided from 0 to 4000 IU D₃/kg, the requirement reported as might be approximately 2,800 IU of D₃/kg in feed. Świątkiewicz et al. (2016) reported in their extensive review that the level of vitamin D for high-performing poultry requires roughly 3000 IU/kg feed of vitamin D to increase mineral digestibility, productivity, immune defense, eggshell quality, and bone health which is much higher than NRC (1994) recommendation. They suggested that the form of 25-hydroxycholecalciferol (25-OH-D₃) is more effective than vitamin D₃ (cholecalciferol) for commercial poultry nutrition. According to research by Abraham et al. (2023), layer diets normally contain basal levels of vitamin D₃ 2760 IU/kg. Then vitamin D₃ was increased to 5520 IU/kg. There was no significant difference reported.

Calcium absorption is regulated by vitamin D and is essential for the development of eggshells. A layer diet with optimal vitamin D levels ensures that dietary calcium is properly utilized, which improves the quality of the eggshell (Gao et al., 2024). Over time, long-term vitamin D₃ supplementation improves eggshell quality indirectly by having a favorable impact on mineral homeostasis and bone growth (Chen et al., 2020). Wen et al. (2019) study found that dietary vitamin D₃ supplementation of up to 35014 IU vitamin D₃/kg feed maintained, if not improved, laying hen performance and improved the quality of the yolk vitamin D₃ content and eggshells in pullets and laying hens. They did note, however, that feeding pullets at a greater level of 68348 IU of vitamin D₃ reduced the laying hens' growth and, eventually, their performance. It's interesting to observe that Hy-line W36's eggshell and bone quality were unaffected by greater vitamin D₃ supplementation (2200 to 102200 IU/kg of feed). Adequate levels of vitamin D among layers can help them withstand diseases by boosting their resistance against certain ailments or reducing their impact as reported by researchers (Shojadoost et al., 2021). The expression of VDR which is the vitamin D receptor on activated T and B cells goes up significantly showing that Vitamin D plays a vital role in

governing these types of cells (Shojadoost et al., 2021). According to Geng et al. (2018) unavailability of vitamin D₃ decreased the lay rate and quality while increasing feed intake as well as feed conversion ratio. There was no difference in any parameter except for shell strength between all three treatments with dietary vitamin D supplementation at 500, 1500, and 3000 IU per kg of feed. A vitamin D₃ deficiency was associated with elevated levels of serum hormones (progesterone, calcium, parathyroid hormone, and estradiol), and an up-regulated population of blood CD3⁺ T cells.

Vitamin D levels correlate significantly with sperm parameters, particularly sperm motility in human models (de Angelis et al., 2017; Pilz et al., 2018). However, there is no direct data is reported related to the tie between vitamin D and fertility in poultry breeders. Further exploration of the specific impact of vitamin D on fertility in poultry breeders may require additional research studies or reviews specifically addressing this aspect.

A partial association between elevated blood testosterone and vitamin D levels in human models is shown by observational studies, which show a substantial relationship between vitamin D levels and sperm parameters, with a focus on sperm motility (Fouad et al., 2020). Vitamin D plays a critical role on the characteristics of sperms particularly the motility (Blomberg Jensen et al., 2018). Several studies have recommended that vitamin D supplementation may enhance the fertility benefits in male influencing testicular levels of reproductive hormones and the semen quality through direct non-genomic and genomic impacts (de Angelis et al., 2017). Finally, this results in enhanced seminal plasma quality mainly through non-hormonal effects and improves sperm motility (Cito et al., 2020). Studies have shown a that there is a direct association between vitamin D and sperm mobility, concentration and testosterone. This brought to spice up the constructive impact of vitamin D and its function in serving reproductive health by having an essential role in regulating male fertility (Rezayat et al., 2022; Wadhwa et al., 2020). Similarly, vitamin D supplementation has been found to be beneficial in this regard. A number of factors, including genetics, stress conditions, aflatoxicosis, and age of poultry, have been linked to decreased fertility in male poultry and impaired semen characteristics (Fouad et al., 2020). No study has been conducted so far that would effectively examine how vitamin D impact male and female fertility in layer hens. In order to properly

understand the role of vitamin D in both the male and female reproductive systems of layers, more investigations need to be carried out.

2.3. Vitamin E

In the case of breeders, vitamin E enhances the quality of the diet without any doubt. A literature review by broiler and layer researchers attributed the role of vitamin E as primary for enhancing the health and production of layer breeders due to its ability to boost immunity, cut on the oxidative stress as well as reproductive performance. Incorporation of vitamin E in broiler diet results proved the hypothesis that broiler chickens would experience an enhanced immune response and their antioxidant status is improved with reduced oxidative stress as concluded by (Khan et al., 2023). Also, some investigations have established that vitamin E plays a role in reducing the negative impact of hot environment on layer hens, thus, maintaining homeostasis and reducing stress (Ajakaiye et al., 2010). Abd El-Hack et al. (2019) reported that the feed conversion ratio and hematological parameters were increased with supplementation of 16000 IU of vitamin A, and 500 mg of vitamin E per kilogram of laying hens feed during the summer.

The specific level of vitamin E for layer breeders is not mentioned in most recent studies by Kakhki et al. (2020), Zhu et al. (2021), and Ali and Hassan (2022). It has been recommended by NRC to a minimum daily intake of 4–5 mg/d of Vitamin E for animals, including layer breeders, in case the diet has a low level of polyunsaturated fatty acids (Raederstorff et al., 2015). According to the research conducted by Ali and Hassan (2022) vitamin E levels were recommended as 200 and 400 mg/kg for broiler breeder's diet. Jena et al. (2013) found that broiler breeder hens' antioxidant state was enhanced during the summer when they received a 250 mg/kg vitamin E supplement, either on their own or with 200 mg of vitamin C. The study's findings demonstrated that the combining of lower dosages of vitamin C (200 mg/kg) and vitamin E (250 mg/kg) improved the erythrocyte antioxidant status in colored broiler breeder hens more effectively than particularly vitamin addition. These results suggest that layer breeders would benefit from giving 250 mg/kg of vitamin E to lower oxidative stress and boost antioxidant levels.

The effects of vitamin E on reproduction function were first reported by Evans and Bishop in rats (Evans & Bishop, 1922), since then, other researchers have shown that feed addition adding vitamin E improves fertility in various poultry species for example, the study by Rengaraj and Hong (2015), offers insightful information about how dietary vitamin E affects reproductive functions in chicken species, especially layer male breeders. This suggests that by serving as an antioxidant and reducing oxidative damage, vitamin E supplementation to breeders' diets may have a positive impact on layer males and females by reducing lipid peroxidation in eggs, and semen. Vitamin E is recognized as a chain-breaking antioxidant, effectively neutralizing freed radicals and preventing the oxidation of lipid membranes (Raederstorff et al., 2015). The quality of the sperm and semen is greatly endangered by a number of circumstances (Nawab et al., 2018; Rengaraj & Hong, 2015). By reducing lipid peroxidation, antioxidant feed supplementation mitigates these effects (Richard et al., 2008). Wahyuni et al. (2018) established that feed supplementation of Kedu breeder chickens with vitamin E enhanced the fertility and hatchability of the chicken eggs. According to the findings, this fertility function of chicken breeders may be improved by a 200–500 mg/kg vitamin E supplement mainly owing to the enhancement of the antioxidant capacity (Surai et al., 1998). In experiments conducted by Biswas et al. (2009), the birds were given 100 mg of vitamin E per kilogram of the food and the birds' better-quality spermatozoa and semen quality was better than in birds given 10 mg/kg of vitamin E. Amevor et al. (2021) examined the impact of 200 mg of vitamin E and 0.4 mg quercetin dietary antioxidant potential and seminal characteristics of elderly male Japanese quail breeder. The research consisted of such keywords as cytokines, biology, food, sperm, animal feed, quercetin, inflammation, and antioxidants. The results identified conclusively that the effect of vitamin E through the feeding of the male Japanese quail offered the qualities of sperm improvement. Based on this study it can be concluded that vitamin E can help improve the reproductive health and sperm quality of male layer breeders.

Including maternal diet vitamin E has been seen to affect the antioxidant state of progeny chicks with impression of a generational interaction of antioxidant vitamin E through maternal supply (Yang et al., 2021). Researchers Zaghari et al. (2013), Urso et al. (2015), and Yang et al.

(2021) showed improvements in hatching rates, egg weight, and the general quality of newly hatched chicks when vitamin E was added to the breeders' feed.

All of these results point to the potential benefits of vitamin E supplementation in the diet for layer breeders' reproductive processes. There is no specific level of vitamin E recommended for layer breeders, further research or references focusing on vitamin E supplementation in poultry diets would be necessary.

2.4. Vitamin C

Birds do not require dietary sources of vitamin C since they can synthesize (İpek et al., 2006). However, it has been noted that some environmental stressors may change how chickens synthesize or use ascorbic acid (Pardue & Thaxton, 1984). Ascorbic acid synthesis is insufficient in humans under stressful situations including low or high ambient temperatures, high rates of productivity, and humidity (McDowell, 1989). It is important to note that much of the existing literature does not specifically target vitamin C as a diet component of poultry breeders. Admittedly most of the experiments that run with broiler breeders are conducted on these birds. For instance; Setiyaningsih et al. (2023) assessed the effects of approved vitamin D3 (25(OH)) with vitamin C at 0, 100, 200 and 400 mg/kg Nutricell HyC® in broiler breeders on the blood profile, egg quality and hatchability. The results indicate a notable difference between the treatments and control regarding hatch performance and egg quality. Similarly, Jena et al. (2013) added different doses of vitamin E (250 or 500 mg/kg) or vitamin C (200 or 400 mg/kg) individually or as a combination for 8 weeks in broiler breeders diet. All groups of patients, which received vitamin supplements, had lower levels of malondialdehyde (MDA) in erythrocytes, higher activities of such enzymes as catalyze (CAT) and superoxide dismutase (SOD), as well as increased ferric reducing antioxidant power (FRAP) activity. Hence, the present study suggested that the effectiveness of combined vitamin supplementation was higher than the individual supplementation in a combating environmental stress in chickens. Therefore, combined vitamin supplementation proved more effective than individual supplementation in alleviating environmental stress in chickens. Akinyemi and Adewole (2021) reported in their recent review

use of vitamins, particularly vitamin C, in conjunction with other nutritional techniques is recommended to lessen negative impact of environmental stress on chickens. It has been advised to add vitamins to chicken feed, particularly during stressful conditions since the birds might not be able to synthesize enough of them. For instance, Sahin and Sahin (2001) assessed the effects of supplementing laying hens raised at a low ambient temperature (6.2 °C) with 250 mg of ascorbic acid and 400 µg of chromium picolinate/kg. The hens produced the best results in terms of feed consumption and feed conversion ratio when compared to the control group.

Shojadoost et al. (2021) have presented a relevant well-summarized review regarding vitamins A, D, E, and C and the chicken immune system. This review describes where these vitamins locate and the significance that it has in the immune system as for the immune responses, antioxidant activities, and immune regulation. Gan et al. (2020) examined the impact of increasing the dietary water and lipid-soluble vit concentrates from 0 to 3 g/kg on old layer's intestinal microbiota, production and immune responses. Hypothesis derived studies revealed that increased level of dietary vitamin addition exerted a huge influence on the composition of the intestinal microbiome. Specifically, there was an elevated ileal lactobacillus abundance and a decreed richness of cecal Feacalibacterium, ileal Turicibacter, and Romboutsia ($P<0.005$) in the water-soluble vitamin treatment. Additionally, the lipid-soluble vitamin group was raised cecal Phascolarctobacterium, and Megasphaera ($P<0.05$) compared to the control group. Kesariya et al. (2023) investigated the effects of in ovo feeding of nano zinc of 5 to 10 ppm along with vitamin C at the rate of 150 mg/1.5 ml on poultry embryonic development and its repercussions on hatchability, chick growth and antioxidant position in the commercial poultry. The study showed nano zinc and vitamin C supplementation had ($p<0.05$) improvements in hatchery performance and antioxidant status of offspring of poultry. Min, Niu, et al. (2018) investigated the effects of adding vitamin E (300 mg/kg) and vitamin C (200 mg/kg), individual or their combination to roosters' diet on immune response, and antioxidant status in breeder roosters under oxidative stress. They suggested that these supplements individually or as a combination enhance antioxidant capacity and immune response by up-regulating the expression of the glutathione peroxidase gene. According to the finding of

Amakye-Anim et al. (2000), the supplementation of the diet of chicken with ascorbic acid at a concentration of 1000 ppm had positive impact on performance and immunological response regarding Inflammatory bowel disease (IBD) vaccination. In addition, a study by Sanda and Oyewole (2015) highlighted the effects of vitamin A and C supplementation on immunological outcome, hematological profile and performance of broiler immunized against Newcastle Disease (ND) The combination of vitamins A and C boosted feed conversion ratio and strengthened their immunity when ND-vaccinated birds were exposed to Newcastle disease.

Based on available data, vitamin C supplementation may improve hens' immunological responses and increase their generation of antibodies after receiving a vaccination against a particular virus.

3. MINERALS

For layer breeder nutrition macro and microelements are very important for their growth, bone mineralization, reproductive performance, and health. Macro and micro elements in all breeders' diets have been shown to have significant effects on various aspects of breeder physiology and productivity. However, it difficult to identify the level of trace elements to be used in the diet of layer breeders since the most references do not give specific information on use of the elements in the diets of layer breeders. However, the relevant information should be sought from works or recommendations, which target only on the layer or broiler breeders. For layer breeder nutrition macro and microelements are very important for their growth, bone mineralization, reproductive performance, and health. Macro and micro elements in all breeders' diets have been shown to have significant effects on various aspects of breeder physiology and productivity. Saber and Kutlu (2019) reported that egg quality, yolk mineral content, and laying performance could be affected by the sources of their diet. Supplementation of minerals to layer diets might play a critical role in enzymatic systems enhancing antioxidant ability and immune response (Wang et al., 2020). The positive effects of breeders' diets including organic trace minerals lead to superior embryonic bone mineralization compared to inorganic trace minerals (Torres & Korver, 2018). Similarly, Güz et al. (2022) reported that using organic minerals in slow-growing broiler diets increased higher concentration

of iron (Fe) and selenium (Se) in eggs and offspring tissues. Additional higher hatchling body weight ($P=0.03$), advanced tibia development, greater thickness, and mineral density ($P=0.03$) were recorded in the slower-growing broiler offspring. However, these effects were not observed in fast-growing broiler offspring.

3.1. Calcium (Ca) and Phosphorus (P)

In chicken production, eggshell quality and the offspring's performance are greatly influenced by the amount of calcium (Ca) that layer breeders' diets include (Akinola & Obene, 2018). There is no specific recommended level of Ca in the diet of layer breeders by NRC (1994), it has been specified that the appropriate level of Ca is 3.5% for white egg layer. According to a review of research done by Roland (1986) covering the years 1975 to 1985, leghorn birds need closer to 4.1% of their diet in calcium. According to Berto et al. (2013), layer meals containing 3.7% calcium can achieve comparable outcomes to those advised with 3.1% calcium. Huang et al. (2016) suggested that local Linwu ducks needed different Ca levels ranging from 2.79 to 3.50% Ca in order to enhance their egg quality and laying performance. Similarly, Xia et al. (2015) reported that the Ca level for the optimal laying performance and bone quality in Longyan shelducks throughout the peak-laying phase could be 3.2-3.6%. Literature has shown that certain calcium content in the layers' diets affect blood uric acid level as well as uric acid/calcium ratio and bone quality (Berto et al., 2013).

The study by Santos et al. (2022), showed that eggs obtained from younger breeder flock at 32 weeks of age have a thicker egg shell and they are also richer in mineral content (calcium, phosphorus, zinc, potassium, copper, calcium manganese iron and magnesium) in yolk than a breeder flock at 42 and 52 weeks of age is significantly different ($p<0.05$). However, the deposition of these elements from yolk sac to freshly hatched chicks did not affect the age of the breeder ($p>0.05$). However, the transport of Ca from egg yolk via yolk sac membrane to progeny is dependent on age, suggesting a clear correlation between the traits of the breeder and the development of the offspring. However, breeder's age influences the deposition of Ca in eggshell (Rayan et al., 2022). Wang et al. (2014) looked at the effects of particle size (less than 0.1 mm versus 0.85 up to 2 mm) of calcium sources (limestone

versus oyster shell) on egg quality, bone characteristics, and laying performance in Longyan duck layers. The outcomes demonstrated that excellent eggshell and bone quality may be obtained by supplementing a diet with big particle sizes of limestone. Furthermore, studies have explored the effects of dietary supplementation with 25-hydroxycholecalciferol and canthaxanthin on the performance of duck breeders, showing impacts on egg quality and serum phosphorus levels (Ren et al., 2016). Commercial layers' eggshell quality has been found to improve when breeders get supplemental nutrients such as 25-hydroxycholecalciferol (Torres et al., 2009). Jiang et al. (2013) found that in contrast to the control group (3.7% Ca), layers fed with high levels of Ca (4.4%) were associated with a reduction in the quality (shell thickness) of eggshells. An et al. (2016) reported that seventy-week-old Hy-Line brown layers were given varying concentrations of calcium (3.5%, 3.8%, 4.1%, 4.4%, or 4.7%) for ten weeks. Egg weight, egg production, and feed consumption did not change significantly. However, there was a linear decline in the quantity of broken eggs as dietary calcium levels rose to 4.7%. ($p < 0.01$). Dietary Ca levels were observed to significantly and linearly ($p < 0.01$) enhance eggshell thickness and strength, which explains why tibial breaking strength increased considerably with Ca levels. The recommended calcium level in the diet of Lohmann Brown laying during the late phase of production (aged between 58 to 73 weeks) is typically studied within a range of 3.5%, 3.8%, 4.1%, 4.4%, or 4.7% Ca, for 10 weeks by Safaa et al. (2008). Results showed that when Ca levels increased to 4.7%, there was a linear decrease in the cracked eggs ($p < 0.01$) and a linear increase eggshell thickness and strength ($p < 0.01$). But the tibial breaking strength rose, somewhat when dietary Ca level increased.

Notably, the ration may disturb metabolism and affect breeder birds' health when calcium and phosphorus (P) levels are disproportionate. Various research has proven that the provision of Ca/P ration in the diet of breeder has the potential to influence bone mineralization as well as turn over which justifies the need to have the correct Ca/P ration in breeder diets. Furthermore, research has demonstrated that dietary supplementation with nano dicalcium phosphate can influence eggshell texture and the utilization of phosphorus and calcium in breeder hens (El-Sheikh & Abou-Elnaga, 2018). Higher

phosphorus content in eggshells has been associated with increasing calcium levels in layer diets (Akinola & Obene, 2018).

Pelicia et al. (2009) recommended Ca and P levels as 45 g/kg and 2.5 g/kg respectively, for optimal performance in brown egg layers. According to Pelicia et al. (2011), diets containing 3.75% calcium are required for sufficient egg production and blood levels of calcium. However, other research indicates that feeding layers diets containing as little as 2.8% calcium during a laying cycle have not demonstrated negative effects on performance (Berto et al., 2013). Although there are some reports on broiler breeders' P requirements (Harms et al., 1964; Wilson & Harms, 1984; Wilson et al., 1980) and several studies have been conducted on White Leghorn layers (Keshavarz, 2003; Keshavarz & Nakajima, 1993; Panda et al., 2005; Rao et al., 1999; Roush et al., 1986) to provide the ideal level of non-phytin phosphorus (NPP) in diet there are just two or three reports on related layer breeders. The P need for breeder hens has to be reexamined due to the elevated expense of P sources and public anxiety about the role that chicken excrement plays in environmental degradation. It has also been discovered that the proportion of calcium to accessible phosphorus in layer diets affects both productivity and egg quality (Pastore et al., 2012). According to Pastore et al. (2012), diets with 39 g/kg of calcium and a 12.12:1 calcium to phosphorus ratio, which translates to an increase of 3.51 g/bird/day in calcium and 289 mg/bird/day in available phosphorus, are sufficient to meet the needs of white egg layers during the 42–58 week age range. Hu et al. (2020) who found that a greater Ca-to-P ratio linearly reduced calcium and phosphorus digestibility, approved these results. According to El-Sheikh and Abou-Elnaga (2018), adding nano dicalcium Phosphate (NDCP) to a layers' diet could assist address issues with aged hen productivity or poor egg shells at the end of the egg-laying season. Up to 400 g/ton of NDCP supplementation, there was a considerable rise in serum Ca and P content, tibia Ca, tibia ash, tibia fresh weight, and P and Ca retention. After that, there was a tendency for these values to decrease. Additionally, when NDCP levels increased, there was a notable decline in the buildup of yolk mineral content (Ca and P).

3.2. Selenium (Se)

As it is already mentioned selenium is involved in spermatogenesis, and being a trace element it is necessary to add it to the diet of breeders to preserve the reproductive processes (Pardyak et al., 2022). Consequently, the deficiency of Se results in low sperm movement, sperm count and competence yet it raises sperm abnormality and impair reproductive capacity in male species (Rengaraj & Hong, 2015; Seema et al., 2007). Selenium and vitamin E combination increased Se accumulation in semen and tissues and also improved reproductive parameters in poultry (Bălăceanu et al., 2022) by reducing lipid peroxidation. Se plays a crucial role in testicular development (Jerysz & Lukaszewicz, 2013) protects sperm DNA against oxidative stress (Bălăceanu et al., 2022; Skoracka et al., 2020), and contributes to sperm chromatin stability (Conrad et al., 2005) which can enhance fertility by protecting cell from oxidation (Sun et al., 2023). Surai et al. (1998) examined the effects of adding vitamin E (20 or 200 mg/kg) and Se (0.3 mg/kg) to breeder diet. They found that these supplements boosted the glutathione peroxidase activity in sperm, semen, and testes.

The two forms of selenium sources are organic and inorganic. These sources are available as supplements for poultry diets. Dietary organic selenium has been shown to raise the selenium content of developing embryos (Paton et al., 2002) and eggs (Cantor et al., 2000; Payne et al., 2005). Therefore, organic Se sources have been shown to offer positive advantages over inorganic Se. In several studies, organic selenium has been shown to have better uptake in poultry and generally, lead to better growth rate, more efficient tissue distribution of selenium (Anizoba et al., 2024), and better antioxidant status as shown by enhancement of glutathione peroxidase activity in liver and serum (Li et al., 2018) compared to inorganic forms of selenium (Fisinin et al., 2008; Surai & Fisinin, 2014; Surai, 2006). Further, organic Se has been suggested to increase immune ability in poultry (Karadas et al., 2016) and it may increase resistance against regular viral and bacterial diseases (Abo-Al-Ela et al., 2021). For instance, Thanabalan and Kiarie (2021) observed that layer breeders fed with omega-3 PUFA improved progeny immunocompetence by the status of passive immunity and antibody titers from vaccination.

Recent research has shown that organic Se sources increased oxidative stability of chicken eggs and meat compared to organic sources. Supplementation of organic Se to layer's diet increased Se accumulation in eggs (Jlali et al., 2013; Tufarelli et al., 2016). This enhances internal egg quality (Hough unit) by activating methionine sulfoxide reductase B (MsrB), a selenium-containing protein that prevents protein oxidation and preserves albumen's ability to hold water (Surai & Fisinin, 2014). Reproductive performance and accumulation of Se in various tissues including eggs and developing embryos of broiler breeders positively influenced by Se supplementation (Yuan et al., 2014).

Egg hatch rates from chickens fed with less than 0.1 mg/kg Se-containing diet versus 0.5 mg/kg Se-containing diet did not differ, according to Pappas et al. (2006). However, Ort and Latshaw (1978) found that hatch rates were only lowered when selenite was added at extremely high concentrations. These findings are related to the hatch of viable eggs and are thought to be unaffected by any. Furthermore, Urso et al. (2015) investigated the effects of two selenium sources (zinc-L-selenomethionine and sodium selenite) and varying dietary levels of vitamin E (30 and 120 mg/kg) on the reproductive and productive performance of broiler breeder diets. Findings indicated that egg production was not affected by vitamin E concentration or selenium sources ($P>0.05$). These consists of organic treatment and 120 mg/kg vitamin E supplemented treatments, heavier eggs and albumen were reported. Based on the present study, breeders fed with 120 mg vitamin E/kg feed had better hatchability than the breeders which were fed with 30 mg of vitamin E at 29 weeks. Organic selenium supplementation increased hatchling weight till the peak of egg production (33 weeks), but it did not affect hatchling quality. Similarly supplement of organic Se to quail breeders feed has been shown to affect the accumulation of Se in tissues and it is transferred to the offspring (Surai, 2006).

Studies have shown that both breeder and their progeny selenium supplementation are vital for the best health and antioxidant defending of the progeny (Pappas et al., 2019) during incubation and early postnatal life (Surai, 2000; Surai et al., 2016; Wang et al., 2011). However maternal Se deficiency during incubation is associated with impaired fetal growth and altered offspring glucose metabolism, thyroid status, and skeletal muscle

development in sexually dimorphic manner (Hofstee et al., 2020). Se supplementation can have long-term effects on the growing embryo and the chicks that just hatched, impacting their growth and development (Surai et al., 2016). Gao et al. (2018) fed Ross 308 breeders of broilers at 24 weeks of age with different sources of 0 g/ton Se, 0.5 g/ton inorganic Se, and 0.5 g/ton organic Se for 8 weeks. Results confirmed that organic Se treatment had the highest Se accumulation in the albumen, egg yolk, and increased breast muscle weight, serum insulin-like growth factor-1 (IGF-1), and insulin content of offspring ($P<0.05$). However, the development of the skeletal muscles in the progeny after hatch was not significantly affected by either organic or inorganic selenium treatment. According to research by Xia et al. (2022), supplementing the diet with maternal selenium enhanced the body weight of progeny; however, diet-related selenium supplementation to both the mother and progeny had no impact on the body weight gain of ducklings after hatch, aged between 0 and 2 weeks. Supplementing duck breeders' diets with an extra 0.16 mg/kg of Se showed positive effects, especially on the antioxidant capacity of ducklings. Zhao et al. (2009) fed breeders with a basal diet consisting of maize and soybeans (0.13 mg/kg Se) supplied with 0.32, 0.40, or 0.54% methionine (Met) and 0, 0.30, or 0.60 mg/kg selenium from Sel-Plex for 70-d experiment period. The concentration of Se was raised, and the muscle lipids' oxidative stability was enhanced upon supplementing with Se or Met, according to the results. Se supplementation reduced the amount of Se deposition in the thigh of offspring hens fed a diet low in Met. The muscle lipids' oxidative stability was reduced by 0.30 mg/kg maternal dietary Se, while 0.6 mg/kg supplementation of Se did not vary from the control. Wang et al. (2011) compared 0.30 mg selenium/kg selenomethionine or sodium selenite in maternal diet. The results indicated that organic selenium improved hatchery performance and selenium accumulation in the tissues and serum of broiler stocks, as well as in the egg albumen, yolk, and tissues of 1-day-old chicks. Additionally, organic selenium raised the progeny's performance during the eight weeks following hatching, surpassing that of sodium selenite.

Recently, there has been increased interest in a novel type of selenium, known as nano mineral selenium, in poultry diets. Using various doses of selenium, 0.1, 0.2, and 0.3 mg of Nano-Se/kg diet and 0.3 mg of organic-Se/kg diet, were investigated respectively (Elfiky et al., 2021). Thus, the

results showed Nano-se supplemented hens to have a significantly better number and weight of eggs, or mass of all eggs along with a better feed conversion ratio if they were compared with the hens fed a control diet ($P \leq 0.05$). Therefore, the hot desert reared hens that received 0.2 mg of nano-selenium per kg of feed may be able to alleviate the severe effects of heat stress, thus improving the chances of laying good quality eggs, with efficiency contributing to economic profits while being relatively cheaper. In comparison to the control, the addition of source selenium considerably increased the shell thickness, egg width, yolk width, and yolk height ($P \leq 0.05$). Sayed (2018) studied the effects of feeding various selenium sources and levels to Silver Montazah parents raised in houses with open systems over the winter on cock fertility, hatching chick performance, and mortality percentage. The two selenium sources under investigation were sodium selenite (SS) and nano selenium (Nano-Se), with three additional levels of each source being 0.10, 0.25, and 0.40 mg/kg. The utilization of 0.25 mg/kg Se was found to improve overall semen quality, physiological parameters, hatchability, and chick performance; the use of Nano Se enhanced the growth performance, volume of sperm ejaculate, and sperm motility of hatched chicks throughout the initial two weeks of life, as well as their dressing percentage and packed cell volume (%) at eight weeks of age. All experiment treatments combined yielded excellent results, with Nano-Se at 0.25 mg/kg diet having the greatest effect on cocks' overall.

3.3. Zinc (Zn)

In all biological systems, including human and animal systems, zinc is one of the important like other trace element. It is vital to several bodily metabolic functions (Frassinetti et al., 2006), and for hens, it is essential for the development of eggshells. Zinc influences the mechanical and ultra-structure characteristics of the shell and has a role in calcium deposition (Li et al., 2019; Min, Liu, et al., 2018) as a catalyst for the hydration of metabolic carbon dioxide to hydrogen carbonate and for providing an initial form of eggshell carbonate as a co-factor of carbonic anhydrase (Zhang et al., 2017). Therefore, using inorganic zinc in diet even can increase shell thickness but cannot improve breaking strength in contrast to organic form of Zn (Zhang et al., 2022)

Zhang et al. (2020) examined the effects of varying dietary zinc ($\text{ZnSO}_4\cdot\text{H}_2\text{O}$) levels on laying duck breeders' plasma antioxidant indices and biochemical, egg quality, tibial features, productive and reproductive performance, and zinc deposition. Breeders of Longyan ducks, aged 21 weeks, were randomly assigned to six groups and fed either a basal diet (containing 27.7 mg/kg of zinc) or a basal diet supplemented with zinc (as $\text{ZnSO}_4\cdot\text{H}_2\text{O}$) at feed weights of 10, 20, 40, 80, or 160 mg/kg Zn over 20 weeks. According to their findings, layer duck breeders' zinc depositing, tibial features, eggshell thickness, antioxidant levels of plasma, and egg production performance were all affected by dietary zinc supplements up to 160 ppm diet. Additionally, they stated that for laying duck breeders, supplementing the base diet (27.7 mg/kg Zn) with an additional 70 to 80 mg/kg Zn was sufficient during the laying phase.

Zinc-methionine supplementation in broiler breeder diets improved cellular immunity in progeny, indicating the positive effects of specific zinc sources (Moghaddam & Jahanian, 2009). This is consistent with the findings of, who demonstrated that supplementing broiler breeder diets with zinc-methionine enhanced the cellular immune response of progeny (Soni et al., 2013). Zinc oxide (ZnO) nanoparticles showed significant antibacterial efficacy against strains of bacteria including *S. aureus*, *K. pneumoniae*, *E. coli*, and *S. aeruginosa*. In the cytotoxicity investigation, there was a dose-dependent, substantial reduction in cell viability. Based on the observed cytotoxicity at the prescribed dose and the broad-spectrum antibacterial activity, it may be inferred that nano ZnO powder presents a viable alternative zinc supplement for animals (Geetha et al., 2020). A study by Abedini et al. (2018) explored the effect of dietary supplementation with zinc oxide nanoparticles (ZnO -NPs) on a range of aspects connected with layer performance, such as egg quality, Zn concentration, immunity response, superoxide dismutase activity, malondialdehyde content in eggs and serum parameters. They reported that zinc oxide nanoparticles can improve zinc absorption in the elderly layers' intestinal tract, making them a more effective source of zinc compared to regular zinc oxide in feed. Bahakaim et al. (2014) investigated the effects of various zinc sources, including organic zinc (Zn-Met) and inorganic zinc ($\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$), at varying levels (0, 50, 100, and 150 mg/kg Zn supplemental diet), and their interactions with regard to egg zinc

concentration, laying hens' blood parameters, egg quality, and productive performance. From the findings 0 to 150 mg/kg levels of Zn increased the concentration of egg zinc without affecting negatively on egg production whether organic or inorganic. The highest amount of zinc was found in eggs laid by layers fed food containing 150 mg/kg diet zinc as an organic supplement. The egg production was greatly boosted when the organic form was added to laying hens' diet.

The addition of zinc to layer diets enhances the content of copper-zinc superoxide dismutase, which removes radical superoxide and inhibits reduced glutathione's auto-oxidation (Abd El-Hack et al., 2018). According to Wang et al. (2022), provision of zinc-glycine to maternal diet can improve productivity among her offspring broiler breeders. This means that it enhances the growth rate, decrease mortality rates and reduce stress level amongst their children which is achieved through raising their Zinc level in body system. Hence, this research suggests how maternal zinc supplementation could be useful for maximizing progeny performance and health among producers.

4. CONCLUSION

More research and literature review on recent developments in poultry nutrition and supplementation practices would be needed to answer the specific query regarding whether mineral and vitamin supplementation based on National Research Council (NRC) recommendations has changed over time. The comparison of historical NRC guidelines with current supplementation practices could help in understanding how mineral and vitamin supplementation strategies have evolved in poultry production.

Mineral and vitamin nutrition is vital in appropriate embryonic development and offspring performance as well as optimizing egg production efficiency for layer breeders. The supplementation of bio-available minerals at proper levels is necessary to preserve the nutritional status of minerals of layer breeders throughout their life stages. There are positive effects of high-dose microelement supplementation in modern layers facing different stresses in commercial production. Additionally, the use of organic mineral sources and phytase enzymes can help reduce antagonistic interactions between minerals and anti-nutritional factors in layer feed formulations.

Establishing accurate databases of mineral requirements for specific layer genotypes under various rearing factors, such as environmental stressors and diets enhanced with phytase, vitamin D, and organic sources, is crucial for optimizing mineral nutrition in layer breeders' production.

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CHAPTER 5

MINERALS IN BROILER NUTRITION

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INTRODUCTION

Nutraceuticals are dietary constituents that hold both nutritional and pharmaceutical significance, contributing to disease prevention, increased productivity, immunomodulation, and health improvement Dhama et al., 2015; Aronson, 2017; Helal et al., 2019; Waheed Janabi et al., 2020). These encompass a wide range of nutrients and non-nutrients such as minerals, vitamins, enzymes, amino acids, fatty acids, organic acids, prebiotics, probiotics, synbiotics, pigments, medicinal herbs, herbal extracts, flavoring agents and antioxidants (Narahari, 2014; Alagawany et al., 2018a; Elgeddawy et al., 2020). Minerals, an essential category of nutrients, are pivotal for various biochemical and physiological functions, facilitating optimal growth, production, and reproduction in animals (Park et al., 2004). They play crucial roles in metabolism, acting as enzyme cofactors and regulating free radicals within the body (Goff, 2018). Integral to our diet, minerals support bone formation, muscle and nerve function, acid-base balance, and osmotic homeostasis (sodium (Na), potassium (K), and chloride (Cl)) (Kim et al., 2013; Ravindran, 2010). Additionally, they are constituents of hormones, enzymes, and other biologically active compounds, contributing significantly to the immune system's functionality. Adequate mineral supply can modulate infection susceptibility and influence chronic disease development (Weyh et al., 2022). The bioavailability of minerals in feeds is generally low, necessitating substantial supplementation to meet cellular requirements (Ghosh et al., 2016; Suttle, 2010). Ensuring comprehensive mineral nutrition is crucial for broiler chicken health, particularly for maintaining skeletal integrity (Kleyn & Ciacciariello, 2021). Unlike inorganic minerals, organic minerals are required in smaller quantities in poultry diets and are more effectively assimilated (Nollet et al., 2007; Ravindran, 2010). Similarly, compared to coarse minerals, mineral nanoparticles have higher accumulation capacities in the animal tissues, and are generally absorbed more efficiently from the gut system, and exhibit pronounced biological effects in the target. These nanoparticles are effectively taken up through the gastrointestinal system and subsequently transported via the bloodstream to essential organs. They possess low toxicity and exceptional bioavailability, which contributes to improved growth performance and nutritional value (Ahmad et al., 2022).

Poultry diets must include both macro and trace elements. Macro-minerals like calcium (Ca) and phosphorus (P) are the most prevalent, with others such as chloride (Cl), magnesium (Mg), potassium (K), sodium (Na), and sulfur (S) also necessary in amounts exceeding 100mg per kilogram of feed (Ravindran, 2010). Microminerals include zinc (Zn), manganese (Mn), selenium (Se), copper (Cu), and iron (Fe), and these are crucial for metabolic pathways, enzyme cofactors, and larger molecular structures, required in trace amounts (about 0.01%) (Faria et al., 2020; Ravindran, 2010). These minerals are indispensable for growth performance, immune system, reproduction, immune function, energy metabolism, and bone growth and development (Bao et al., 2007; Dibner et al., 2007).

This chapter aims to elucidate the mineral requirements in broiler chicken nutrition and their impact on broiler performance.

MINERAL CLASSES

There are 118 experimentally known elements, with ninety-two occurring naturally, ranging from hydrogen (1) to uranium (92) (Schwerdtfeger et al., 2020; Scerry, 2013). Of these 118 elements, 26 are deemed essential for animals (Underwood, 1977). The essential elements include 11 macro elements: carbon, hydrogen, oxygen, nitrogen, sulfur, calcium, phosphorus, potassium, sodium, chlorine, and magnesium, and 15 microelements: iron, zinc, copper, manganese, nickel, cobalt, molybdenum, selenium, chromium, iodine, fluorine, tin, silicon, vanadium, and arsenic (Vieira, 2008).

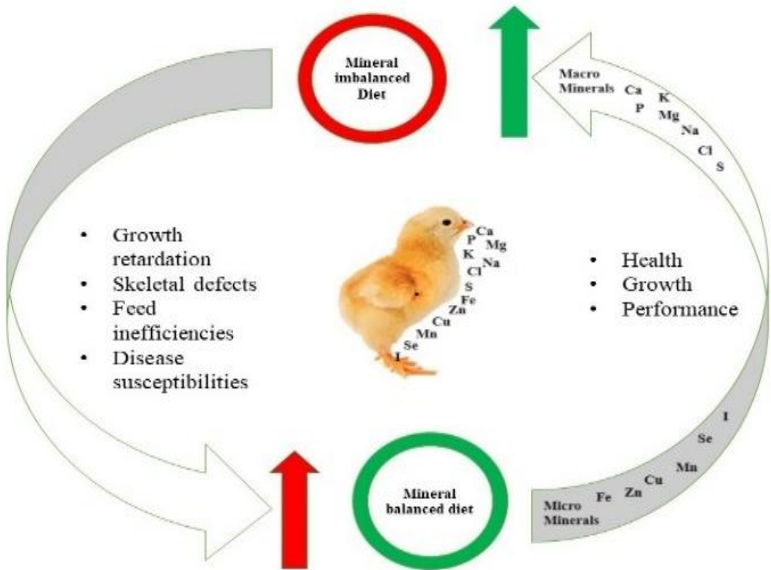


Figure 1. Shows macro and micro-minerals effects in broiler.

Difference between macro and micro minerals

Minerals are indispensable for various physiological functions in animals, constituting approximately 3.5% of an animal's body weight (McDowell, 2003). Macroelements, typically expressed as a percentage of feeds or tissues, predominantly participate in structural roles. In contrast, microelements, or trace minerals, are present in very low concentrations in animal tissues, making their analysis challenging due to equipment and technique limitations. Despite their low concentrations, microelements perform diverse and essential functions (Vieira, 2008). Macrominerals are required at levels exceeding 100 mg/kg, whereas trace elements are found in concentrations not greater than 50 mg/kg and are needed at less than 100 mg/kg in the diet (McDonald et al., 2010). Table 1 highlights the essential mineral elements and their approximate concentrations in the animal body.

Table 1. Essential minerals and their approximate concentration in the animals (McDonald et al., 2010)

Major elements	g/kg	Trace elements	mg/kg
Ca	15	Fe	20-80
P	10	Zn	10-50
K	2	Cu	1-5
Na	1.6	Mb	1-4
Cl	1.1	Se	1-2
S	1.5	I	0.3-0.6
Mg	400	Mn	0.2-0.5
		Co	0.02-0.1

MACRO-MINERALS

CALCIUM AND PHOSPHORUS

Calcium (Ca) and phosphorus (P) are principal macroelements in living organisms, primarily serving as structural materials. Notably, 99% of the body's calcium is stored in the skeleton structure, where, along with phosphorus, it forms hydroxyapatite (calcium phosphate) (Bello et al., 2014). The skeleton not only supports the body and maintains its shape but also protects delicate internal organs. Additionally, the skeleton, along with attached muscles, forms the locomotor system, enabling movement and indicating growth potential (Suttle, 2010). A smaller fraction of calcium (1%) is found outside the skeletal system, playing vital roles in various physiological processes, including blood coagulation, biomolecular adhesion, cell proliferation, nervous signal transmission, and muscle contraction (Brini et al., 2013). Calcium is crucial for enzyme activities that hydrolyze polysaccharides, phospholipids, and proteins, affecting cytoplasm viscosity, membrane permeability, hormone secretion, and cell apoptosis (Matuszewski et al., 2020). Phosphorus, predominantly found in bones (80%), also has critical biological functions. It is involved in glucogenesis, transport of fatty acids, amino acids, proteins, and is a constituent of nucleic acids essential for cell differentiation and growth. Phospholipids, which contribute to cell membrane fluidity and integrity, also play a role in maintaining osmotic and

acid–base balance (Mykytczuk et al., 2007; Suttle, 2010). Additionally, phosphorus is involved in energy metabolism, particularly in the functions of adenosine monophosphate (AMP), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) (Adeola et al., 2005). The bone undergoes continuous changes as calcium and phosphorus are resorbed into the bloodstream and other tissues. This dynamic exchange is particularly significant in high-producing animals like dairy cows and laying hens, which have substantially higher calcium demands (McDonald et al., 1995). The metabolism of calcium and phosphorus is regulated by hormones, primarily parathyroid hormone (PTH) and calcitonin. PTH, secreted during hypocalcemia, increases calcium release from bones and renal reabsorption, while calcitonin inhibits osteoclast activity, reducing bone resorption and calcium release (Frandsen & Spurgeon, 1992; Koreleski & Świątkiewicz, 2005). Recent research also highlights the roles of fibroblast growth factor 23 (FGF23) and Klotho proteins in calcium and phosphorus metabolism (Kuo-o, 2006; Li et al., 2017b; Erben, 2018).

Calcium and phosphorus requirements for broiler chickens

Calcium (Ca) is vital for skeletal development and numerous physiological functions in broilers (David et al., 2023). Besides its skeletal role, calcium in extracellular fluid, plasma, and within cells is crucial for metabolism, blood clotting, enzyme activation, neuromuscular function, muscle contraction, cell adhesion, and intracellular signaling (Veum, 2010). The Nutrient Requirements of Poultry (NRC, 1994) established essential dietary calcium and phosphorus levels, which are often supplemented in broiler diets to enhance bone mineralization and prevent skeletal diseases (Waldenstedt, 2006). The interdependence of calcium and phosphorus necessitates their combined consideration in dietary formulations. An imbalance in one can lead to a deficiency or excess of the other (Al. Masri, 1995). Excess calcium can form insoluble calcium phosphates in the intestines, causing phosphorus deficiency (Sobhi et al., 2020). Broiler chickens require more calcium for mineralization than for weight gain, with an optimal calcium-to-phosphorus ratio generally set between 1:1 and 2:1 (Bar et al., 2003). This ratio can vary with chicken age and genotype, with higher ratios recommended for fast-growing breeds (Williams et al., 2000).

Calcium and phosphorus requirements differ with age. For instance, broiler chickens up to six weeks old require 1.04% calcium and 0.36% available phosphorus, whereas older birds need higher levels (Huyghebaert, 1996; Rama Rao et al., 1999; Bar et al., 2003). Recent studies suggest that 6.5 g/kg of calcium and 3.5 g/kg of phosphorus in starter feed mixtures without phytase supplementation are suitable for young broilers, with requirements decreasing slightly as they age (Li et al., 2017a). Phytase supplementation can enhance performance by improving phosphorus utilization, particularly at lower phosphorus levels. Vitamin D3 (cholecalciferol) supplementation is essential for optimal calcium and phosphorus absorption and bone mineralization (Driver et al., 2005; Kasim et al., 2006). Vitamin D3 stimulates the production of calcium-binding proteins in the mucosa, facilitating calcium absorption and enhancing plasma calcium levels, which are crucial for various biological processes (Santos, 2006). Establishing a precise calcium-to-phosphorus ratio remains complex due to their varied metabolic effects.

Sources and availability of Ca and P

Among plant sources, few are rich in calcium, with extracted rapeseed meal being an exception, containing approximately 0.65% Ca (Nwokolo & Bragg, 1980). However, 20-30% of the calcium in plants is in the form of oxalates, which are not absorbable by birds (Francesci & Nakata, 2005). Another limitation is phytic acid, predominantly found in seeds, which hinders calcium absorption. Hence, poultry diets require supplementation with inorganic calcium sources, such as limestone (about 97% calcium carbonate, CaCO_3) and mono- and di-calcium phosphates, which also provide phosphorus (Walk et al., 2012). Limestone, commonly used in poultry feed, has a smooth texture and is available in fine and coarse grains, with coarse grains being preferred. Organic calcium sources, such as disintegrated oyster shells, are highly absorbable, with almost 100% absorption in birds. Other organic sources include snails and clam shells (Ajakaiye et al., 2003; Rama Rao et al., 2006; Oso et al., 2011) and seaweed (Bradbury et al., 2016a). Phosphorus is primarily sourced from cereal seeds and by-products of oil and cereal processing. High phosphorus content is found in extracted soybean or rapeseed meals, approximately 4-15 g/kg dry matter (Jeroch, 1994). In feed materials, 50-90% of phosphorus is in phytate form (Oloffs et al., 2000),

necessitating the presence of the enzyme phytase for its release and absorption. Exogenous dietary phytase enhances growth performance, bone parameters, and phosphorus retention in broilers, even with low-phosphorus diets (Baradaran et al., 2017). Phytase increases phosphorus availability for biochemical functions (Abd El-Hack et al., 2018a).

Phosphorus plays a pivotal role in both soft and hard tissues (Underwood & Suttle, 1999). Rao et al. (2006) noted that calcium and phosphorus often co-exist in biological functions, but their dietary requirements are interdependent. Phytase enzyme, essential for hydrolyzing phytic compounds in the gastrointestinal tract, is produced in limited amounts endogenously by chickens (Maenz & Classen, 1998). Cereals like rye, triticale, and wheat contain phytase, though their activity varies by species and processing treatments (Cossa et al., 1999; Oloffs et al., 2000). Consequently, commercial phytases derived from *Aspergillus* fungi or bacteria have been utilized in poultry feed for years (Jayaprakash et al., 2016). Inorganic phosphates, such as mono- and di-calcium, defluorinated, or magnesium phosphates, are common supplements (Applegate & Angel, 2008). Monocalcium phosphate contains 22.7% Ca and 18% P by weight, while dicalcium phosphate contains 18% P and 24% Ca in its hydrated form, and 20-21% P and 25-29% Ca in its anhydrous form (Grochowicz, 1996). However, the use of phosphates is declining due to environmental concerns, such as eutrophication, and the increased use of phytase to improve phosphorus absorption from plants (Ptak et al., 2013; Valable et al., 2018).

Calcium solubility and particle size

The solubility of calcium in the acidic environment of the gastrointestinal tract (GIT) is critical for absorption. In the acidic medium of upper GIT, calcium carbonate (limestone) is approximately 80% soluble and absorbable (Walk et al., 2012). This statement presents that calcium solubility is more effective in the proventriculus and ventriculus (pH-1.5-4) as compared to the higher pH of the small intestine (Svihus, 2011). The particle size of calcium sources also affects absorption. Early studies primarily on laying hens (Scott et al., 1971; Rao & Roland, 1990; Skrivan et al., 2010) showed that calcium solubility in chickens is influenced by its interaction with phytate phosphorus. Manangi and Coon (2007) reported that chickens fed

with phytase could absorb calcium more effectively from larger limestone particles, which dissolve less readily in the crop and upper gastrointestinal tract. This slower dissolution may enhance phytate phosphorus hydrolysis by phytase in the intestines. Conversely, smaller calcium particles dissolve more readily throughout the gastrointestinal tract, potentially forming mineral-phytate complexes that inhibit phytase activity. A study with broilers demonstrated that medium-sized calcium particles (0.25-0.85 mm) yielded better bone ash content than larger particles (2.36-3.35 mm; McNaughton et al., 1974). Another study found that medium-sized calcium carbonate particles (0.137-0.388 mm) resulted in higher final body weights and tibia ash content compared to smaller or larger particles (Manangi & Coon, 2007). Other research suggests that highly soluble calcium sources improve production parameters and bone mineralization (Walk et al., 2012).

Optimal calcium and phosphorus ratios

The optimal calcium-to-phosphorus ratio in broiler feed is 2:1 due to strong interactions between these elements (Coto et al., 2008). Highly soluble calcium sources and phytase supplementation can reduce the need for additional calcium. Bintvihok and Kositcharoenkul (2006) noted that dietary calcium addition positively affects body weight gain (BWG). Driver et al. (2006) reported that a diet containing 0.80% calcium improved both the quantity and quality of poultry carcasses. A deficiency of calcium ions in bones leads to skeletal deterioration and reduced bone strength (Kwiatkowska et al., 2017). In conclusion, the optimal particle size of calcium sources for broiler chickens varies with age, but the source and concentration of calcium have a lesser effect on production results than ensuring adequate phosphorus levels and effective phytase supplementation.

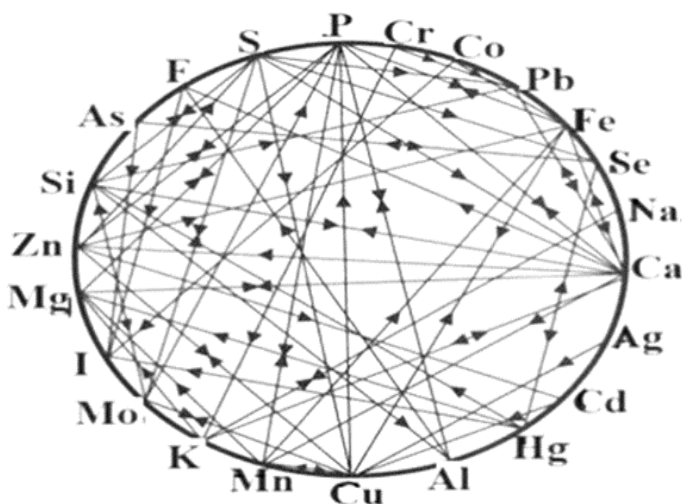


Figure 2. Minerals interactions (Vieira, 2008).

- * Mineral synergy is represented by arrows pointing in the same direction.
- * Mineral antagonism is represented by arrows pointing in the opposite direction.

Calcium and phosphorus deficiency

Dietary deficiencies of calcium (Ca) and phosphorus (P) can severely impair bone formation, leading to skeletal abnormalities and the presence of bloody meat during carcass processing (Chen & Moran, 1995). An imbalance between these minerals can result in rickets in growing birds and osteomalacia or osteoporosis in mature animals, presenting symptoms such as paralysis, fractures, and skeletal deformities. Conversely, an excess of calcium can cause nephropathy, characterized by renal fibrosis, atrophy, and visceral gout. Additionally, excessive phosphorus intake can increase bone fragility in birds (Klasing, 2013).

MAGNESIUM

Magnesium (Mg) is frequently employed as a laxative in humans (Schiller, 2001) and as an osmotic agent in animal diarrhea models, with supplementation of 2 g·kg⁻¹ magnesium sulfate (MgSO₄) consistently inducing diarrhea in rats (Antonisamy et al., 2009; Ikarashi et al., 2011). As a vital dietary element, magnesium was first reported as essential for animal growth and survival by Kruse et al. (1932). Magnesium is the fourth most abundant cation in living organisms (Wolf & Cittadini, 2003), second to

calcium in bone tissues (Morii, 2007), and to potassium in soft tissues (Suttle, 2010). The deficiency of magnesium can lead to remarkable biochemical and symptomatic changes (Coudray et al., 2005), with symptoms described in laying hens (Cox & Sell, 1967), growing chicks (Shastak & Rodehutsord, 2015)), and ducks (Van Reen & Pearson, 1953). Magnesium deficiency in growing poultry birds is indicated by stunted and poor growth, shoddy feathering, reduced muscle tone, incoordination, tremors, convulsions, coma, and death (Shastak & Rodehutsord, 2015). Magnesium is embroiled in the metabolism of sugars, fats, amino acids, bone calcium, as well as vitamin D metabolism (Morii, 2007a). Common feedstuffs provide sufficient magnesium to encounter the requirements of birds and make deficiency of Mg rare, which is why research on magnesium metabolism in poultry is limited (Suttle, 2010). However, recent studies suggest that supplemental magnesium can positively affect meat quality and growth and prevent heat stress-induced oxidative damage in poultry under certain conditions (Guo et al., 2003; Gaal et al., 2004; Sahin et al., 2005; Yang et al., 2012). Maintaining a balanced ratio of calcium, magnesium, and phosphorus is indispensable for normal body function and growth (Haag & Palmer, 1928). Determining the phosphorous requirements becomes complicated if there is a failure to monitor the magnesium requirements of poultry birds (Lee & Britton, 1980). Calcium, magnesium, phosphorus, and vitamin D are critical for bone development and cellular energy metabolism. These nutrients not only support bone health but also optimize cardiac, respiratory, and neurological performance (Taylor, 2021). Calcium is crucial for cytoskeletal stability and intracellular enzyme regulation, playing a role in neuronal conduction through ion channels (Beto, 2015). Magnesium is essential for muscle contraction, blood pressure regulation, insulin metabolism, cardiac excitability, vasomotor tone, nerve transmission, and neuromuscular conduction (Reddy et al., 2018). Its roles as a natural calcium antagonist, N-methyl-D- aspartate (NMDA) receptor blocker, vasodilator, antioxidant, and anti-inflammatory agent offer therapeutic benefits (Mathew & Panonnummal, 2021). Abnormal magnesium levels can affect DNA/RNA stabilization, enzyme activities, ion channel function, and cell protection against oxidative stress, leading to pathological conditions (Zhu et al., 2016).

Dietary magnesium supplementation, though uncommon in practical farming, can enhance hepatic catalase activity, reduce lipid and muscle tissue peroxidation, and improve meat quality (Liu et al., 2007; Guo et al., 2003). However, high magnesium levels can negatively impact bone calcification (Atteh & Leeson, 1983) and induce diarrhea (Lee & Britton, 1987) in broilers. A study conducted by Van der Hoeven-Hangoor et al. (2013) presented that adding magnesium to broiler diets increased digesta and excreta moisture content in a linear manner, with the highest increase observed for magnesium chloride, followed by magnesium sulfate and magnesium oxide.

Magnesium sources

Magnesium salts incorporated into diets can be categorized as either organic or inorganic. Organic salts include magnesium acetate, aspartate, citrate, gluconate, lactate, and pidolate, whereas inorganic salts comprise magnesium chloride (MgCl_2), magnesium carbonate (MgCO_3), magnesium oxide (MgO), and magnesium sulfate (MgSO_4) (Maier et al., 2020).

Absorption and excretion of magnesium

Magnesium absorption takes place through the intestinal epithelium by passive diffusion, active transport, and solvent drag mechanisms (Kimura, 2007). Studies on rats have shown that the transport mechanism transitions from primarily passive in juvenile animals to carrier-mediated in adults (Meneely et al., 1982). Moreover, cholecalciferol and its metabolites help to enhance the absorption of magnesium due to the chemical similarity between magnesium and calcium (Kimura, 2007). Despite this, there is no substantial evidence that the intestine adapts to prolonged low or high magnesium intake, unlike calcium (Fine et al., 1991). In mature chickens, dietary magnesium absorption predominantly takes place in the duodenum (34%), followed by the ileum (16%) and colon (13%), and a lesser percentage (3%) is absorbed in the jejunum (Guenter & Sell, 1973). Endogenous magnesium excretion occurs via urine and feces, with urinary excretion being the primary regulator of magnesium homeostasis in the body (Taylor & Kirkley, 1967; Kimura, 2007). Magnesium secretion into the intestine primarily occurs in the duodenum and upper ileum, with additional secretion in the rectum (Guenter & Sell, 1973; Van der Klis et al., 1990).

Interaction with calcium and phosphorus

Magnesium metabolism is closely linked with calcium and phosphorus metabolism (McDonald et al., 2011). Shastak and Rodehutsord (2015) observed that the utilization of calcium and phosphorus compounds is significantly influenced by their relative ratios to magnesium in the diet. The intake of soluble magnesium increases calcium excretion in adult animals and inhibits its deposition in younger ones (Malcolm, 1905). Buckner et al. (1932) stated that the addition of Mg during the first six weeks of life of chicks can lead to disruption of the balance of Ca and P that is necessary for normal. Nugara and Edwards (1963) demonstrated that an increment in dietary Ca and P content enhanced the requirement of Mg for optimal body weight gain. Supplementation of commercial diets for chickens under three weeks of age with magnesium can result in malformation of the leg bone and porosis-like symptoms (Gaal et al., 2004). Elevated dietary magnesium levels can impede calcification, increase leg issues (Atteh & Leeson, 1983), and cause reduced bone ash content in chicks (Nugara & Edwards, 1963). The decrease in bone ash content may result from excessive cartilage proliferation, increased osteoclastic resorption, or insufficient mineralization (Lee et al., 1980). Furthermore, higher Mg consumption results in decreased phosphorus absorption, perhaps due to the formation of insoluble magnesium-phosphate complexes (Fine et al., 1991; Kimura, 2007).

SODIUM

Dietary sodium (Na), primarily consumed as sodium chloride (NaCl), is crucial for numerous physiological processes, including electrolyte homeostasis, nutrient absorption, and maintenance of plasma volume, acid-base balance, nerve impulse transmission, and normal cell physiology. Sodium is pivotal for establishing membrane potential in most cells and is directly involved in action potentials required for nerve transmission and muscle contraction (Kaushik et al., 2018). It is the predominant cation in extracellular fluid, essential for the life cycle and metabolism of both plants and animals, making up about 93% of the total cation content in blood plasma (Leeson & Summers, 2001). Its significance has been recognized since 1881. In fast-growing chickens, adequate sodium intake enhances feed consumption and growth rate (Borges et al., 2004), while excessive sodium and chloride

levels increase litter moisture (Vieira et al., 2003). Studies indicate that increasing dietary sodium to 2-3 g/kg improves bird growth performance beyond NRC (1994) recommendations (Oviedo-Rondon et al., 2001; Mushtaq et al., 2007), and a dietary sodium level of 3 g/kg improves breast muscle yield and reduces abdominal fat deposition (Mushtaq et al., 2005).

Physiological role of sodium

Sodium plays multiple roles: it maintains acid-base balance and optimal osmotic relationships, participates in regulating body fluid volume and muscle contractions, is closely related to adrenal gland functions, and aids in carbohydrate absorption and energy turnover. Sodium content in poultry ranges from 0.1 to 0.14% of body mass, with 30-40% bound in the skeleton, rendering it less available for immediate needs. It is mainly found in blood plasma, maintaining pH, with chicken blood plasma containing 8.4 mg/ml sodium (Leeson & Summers, 2001).

Sodium deficiency

Sodium deficiency disrupts osmotic pressure and acid-base balance. Severe deficiency symptoms include heart failure, low blood pressure, and increased hematocrit, decreased subcutaneous tissue elasticity, and impaired adrenal function, leading to elevated uric acid and potential shock or death. In chickens, mild deficiency results in poor growth, soft bones, corneal keratinization, and reduced plasma volume. Laying hens show decreased egg production, impaired growth, and sometimes cannibalism. Sodium-deficient poultry diets contain less than 0.012–0.050% sodium (Puls, 1990). Sodium is the primary limiting mineral in salt-deficient diets due to its lower concentration compared to chlorine (Andriguetto et al., 1990).

Excess sodium

Sodium levels above 0.5% in poultry feed are toxic. Even 0.35% sodium stimulates increased water intake, causing electrolyte imbalance and water toxicity (Puls, 1990; Leeson & Summers, 2001). Ducks and muscovy ducks are particularly sensitive to sodium deficiency, which can cause over 60% mortality when levels fall below the recommended minimum (Dean et al., 1973). High dietary sodium intake leads to increased water consumption

and litter moisture, raising disease risks such as foot pad dermatitis (FPD) (Juśkiewicz et al., 2009). Consequently, the German Society of Nutrition Physiology (GFE, 1999) recommends lower sodium intake levels of 1.3, 1.1, and 0.9 g/kg for successive growth stages, much lower than NRC (1994) and other recommendations (Smulikowska & Rutkowski, 2005; Oviedo-Rondon et al., 2001; Mushtaq et al., 2007).

Sodium requirements for poultry

For young birds, sodium requirements are 0.20% (NRC). Murakami et al. (1997) recommend 0.25% sodium for 21-day broilers. A linear relationship exists between growth rate and dietary sodium level in young poultry, with the limit often determined by manure consistency.

POTASSIUM

Potassium (K) is the principal intracellular cation within body tissues and is the third most abundant mineral element in the body (NRC, 2001). Unlike sodium, potassium is primarily found inside cells. Potassium levels in blood cells are about 25 times higher than in plasma cells, with high concentrations particularly in muscle and nerve cells—approximately 4 mg/kg in muscles compared to 0.1 mg/ml in blood plasma (Leeson & Summers, 2001).

Physiological role of potassium

The functional role of potassium is similar to that of sodium, but it operates inside the cell. Key roles of potassium include: i) Maintaining acid-base balance and optimal osmotic relationships, ii) Activating various intracellular enzymes, iii) Participating in protein and carbohydrate metabolism., iv) Ensuring normal heart function by decreasing cardiac muscle contractility and promoting heart muscle relaxation, v) Increasing cell membrane permeability, vi) Promoting the absorption of free neutral amino acids, such as glycine (NRC, 2001; Harrison et al., 2011; Tuckers et al., 1991).

Potassium deficiency and excess

Potassium deficiency, or hypokalemia, manifests as generalized muscle weakness, decreased intestinal tonus with distension, cardiac insufficiency, and respiratory failure. Hypokalemia can result from severe stress, which increases plasma proteins and triggers adrenaline-mediated renal excretion of potassium (Castro & Sharma, 2024). During stress adaptation, blood flow to muscles improves, restoring lost potassium (Baloš et al., 2016). Drinking water with 0.9% KCl can cause severe hyperkalemia, marked increases in Ca^{2+} , Cl, Na, osmolality, and significant metabolic acidosis, especially in younger birds (Ait-Boulahsen et al., 1995). Higher potassium concentrations beyond NRC requirements can lead to hyperkalemia due to reduced renal excretion or excessive diffusion from cells to extracellular fluid (Zarrin-Kavyani et al., 2018).

Potassium requirements for poultry

The NRC (1994) recommends 0.3% potassium for broilers at different life stages. Potassium deficiency is rare in practice as standard poultry feed mixes usually contain over 1% potassium, with feed below 0.1% potassium considered deficient (Puls, 1990). Potassium content in feed should range between 0.4% and 0.6%. Nutritional needs vary with the poultry's age, and requirements increase by 63% when ambient temperature rises from 24°C to 35°C (Baloš et al., 2016; Puls, 1990).

CHLORIDES

Chlorides (Cl^-), primarily found in extracellular fluids, are also present in red blood cells and other tissues. These elements are absorbed in the small intestine, with excess chloride excreted in the urine, often associated with sodium and potassium.

Physiological role of chlorides

While closely linked with sodium, chlorides have distinct functions (Baloš et al., 2016):

i) Chlorine, as the major anion of gastric juice, combines with hydrogen ions to form gastric acid.

ii) It participates in carbon dioxide transport in the blood, increasing plasma bicarbonate content.

Chloride metabolism and deficiency

Chloride ions have a weak affinity for binding to protein ions and enter cells alongside potassium. They are actively transported, particularly via gastric mucosa cells. Chloride deficiency in chickens is associated with poor growth, limb weakness, poor bone mineralization, high mortality rate, dehydration, reduced blood chloride levels, and hemoconcentration (Leach & Nesheim, 1963). Chlorine-deficient chicks exhibit nervous conditions resembling tetany and may fall forward with legs extended backward when startled by a sharp noise (NRC, 1994). Feeds with less than 0.05% sodium chloride (0.03% chlorine) are considered deficient, a condition more common in herbivores due to the low salt content in forage and grains.

Chloride requirements for poultry

Chloride levels in poultry diets must be balanced with sodium and potassium levels. Typically, chloride concentration in feed should be 10-15% higher than sodium. The NRC (1994) states that optimal chlorine levels for maximal growth rate in broiler chickens are 0.12%-0.20%, and for turkey poults, 0.12%-0.15%. Puls (1990) suggests optimal levels of 0.30% for chickens (ranging from 0.20% to 0.40%) and 0.25% for turkey poults. Oviedo-Rondon et al. (2001) found that the Na^+ and Cl^- requirements for optimal performance in young broilers are 0.28% and 0.25%, respectively. Practically, a level of 0.2% chloride is recommended (Teeter & Wiernusz, 2003). An optimal Na/Cl ratio of 1:1 (w/w), corresponding to 1.5:1 on a molar basis, supports chick growth, with a dietary requirement of about 0.13% for each mineral, and there is a need to reevaluate the Cl requirements to avoid excess of dietary Cl (Hurwitz et al., 1974; Zhang et al., 2022)

Electrolyte balance

When devising diet plans for poultry birds, it is possible to avoid severe electrolyte imbalance by taking the cation: anion ratio into account. It is imperative to acknowledge that diet is not the sole factor contributing to potential imbalance; bird management and welfare also assume paramount

significance. Na+K+Cl in the feed is typically into account for electrolyte balance, and in most dietary scenarios, this seems like a sensible simplification. Electrolyte balance is commonly described in terms of mEq for each of the different electrolytes; Molecular weight (Mwt)÷1000 is the calculation used for each individual electrolyte. The majority of minerals are relatively low in feed, which is why this equation is applied. The following formula can be used to calculate electrolyte balance. Here is an example to find the mEq for a diet containing 0.17% Na, 0.80% K, and 0.22% Cl.

Sodium: Mwt = 23.0, Eq = 23g/kg, mEq = 23mg/kg

Diet contains 0.17% Na = 1,700 mg/kg = 1700/23 mEq = 73.9 mEq

Potassium : Mwt= 39.1, Eq = 39.1g/kg, mEq = 39.1mg/kg

Diet contains 0.80% K = 8,000 mg/kg = 8,000 /39.1 mEq = 204.6 mEq

Chloride : Mwt = 35.5, Eq = 35.5g/kg, mEq = 35.5mg/kg

Diet contains 0.22% Cl = 2,200 mg/kg, = 2,200/35.5 mEq = 62.0 mEq

Overall diet balance becomes Na + K – Cl = 73.9 + 204.6 – 62.0 = 216.5 mEq
(Leeson and Summers, 2009)

MICRO-MINERALS/TRACE MINERALS

Essential trace minerals include manganese (Mn), zinc (Zn), iron (Fe), copper (Cu), molybdenum (Mo), selenium (Se), iodine (I), and cobalt (Co). Other elements, such as fluorine, nickel, silicon, tin, vanadium, and chromium, have shown beneficial effects in chicken nutrition, although the mechanisms remain unclear (Bao & Choct, 2009).

Manganese, zinc, and copper are vital structural components and catalysts of the antioxidant enzyme superoxide dismutase (SOD), and they play a crucial role in the functionality of immunity mediators such as cytokines, thymus peptides, and various enzymes (Silva et al., 2015). Manganese and zinc also act as co-factors in the formation of mucopolysaccharides and carbonates, which are vital for the genesis of bones (Świątkiewicz et al., 2014).

ZINC

Zinc (Zn) is an indispensable mineral for various metabolic processes for including protein synthesis, and serves as a co-factor for over 300 metalloenzymes (Salim et al., 2008). It is vital for enhancing growth

performance (Liu et al., 2011), maintaining immune functions (Kidd et al., 1996), and supporting the development of the skeletal system in the broilers (Alagawany et al., 2021). A study has shown that zinc possesses antioxidant properties and has a role in various hormone functions of growth and sex hormones as well as in pancreatic hormones, i.e., insulin and glucagon (Abd El-Hack et al., 2017). Research by Zhang et al. (2018) indicated that supplementing the starter and grower diets of broilers with 40 and 32 mg/kg of zinc, respectively, resulted in improved growth performance and reduced zinc excretion in the environment. Sahin et al. (2015) demonstrated that zinc safeguards the pancreatic tissues from oxidative damage and stimulates the secretion of digestive enzymes, hence resulting in the enhancement of nutrient digestion. Being part of the antioxidant defense system, zinc limits the production of free radicals and prohibits membrane oxidation (Zago & Oteiza, 2001). Zinc, as a component of cytosolic Cu/Zn SOD, plays a critical role in the cellular defense against oxidative stress and is directly implicated in metabolic pathways (McDowell, 2003). Zn is also essential for synthesizing various enzymes and proteins, including collagen and keratin, which are crucial for structural integrity in hooves, feathers, skin, beaks, claws, cartilage, and bones (Underwood & Suttle, 1999).

Zinc deficiency can lead to bone abnormalities, poor feathering, decreased tissue strength, and dermatitis due to decreased synthesis rates of collagen and keratin (Leeson & Summers, 2001). Zinc-dependent enzymes, such as collagenases/matrix metalloproteinases (MMPs), also play a role in collagen turnover, which is affected by zinc deficiency (Starcher et al., 1980; Pardo & Selman, 2005).

Zinc requirements

The NRC recommends 40 mg of zinc per kg of diet dry matter for broiler chickens, but other sources suggest higher levels. The German Society for Nutrition Physiology (GFE, 1999) and the Bureau of Indian Standards (BIS, 2007) recommend 50 and 80 mg/kg, respectively. Some studies suggest that Zn supplementation up to 150 mg/kg could further benefit broiler health (El-Wahab et al., 2013). Current investigations describe that the use of nano Zn in broilers' diets can reduce the dietary Zn requirements (Navidshad et al., 2016).

COPPER

Copper (Cu) is an essential element in metalloenzymes, playing a pivotal role in antioxidant defense, cellular respiration, lipid and carbohydrate metabolism, bone formation, immune functions, connective tissue development, spinal cord myelination, and tissue keratinization. It is a pro-oxidant and also a vital component of SOD and it protects the living cells from the reactive oxygen species (Diplock et al., 1998; Shamsudeen & Shrivastava, 2013). In order to reduce the amount of copper being excreted into the environment, broiler diets must have more accessible sources of copper. In both humoral and cellular immunity, copper is an essential element for the production of antibodies and assisting in the removal of harmful organisms. It stimulates the immune system and aids in the preservation of optimal microbial balance in the digestive system (Makarski et al., 2014). Copper, because of possessing microbiological properties and the capacity to enhance body weight, is therefore frequently utilized as a nutritional supplement in broiler chicken production (Wang et al., 20008). According to Ruiz et al. (2000), copper, being a feed additive, has a favorable effect on body weight (BW), feed conversion ratio (FCR), and regulation of gut bacterial ecology. The addition of copper sulfate up to 200mg/kg in the diet of broilers resulted in improved growth performance (Hashish et al., 2010). Copper sulfate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) is commonly used in poultry diets due to its affordability and availability. However, organic copper and nano-sized copper have shown higher bioavailability than copper sulfate pentahydrate (Gonzales-Eguia et al., 2009).

According to research conducted by Xia et al. (2004), gut pathogenic microbes were greatly reduced, and body weight was improved by the supplementation of copper sulfate up to 150mg/kg of feed. In other studies, it was observed that the addition of copper to broilers' diet led to enhanced growth performance and decreased plasma levels of cholesterol, triglycerides, and meat cholesterol (Samanta et al., 2011; Kumar et al., 2013). Copper is also vital for iron transport and metabolism and erythrocyte production and function (Ross et al., 2020). Copper is also crucial for numerous health and performance functions in all animal species, with copper-dependent enzymes often enhancing zinc functions. For example, lysyl oxidase, a copper-dependent enzyme, crosslinks collagen subunits into mature protein forms,

hence increasing their strength (Rucker et al., 1998). Because of its role in collagen crosslinking, copper enhances bone, skin, intestinal, and tendon strength. Poultry experiments have shown that bone-breaking strength strongly correlates with collagen crosslinking. Moreover, elastin, a structural protein found in connective tissues like the cardiovascular system and intestines, is also crosslinked by lysyl oxidase (Rath et al., 1999). Collagen and elastin may not withstand the mechanical stresses of the cardiovascular or skeletal systems in copper-deficient animals (O'Dell et al., 1961; Guenther et al., 1978).

In excessive copper deficiencies, aortic ruptures and brittle and easily breakable bones can be observed (Guenther et al., 1978; Opsahl et al., 1982; Underwood and Suttle, 1999). Therefore, copper is a micronutrient essential for numerous physiological processes and immunity, crucial for the optimal health and growth of poultry.

Copper requirements

The National Research Council (NRC, 1994) established that the minimum copper requirement for broilers is 8 mg/kg of diet. However, the copper requirement can be variable depending upon the degree, extent, and intensity of stress experienced by animals. Research indicates that poultry performance can be enhanced by supplementing copper above the minimum requirements (Pesti and Bakalli, 1996; Zhang et al., 2009a). Growth performance of broiler chicks was enhanced by the addition of copper at 300mg/kg. Various organizations have recommended different dietary copper levels for broilers. The GFE (1999) recommended 7 mg Cu/kg diet, slightly lower than the NRC (1994) value, while the Bureau of Indian Standards (BIS, 2007) proposed a higher level of 12 mg/kg diet.

MANGANESE

Manganese (Mn) is crucial for regulating blood sugar levels, immune function, cellular energy production, reproduction, digestion, and bone growth. Additionally, it plays a significant role in cellular defense against free radicals and is essential for blood clotting and hemostasis alongside vitamin K (Hassan et al., 2020). Many important enzyme systems are Mn-dependent, including lyases, phosphoenolpyruvate carboxylase, transferases, lyases,

hydrolases, oxidoreductases, isomerases, and ligases, encompassing arginase, glutamine synthetase, and Mn-superoxide dismutase (Aschner & Aschner, 2005). A deficiency in dietary manganese can result in growth retardation, skeletal defects, reduced bone development, birth abnormalities, decreased fertility, and impaired carbohydrate and lipid metabolism, leading to abnormal glucose tolerance (Chegeni et al., 2019). Manganese is indispensable for the metabolism of lipids, carbohydrates, and amino acids (Crowley et al., 2000; Suttle, 2010). It activates several enzymes, including glycosyl transferase, SOD, and pyruvate carboxylase (Suttle, 2010), and plays a predominant role in the growth, bone development, optimal eggshell quality, and the prevention of perosis (Lu et al., 2007; Olgun, 2017). Cellular oxidative stress can be prevented by the Mn-SOD (Li et al., 2011). The dietary supplementation of Mn at 90 mg Mn/kg feed prevented various disorders and maintained normal broiler growth and development (Olgun, 2017).

It has been evidenced that supplementation of Mn at 12mg/kg of feed broiler diets both in organic and inorganic forms found to adequate for optimal growth performance (Mwangi et al., 2019). Manganese is essential for proper bone growth, lipid and carbohydrate metabolism, immune and nervous system function, and reproduction in broilers (Power, 2003). A study regarding the effects of Mn deficiency on the proximal tibia microstructure and OPG/RANKL gene expression in chicks was conducted by Liu et al. (2015); they manifested that Mn deficiency caused metaphyseal osteoporosis and adversely affected the tibial development in broiler chicks, might be due to reduced OPG/RANKL mRNA expression. Chicks fed 60 mg Mn/kg exhibited the best tibia development (Navidshad et al., 2019). Therefore, manganese is essential in broiler nutrition due to its role in bone development and nutrient metabolism.

Requirements of manganese

The recommended level of Mn by NRC (1994) for broiler chicken is 60 mg/kg. The upper limit of Mn in poultry is 2000mg/kg DM, while toxicity appears when the level exceeds 3000mg/kg of diet (NRC, 2005). The GFE (1999) suggested a slightly lower requirement of 50 mg Mn/kg, while the BIS (2007) recommended a higher value of 100 mg Mn/kg.

IRON

Iron (Fe) is indispensable for maintaining homeostasis in poultry and is routinely added to their diets. It plays a pivotal role in various enzymes as a cofactor and as a structural cofactor for numerous proteins (Lozoff et al., 2006; Scott et al., 2008). It is essential for oxygen transport, health maintenance, and the regulation of cell growth and differentiation (Saki et al., 2014). Iron is fundamental in the synthesis of hemoglobin, myoglobin, and other components of red blood cells, which are crucial for oxygen transport. It aids enzymes throughout the tricarboxylic acid cycle and helps eliminate harmful metabolic byproducts through iron-containing peroxidases and catalase (Hassan et al., 2020). Additionally, iron-activated hydroxylases are crucial for connective tissue development (Nikonov et al., 2011). Iron has diverse functions in animals, including being part of blood hemoglobin and muscle myoglobin, neurotransmitter synthesis, energy metabolism, synthesis of DNA, collagen, bile acids, and phagocyte antimicrobial activity (Jia et al., 2015). Iron is vital for oxygen storage and transport, energy supply, protein metabolism, body immunization, and antioxidation processes (Drygalski & Adamson, 2013; Abbaspour et al., 2014). Common Fe supplements in poultry diets include sulfates, oxides, and carbonate salts, with more bioavailable organic Fe components now available (Bao et al., 2007).

Iron deficiency and supplementation

Anemia is the primary symptom of iron deficiency, resulting in decreased physical activity due to lower hemoglobin and myoglobin levels, as well as declined functionality of Fe-dependent cytochromes, which diminishes cellular ATP and impairs immune system functions and performance (Van Paemel et al., 2010). The iron requirements of chickens are influenced by factors such as growth rate, immune status and meat quality (Yang et al., 2011). Fe is used a supplement in poultry diets (Xie et al., 2019). Supplementation of Fe at 60 mg/kg in broiler breeder diets can provoke productive performance (Bess et al., 2012). Research studies have suggested that organic iron supplements in poultry feed can improve antioxidant capacity and immunity (Xie et al., 2019a, 2019b). A study has revealed that *in-ovo* iron injection provoked body weight and body weight gain, reduced the serum total lipids and cholesterol in broiler chicken, and produced healthy

food for consumers (Mogahid et al., 2019). In conclusion, iron is crucial for oxygen transport and storage, protein metabolism, energy supply, and enhancing immunity and antioxidant capacity.

Iron requirements

National Research Council (1994) recommends 80 mg Fe/kg dry matter in broiler diets, a level reaffirmed by the Bureau of Indian Standards (BIS, 2007). However, the GFE (1999) suggests a higher requirement of 100 mg dietary Fe per kg of feed.

IODINE

Iodine (I) is an indispensable trace element and integral for various biological functions, particularly for the thyroid gland (Van Middlesworth, 1996). It is an essential element of triiodothyronine (T3) and thyroxine (T4) hormones, which are crucial for regulating metabolism, oxidation process, and cellular activity (Lewis, 2004). Additionally, iodine impacts reproductive performance (Travnicek et al., 1997), circulatory and muscular systems, cell development, nervous system efficiency, and pituitary gland function (Delange, 2002; Liu et al., 2001; Lewis, 2004). Furthermore, iodine is essential for circulatory and muscular system health, cell and tissue maturation, reproductive processes, and nervous system operations (Travnicek et al., 1997; Delange, 1998; Liu et al., 2001). In animal-derived products iodine content can be enhanced through dietary iodine sources such as sodium iodide (NaI), potassium iodide (KI), and calcium iodate ($\text{Ca}(\text{IO}_3)_2$) (Słupczynska et al., 2014). Iodine is primarily supplemented via mineral premixes in poultry diets, commonly in the form of KI, $\text{Ca}(\text{IO}_3)_2$, or iodized salt (Opalinski et al., 2012). Supplementing drinking water with iodine at 2 mg/kg feed has been shown to significantly enhance broiler growth performance (Stanley et al., 1989). The involvement of iodine in metabolic regulations and its effectiveness is exhibited by improvement in production performance. Commonly found iodine sources include potassium iodide (KI), sodium iodide (NaI), calcium iodate ($\text{Ca}(\text{IO}_3)_2$), and calcium iodate hexahydrate ($\text{Ca}(\text{IO}_3)_2 \cdot 6\text{H}_2\text{O}$) (Słupczynska et al., 2014).

Iodine requirements

The iodine requirement for broiler chickens, as recommended by the NRC (1994), is significantly lower than other sources and commercial guidelines; it recommends 0.35 mg/kg, while the GFE (1999) and BIS (2007) suggest higher levels of 0.50 mg/kg and 1.2 mg/kg, respectively.

SELENIUM

Selenium was discovered by Jacob Berzelius in 1818; selenium (Se) exists in both organic and inorganic forms, performing numerous essential functions (Sarkar et al., 2015). Selenium is vital for antioxidative defense, male reproduction, thyroid metabolism, muscle development, and anti-carcinogenesis (Rayman, 2005). It is a component of at least 25 selenoproteins, which contain selenocysteine at their active sites (Naziuroğlu et al., 2012; Naziuroglu and Yürekli, 2013). Selenium being a strong natural antioxidant when supplemented in the broilers diet, reduces the oxidative stress and lipid peroxidation by provoking the activities of glutathione peroxidase (GSH-Px), catalase (CAT), and superoxide dismutase (SOD) (Cai et al., 2012). Selenium-dependent iodothyronine deiodinases activate thyroid hormones (Naziuroğlu et al., 2012). According to Surai (2000) small intestine and pancreas can be prevented from oxidative stress by the dietary supplementation of selenium in the poultry diet. Placha et al. (2014) observed significant increases in blood and duodenal mucosa selenium concentrations and enhanced activities of thioredoxin reductase and glutathione peroxidase in broilers fed 0.4 mg Se/kg diet. The deficiency of selenium can be recognized by stunted growth, decreased appetite, and bone elasticity (Fischer et al., 2008; Turan et al., 1997). Due to lack of selenium in common feedstuffs, it is commonly added to poultry. Sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) are typical selenium supplements in poultry rations, but organic forms like selenomethionine and selenium-yeast show higher bioavailability (Choct et al., 2004; Yang et al., 2014). Selenium is crucial for growth and health in humans and animals (Kieliszek & Błazejak, 2016). In poultry, selenium backs high productive and reproductive and productive performance (Papazyan et al., 2006). According to Rizk et al. (2017) and Hussain et al. (2004), organic selenium supplementation improves hatchability and fertility in chickens and boosts bursa and thymus weight, leading to enhanced

immunity. It improves FCR and reduces drip loss, leading to improved meat quality and better economic gain (Deniz et al., 2005). GSH-Px is a Se-dependent enzyme that is vital in the antioxidant system, it is important for immune regulations and biological functions, selenium is also involve in the regulation of GSH-Px gene expression (Ebeid, 2009; Habibian et al., 2014).

It is pertinent to note that selenium is not directly involved in the inhibition of reactive oxygen species (ROS) formation, rather it functions indirectly by acting as a co-factor of enzymes (Horvath & Babinsky, 2018). Research has shown that the incorporation of organic and inorganic forms of Se and Zn, along with pomegranate, rosemary, hydroxytyrosol, grape, and harpagophytum extracts, enhances the shelf life and nutritional quality of chicken products. Moreover, combing Se and Zn with phenolic compounds help to maintain the sensory quality of chicken products by reducing the lipid and protein oxidation and inhibiting the growth of microorganism (Martínez et al., 2020). Therefore, selenium has significant importance in the poultry nutrition due to its valuable effects on the physiological and productive performance of poultry.

Selenium requirements

The NRC (1994), GFE (1999), and BIS (2007) recommend 0.15 mg/kg of selenium for broiler chickens. Recent studies indicate that selenium supplementation beyond the NRC (1994) recommendation benefits broilers. For instance, Sodium selenite (0.2mg/kg) and nan-Se (0.5mg/kg) were compared by Wang (2009) to evaluate their effects on growth rate, tissue Se retention, and GSH-Px activity in broilers. He found that all Se supplementations significantly improved the DWG, FCR, GSH-Px activity, tissue selenium retention, feed efficiency and survival rate.

CHROMIUM

Chromium (Cr) is an essential trace mineral that plays a critical role in enhancing insulin activity, which is important for the metabolism of glucose, proteins and fats (Haq et al., 2016; Das et al., 2022). This makes chromium a valuable dietary supplement. Chromium involvement carbohydrates and lipid metabolism is crucial for animal development ad fattening. Chromodulin, a low molecular weight chromium binding compound, is essential for the

insulin signaling process and requires chromium for its production (Dalólio et al., 2018). Chromium is well known for improving growth performance and carcass quality in livestock (Van Hoeck et al., 2020). It is available in organic and inorganic forms, each with different bioavailability and absorption rates. The common inorganic forms include metallic chromium (Cr0) and trivalent chromium (Cr3+) and hexavalent chromium (Cr6+) (Haq et al., 2016). Trivalent chromium is the most stable and safest form, whereas hexavalent Cr is toxic, mutagenic, and carcinogenic (Chowdhury et al., 2003). In poultry chromium enhances growth performance, acts as an antioxidant, reduces cholesterol levels, increases high density lipoproteins cholesterol, and improves nutrient digestion (Haq et al., 2016). Chromium supplementation has been shown to bolster immune response and antioxidant defense systems (Farag et al., 2017). It also improves feed conversion ratio (FCR), promotes weight gain, and supports muscle development (Haq et al., 2016). Additionally, chromium positively influences reproductive and productive performance and physiological traits (Sahin et al., 2005).

Chromium helps mitigate the effects of various stressors, including environmental, nutritional, and physiological stress, in poultry production (Chandrasekar & Balakrishnan, 2019). Arif et al. (2019) demonstrated that adding chromium propionate to broiler diets at 400 ppb improved performance and weight gain. Dietary chromium supplementation also enhanced the immune functions of chickens vaccinated against the Avian Influenza Virus (Lu et al., 2019). Chromium deficiency disrupts carbohydrate and protein metabolism (Haq et al., 2016). Although chromium is not typically considered an essential trace mineral in the broiler industry (Amata, 2013), studies indicate it can enhance weight gain, FCR, high-density lipoprotein levels, lean muscle development, and nutrient digestion (Sahin et al., 2003; Al-Bandr et al., 2010).

Chromium's benefits are more pronounced under conditions of environmental, dietary, and hormonal stress (Amata, 2013). While the National Research Council (NRC, 1994) does not provide specific chromium recommendations for broiler diets, numerous studies suggest that chromium supplementation improves broiler growth performance (Toghyani et al., 2012; Mohammed et al., 2014; Huang et al., 2016). Samanta et al. (2008) reported that supplementing diets with CrCl₃ at 0.5 mg/kg significantly improved

weight gain and FCR compared to control groups, while Zheng et al. (2016) found that 0.4 mg/kg of CrCl₃ positively affected carcass characteristics but not growth performance. Other researchers noted no significant effects on average daily gain (ADG), average daily feed intake (ADFI), or FCR from CrCl₃, Cr-yeast, or Cr picolinate (CrPic) treatments (Król et al., 2017; Lu et al., 2019).

However, several studies indicate that chromium supplementation, regardless of its source, enhances growth performance and carcass traits under heat stress conditions (Toghyani et al., 2012; Huang et al., 2016). Zha et al. (2009) found that while inorganic chromium did not affect growth performance under heat stress, organic chromium did. Uyanik et al. (2010) observed that dietary chromium supplementation (as chromium chloride) at 20 ppm reduced feed consumption by 18.57% and improved FCR by 16.77%. However, higher chromium levels (40 and 80 ppm) did not significantly affect feed consumption or FCR. The U.S. Food and Drug Administration Center for Veterinary Medicine (FDA) approved a food additive petition in June 2016, allowing the use of Cr propionate as a source of supplemental Cr in broiler diets (FDA, 2016). Sahin et al. (2002) noted that increasing supplemental chromium levels in broiler diets (200, 400, 800, and 1200 µg/kg as chromium picolinate) under heat stress conditions improved carcass traits. Similarly, Sahin et al. (2005) found that supplementing growing Japanese quail diets with 400 µg/kg of chromium picolinate significantly increased cold carcass percentage under heat stress. Dietary Cr picolinate (800, 1600, or 3200 µg/kg) increased high-density lipoprotein and decreased low-density lipoprotein and very low-density lipoprotein in broilers (Lien et al., 1999), with serum glucose concentrations reduced at levels of 1600 and 3200 µg/kg of chromium.

Chromium supplementation also decreased total lipids, total cholesterol, very low-density lipoprotein, and low-density lipoprotein, while increasing high-density lipoprotein and triglycerides in serum (Taha et al., 2013). In heat-stressed Japanese quail diets, chromium supplementation reduced MDA levels in serum (Sahin et al., 2002). In conclusion, chromium is essential for improving productive performance in poultry due to its significant roles in growth, metabolism, and the reduction of lipid and protein peroxidation.

Chromium requirements

There are no specific chromium requirements for broilers recommended by the NRC.

Comparison of macro and micro minerals requirements of broilers in different phases of life from NRC (1994), Cobb500 (2022), Ross 308 (2022), and Hubbard (2022) guides are shown in Table 2, while the inorganic source of macro and micro minerals is shown in Tables 3 and 4, respectively.

Table 2. Comparison of macro and micro minerals requirements of broilers in different phases of life from NRC (1994), Cobb500 (2022), Ross 308 (2022), and Hubbard (2022) guidelines

	NRC	C*	R**	H***		NRC	C*	R**	H***
Starter									
Macro (%)					Micro (mg)				
Ca	1.00	0.98	0.95	1.00-1.05	Mn	60	100	120	80
P	0.45	0.58	0.50	0.50	Zn	40	100	120	80
Mg	0.06	--	0.05-0.30		Fe	80	40	20	60
Na	0.20	0.16-0.23	0.18-0.23	0.16-0.18	Cu	8	15	16	10
Cl	0.20	0.16-0.30	0.18-0.23	0.15-0.20	I	0.35	1	1.25	1.0
K	0.30	0.60-0.95	0.60-0.90	0.85	Se	0.15	0.35	0.30	0.2
Grower									
Macro (%)					Micro (mg)				
Ca	0.90	0.80	0.75	1.00-1.05	Mn	60	100	120	80
P	0.35	0.40	0.42	0.45	Zn	40	100	120	80
Mg	0.06	--	0.05-0.30		Fe	80	40	20	60
Na	0.15	0.16-0.23	0.18-0.23	0.16-0.18	Cu	8	15	16	10
Cl	0.15	0.16-0.30	0.18-0.23	0.15-0.20	I	0.35	1	1.25	1.0
K	0.30	0.60-0.95	0.60-0.90	0.80	Se	0.15	0.35	0.30	0.2
Finisher									
Macro (%)					Micro (mg)				
Ca	0.80	0.74	0.65	0.85-0.90	Mn	60	100	120	80
P	0.30	0.37	0.36	0.40	Zn	40	100	120	80
Mg	0.06	--	0.05-0.30		Fe	80	40	20	60
Na	0.12	0.16-0.23	0.18-0.23	0.16-0.18	Cu	8	15	16	10
Cl	0.12	0.16-0.30	0.18-0.23	0.15-0.20	I	0.35	1	1.25	1.0
K	0.30	0.60-0.95	0.60-0.90	0.75	Se	0.15	0.35	0.30	0.2

*Cobb500, ** Ross308, *** Hubbard

Table 3. Macro-minerals' inorganic sources and corresponding mineral content (NRC, 2012)

Source	Calcium (%)	Phosphorus (%)	Sodium (%)	Chloride (%)	Potassium (%)
Bone meal, steamed	29.8	12.5	0.04	---	0.2
Calcium carbonate	38.5	0.02	0.08	0.02	0.08
Calcium phosphate (monocalcium)	16.9	21.5	0.20	---	0.16
Calcium phosphate (dicalcium)	24.8	18.8	0.20	0.47	0.15
Calcium phosphate (tricalcium)	34.2	17.7	6.0	---	---
Limestone	35.8	0.01	0.06	0.02	0.11
Phosphate, defluorinated	32.0	18.0	3.27	---	0.10
Phosphate(rock curacao, ground)	35.1	14.2	0.20	---	---
Phosphate (rock, soft)	16.1	9.05	0.10	---	---
Potassium chloride	0.05	---	1.0	46.9	51.4
Sodium chloride	0.30	---	39.5	59.0	0
Sodium carbonate	---	---	43.3	---	---
Sodium bicarbonate	0.01	---	27.0	---	0.01

Table 4. Micro-minerals' inorganic sources and corresponding mineral content (NRC, 2012)

Inorganic mineral	Source	Mineral content (%)	Relative bioavailability (%)
Copper	Cupric sulfate (pentahydrate)	25.2	100
	Cupric chloride, tribasic	58.0	100
	Cupric oxide	75.0	0-10
	Cupric carbonate (monohydrate)	50-55	60-100
	Cupric sulfate (anhydrous)	39.9	100
Iodine	Ethylenediamine dihydroiodide (EDDI)	79.5	100
	Calcium iodate	63.5	100
	Potassium iodide	68.8	100
	Potassium iodate	59.3	---
	Cupric iodide	66.6	100
Iron	Ferrous sulfate (monohydrate)	30.0	100
	Ferrous sulfate (heptahydrate)	20.0	100
	Ferrous carbonate	38.0	15-80
	Ferric oxide	69.9	0
	Ferric chloride (hexahydrate)	20.7	40-100
	Ferrous oxide	77.8	---
Manganese	Manganous sulfate (monohydrate)	29.5	100
	Manganous oxide	60.0	70
	Manganous dioxide	63.1	35-95
	Manganous carbonate	46.4	30-100
	Manganous chloride (tetrahydrate)	27.5	100
Selenium	Sodium selenite	45.0	100
	Sodium selenate (decahydrate)	21.4	100
Zinc	Zinc sulfate (monohydrate)	35.5	100
	Zinc oxide	72.0	50-80
	Zinc sulfate (heptahydrate)	22.3	100
	Zinc carbonate	56.0	100
	Zinc chloride	48.0	100

APPLICATION OF NANOTECHNOLOGY IN POULTRY

Nanoparticles (NP) offer a promising alternative to traditional chemoprophylactic drugs in poultry. Over the past decade, NPs have seen extensive application in various medical fields, including vaccines (Adair, 2009), drug delivery (De Jong & Borm, 2008), and diagnostics (Bentolila et al., 2009). As an emerging scientific field, nanotechnology manipulates materials at dimensions between 1 to 100 nanometers, conferring unique properties and innovative uses. The nanometer concept, describing particle size, was introduced by Nobel Laureate Richard Zsigmondy (Hulla et al., 2015). Materials at the nanometer scale exhibit distinct properties compared to their bulk counterparts and isolated atoms (Albrecht et al., 2006). The origin of nanotechnology date back to 1956, when American physicist Richard Feynman introduced his groundbreaking ideas (Rajendran et al., 2013). This technology is now applied across disciplines such as physics, chemistry, engineering, medicine, and recently, livestock and agriculture. Nano-sized materials are ubiquitous in nature, with biological systems relying on nano-scale structures like proteins, DNA, and enzymes. Naturally occurring nano-sized particles also exist in the atmosphere (Albrecht et al., 2006). Nanotechnology is among the most innovative fields, enabling the production of materials and elements with altered structures, textures, and enhanced quality at the molecular level (Mahmoud, 2012). It significantly contributes to the production, processing, storage, transportation, traceability, security, and safety of food (Otles & Yalcin, 2008). Nanoparticles, created using various techniques, display distinct characteristics and are classified into categories such as organic, inorganic, nano-clays, dispersions, and emulsions (Marappan et al., 2017). In poultry, nanoparticles of minerals such as silver (Vadalasetty et al., 2018), zinc oxide (Fathi, 2016), copper (Ognik et al., 2016; Abdullah et al., 2022), iron (Abdel-Rahman et al., 2022), and selenium (Rana, 2021) have been utilized.

Nanoparticles of trace elements enhance mineral bioavailability, mitigate mineral antagonism in the intestine, and improve absorption while reducing environmental excretion. These nanoparticles also potentially enhance immune responses and digestive efficiency in birds, leading to improved feed efficiency (Gopi et al., 2017). Hamza et al. (2019) documented the use of yeast cell wall beta-glucan encapsulated humic acid nanoparticles

as a binder for aflatoxin B1 mycotoxin. Despite its potential, the application of nanotechnology in the poultry industry remains underexplored due to insufficient knowledge and research. Ongoing advancements in nanotechnology are expected to enable scientists to increase meat production, enhance the bioavailability of nutrients, and reduce antagonistic behavior in the gut, thereby aiding developing countries and enhancing the poultry industry (Carmen et al., 2003; Marappan et al., 2017).

Manufacturing of nanoparticles (NP)

The manufacturing and preparation of nanoparticles (NPs) primarily follow two key principles (Yonzon et al., 2022):

Top-down method

This physical approach involves mechanically grinding bulk material into nanoscale particles. Stabilizers are then employed to prevent the newly formed NPs from aggregating back into microscale or macroscale structures.

Bottom-up method

This method involves constructing NPs from smaller units through processes such as sonodecomposition, simple chemical reduction, and electrochemical reduction.

Classification of nanomaterials

Nanomaterials can be classified based on several criteria:

- a. Shape:** NPs exhibit various shapes, such as spherical, cubic, needle-like, triangular, and rod-shaped (Gour & Jain, 2019). Figure 2.
- b. Form:** Nanomaterials are categorized into various forms including nanoclusters, nanotubes, micelles, liposomes, carbon tubes and fibers, and dendrimers (Abdullaeva, 2017).
- c. Size:** Nanoparticles (NPs) range in size from 1 to 100 nm. Smaller particles have a larger surface area, which influences their functionality and toxicity (Saleh, 2020; Lang et al., 2021).

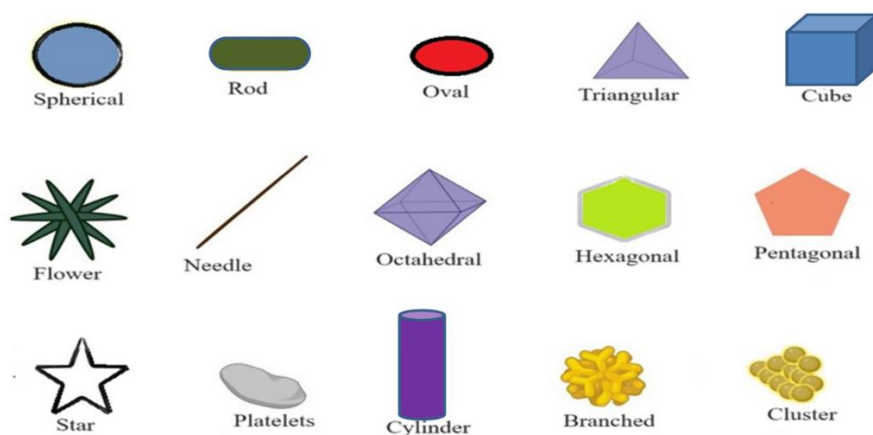


Figure 3. Various shapes of nanoparticles (Gour & Jain, 2019)

- d. **Application:** NPs have diverse applications including therapeutic, diagnostic, theranostic, vaccine production, and nutritional uses (Abd El-Ghany et al., 2021).
- e. **Surface Modifications:** Surface functionalization of NPs includes PEGylation, carboxylation, and amination, which involve linking functional groups or adding fatty acids, thiols, or anionic compounds. Surface charge modifications can also be made, either negatively or positively (Saleh, 2020).
- f. **Composition:** Nanomaterials can be synthesized from a single material or as composites made from at least two different NPs. Composites are created to enhance properties, achieve new functionalities, and provide multiple functionalities not possible with single-component NPs (Buzea & Pacheco, 2017; Du & Yuan, 2020).
- g. **Nature:** NPs are classified as inorganic, organic, and carbon-based.
- i) **Inorganic NP** are composed of metals or metal oxides. Examples include gold, silver, cadmium, aluminum, cobalt, copper, iron, and zinc NP (Reverberi et al., 2016). Iron NPs are highly reactive and oxidize quickly in the presence of oxygen at room temperature, thus requiring coating to remain stable and prevent aggregation (Ali et al., 2016). Metal-oxide NPs are primarily synthesized for their reactivity and efficiency (Tai et al., 2007).

ii) **Organic NP** encompass dendrimers, micelles, ferritin, liposomes, proteins, and peptide-based NPs. These are non-toxic, biocompatible, and biodegradable. Nano-capsules such as liposomes and micelles possess a central hollow core and an outer shell, making them ideal for drug delivery and controlled release applications (Osama et al., 2020; Sahoo et al. 2020). Figure 4

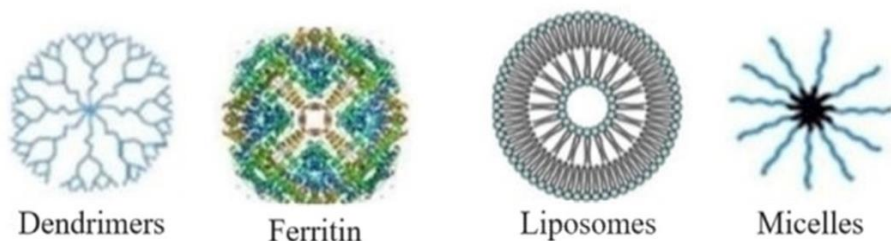


Figure 4. Structure of organic nanoparticles (Sahoo et al.,2020)

iii) **Carbon-based NPs** include fullerenes, graphene, carbon nanotubes, carbon nanofibers, and black carbon (Trong et al., 2020), Figure 5.

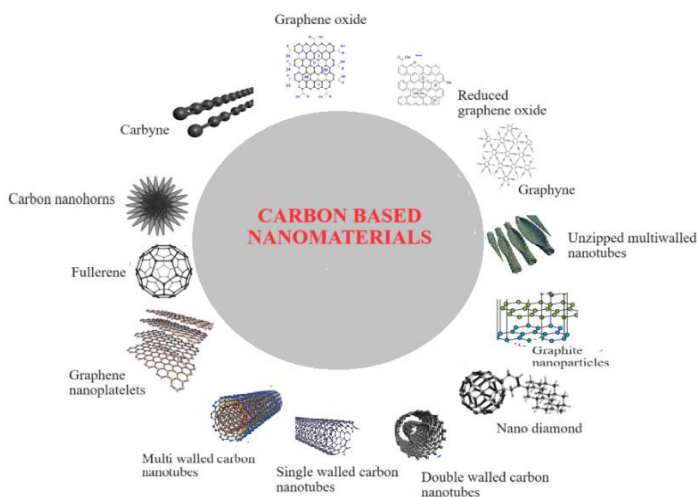


Figure 5. Common allotropes of carbon nanomaterials (Trong et al., 2020)

NANOPARTICLES APPLICATION IN BROILER NUTRITION

NANO CALCIUM AND PHOSPHORUS

Nanoparticles (NPs) demonstrate significant potential at lower doses compared to traditional inorganic or organic mineral sources such as calcium (Ca) and phosphorus (P) (Swain et al., 2015). The pursuit of more efficient macromineral sources for poultry has heightened interest in nanotechnology. Recent investigations have focused on Ca-P nanoparticles in feed, including calcium phosphate (Vijayakumar & Balakrishnan, 2014, 2015; Samanta et al., 2019), dicalcium phosphate (Hassan et al., 2016; Mohamed et al., 2016), calcium carbonate (Salary et al., 2017), and hydroxyapatite (Sohair et al., 2017). Nanoparticles are considered viable alternatives to conventional mineral sources, aiming to reduce the quantity of required elements through enhanced bioavailability and absorption efficiency (Gonzales-Eguia et al., 2009). Initial studies on poultry examined the effects of calcium phosphate nanoparticles, noting no significant adverse changes in biochemical blood markers such as glucose, albumins, triglycerides, cholesterol, creatinine, or hepatic enzymes compared to broilers fed traditional dicalcium phosphate (Vijayakumar & Balakrishnan, 2014). Similar results were observed for hematological parameters (Vijayakumar & Balakrishnan, 2015).

Mohamed et al. (2016) found no significant differences in the mineral content of the gizzard, heart, and liver when diets included only 25% of the required non-phytate P from nanoparticles instead of 100% from conventional sources. They observed higher final body weight, improved feed conversion ratio, and increased daily weight gains in birds fed nano dicalcium phosphate. Additionally, birds fed nanoparticle sources exhibited a 50% reduction in Ca and P excretion in feces (Hassan et al., 2016). Vijayakumar and Balakrishnan (2015) found no significant differences in tibia morphometry and mineralization when comparing 100% dicalcium phosphate (control) with increasing proportions of nanoparticle dicalcium phosphate. These findings suggested that nanoparticles had 200% better bioavailability of P and Ca than conventional forms, potentially benefiting environmental protection. Samanta et al. (2019) demonstrated that Ca phosphate nanoparticles, whether used alone or in combination with standard sources, did not affect cumulative feed consumption, feed conversion ratio, biochemical parameters, or carcass

characteristics. However, growth performance improved in birds fed nanoparticles at the 50% level. Studies on hydroxyapatite nanoparticles indicated they could be an adequate Ca and P source in broiler feed, enhancing body weight gain and feed intake by 6% and 8%, respectively, without affecting the feed conversion ratio (Sohair et al., 2017).

Injecting Ca carbonate nanoparticles *in ovo* resulted in higher body and organ weights (spleen, bursa of Fabricius) in broiler hatchlings (Salary et al., 2017). Although weight gain, feed conversion ratio, and feed intake did not differ significantly among groups reared to 21 days post-hatch, these studies mainly focused on nanoparticle administration in water or feed to enhance mineral absorption and bird health, including *in ovo* injections. Few studies have examined the impact of Ca and P nanoparticles on the skeletal system. Mohamed et al. (2016) compared the effects of nanoparticle and conventional dicalcium phosphate on carcass traits and other parameters in 26-day-old broilers, finding that nanoparticles positively influenced tibia morphometry, including weight, length, width, and breaking force, even at significantly lower doses. Bone development was also enhanced by *in ovo* application of Ca carbonate nanoparticles, resulting in higher Ca and Cu, but not P, concentrations in tibia bones of broiler hatchlings. This study also demonstrated increased serum alkaline phosphatase activity in *in ovo*-injected groups, underscoring its role in bone ossification and calcification. Further research is necessary to fully explore the potential of Ca and P nanoparticles in poultry nutrition.

NANO-ZINC

Zinc is one of the essential trace elements that is involved in the proper feather development, skin quality, bone structure, growth, and reproduction in birds (Pandy & Puranik, 2015). It also enhances disease resistance and boosts immune functions (Kidd et al., 1996). Lina et al. (2009) found that 40 ppm of nano-ZnO led to better growth and dressing percentage in broiler chickens compared to 80 and 120 ppm. Studies comparing the Zn nanoparticles with other forms, such as inorganic, organic, or chelated Zn, showed that birds fed nano-ZnSO₄ had reduced feed intake and body weight gain, but the feed conversion ratio remained unchanged. Nano-ZnO improved carcass traits by increasing the dressing percentage and the weights of the pancreas and

proventriculus without significantly affecting lymphoid organs (Mohammadi et al., 2015). Diets having 20 and 60 ppm of nano-ZnO led to higher live body weight gains and better FCR than the control group and 100 ppm reduced the live weight after four weeks (Hassan et al., 2020). According to Ibrahim et al. (2017), the replacement of traditional inorganic ZnO with nano-ZnO or combining it with Zn methionine enhances broiler growth, zinc absorption, and antioxidant status without adversely affecting mineral distribution in tissues. Additionally, supplementing broilers with organic nano-Zn at the dose rate of 20ppm improved body weight, weight gain, FCR, Zn levels in serum and tissues, and increased profitability (Badawi et al., 2017). Zinc nanoparticles in chicken feed have been shown to boost growth, reproduction, and immunity (Swain et al., 2016).

NANO –SELENIUM

Oxidative stress can damage cells due to the continuous release of reactive oxygen species caused by various biotic (fungi, bacteria, viruses) and abiotic stressors. Selenium, known for its antioxidant properties, is widely used as a feed additive to mitigate oxidative stress (Back, 2013). Because of its small size and high bioavailability, nano-Se results in greater selenium retention and increased glutathione S-transferase activity (Peng et al., 2007). Nano-Se has manifested better results in body weight gain than sodium selenite and basal diet in broiler chickens (Kumaran et al., 2015). Similar results were noticed with a basal diet supplemented with nano-Se, Se-yeast, or sodium selenite (Liu et al., 2015). The addition of Nano-Se at 0.2 to 0.5 ppm in broilers diets improved growth performance, carcass traits, and immune functions without affecting internal organs (Ahmadi et al., 2018). Combining probiotics with selenium nanoparticles improved growth, the fatty acid profile of muscles, and alpha-tocopherol in the serum of the broiler chickens (Saleh et al., 2014). Nano-Se also raised the IgG and IgM levels and enhanced the antioxidant status compared to inorganic and organic selenium under oxidative stress (Boostani et al., 2015). The supplementation of nano-Se in broilers reared under high temperatures resulted in improved growth, antioxidant functions, and immune responses (Mahmoud et al., 2016). Studies comparing different selenium sources found that nano-selenium and organic

selenium improved growth performance and meat and carcass traits more than inorganic selenium (Selim et al., 2015).

Surai et al. (2017) reported that the addition of Se in broilers diet resulted in increased weight gain, livability, and better feed conversion ratio. While different selenium sources did not affect growth performance these improved antioxidant capacity (GSH-Px in breast meat and malondialdehyde in serum) and the meat quality with nano and organic selenium (Hulla et al., 2015). Some of the studies found no significant effects of nano-Se on growth or carcass color and immune organ index but exhibited significant antioxidant benefits in broiler chickens (Csi et al., 2012). Abd-El-Ghany (2019) concluded that nano-selenium offers a broader optimal-to-toxic dietary range with better retention improved growth and survivability rates, and a feed-to-gain ratio.

NANO-SILVER

Silver nanoparticles (nano-Ag) are beneficial in poultry diets due to their ability to penetrate physiological barriers and target molecular mechanisms (Chang, 2010). Nano-Ag enhances immune response and metabolic activity and serves as an antibiotic, widely used across scientific fields (McShan et al., 2014). Although different nano-Ag concentrations in diets usually do not change growth performance, they often improve immunity and antioxidant capacity. A diet with 4 ppm nano-Ag per kilogram resulted in the best FCR, lower serum lipid and cholesterol levels, and increased antioxidant capacity (Hulla et al., 2015). Ag-NPs, when administered orally, regardless of size or lipid coating, did not affect growth or carcass traits but altered gut microflora, increasing aerobic bacteria and reducing coli group bacteria (Ognik et al., 2016). Nano-Ag also reduced pathogenic gut microorganisms, enhancing nutrient absorption, feed intake, weight gain, and feed efficiency (Andi et al., 2011). Ahmadi (2012) found that 20, 40 and 60 ppm nano-Ag supplementation in feed reduced lymphoid organ weights due to its antimicrobial properties. Adding nano-Se and nano-Ag to diets reduced oxidative stress through antioxidant activities (Aparna and Karunakaran, 2016).

NANO-COPPER

Copper is also a crucial trace element in the diet of poultry birds, necessary for various biological processes. It is part of several metalloenzymes and proteins (Tapiero et al., 2003). Diet supplemented to broiler chickens with 100 ppm nano-chitosan Cu enhanced the body weight gain, beneficial gut microbes with reduced coliforms, as well as increased the immunoglobulin level and complement components (Wang et al., 2011). Growth performance of broilers was increased when these were injected with intramuscular injections of nanoparticles solution (Scott et al., 2016). Nano-Cu has shown strong antibacterial properties against *Campylobacter* and *Salmonella* in poultry birds and has increased hemoglobin, protein, red blood cells, and copper levels in the serum of broilers chickens (Duffy et al., 2018; Hassan et al., 2020). Mroczek-Sosnowska et al. (2017) found such significant effects on chick development and hematological parameters with nano-Cu and CuSO₄ injections in broiler eggs. Different sources of Cu did not significantly impact oxygen (O₂) consumption, but higher concentrations (50 ppm) did lead to an increase in O₂ consumption. Research revealed a dose-dependent increase in serum Cu levels when nano-Cu was given to broilers in their drinking water at 5, 10, and 15 ppm (Ognik et al., 2016). Although intestinal copper accumulation did not impact iron absorption, it did decrease Ca and Zn absorption (Hassan et al., 2020). Supplementing with nano-Cu improved the weight-to-volume ratio of femoral bones, their fracture resistance, and the presence of proliferating cell nuclear antigens in chickens (Scott et al., 2018). The *in-ovo* feeding of nano-Cu was examined for its effects on hatchability, post-hatch performance, and immunity. Broilers responded well to *in-ovo* injection of nano-Cu, though it did not significantly affect the expression of immune-related genes (Lee et al., 2016). *In-ovo* feeding of Nano-Cu (4µg/egg) did not alter the hatchability but enhanced the FCR and breast meat percentage in broiler chickens (Joshua et al., 2016). Copper silicate nanoparticles positively modified the intestinal microbiota by increasing *Lactobacillus* count and reducing *E.coli* (Shi Minglei et al., 2013).

Additionally, Miroshnikov et al., (2015) reported that intramuscular injection of Cu-NPs enhanced the growth, elevated serum copper and protein levels, increased hemoglobin levels, and raised arginine content in the chicken liver.

NANO-IRON

Nano-iron (nano-Fe) has manifested positive effect in broilers. When iron was injected as nano or microparticles in broilers, an increase was observed in body weight gain and protein deposition in tissues (Sizova et al., 2015; Hassan et al., 2020). In ovo injections of iron, including nano-fe (25, 75 and 125 ppm), Fe alimet (methionine hydroxyl analogue) chelate (50, 100, and 150 ppm), and nano-Fe alimet chelate (50, 100 and 150 ppm), resulted in higher egg and chick weight and improved weight ratios compared to control diet (Saki et al., 2014a). Birds fed diets with 25 and 100 ppm nano-Fe alimet chelate, as well as 150 ppm Fe alimet chelate showed increased serum iron levels and liver weight. Additionally, blood hemoglobin levels were higher in birds fed 150 ppm Fe alimet chelate and 100 ppm nano-Fe alimet chelate (Hassan et al., 2020). Including nano-Fe in the diet of broilers improved body weight gain in the supplemented groups, though it did not significantly affect liver weight, thigh and breast composition (Sizova et al., 2016). Various studies have reported increased body weight gain in broilers with dietary nano-Fe supplementation (Miroshnikov et al., 2015; Rahmatollah et al., 2017). However, inoculating nano-iron (50-100ug/ml) in chicken embryos led to low weight chicks with neuronal degeneration, while a higher concentration (200ug/ml) resulted in 100% embryo mortality (patel et al., 2017). Conversely, Fe-NPs injections in 15 and 29-day old broiler chickens combined with amino acid mixtures in their diets improved weight gain by 20% (Yausheva et al., 2016). In ovo-injection of Fe-NPs did not impact hatchability, chick weight, hemoglobin content, or blood and liver Fe concentrations compared to coarser particle size (Saki et al., 2014).

NANO-MANGANESE

Manganese (Mn) is an essential trace element found in all body tissues and is crucial for metabolizing carbohydrates, lipids, proteins, and amino acids (Tufarelli & Luadadio, 2017). Lotfi et al. (2014) observed increased tibial bone weight from Mn-NPs supplementation. It was also found that Mn-sulfate-NPs improved tibial length, volume, breaking strength, and diameter compared to Mn-sulfate coarse particles. Furthermore, supplementing diets with nano-Mn increased both fresh and dry tibia weights during the starter and finisher phases and enhanced tibia breaking strength, although it had no

significant effect on ash percentage, tibial volume, and density. In turkeys, nano-Mn also increased aminopeptidase activity (Jozwik et al., 2018). Additional research examined broilers given Mn in their diets at levels of 50, 100 and 150 ppm in macro and nano-particle form (Ognik et al., 2018). Chegeni et al. (2019) studied broilers injected *in-ovo* with Mn-sulfate, Mn-NPs, Mn-methionine and Mn-methionine-NPs at 1 ml per bird, finding increased feed intake, average daily gain, and lower FCR or Mn-sulfate compared to Mn-NPs. However, no significant differences were noted between Mn-methionine, and Mn-methionine NP concerning carcass traits and tibial breaking strength, with no impact on ash percentage, volume and density. These findings suggest that Mn NPs may have a modest effect on growth performance but could enhance antioxidant and certain bone traits in broilers, depending on the chemical forms and sizes of the nanoparticles used.

NANO-CHROMIUM

Chromium (Cr) a trace mineral, although needed in small quantities in poultry feed, has attracted attention for its potential benefits. Recognized as an essential element for approximately five decades, Cr is toxic in large amounts but is vital for metabolizing fats, carbohydrates, proteins and nucleic acids (Hassan et al., 2020). It acts as a biological modulator of insulin and is recommended for preventing and treating insulin resistance (Anderson, 1987). Chromium also activates enzymes involved in protein stabilization and various biological pathways (NRC, 1997). Currently, there are no specific recommendations for Cr in poultry feed (Hajjalizadeh et al., 2017). Supplementation with chromium picolinate and nano-Cr has significantly improved FCR weight gain, and antibody titres against avian influenza in heat-stressed broiler chickens (Sirirat et al., 2012). Different sources of chromium (Cr-Cl, nano-Cr and Cr-picolinate) at a dose of 500 ppb showed that both Cr-picolinate and nano-Cr increased body weight, FCR, carcass and lean yield while reducing abdominal fat. Unique to nano-Cr, it increased protein content in the breast and thigh and lowered cholesterol, without affecting Cr content in serum, liver and kidney (Lin et al., 2014). Additionally, diets containing Cr picolinate and nano-Cr led to decreased feed intake, serum low density lipoproteins cholesterol, and serum Cr concentration in broilers (Sirirat et al., 2013). Chromium supplementation has

been shown to enhance weight gain, feed efficiency, lean muscle development, lower abdominal fat and cholesterol, increase high density lipoprotein, and alleviate environmental stress effects (Farak et al., 2017). In heat-stressed broiler chicks, supplementation with Cr-NPs and Cr-picolinate improved average daily gain, feed efficiency, and lean muscle mass while reducing abdominal fat, outperforming inorganic Cr at the same dose (Zha et al., 2009). However, a study by Hamidi et al. (2016) found no dose-wise effect on growth performance in heat-stressed broiler chickens with three doses (0.5, 1.0 and 1.5 mg/kg) of Cr-picolinate compared to Cr-picolinate nanoparticles.

ORGANIC MINERALS

The Association of American Feed Control Officials defines various organic mineral products (Spears, 1996).

Metal amino acid complex

This product is derived from the complexation of a soluble metal salt with amino acids. Examples include zinc methionine, zinc lysine, manganese methionine, iron methionine, and copper lysine.

Metal amino acid chelate

This product results from the reaction between a metal ion from a soluble metal salt and amino acids in a molar ratio of one metal to one to three (ideally two) amino acids, forming coordinate covalent bonds. The average molecular weight of hydrolyzed amino acids should be about 150, and the chelate's molecular weight should not exceed 800. Chelation involves a specific complex formation between a ligand and a metal ion. For a ligand to be classified as a chelate, it must have at least two functional groups (such as oxygen, nitrogen, amino, or hydroxyl) capable of donating electron pairs to form coordinate covalent bonds with the metal, creating a heterocyclic ring structure (Kratzer & Vohra, 1986). Not all metal complexes qualify as chelates. Examples include amino acid chelates for zinc, copper, iron, manganese, and cobalt, along with macrominerals like calcium and magnesium.

Metal proteinate

This product forms from the chelation of a soluble salt with amino acids and/or partially hydrolyzed proteins. Commercially available proteinate forms include those for copper, cobalt, iron, manganese, and zinc.

Metal polysaccharide complex

This product is created by complexing a soluble salt with a polysaccharide solution, with examples including manganese and zinc polysaccharide complexes. Historically, trace minerals in poultry diets have been used in inorganic forms such as oxides and sulfate salts. However, these forms often suffer significant losses during passage through the gastrointestinal tract (GIT) due to interfering substances in the diet. Consequently, inorganic minerals are typically supplemented at levels two to ten times the National Research Council (NRC) standards for poultry (Council, 1994) because of their low retention rates and broad safety margins (Varun et al., 2017). This high supplementation leads to mineral wastage and environmental pollution from excessive excretion by birds (Leeson, 2003). Enhancing stability in the small intestine can improve bioavailability and efficacy, thus mitigating environmental pollution (Bao & Choct, 2009; Bao et al., 2007). For instance, under normal commercial dietary condition in poultry 94% of ingested zinc is excreted, contributing to soil phytotoxicity, so Zn excretion can be reduced and related problems can be avoided by utilizing low concentration of Zn or using other organic sources of Zn in chickens (Bolan et al., 2004).

Chelated mineral complexes comprise a central atom and a ligand (proteins, carbohydrates, lipids, or amino acids) with at least one ligand atom (sulfur, oxygen, or nitrogen) that possesses a pair of free electrons (Swinkels et al., 1994). The ligand atom forms a coordinate bond with the metal atom by donating an electron pair. The inclusion of low levels of organic minerals is favored due to their physiological and ecological benefits. However, data on poultry response to these minerals are limited due to their reduced dietary levels (Saripinar-Aksu et al., 2012). When supplementing trace minerals in poultry diets, availability differences and contamination are significant concerns. For example, copper sulfate and zinc oxide, common inorganic sources of zinc for poultry, are often derived from the steel industry and may

contain contaminants such as fluorine and cadmium, which can enter the feed (Lopes et al., 2017). Additionally, mineral absorption can be impeded by antagonism, leading to reduced metabolism and absorption rates. In contrast, chelate complexes of metals with amino acids are inert due to ionic and covalent bonding between the ligand and mineral, remaining unaffected by factors that cause precipitation reactions in inorganic minerals after solubilization (Bao & Choct, 2009). Chelation reduces size and enhances stability, allowing the complex to pass through the GIT intact and be absorbed without degradation of its amino acids (Bao et al., 2007). Various research experiments are investigating the role and impact of different minerals in chelated or organic forms on poultry performance compared to their inorganic counterparts.

ORGANIC MINERALS CLASSIFICATION AND ABSORPTION IN THE GUT

Organic minerals are classified into two categories: natural and synthetic. Natural mineral complexes form during digestion, absorption, and metabolism within living systems. These complexes can either enhance or reduce the effectiveness of ingested minerals. Herrick (1989) categorized natural organic minerals into three types based on their biological functions: (1) those that transport and store metal ions, (2) those essential for physiological activity, and (3) those that interfere with metal ion utilization. Important metal-binding and transporting agents in the gastrointestinal tract, such as amino acids, EDTA (ethylene diamine tetraacetate), and other synthetic ligands, enhance the uptake of metal ions from the intestinal lumen into mucosal cells. For example, transferrin is crucial for the gut absorption, transport, and storage of iron. Additionally, metal complexes formed in biological systems facilitate the physiological activity of certain compounds, such as hemoglobin, which contains iron, and vitamin B12, which contains a central cobalt atom. Conversely, synthetic mineral complexes, often used as dietary supplements, aim to increase mineral utilization efficiency by complexing metal ions with various organic ligands, thus enhancing mineral absorption across the intestinal mucosa. Chelated minerals, upon intake, can be absorbed in any part of the small intestine, whereas inorganic metals are usually absorbed in the duodenum. After hydrolysis in the gastric region,

ligand atoms covalently bonded with metal atoms function as transporters and protect the mineral atoms from various antagonists such as oxalic acid, gossypol, and phytates. Subsequently, these complexes are absorbed by enterocytes in the intestine. In contrast, inorganic forms are absorbed only when they have transporters in the form of inorganic metals; otherwise, they are excreted (Świątkiewicz et al., 2014).

APPLICATION OF ORGANIC MINERALS IN BROILER NUTRITION

ORGANIC ZINC

Organically complexed zinc demonstrates higher bioavailability than its inorganic counterpart, proving more beneficial for birds (Salim et al., 2011). Numerous studies have investigated the bioavailability of organic zinc sources. For example, a study by Star et al. (2012) compared the bioavailability of zinc complexes with amino acids against zinc sulfate in broilers, reporting higher bioavailability with zinc propionate (Brooks et al., 2013). A relative slope assay, body weight gain, and tibia zinc concentration were used as response parameters, indicating that the relative bioavailability of zinc propionate was 119%, 116%, and 116%, respectively, compared to zinc sulfate (Brooks et al., 2013). Similarly, another study found that Zn proteinate positively affected feed intake, body weight gain, and Zn concentration in tibia ash and plasma of broilers. However, no significant difference in bioavailability between Zn proteinate and zinc sulfate was reported, possibly due to the low chelation bonding strength of Zn proteinate (Liu et al., 2013). Dong et al. (2023) examined the optimal dose of methionine hydroxyl analogue chelated zinc (MHA-Zn) in broiler diets with a 1.5% reduction in crude protein. They found no significant difference in the edible parts of broilers between the low-protein diet group (90 mg/kg MHA-Zn) and the normal diet group. Additionally, adding 90 mg/kg MHA-Zn to the low-protein diet significantly improved ileum morphology and apparent total tract digestibility of nutrients ($p < 0.05$). A 16S rRNA sequencing analysis indicated that supplementing the low-protein diet with 90 mg/kg MHA-Zn promoted beneficial bacteria in the cecum, such as *Lactobacillus*, *Butyricoccus*, and *Oscillospira*. Dietary organic Zn supplementation in broilers resulted in improved growth, nutrient digestion, antioxidant qualities,

humoral immunity, raw meat Zn content, and reduction in lipid peroxidation in meat. Moreover, the addition of organic Zn in broilers' diets resulted in higher levels of immunoglobulins A, M, and G, hence leading to the strengthening of the immunological capacity of birds (Moghaddam & Jahanian, 2009; Feng et al., 2010). Zn-methionine supplementation in poultry birds led to reduce low density lipoproteins (LDL), cholesterol, and blood triglycerides while enhancing the Zn level (Abd El-Hack et al., 2018)

ORGANIC MANGANESE

Manganese is an essential trace element for all living organisms and plays a critical role in various enzymatic functions, including defense against free radicals, protein metabolism, and bone formation. Key enzymes that require manganese include superoxide dismutase, ligase, transferase, and hydrolases (Keen et al., 2000). Manganese propionate is a common commercial source of organic manganese. Research has demonstrated that manganese propionate enhances bioavailability compared to inorganic manganese sulfate. However, at the molecular level, there are no significant differences between inorganic and organic sources regarding manganese superoxide dismutase activity (Wang et al., 2012). Conversely, Brooks et al. (2012) found that the bioavailability of manganese proteinate was 139% higher than inorganic manganese sulfate when added to a corn-soybean-based diet with increased levels of calcium and phosphorus. Two studies by Bai et al. (2012) explored the impact of different manganese sources on manganese transport by activating the divalent metal transporter gene in the small intestine of broilers. They discovered that chelated manganese with amino acids significantly increased the mineral's bioavailability and transport. Additionally, stronger chelation improved transport safety (Bai et al., 2012). Similarly, Li et al. (2010) observed positive effects of superoxide dismutase gene expression on manganese chelated with amino acids at both transcriptional and translational levels in the chicken heart.

Since 1994, organic Mn supplements have become more commercially available, with studies confirming their higher effectiveness compared to mineral Mn. Berta et al. (2004) conducted research on cockerel chicks, supplementing a basal corn-soybean diet containing 23 mg/kg Mn with levels of 0, 30, 60, and 240 ppm Mn from inorganic (MnO) or organic (Mn

fumarate) sources. The Mn source did not affect body weight, feed efficiency, or mortality rate. Li et al. (2005) used a basal diet with high calcium levels (Control: 18.5 g Ca/kg and 20 mg Mn/kg) and supplemented it with 60, 120, or 180 mg Mn/kg as Mn sulfate, Mn methionine E (with weak chelation strength), Mn amino acid B (with moderate chelation strength), or Mn amino acid (with strong chelation strength). The results indicated higher relative bioavailability for organic Mn sources with moderate or strong chelation strength, showing acceptable resistance to high dietary calcium interference during digestion. Mn accumulation in the tibia was higher in Mn-supplemented diets compared to the control group, and liver accumulation significantly increased only with 60 and 240 ppm supplements, regardless of the Mn source. Supplementation of organic Mn was found to be more effective than inorganic Mn in reducing lipoprotein lipase (LPL) activity in the abdominal fat of broilers.

It was also observed that dietary Mn reduced abdominal fat deposition by decreasing LPL and malate dehydrogenase activities or increasing hormone-sensitive lipase activity in abdominal fat tissue. Additionally, dietary Mn upregulated muscle MnSOD gene expression, resulting in lower malondialdehyde levels in leg muscle (Lu et al. (2007). Xia et al. (2022) conducted a study on Arbor Acres broilers with four treatment groups: control group (Mn sulfate, 60 mg/kg), Mn deficiency (22 mg/kg), Mn glycinate (60 mg/kg), and Mn proteinate (60 mg/kg). The study results indicated that Mn is essential for normal tibia development and the maintenance of redox homeostasis in broilers. Organic Mn supplementation, particularly Mn proteinate, enhanced tibia development, absorption efficiency, and overall oxidative stress status of broilers, demonstrating greater bioavailability than inorganic Mn. Therefore, the application of organic Mn sources may effectively reduce economic losses and address animal welfare concerns related to tibial dyschondroplasia in commercial poultry farming.

ORGANIC SELENIUM

Selenium is essential for growth and plays a crucial role in maintaining the normal development and production of animals (Liu, 2019). Selenium deficiency in poultry can lead to various health issues such as oozing diathesis, pancreatic fiber degeneration, muscle nutritional atrophy, reduced

reproductive performance, thyroid dysfunction, decreased immune function, and diminished stress tolerance (Su, 2016). Selenium deficiency is also linked to Keshan disease, Kashin–Beck disease, hypothyroidism, and a weakened immune system. Beyond meeting basic nutritional needs, selenium offers potential health benefits (Pan et al., 2007). Broiler chicks, known for their rapid growth, are particularly susceptible to dietary selenium deficiency. Classical selenium deficiency diseases in chickens include exudative diathesis, pancreatic atrophy, and nutritional muscular dystrophy (Huang et al., 2011). Selenium deficiency results in pathological changes in farm animals, leading to significant financial losses annually. Low selenium levels can cause nutritional muscular dystrophy, or white muscle disease, in young lambs, kids, foals, calves, and poultry (Żarczyńska et al., 2013). Additionally, selenium deficiency may lead to exudative diathesis in poultry and dietary necrotic liver degeneration, and mulberry heart disease in pigs. The selenium concentration in foodstuffs is determined by the selenium content in the soil. Animals may suffer from selenium deficiency due to low soil selenium levels, primarily affecting cattle and sheep, which are directly linked to soil via roughage. The risk of selenium deficit in pigs and poultry is relatively lower due to reduced dependence on local soil selenium levels and selenium fortification in feed mixes (Suchy et al., 2014).

Selenium supplementation for livestock and poultry offers two significant benefits: it improves the health and performance of animals and enhances the quality of animal products (e.g., meat, milk, and eggs), thus benefiting human health (Lymbury et al., 2008). While selenium is a nutritive functional additive, it is also a highly toxic mineral element. The limit of selenium in mixed feed for poultry is 0.5 mg/kg (Qi et al., 2019). Selenium sources can be divided into organic and inorganic forms. Inorganic selenium, mainly used as sodium selenite, is prevalent in livestock diets. However, its strong toxicity, low bioavailability, and oxidation potential can adversely affect animals and the environment (Chen et al., 2003). Organic selenium, typically in the form of selenium yeast or selenomethionine, has higher bioavailability and better absorption rates. Selenomethionine is actively absorbed in the small intestine via the neutral amino acid transport system in animals (Si, 2019). Organic selenium, often in the form of selenium yeast, can be directly absorbed, converted, and utilized by animals (Zhang, 2010; Wang

& Xie, 2003). It enhances antioxidant capacity, production performance, immunity, anti-stress capacity, and overall growth and development (Lei et al., 2020). Supplementing broilers with organic selenium, such as selenium yeast, significantly reduces water loss, improves feather quality, and boosts production performance by 2–4%. Additionally, the growth rate of broilers improves with selenium yeast supplementation (Ge et al., 2013).

Abd El-Hack et al. (2023) found that selenium nanoparticles (SeNP) supplementation in broilers' diets led to greater body weight, weight gain, and performance indicators compared to the control group after 38 days. Dietary interventions did not affect the feed conversion ratio (FCR). An et al. (2023) demonstrated that supplementing 0.4 ppm of organic selenium and selenomethionine in broiler feed resulted in the highest selenium deposition in breast meat, improved uric acid levels, total antioxidant status (TAS), jejunum morphology, and meat quality. Organic selenium supplementation enhances the activities of antioxidant enzymes and hepatic expression of selenoprotein genes in broiler chickens. However, neither organic nor inorganic selenium supplementation significantly affected growth performance and immune variables in broiler chickens (Prasad et al., 2023). Organic selenium in broilers is associated with higher fertility, hatchability, enhanced performance, lower mortality, and better meat quality (Surai, 2006; Ravindran & Elliott, 2017). Moreover, organic selenium helps to maintain tissue integrity and prevent oxidative stress-related diseases (Pappas et al., 2008). These studies underscore the benefits of organic selenium supplementation in broiler chickens.

ORGANIC COPPER

Inorganic copper exhibits strong oxidative effects in the absence of chelation with proteins, which can lead to the peroxidation of fats in the intestinal tract and feed (Surai, 2005). In contrast, the organic form does not have this pro-oxidant activity, thereby improving copper status in birds. Liu et al. (2012) compared the bioavailability of organic copper as copper proteinate with copper sulfate. The study indicated that the bioavailability of organic copper was 79.3% compared to 100% for inorganic copper sulfate, based on bile concentration of copper and daily copper intake in broilers fed a corn-soybean-based diet. However, this difference was not statistically significant.

On the other hand, substituting inorganic copper with organic copper (Cu proteinate) positively impacted body weight gain, feed conversion ratio, and nutrient utilization in broilers. Improved nutrient utilization was associated with better digestibility of dry matter, organic matter, sugars, and nitrogen-free extracts (Das et al., 2009).

Shamsudeen and Shrivastava (2013) studied the effects of aflatoxins during the supplementation of organic and inorganic copper sources, finding that organic minerals alleviated the negative effects of aflatoxins, thereby improving overall broiler performance. Furthermore, Kim et al. (2011) tested the efficacy of high doses of two different organic copper sources (copper proteinate and copper methionine) compared to antibiotics in broilers. They observed that organic copper had a more positive influence on various growth parameters than the tested antibiotic (avilamycin), with an increase in lactobacilli population and a reduction in *E. coli* in the intestinal lumen. This suggests that organic minerals can effectively replace antibiotics. However, Kwiecień et al. (2014) reported no significant differences in efficacy between copper sulfate and organic copper chelate with glycinate in broilers, though they noted positive effects on the biochemical characteristics of the femur. Lee and Kim (2024) found higher apparent ileal digestibility (AID) and standardized ileal digestibility (SID) of copper in corn compared to soybean meal, corn gluten meal, and fish meal. Deo et al. (2023) examined the effects of copper sulfate (CuS), copper chloride (CuCl), and copper propionate (CuP) at four different concentrations (8, 100, 150, and 200 mg/kg) in broiler chickens. They found no significant changes in body weight gain and feed intake, but a CuP-supplemented diet significantly improved the feed conversion ratio between 4–6 and 0–6 weeks ($P \leq 0.05$). The Cu sulfate-supplemented diet had a higher excreta zinc content ($P \leq 0.01$), while the Cu propionate-supplemented diet had the lowest. Excreta with higher iron concentrations were found in diets supplemented with copper sulfate and copper chloride ($P \leq 0.05$) compared to those supplemented with copper propionate.

CONCLUSION

In conclusion, both macro and micro minerals are vital for the growth performance and health of broilers, owing to their significant roles in

immunity, antioxidant activity, gut health, and various metabolic and enzymatic processes. Emerging trends in mineral supplementation for broilers highlight the use of nanoparticles and organic minerals, which demonstrate greater efficiency compared to traditional inorganic minerals. Scientific research has validated the benefits of these innovative supplements, noting their positive impact on broiler performance and health, as well as their reduced environmental excretion, thereby minimizing pollution. Future research should continue to explore the advantageous effects of minerals in nanoparticle, organic, and inorganic forms on broiler chickens to optimize their health and environmental sustainability.

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CHAPTER 6

VITAMINS IN BROILER NUTRITION

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INTRODUCTION

General Information About Vitamins

Vitamins are complex organic compounds that are necessary for the normal functioning of metabolism, are found in small amounts in the natural state of foods, and deficiency in the diet can cause diseases. The term vitamin was defined by Polish biochemist Casimur Funk in 1912 and is classified as either fat- or water-soluble. In their classification, their functions, not their chemical properties, are taken into consideration. They are separated from trace elements due to their organic structure. Daily consumption of micrograms and milligrams is needed in nutrition for living things to maintain their health, growth and reproductive abilities. Removing any of the vitamins from the diet may lead to deficiency symptoms. Most vitamins function as coenzymes that act as metabolic catalysts (McDowell, 2000;2004).

In the field of animal nutrition, feed factor is an element that should be evaluated within the scope of health, efficiency and economy. Among these elements, vitamins, which are in the additive class, are organic compounds that are vital for life and have catalytic, developmental and protective roles in the organism. Additives, including vitamins, constitute 1-2% of feed costs, and vitamin additives have an important statistical slice in this economic chart. In general, the potential for ration raw materials to be affected by the economy also affects vitamins, and the increase in the cost of vitamin additives has the opposite effect, potentially increasing ration costs. As a matter of fact, the serious decrease in the global vitamin supply in late 2017 even created a situation that could cause a significant increase in prices and inaccessibility problems for some regions. The situation that has emerged has also created pressure that may cause changes in vitamin supplement levels in broiler chicken feeds. Indeed, it should not be forgotten that even vitamin deficiencies at levels that are not taken into account can lead to more costly results than other acute raw material deficiencies. Seemingly insignificant deficiencies can have consequences on a wide scale, from a slight decrease in target body weight to a 1-2 point increase in feed conversion ratio or a decrease in egg production (Ward, 2022).

Poultry are raised in intensive production systems and are very sensitive to vitamin deficiencies. Insufficiency of microbial vitamin synthesis in the poultry gastrointestinal tract, normally high vitamin requirements and

stress caused by intensive feeding programs are the main reasons for sensitivity. Cereal oilseed meals (e.g. corn-soybean meal) in the diets of poultry should be supported by fat- and water-soluble vitamins (Scott, Nesheim and Young, 1982). Substances in the vitamin group such as thiamine, vitamin B6, biotin and folacin can generally be found in sufficient amounts in corn-soybean meal-based poultry rations. However, it should be known that vitamins A, D, riboflavin and B12 are insufficient due to the raw material content. Adding other vitamins to poultry diets, mostly according to preventive medicine and feeding procedures, is a practice done to take precautions.

The effectiveness of coprophagy is quite limited in birds living in cages due to ergonomic and physical conditions. Therefore, due to this deficit, which can only be achieved through coprophagy activity, they need more vitamins K and B than the rations of animals reared on the ground (Mc Dowell, 2006).

The proportions of additives to be included in the ration in broiler breeding are decided as a result of the age of the bird, its life and productivity period, and the experience and data obtained from the field and research. The rapid change in broiler genetics plays an important role in increasing the diversity and usage possibilities of additives (Ward, 2022).

An increase in broiler growth rates (approximately 3-4%) is achieved each year by obtaining more product with less feed consumption (Zuidhof, Schneider, Carney, Korver and Robinson, 2017). As a result, the feed conversion ratio (FYO) appears to have improved by 2 points in the last 10 years and the time to reach the appropriate slaughter weight has decreased from 52 days to 40 days in the last 25 years. Although this seems like a positive development, the shorter time has also shortened the time to detect and correct errors in nutrition, management or health programs (Agri Stats, 2022). During the rapid development process, problems such as mortality, bone deformity, contact dermatitis and negative effects on meat quality began to appear. Despite the rapid and high performance of muscle mass and bone development, the need for feed additives and vitamins has increased in order to protect from these negativities (Jiang et al., 2011; Mejia, Turner, Ward, Burnham and DeBeer, 2014). Today, as in other species, specific vitamins are used against acute problems in poultry. In broiler nutrition, not only the post-

hatching period but also the embryonal period should be taken into consideration. Therefore, it becomes important to know the vitamin needs and metabolism dynamics of living things during the egg stage. Vitamins other than the currently known vitamin C are found in eggs (Combs, 2012) and play important roles in embryonal development. Nutrition and absorption rates and vitamin interactions play an active role in the transmission and rate of vitamin transfer from the blood to the egg. Among these vitamins, vitamin D3 can reach the highest level in eggs (Yao et al. 2013), while others are known to remain at more limited levels (Ward, 2017).

In the classification of vitamins, their solubility in fat solvents or water is decisive. Fat-soluble vitamins are formed together with lipids in foodstuffs and are absorbed together with dietary fats. The absorption of fat-soluble vitamins is also facilitated in the presence of adequate bile flow, which is necessary for fat absorption, and appropriate micelle formation (Scott, Nesheim and Young, 1982). Water-soluble vitamins have no relationship with fats and their absorption is not affected by changes in fat absorption.

The main source of vitamins is plant tissues, and vitamins are found in animal tissues through the consumption of plants and/or the activity of microorganisms that synthesize vitamins in the animal body. An example of this is the presence of fat-soluble vitamins A and D in plant tissue as provitamins and their conversion into a vitamin in the animal body. However, provitamins have not been defined for water-soluble vitamins; only niacin is privileged in this group and can be obtained from tryptophan, an amino acid in many living species. The accumulation status in tissues is also decisive in the distinction between water- and fat-soluble vitamins. While water-soluble B vitamins can be found in all tissues, fat-soluble vitamins are almost completely absent in some tissues (McDowell, 2000).

Fat-Soluble Vitamins

The distinction between vitamins as fat-soluble and water-soluble vitamins in terms of their chemical structure and course in metabolism is evaluated in terms of nutritional dynamics. The choice of vitamin form is important in terms of its inclusion in the diet, its interaction with the diet, and

the quality of its absorption and availability by the organism. Fat-soluble vitamins are known as vitamins A, D, E and K.

Fat-soluble vitamins are hydrophobic and emulsify in the small intestine with the help of bowel movements. Fat-soluble vitamins are transported to the microvilli, where absorption occurs, through micelles consisting of a mixture of free fatty acids, monoglycerides and bile acids, and are subsequently involved in metabolism (Ward, 2022). The extent of interactions that occur between fat-soluble vitamins is closely related to their current levels in the diet (Abawi and Sullivan, 1989).

Water Soluble Vitamins

Vitamins B and C are known as water-soluble and/or water-soluble vitamins due to their chemical properties. These vitamins dissolve in the small intestine lumen and are absorbed through microvilli. Their absorption may vary depending on their molecular weight, ionization capacity, and ambient pH value, especially for vitamins B (Basu and Donaldson, 2003). Another factor that facilitates the absorption of vitamins from the intestines is their small molecular structure and weak ionic character. Due to these properties, niacin (niacinamide), pyridoxine, biotin and vitamin C are easier to absorb, while thiamine and vitamin B12 are relatively more difficult to absorb. A mechanism involving a transporter is required for the absorption of thiamine, B12, folic acid and vitamin C. A facilitating way here can be to resort to a simple diffusion mechanism for absorption depending on the high vitamin level in the diet (Basu and Donaldson, 2003; Ward, 2022).

The biological mechanisms in which B vitamins are involved are related to energy metabolism, carbohydrate and fatty acid metabolism, and DNA repair (Ward, 2022). Tufarelli et al. (2021) showed that folic acid, riboflavin, pyridoxine, thiamine and vitamins B12 made a positive contribution to hatchability, hatching weight and chick quality with in ovo injection application. Its importance in breeding chicken farming has been demonstrated by showing that methionine/cysteine metabolism is impaired in 18-day-old embryos in cases of pyridoxine or folic acid deficiency.

Vitamin A (C₂₀H₃₀O)

Although the importance given to all vitamins in living life and nutritional dynamics is at a similar level, the importance of vitamin A comes

to the fore in nutritional science. Vitamin A is found in plants as its precursor form, carotenoids (provitamin A). This form is converted to true vitamin A by an enzymatic reaction in the intestinal walls (Ensminger and Olentine, 1978).

Vitamin A is a type of unsaturated alcohol that is generally colorless, fat-soluble, and has a long chain and five double bonds in terms of its chemical properties. It consists of isoprene units with conjugated β -ionone rings in their side chains. Vitamin A is found in various forms in animal tissues. The alcohol form of vitamin A is retinol, which is also easily transferred to eggs in poultry. Retinal is formed by replacing the alcohol group with an aldehyde group. Retinoic acid is obtained by replacing it with an acid group. The esters of retinol are retinyl esters, and long-chain fatty acid esters are mostly found in animals (McDowell, 2000).

Vitamin A and carotenoids exist in protein structures. In order to be released from here, pepsin released from the stomach and proteolytic enzymes in the small intestine are needed (Ong, 1993; Ross, 1993). Bile salts, which act in the small intestine, turn fat globules formed by carotenoids and retinyl esters into micro-sized lipid clusters, so that digestive enzymes (pancreatic lipase, retinyl ester hydrolase and cholesteryl ester hydrolase) can act more effectively. Unlike other species, in poultry, only the unaltered hydroxy carotenoids are absorbed and stored in the tissues. Hydrocarbon carotenoids structurally show provitamin A activity. These provitamins are converted to vitamin A in the poultry intestine (McDowell, 2000) and are absorbed from the proximal jejunum. While the absorption level is 80% to 90% for vitamin A, this rate is approximately 50 to 60% for β -carotene (Olson, 1991).

Vitamin A requirement in broilers is determined as 1500 IU/kg according to NRC (1994) records. When determining the level of vitamin A to be added to the diet, β -carotene from natural sources should also be taken into account. If the level of vitamin A in the diet is high, the conversion from β -carotene decreases (Van Vliet, Van Vlissingen, Van Schaik and Van den Berg, 1996). The conversion rate of 1 mg β -carotene to vitamin A was determined as 1:1667 (Tuncer, 2020). As the level of β -carotene increases, the conversion efficiency decreases from 2:1 to 5:1 in poultry (Bauernfeind, 1972).

Corn, which is widely included in broiler diets, is a good source of β -carotene, but it should not be forgotten that the rate of carotene loss may be

high depending on storage conditions. In a study conducted by Quackenbush (1963), it was shown that carotene loss in corn could be 50% in 8 months and $\frac{3}{4}$ in 36 months at 25°C ambient temperature, whereas this rate would be much less in colder conditions (7°C).

Stabilized vitamin A supplements are available in commercially prepared diets, but losses are still likely to occur. It is known that to ensure stability, antioxidants, gelatin and emulsifying agents and sugar are used in esterification, spraying and drying, bead-shaped or prilled products (Shields Campbell, Hughes and Dillingham, 1982); However, long-term storage of vitamin A supplements before serving to animals may be unsafe.

Another indicator of the level of vitamin A taken in the diet is its concentration in egg yolk. In a study by Hill and Baker, (1961), it was reported that vitamin A concentrations in the diet (1,760, 4,400 and 22,000 IU/kg) caused a directly proportional increase in egg yolk (0.9, 6.3 and 16.3 IU/g).

The interaction of vitamin A with vitamin E in metabolism has been known for a long time and is an issue that should be taken into consideration in poultry breeding (Davies and Moore, 1941). It is known that deficiency symptoms occur as a result of suppression of the activity of vitamin E in poultry when fed with a diet containing high levels of vitamin A (Combs and Scott, 1974, Sklan and Donoghue, 1982). On the other hand, it has been shown that the problem of hypervitaminosis A can be prevented if the diet is high in vitamin E (tocopherol) (McCuaig and Motzok (1970). Vitamin E deficiency is explained by the oxidation in the duodenum and the decrease in the level of tocopherol in the later parts of the digestive system (Sklan & Donoghue, 1982). This was also demonstrated by the significantly reduced plasma α -tocopherol levels in chicks fed high levels of vitamin A (retinyl palmitate) for 24 days compared to chicks fed vitamin A in the control group. Another effect of dietary vitamin A is to increase the enterohepatic secretion of tocopheryl glucuronides into the duodenum. The production and excretion of glucuronides represents an important drain for vitamin E, as it shows little reabsorption into the tocopherol pool (Sklan and Donoghue, 1982).

In many species, if the diet is inadequate in terms of vitamin A, significant problems in reproductive metabolism can occur. Incubation ability in the field of poultry breeding is also affected by this situation. This problem

can be a potential source of trouble, especially for breeding broilers (McDowell, 2000). If puppies from breeders that are insufficiently nourished in terms of vitamin A continue to be fed in a similar manner, symptoms of deficiency begin to appear in their first weeks. On the other hand, vitamin A, which the mother hen receives at sufficient levels in the diet, can be effective in preventing any deficiency symptoms in the first 6-7 weeks of the chicks, even though there is no vitamin A in the diet (Scott, Nesheim and Young, 1982). This situation can be said to show that vitamin A has maternal functionality in the offspring.

Among the first symptoms of vitamin A deficiency (hypovitaminosis A), depending on the severity of the course in adult animals, there is loss of appetite, decline in live weight, fluffy feathers, eye lesions, muscle coordination disorder, ataxia, a significant decrease in egg production in breeders and a prolongation of incubation periods. Embryonic deaths due to vitamin deficiency in obtaining embryonic eggs, which is a very important factor in the production line, may cause great losses in this field. In later stages of the disease, discharge from the nose and eyes and stickiness on the eyelids may be observed (Jensen, 1965; Scott, Nesheim and Young, 1982; Ergün, 2020).

The basis of secondary infections seen in vitamin A deficiency is the replacement of the mucosal epithelium by stratified squamous and keratinized epithelium (keratinization) and the invasion of bacteria and other pathogenic microorganisms with the disintegration of the mucosa membrane. Pathological conditions can be seen in the mucosa of the nose, mouth, esophagus and pharynx, and the presence of white pustules in these areas draws attention (Scott, Nesheim and Young, 1982; Ergün, 2020).

Due to the loss of membrane integrity, changes in water retention, hypertrophy due to uric acid accumulation in the kidneys (Lopez, Phillips, Nockels and Faulkner, 1973), exudate production by affecting the epithelium of the eye, and eventually xerophthalmia (Ergün, 2020) may occur. Conditions that develop due to membrane disorder may also have negative effects on the immune system of animals, resulting in immune system weakness that may lead to the development of other diseases (Sijtsma, West, Rombout and Van Der Zijpp, 1989; Semba, 1998). The effect on T cell antigens is remarkable in problems seen in the immune system (Ross, 1992).

Since vitamin A is insufficient in poultry rations in terms of raw material composition, it is a vitamin that must be added to the ration (Tuncer, 2020). They are products that can be found commercially in different forms (liquid and oily, micro-emulsified, dry powder and granule), are mostly orange in color and have a shelf life of 1 year in production form (Blum et al., 2014).

Vitamin D (C₂₇H₄₄O)

Since vitamin D₃ is produced by the effect of UV light on 7-dehydrocholesterol in case of exposure to direct sunlight at a sufficient level and duration, many animal species and humans do not need to take vitamin D through food and feed. In broiler feeding, if there is not enough direct sunlight in closed environments (intensive livestock farming) (Tuncer, 2020), this need is tried to be met with rations and UV lights. Other factors affecting the vitamin D requirements received through ration feed raw materials include the Ca and P level in the ration, their ratio and availability to each other, the species and physiological characteristics of the animal (McDowell, 2000). The amount of vitamin D that broilers should receive as a diet within 0-8 weeks is 200 ICU/kg (Tuncer, 2020).

In order for vitamin D to be activated, it must undergo a hydroxylation process. While this process occurs mainly in the liver in mammals, it occurs in birds by D-25-hydroxylase enzymes in the extrahepatic tissues of the liver, kidney and intestines (McDowell, 2000). In broilers, the level of 7-dehydrocholesterol (provitamin D₃) is 30 times higher in the leg and foot skin than in the body skin. Circulating vitamin D₃ can reach 4-fold levels within 30 hours under the influence of light. As in all areas of poultry nutrition, vitamin D₃ is preferred over vitamin D₂ in broiler rations (Tian, Chen, Liu, Shao and Holick, 1994; McDowell, 2000; Tuncer, 2020).

Vitamin D deficiency symptoms are more likely to affect the health of broodstock in broilers due to lack of long-term feeding. The main symptoms that are generally seen similarly in all birds are the decrease in growth rate, egg production and hatchability. Vitamin D is a vitamin that is not present in grain and soybean-based natural feed raw materials that are added to poultry rations in high amounts. Since natural sunlight cannot be provided in closed growing systems, vitamin D must be one of the vitamins that should be added

to the ration in places where this type of cultivation is carried out (McDowell, 2000).

Vitamin D is essential for normal calcification of bones. Apart from growth retardation, which can be seen as a general symptom of deficiency, the first symptom in chicks is severe weakness and rickets in the legs (Tuncer, 2020). Hypocalcemia, which is seen together with vitamin D deficiency, causes the cartilage in the epiphyses of long bones to expand and the bodies to weaken, which disrupts skeletal development (NRC, 1994). Animals tend to squat and rest frequently. There is limping and stiffness in the leg muscles. It can be distinguished from vitamin A deficiency by the presence of a “limping” movement instead of ataxia. Problems in bone metabolism also include signs of beak and nail softening. This softening can be noticeable at 2-3 weeks of age. In internal examination, the formation of bead-like structures at the junction of the ribs and the spine in the bone structure is known as a typical symptom of vitamin D deficiency. In more advanced cases, curvature of the spine may be seen near the tail region (Scott, Nesheim and Young, 1982).

One of the symptoms of vitamin D deficiency in the diet, whose effects on bone metabolism are well known, is endochondral ossification defects (EOD). This problem begins to be seen on a global scale in broilers within the first few weeks following hatching, and it is seen to cause deformations in the bones, signs of fractures in later cases, and limping gait. A condition called rickettsia, which causes inappropriate bone formations, can be encountered together with Ca and P deficiency in the organic matrix of bone and cartilage. It is reported that the amount of bone ash is reduced and $1.25\text{-(OH)}_2\text{D}_3$ level is low in animals with this problem. On the other hand, it is known that mineralization of growth plates and bone maturation are better with high doses of vitamin D administered systemically in the first 2 weeks of broiler chickens (Vaiano, Azuolas, Parkinson and Scott, 1994).

Among the symptoms of vitamin deficiency, apart from poor feathering (Tuncer, 2020) and fluffy feather appearance, colour change is also noticeable. This change manifests itself with abnormal black pigmentation and if the deficiency is severe, pigmentation may become widespread (NRC, 1994). This reaction is reversible and the colour may return to its previous

state with sufficient vitamin intake, but the blackness may continue in areas that are normally colourless (McDowell, 2000).

The level of vitamin D in the diet consumed by the brood hen has the capacity and effect to affect the health and developmental status of the chicks in the initial period. The positive effect of vitamin D on egg production, hatching rate and shell quality is known (Tuncer, 2020). Therefore, the nutrition of brood hens also affects the chicks in terms of vitamin D (McDowell, 2000).

The clearly demonstrable effects of vitamin D in living things are related to biochemical reactions occurring in the intestines, bones and kidneys (Tuan and Suyama, 1996). Vitamin D, which is also known to be necessary for the embryonic development of chicks, has an effect that stimulates Ca mobilization in the egg yolk. It performs this effect through calbindin, a Ca-binding protein in hormonal metabolism (Elaroussi, Uhland-Smith, Hellwig and DeLuca, 1994). Calbindin is a protein that is deficient in the intestines of birds with rickets. Its presence and effectiveness in the intestines can be restored with the application of vitamin D (McDowell, 2000).

It has been determined that vitamin D (25-OH D₃) increases the number of mitotic satellite cells, which are important in skeletal muscle growth, when given to the mother hen and/or the chick at hatching (Avila et al., 2022). In another study showing its effects on microflora (Guo et al., 2022), it was reported that laying hens with vitamin D₃ deficiency were more susceptible to *Salmonella enterica* infections and intestinal mucosa damage, and *Escherichia*, *Enterobacteriaceae* and *Clostridia* species increased, whereas *Lactobacillus* and *Bacilli* species became dominant in animals whose diets were supplemented with 3,000 IU/kg of vitamin D₃. Similarly, there are findings that dietary 25-OH vitamin D₃ is beneficial in creating a wider spectrum of bacterial populations, increasing intestinal functions and intestinal indices, and increasing oxidative capacity indices and villus height (Wang et al., 2021). Commercially, 25-hydroxyvitamin D₃ preparations are available in thin form, beige to brown in color. It is also available in free-flowing or spray-dried powder form. Its stability lasts up to 1 year as long as it is stored in its production form (Blum et al., 2014).

Vitamin E (α – tocopherol, $C_{29}H_{50}O_2$)

Vitamin E, which was first introduced commercially in the 1930s after its effects on the reproductive system and fertility were noticed in some random experiments conducted on rats, is in the group of fat-soluble vitamins (Shastak, Obermueller-Jevic, and Pelletier, 2023). Vitamin E, which is the name of a group that has a close relationship and contains active compounds, has 8 different forms in nature, 4 of which are tocopherols and 4 of which are tocotrienols (Tuncer, 2020). It was reported by Mattill (1927) that vitamin E is better soluble, especially in unsaturated fats. The antioxidant effect of vitamin E, especially in membranes (Tappel, 1962), was revealed by Mattill (1927) and Cummings (1931) in the same years. Vitamin E is the second most important vitamin in terms of the amount used in animal nutrition today.

Vitamin E ranks second among the most commonly used vitamins in animal nutrition. In a recent market research conducted to reflect the current situation, it was determined that vitamin E consumption in animal nutrition corresponded to approximately 65,000 tons in the world in 2020. It has been reported that with this value, it ranks second after choline chloride (Hackett, 2021).

To summarize the stages of vitamin E preventing lipid peroxidation (Burton and Ingold, 1989): Radical (RO^*) formation is observed in fatty acids exposed to heat, light or trace elements. The RO^* formed reacts with oxygen and forms a reactive peroxyradical (ROO^*). A chain reaction begins as the ROO^* formed oxidizes other fatty acids. As the chain reaction gradually increases, vitamin E reacts with ROO^* and prevents the chain reaction from continuing.

Vitamin E is a vitamin that supports the immune system. The reason for this effect lies in its potential to affect leukotriene, thromboxane and prostaglandin synthesis, as well as arachidonic acid metabolism. Under stress conditions, which are important for broilers, this reaction becomes important when these compounds are synthesized endogenously in the body or negatively affect immune cell functions with their increased levels upon entry from outside (Hadden, 1987).

Other substances with which vitamin E can exhibit these properties together in a synergistic effect are vitamin C (Kazmierczak, Boguszevska, Adamus and Karwowski, 2020) and selenium, known as a trace mineral.

Selenium (Walsh, Kennedy, Blanchflower, Goodall and Kennedy, 1993) also contributes to the formation of this synergy by increasing the absorption of vitamin E from tissues. The complex collaboration between vitamin E, vitamin C, and selenium appears to optimize cellular protection against oxidative damage by harmonizing their antioxidant functions. A balanced diet that provides adequate levels of these micronutrients is necessary to reduce the risks associated with oxidative stress while maintaining optimal animal health and performance. Increasing the welfare and productivity of animals is among the important goals in order to achieve strategically better feeding procedures. In this regard, it may be useful to better understand the specific roles and interactions of antioxidants (Shastak, Obermueller-Jevic and Pelletier, 2023). These properties of vitamin E may also close important gaps in broiler breeding.

The effect of vitamin E on heat stress in broilers is known. Calik et al. (2022) reported that chickens exposed to heat stress showed less stress symptoms with the combined use of vitamin E and selenium, supporting this information. With the effects it provides, an increase in the growth performance and carcass yield of animals can be observed, while a decrease in mortality rates due to increase in environmental temperature, balance in body internal temperature and improvement in bone mineral density in the skeletal system can be observed. There is a synergetic effect created by vitamin E with selenium, but heat stress does not necessarily have to occur for this effect to be seen. Under normal growing conditions, it has a significant improving effect on production parameters without causing any negative effects on the general health condition. Likewise, it has a positive effect on growth hormone receptor (GHR) and insulin-like growth factor 1 (IGF1) gene expressions in the liver. These genes are effective in growth performance (Khalifa et al., 2021). Oxidative stress and histopathological formations in the duodenum and jejunum regions of the small intestines, which are indicators of infections caused by New Castle Virus, can also be prevented by vitamin E applications. Rehman et al. (2018) obtained results confirming this finding with the use of vitamin E at a level of 50 - 100 IU/day/kg body weight.

After the use of antibiotics for growth promotion in animal nutrition within the scope of the European Union *acquis* was banned in order to prevent antibiotic resistance and residues, different alternatives have been sought

(Genç and Ergün, 2018). The effects of intestinal microflora on growth promotion as well as combating diseases are known. In this context, there is a study (Luo et al., 2013) emphasizing that vitamins have positive effects on intestinal flora in birds, as in mammals (Pham, Dold, Rehman, Bird and Steinert, 2021). In this study, it was reported that the caecal beneficial bacterial diversity decreased by increasing the pathogenic bacterial population in 28-day-old broiler chickens that were deficient in vitamins. According to this result, it is possible to talk about the necessity of vitamins in ensuring intestinal homeostasis in poultry. In this context, it is thought that vitamin E may reduce the need for antibiotics and other pharmaceutical agents in the field due to its disease-preventive effects and may also have a growth-promoting effect (Hosain, Kabir, and Kamal, 2021).

The effects of vitamin E used during the production of animals on food products after their lives are also of great importance. The preventive effect of vitamin E on lipid oxidation, which shortens the shelf life of meat and causes off-flavors (Zdanowska et al., 2016, Trombetti et al., 2022) can be given as an example in this regard. Vitamin E is an element that can remain in broiler meat and can be used by humans when used as consumer food. The amount of vitamin E carried with meat in the broiler ration is at the level of 2% (Berges, 1999). In the light of this information, it is seen that vitamin E can be an important factor in the production of broiler meat, which has an important place in closing the protein gap in human food, without compromising food safety and quality (Shastak et al., 2023).

It is known that absorption in vitamin E metabolism occurs mainly in the small intestine and especially in the middle sections. Fat digestion plays a major role in this process, and bile and pancreatic lipase enzymes facilitate absorption (Sitrin et al., 1987), and most of them are absorbed in the form of alcohol. Vitamin E can be stored in all tissues in the body. The most accumulation occurs in the liver, followed by adipose tissue and muscle (McDowell, 2000).

Natural sources of vitamin E include corn, wheat, wheat bran, soy flour, rapeseed meal and sunflower seed meal, which can be used in broiler diets. Vitamin E, which used to be obtained from nettle and wheat germ in the past, is now mostly obtained synthetically. The fact that hundreds of kilos of plants were used to obtain it from nettle and that only the final acetate form could be

obtained by obtaining phytol as a precursor was seen as a disadvantage. Therefore, preparations based on wheat germ oil have come to the fore to obtain vitamin E. These products were initially used against hypovitaminosis and fertility problems, but with the widespread use of synthetic vitamin production (popularity and economic reasons), their biological activity unit was redefined and their use within commercial standards increased (Eggersdorfer et al., 2012). Vitamin E used today can be found in chemical forms such as dl- α -tocopheryl acetate, d- α tocopherol, dl- α tocopherol, dl- δ tocopherol, and dl- γ tocopherol (Arbeitsgemeinschaft für Wirkstoffe in der Tierernährung, 2002). The presence of sufficient vitamin E in rations is generally accepted with the presence of sufficient levels of dl- α -tocopheryl acetate (Darroch, 2000). Tocotrienol forms are not included in animal nutrition due to their negative effects on offspring development and the potential to endanger animal health due to problems such as erythrocyte hemolysis (Shastak et al., 2023). Examples of natural plant sources containing tocopherol derivatives (α , β , γ and δ) are corn, barley, wheat, rye and oats, and the oils obtained from these are wheat germ oil, corn oil, cottonseed oil, safflower oil, soybean oil and palm oil can be given (Ullrey, 1981). Of course, it is important to choose the species and forms that are suitable for use in broiler breeding from these sources.

The stability of all tocopherols found in natural sources is quite variable and weak. Improper processing techniques and storage conditions can cause oxidation and deterioration of the nature of tocopherols. Changes in their natural state also affect their activation. The main factors that cause this oxidation are heat, oxygen, humidity, oxidizing oils and microminerals and the reactions they create (McDowell, 2000). In this case, animals cannot get enough vitamin E they need and deficiency symptoms may occur. Vitamin E also has positive effects on male reproductive potential. In a study conducted by Shabani, Mehri, Shirmohammad and Sharafi, (2022) on Hubbard breed breeding roosters, it was reported that sperm quality improved in roosters fed with a diet containing 250 IU/kg vitamin E with 1% lecithin.

The causes of vitamin E deficiency (E hypovitaminosis) problems in animal nutrition can be listed as feed formulation problems, excess of polyunsaturated fatty acids, health problems that prevent intestinal absorption, parasitic infections, nutrient imbalance or deficiencies in the ration, stress, and

the presence of mycotoxins in the feed (Haga, Ishizaki and Roh, 2021). In its deficiency, disorders and diseases such as encephalomalacia (2-6 weeks), exudative diathesis, muscular dystrophy (with a deficiency of sulfur amino acids), immune deficiency, myopathic disorders, embryonic apoptosis, male infertility, hypoproteinemia, loss of cerebral stiffness, and hemorrhagic diathesis are observed (Tuncer, 2020; Shastak et al., 2023). Especially in the case of exudative diathesis, deaths may occur within 2-6 days after the animals begin to show signs of immobility, inability to access feed and water (McDowell, 2000).

According to NRC (1994) data, the vitamin E requirement for broilers in the 0-8 week period is 10 IU/kg. In dietary vitamin E requirement, the amount of Se with which it is metabolically related should also be taken into consideration. The data of Thompson and Scoot (1969) also confirm this information. In their study, it was reported that the Se needs of chicks fed a ration containing 100 IU/kg vitamin E were 0.01 ppm, while this rate increased to 0.05 ppm in case of vitamin restriction. It is known that vitamin E levels also vary depending on the presence of some other substances. Studies have shown that there is a directly proportional change in poly unsaturated fatty acids (PUFA), vitamin A, oxidizing substances, gossypol carotenoids, or trace minerals, whereas it is inversely proportional to sulfur amino acids and fat-soluble antioxidants (Dove and Ewan, 1990; Nockels, 1990; Hidiroglou, Cave, Atwal, Farnsworth and McDowell, 1992; McDowell et al., 1996).

Different forms of vitamin E are commercially available. RRR- α -tocopherol, All-rac- α -tocopheryl acetate, and RRR- α -tocopheryl are commercially available products. The products can be in the form of light yellow, cream-white, viscous oil and adsorbent powder. Its stability in the produced form can be up to 2 years and its stability in feed and premixes can be at least 6 months (Blum et al., 2014).

Vitamin K (C₃₁H₄₆O₂)

This vitamin, which is in the fat-soluble vitamin class, is known to play a role in important mechanisms through its relationship with proteins in metabolism. Half of the body's vitamin K reserve is kept in the liver (Thijssen, Driitij-Reijnders, and Fischer, 1996). It is also known as antihemorrhagic vitamin, coagulation vitamin and prothrombin factor (Tuncer, 2020). Vitamin

K can be found in nature in two sources and forms. The first of these is phyloquinone (vitamin K1), which is the most abundant in plant sources and in nature (Tuncer, 2020), and the other is menaquinone, which is produced by the bacterial flora of the digestive system (vitamin K2). In order for them to be absorbed, micelle formation is required, and bile salt and pancreatic fluid are required for this. Absorption occurs in the intestine. The vitamin identifies quinone compounds with antihemorrhagic properties and it is known that birds are sensitive to deficiency of this vitamin. This quinone group is a homologous group consisting of 2-methyl-1,4 naphthoquinone. The active compounds of vitamin K, formed by bacterial fermentation in the intestines, are menaquinones and are also known as vitamin K2 (McDowell, 2000). However, vitamin D production in the intestinal tract in poultry is not as efficient as in other species (Scott et al., 1982). The simplest form of synthetic vitamin K is menadione (K3) (McDowell, 2000).

The importance of vitamin K needs of poultry can also be defined by some metabolic deficiencies. Examples include rapid feed passage, short intestinal system and insufficient hepatic vitamin K epoxide reductase enzyme content of chicks. The fact that chicks cannot effectively recycle vitamin K epoxide (phyloquinone 2.3 epoxide) emphasizes the importance of vitamin K requirement (Will and Suttie, 1992). Vitamin K requirement in broilers is at the level of 0.5 mg/kg (NRC, 1994).

In poultry, plasma prothrombin level can decrease to below 2% of normal in poultry in case of vitamin K deficiency. This problem can be seen more severely in newly hatched chicks because this level is normally low. The presence of parental vitamin K deficiency before hatching is one of the main reasons for this. Situations where blood clotting failure can cause problems can only be seen in traumatic cases for poultry. At this point, deaths may occur due to bleeding during beak trimming or wing marking for research purposes. The prolonged clotting process caused by dietary vitamin K deficiency begins at 5-10 days of age and becomes visible at the clinical level within 2-3 weeks (McDowell, 2000).

With borderline levels of vitamin K deficiency, small hemorrhagic spots can be seen in the breast, leg and wing muscles, abdominal cavity and intestinal surface. Even if this situation occurs in the mildest case, it constitutes an important source of economic problems in broiler breeding

(Scott et al., 1982). In fact, this situation is one of the most common problems encountered and complained about by broiler meat consumers.

Causes of vitamin K deficiency in poultry include vitamin K deficiency in the diet, insufficiency in the ration consumed by the mother, insufficiency of synthesis in the intestine, inability to coprophagia, presence of sulfate-containing drugs in the diet and antibiotic applications. Coccidiosis, which affects the intestinal system, damages the intestinal wall and changes the flora, can also cause vitamin K deficiency (Scott et al., 1982).

Commercial forms of vitamin K are available as menadione sodium bisulfite (MSB) and menadione nicotinamide bisulfite (MNB). It can be in the form of a white-brown powder. Its durability in feed and premixes varies between 3 and 6 months (Blum et al., 2014).

Water-soluble vitamins

Thiamine (Vitamin B1, $C_{12}H_{17}N_4OS \cdot NO_3$ Thiamine mononitrate, $C_{12}H_{17}ClN_4OS \cdot HCl$ Thiamine hydrochloride)

The coenzyme or prosthetic group of thiamine, which is in the group of water-soluble vitamins, is thiamine diphosphate and is known to be most effective in oxidative decarboxylation metabolism. It also functions as a coenzyme cocarboxylase in all cells (Tuncer, 2020). Thiamine has a structure consisting of pyrimidine and a thiazole molecule bonded with methylene and containing nitrogen and sulfur. Also known as the first discovered vitamin, thiamine deficiency is rarely encountered in monogastric animals when grain-based nutrition is applied. Eijkman's observation in the 1890's that the symptoms seen in chickens fed with polished rice were similar to those of human beriberi and that the symptoms disappeared in animals consuming bran along with rice led to the understanding that this condition in chickens could develop due to nutritional errors (McDowell, 2000). In this respect, it is not a very common phenomenon to see its deficiency in poultry feeding where grain-based feeding is used.

Thiamine, a vitamin that is slightly soluble in fat and completely insoluble in alcohol, becomes susceptible to change when the pH is above 7 because the thiazole ring opens. Thiamine, which cannot maintain its stability for a long time in a humid environment, can remain intact for several hours even at 100 °C in dry conditions. It should not be forgotten that denaturation

can occur with autoclaving (Maynard, Loosli, Hintz and Warner, 1979). The risk of presence of substances with anti-thiamine effects (sulphur, pyriithiamine, oxythiamine and amprolium), which are widely found in nature and also obtained synthetically, in the ration raw material should be taken into consideration. These substances interact with thiamine in different ways in metabolism and reduce its effectiveness (Barclay and Gibson, 1982).

Due to these properties, the above-mentioned issues should be taken into consideration within the scope of the raw material production, storage and operations to be performed on the rations to be used in broiler feeding.

Adequate gastric hydrochloric acid production is important for the absorption of thiamine. Free thiamine is water-soluble and easily absorbed, while phosphoric acid esters are broken down in the intestine. Absorption of thiamine occurs mostly in the jejunum (McDowell, 2000). In case of insufficient thiamine that can be stored in organs, it can be supplied from these organs (heart, liver, brain and kidneys) (Tanphaichair, 1976). The amount of thiamine synthesis in poultry varies depending on the type of carbohydrate in the diet. It is known that this amount increases in the presence of heat-treated starch (Scott et al, 1982). The requirement for broilers has been determined as 1.8 mg/kg (NRC, 1994). Brewer's yeast, seed embryos and shells are products that can be included in broiler diets as a source of thiamine. The probability of this vitamin deficiency is quite low when a diet containing feed raw materials such as corn and soybeans is fed. However, it should not be forgotten that the vitamin will denature over time, and therefore long-term storage should be avoided or additional vitamins should be added to the ration as a precaution. Thiamine deficiency in broilers may cause loss of appetite and live weight, lethargy, neck opisthotonus, muscle contractions, and advanced findings such as polyneuritis, testicular and ovarian atrophy (McDowell, 2000, Tuncer, 2020).

Thiamine is commercially available as thiamine mononitrate 98% and thiamine hydrochloride 98% in fine granular and powder form in white to pale yellow color, and its shelf life can be up to 3 years in production form (Blum et al., 2014).

Riboflavin (Vitamin B2, C₁₇H₂₀O₆N₄)

Another water-soluble vitamin that may be deficient in animals fed a diet low in animal protein is riboflavin. In poultry fed a corn-based diet, there is a possibility of riboflavin deficiency if purified amino acid sources are not used (Chung and Baker, 1990). Liver, kidney and heart muscle are the most stored organs and are necessary for the enzymes required to utilize carbohydrates, proteins and fats in the diet to function.

The sources of riboflavin are green plants, mushrooms and yeast, but they can also be bacterial (Tuncer, 2020). Chemically, riboflavin is found in the structure of Flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). It has ribose alcohol and dimethyl isoalloxazine nucleus. Due to its physical properties, it is odorless and yellow-orange in color. Its melting point is 280 °C (McDowell, 2000). Since it is rarely found free in nature, it is made free by treatment with acids or enzymes (Scott et al., 1982). There are some points to be considered in its storage. Riboflavin, which has a relatively high stability, is unstable in aqueous solution states against visible and ultraviolet light and its structural properties are naturally affected. This instability increases with heat and alkaline environment. When it dries, its effect from light decreases (McDowell, 2000). The need for riboflavin in poultry decreases with age. The necessity of riboflavin is emphasized for the prevention of leg muscle paralysis seen in poultry rather than for growth (Ruiz and Harms, 1988). Another issue that can cause riboflavin deficiency in poultry is renal riboflavinuria, a hereditary recessive disease known as the absence of riboflavin-binding protein (White, 1996). In the presence of this disease, embryonal development usually does not continue after the fourteenth day of incubation and the chicks do not survive (Clagett, 1971). This condition is also likely to be seen among the problems before egg hatch in broiler breeding. The first symptoms in chicks appear as curling and paralysis of the fingers. The main cause of curling and paralysis is peripheral nerve degeneration and entrapment of the sciatic nerve. In later stages, dermatitis may be seen on the feet, legs, beak area and eyelids (Tuncer, 2020). Affected animals do not want to move (Scott et al., 1982). Other symptoms include growth retardation and diarrhea on the 8th-10th day, and death may be observed in the 3rd week (McDowell, 2000). According to NRC (1994), the riboflavin level that should be present in the ration for broilers during the 8-

week period should be 3.6 mg/kg. This rate should be increased if the amount of protein in the ration is increased for special purposes (scientific, breeding, etc.). Riboflavin is commercially available in powder form, obtained by spray-drying method, containing 80% riboflavin, has an orange-brown to yellow-brown color and has the ability to maintain its durability for up to 3 years in its production form (Blum et al., 2014).

Vitamin B6 ($C_8H_{11}NO_3 \cdot HCl$)

Vitamin B6, which is found in the form of pyridoxine (pyridoxol), pyridoxal and pyridoxamine compounds according to their alcohol, aldehyde and amine groups, is not frequently seen in these animals due to their natural presence in grain raw materials in broiler rations, except for some conditions. While the pyridoxine form is usually found in plant forms, the others are of animal origin. Heat, high pH and light can cause their natural state to deteriorate, in which case insufficient intake from the ration may occur in broilers. While the absorption of vitamin B6 occurs primarily in the jejunum, it occurs in the ileum by passive diffusion in further sections (McDowell, 2000). Vitamin B6 can be stored in very small amounts. The storage location is mostly skeletal muscles. The metabolic activities in which vitamin B6 plays an important role in living things are fatty acid, amino acid and carbohydrate metabolism and the citric acid cycle. It is known that more than sixty enzymes are dependent on vitamin B6 coenzymes (Merrill and Burnhan, 1990). In addition to these metabolic effects, they have been shown to play a role in the development of resistance against viral diseases, uremia, rheumatoid arthritis and tumor formations (Rall and Meydani, 1993) and the formation of B-cell mitogen and interleukin 2 in the immune system (Meydani et al., 1991). Although the risk of vitamin B6 deficiency is not anticipated for broilers due to the nature of the feed raw material in the ration, vitamin B6 supplementation is applied as a precaution. In this direction, the recommended riboflavin requirement for broilers is at the level of 3.0 - 3.5 mg/kg DM. In case of possible deficiency, lack of coordination, convulsions, anemia and live weight loss can be seen in animals (Tuncer, 2020). Taking this precaution may be considered appropriate depending on the possible heat in the raw materials during ration production, changes that may occur during mixing, raw material loss during transfer processes, high crude protein values and the

condition of the special rations used in experimental studies. Pyridoxine is commercially available as pyridoxine hydrochloride 99% in white crystal and/or powder form and is stable for up to 3 years in production form (Blum et al., 2014).

Vitamin B12 (Cyanocobalamin, $C_{63}H_{88}O_{14}N_{14}PCo$)

It is an essential vitamin that is not found in plants because it can only be produced by microorganisms. Considering the plant-based diet of commercial broilers, another vitamin that should be considered in their rations is vitamin B12. It has a complex structure containing hydrogen, oxygen, nitrogen, phosphorus and cobalt, and a cyan group is attached to the cobalt in the middle of the molecule (Tuncer, 2020). Although vitamin B12 is generally resistant to environmental conditions, it has a slight sensitivity to heat, light, moisture and oxygen (McDowell, 2000).

In broiler breeding, in case of vitamin B12 deficiency, growth retardation, decrease in live weight gain, poor feathering, renal problems, heart and liver steatosis, feed consumption and feed utilization rate are generally seen (Tuncer, 2020). Perosis and weakness in the legs are also among the problems that occur secondary to the effect of vitamin B12 deficiency on the nervous system. Similar symptoms can be seen in the deficiency of choline, betaine and methionine, which provide methyl, depending on the relationship of vitamin B12 with methyl groups. In advanced cases, gizzard erosion, anemia, heart and kidney steatosis are the conditions that can be seen (NRC, 1994). The fact that deaths occur on the 17th day in embryonic vitamin B12 deficiency (McDowell, 2000) also shows that it is important for breeding animals to be fed with sufficient vitamin in nutrition. In breeding systems where coprophagy is not possible in poultry, it is particularly important that vitamin B12 is included in the diet at an adequate level (McDowell, 2000). Since its stability in water is very low (Pannevis and Earle, 1994), B12 supplementation should be provided in the diet for broilers. According to NRC (1994) reports, 10 µg/kg DM vitamin B12 should be included in the diet for broilers. It is commercially available as 1% concentrate or 0.1% preparations. The products, which can be found in pale pink-pink liquid or powder form, can maintain their stability for up to 2 years in production form (Blum et al., 2014).

Pantothenic Acid (C₉H₁₇NO₅)

Pantothenic acid is located in the structure of Coenzyme A and acyl carrier protein and is involved in the steps of carbohydrate and fat metabolism. Although it is a vitamin found “everywhere” based on the literal meaning of “pantos”, it must be added to rations in order to ensure performance in broiler feeding. It is also known as chick antidermatitis factor because it was first identified with pellagra-like dermatitis disease in chicks (McDowell, 2000). Chemically, pantothenic acid is an amide composed of β -alanine and pantoic acid, and its commercial form is calcium pantothenate (Tuncer, 2020).

It is a vitamin that is needed more for muscle development in broiler breeding rather than egg production, and according to NRC (1994) data, it is recommended to take 10.0 mg/kg/DM at 0-8 weeks of age. The level of other vitamins it is related to is also important in determining pantothenic acid needs. In this context, it has been observed that pantothenic acid needs increase in cases of B12, biotin and vitamin C deficiency and this relationship is tighter in poultry than in other species. It is also necessary to consider that heat treatments have a negative effect on pantothenic acid levels in feeds (Scott et al., 1982).

When pantothenic acid sources are examined, other than tapioca flour, other plant and animal feeds are at sufficient levels (Tuncer, 2020), raw materials such as rice bran and wheat bran, which are by-products of milling, contain two to three times higher pantothenic acid than many grains (Southern and Baker, 1981) is known.

In its deficiency, growth retardation, mathematical increase in feed conversion ratio, skin lesions, nervous system disorders, gastrointestinal disorders, inhibition of the formation of immune system elements and therefore vulnerability to infections and adrenal system problems are observed in different species, while in poultry, it causes mainly nervous, adrenal and dermal problems. It is known to occur (Scott et al., 1982). Most of the problems in breeding broilers result in decreased egg production and hatchability. Subcutaneous hemorrhages and embryo deaths are also among the conditions seen in chicks. Although disease symptoms are also seen on the beak edges, problems in epidermal formations are more common in the feet. Cracks and peeling can be seen in these areas. In advanced cases, thickening

and callousing of the foot skin occurs. Wart-like formations are seen on the toes. Viscous discharge from the eyes may be seen on the 12th-14th day of the disease. Symptoms in young chicks can be confused with biotin deficiency (McDowell, 2000). In broilers, hypertrophy and yellow discoloration of the liver are observed after slaughter (Scott et al., 1982).

D-pantothenic acid is fluid, pale yellow and hygroscopic. It is commercially marketed as calcium D-pantothenate (at least 98%) and D-panthenol (at least 98%). Its stability in feed varies between 3 and 6 months (Blum et al., 2014).

Niacin (Nicotinamide, Vitamin B3, $C_6H_5O_2N$)

Niacin, also known as vitamin B3, has two forms: nicotinic acid (3-pyridine carboxylic acid) and nicotinamide. Niacin is the vitamin with the simplest structure. The term niacin is known as a general definition of derivatives with the same biological activity as pyridine 3-carboxylic acid and nicotinamide. It can be converted from tryptophan acid in the intestinal wall. It is known for being in the coenzyme forms of niacin, nicotinamide, NAD and NADP. Enzymes containing NAD and NADP play an important role in the metabolism of carbohydrates, proteins and lipids from nutrients and in oxidation-reduction systems. Therefore, niacin is important for energy metabolism reactions. The vitamin is quite stable against heat, light, air and alkaline environment (McDowell, 2000; Tuncer, 2020).

Nicotinamide is the first form of niacin in the organism and is transformed into coenzyme forms in the tissues. When needed, it is released by liver and intestinal glycohydrolases for transfer to NAD-synthesizing tissues (Stein et al. 1994; McDowell, 2000).

Barley, peas, blood meal, brewer's grains, cottonseed and meal, corn, fish meal and liver meal can be given as examples as natural sources of niacin (NRC, 1982). Among the characteristics of niacin that should be considered in animals fed with grain-based diets, the form in which it is present is of great importance. It is known that 60% of niacin in the bound form is bound to polysaccharides and 40% to peptides or glycopeptides (Mason, Gibson, and Kodicek, 1973). The blocking function of these molecules, which prevents digestive enzymes from affecting the niacin macromolecule bond, is shown as the possible reason for niacin deficiency (McDowell, 2000). The niacin

requirement for all broiler breeds and for each period is 25–30 mg/kg (NRC, 1994). It is known that niacin can be synthesized in poultry. However, this synthesis may not be at the required speed for healthy development. The presence of sufficient tryptophan in the diet is also important for niacin adequacy. Tryptophan deficiency is not a very common finding in chicks due to the significant amount of tryptophan found in egg yolk protein. Therefore, niacin deficiency is not one of the conditions that can be seen under these conditions (Ruiz and Harms, 1990a,b; NRC, 1994). In niacin deficiency due to tryptophan deficiency, tibiotarsal joint widening, leg bending, feathering problems and dermatitis cases on the feet and head can be seen. In chicks, loss of appetite, tongue and oral cavity infections and black tongue disease can be seen (McDowell, 2000; Tuncer, 2020). Niacin is commercially available in pure nicotinic acid and nicotinamide forms. It is available as a product in white and off-white granule form and its durability in feed varies between 3 and 6 months (Blum et al., 2014).

Choline (β -hydroxyethyltrimethylammonium hydroxide, $C_5H_{14}NO$)

Choline is a vitamin necessary for the realization of some metabolic processes in living beings. Unlike other B vitamins, it is a vitamin that can be synthesized in the body but still needs to be present in broiler diets (McDowell, 2000). According to NRC (1994) reports, the recommended level for broilers is 1300 mg/kg DM for the first 3 weeks and 750 mg/kg DM for 3–8 weeks, while it should not be forgotten that young animals are more sensitive to deficiency than older animals. It was reported in the study conducted by Derilo and Balnave (1980) that choline can have a toxic effect in case of overdose on broilers, and it was reported that toxicity has a negative effect on live weight gain. An increase in the ration protein level also increases the choline requirement. Choline is found in all living cells, mostly in the form of phosphatidylcholine, lysophosphatidylcholine, choline plasmalogens and sphingomyelin, which are the basic components of all membranes, and choline requirement increases especially in the chick period due to vitamin B12 and folacin deficiency (Welch and Couch, 1955; Zeisel, 1990).

Perosis is one of the diseases that is likely to be seen as a result of the application of incorrect feeding programs in broiler breeding. While there is a

need for bone cartilage matrix development in the prevention of this disease, choline has an important place as a component of the phospholipids required to ensure this (McDowell, 2000).

When the grain sources in broiler nutrition are evaluated and general averages are taken into account, it is seen that it is found at 0.1% in grain seeds and 0.2% to 0.35% in legumes. It is found at 1177 ppm in barley, 620 ppm in corn, 1116 ppm in oats, 1053 ppm in wheat, 1797 ppm in wheat bran and 2916 ppm in soybean meal (DuCoa, 1994). The type of raw materials included in the ration may also have an indirect effect on choline level. An example of this is that the production of trimethylamine in the intestine (resulting from the bacterial degradation of choline) in chicks is higher in animals with rapeseed meal in the diet than in those consuming soybean meal (McDowell, 2000). Miles and Harms (1983) reported in a study that there was no need for additional methionine supplementation for animals fed broiler rations containing 0.11% choline and 0.1% sulfate. Miles et al. (1983) reported that sulfate supplementation given together with choline or methionine in broilers provided better values in live weight gain compared to the application of either one alone. Spires, Botts, and King (1982) found in a study that adding methionine and cysteine to broilers at the rate of 0.3% and 0.43%, respectively, in the starting period and 0.25% and 0.42% cystine in the finishing period, could reduce the need for choline by one third. Similarly, it is known that betaine supplementation (betaine hydrochloride form) (Odle, 1996) to broiler diets has an increasing effect on choline oxidation. Possible symptoms in broilers in case of choline deficiency include perosis, tibiotarsal joint flattening (Scott et al., 1982), fatty liver, hemorrhage in the kidneys, reduced breeding and egg production, growth retardation and sclerosis in the arteries (NRC, 1994; Tuncer, 2020).

Folic Acid (Vitamin B9, C₁₉H₁₉N₇O₆)

Folic acid is a type of B vitamin that must be taken with the diet and can be found in different forms after absorption in the organism (Liu and Ward, 2010; Ratajczak et al., 2021). Muscle structure and percentage in broiler breeding is one of the most important parameters of economic farming. While the muscle structure of broilers constitutes approximately 40% of the total body weight, breast meat muscle constitutes approximately 50% of the

muscle tissue in chickens (Liang et al., 2022). The effect of folic acid on muscle development is known. Amino acid metabolism modulation (Brade, Jarck and Vetter, 1972; Yao et al., 2013) and myogenesis-stimulating Akt pathway activation (Hwang et al., 2015) are important roles in this function. Folic acid is needed for uric acid synthesis in protein metabolism. For this reason, the need for folic acid also increases with increasing dietary protein value (Scott and Weir, 1981).

It has been reported that folic acid, which has been shown to affect genes responsible for muscle growth and development in offspring and to affect skeletal muscle protein metabolism when administered to mothers in mammals (sheep) (Wang et al., 2019), significantly reduced abdominal fat weight and percentage when administered to broiler chickens via perfusion, and no negative effect on carcass weight was observed (Liu, Liu, Ren, Cao and Yang, 2019).

It has been shown that breast meat/carcass ratio can be increased with folic acid application (Liu, Liu, Ren, Cao and Yang, 2019). The same study emphasized the effect of folic acid on gene expression. Gene function and myogenic regulatory factors for muscle protein synthesis are of major importance. This study showed that high levels of folic acid increased MyoG gene expression in breast meat muscle. It was reported that folic acid had a significant ($P<0.05$) positive effect on live weight gain and average daily feed consumption in broiler chickens. In the study, it was reported that the breast meat percentage was significantly ($P<0.05$) higher in the group consuming high levels of folic acid (13 mg/kg), abdominal fat accumulation was significantly ($P<0.05$) reduced in the broiler starter period, and serum ALT and LDL levels were lower than in the control group. These results also emphasized that folic acid has a liver-protective effect. Although folic acid deficiency can be easily created in broilers with research diets, adequacy in terms of folic acid content in the diet is generally achieved in commercial and practical feeding. Feed materials such as corn and soybean meal, which are generally found in rations, are considered rich sources of folic acid. However, this situation can be encountered if sucrose is used as a carbohydrate source and the ration crude protein value is high (Scott et al., 1982). In addition, it is possible to see that they are much more sensitive than other animal species when fed with an incorrect ration that is insufficient in folic acid. Another

advantage of broilers in terms of benefiting from folic acid is that although a significant portion of folic acid in poultry feeds is in conjugated form, these species can benefit from all of the folic acid (McDowell, 2000). According to NRC (1994) reports, the folic acid requirement of broilers is reported as 0.55 mg/kg DM. In case of folic acid deficiency in the diet, decreased feed consumption, developmental delay, macrocytic anemia, perosis, cervical paralysis, poor feathering and pigment loss in feathers are the symptoms that can be seen in animals (McDowell, 2000). Sulfonamides and aflatoxins, which inhibit the intestinal microflora, are known as antagonists of folic acid (Blum et al., 2014). Folic acid is commercially available in anhydrous base at least 96% or 80% and sprayed on a carrier. Common physical properties of commercial folic acid products are yellowish-orange in color and crystalline powder. Its shelf life in production form is at least 24 months (Blum et al., 2014).

Biotin ($C_{11}H_{18}O_3N_2S$)

For a long time, it was thought that there was no need for additional biotin in broiler diets, but as a result of clinical problems experienced in the mid-1970s, it was understood that this judgment was not correct (McDowell, 2000). Biotin, which is resistant to general conditions and room temperature, begins to lose its naturalness intermittently with the decrease in pH and the increase in ultraviolet light (Scott et al., 1982). The development of biotin deficiency in poultry can be shaped by the lack of biotin in the diet.

Absorption occurs from the intestine (Said and Derweesh, 1991). Biotin, which acts as a coenzyme in carbohydrate, fat and protein metabolism, is effective in the transformation of these three structures. Biotin is also effective in regulating normal blood sugar levels when dietary carbohydrate levels are low and from protein and fat metabolism. It is known that polyunsaturated fats and B vitamins in the diet can affect the biotin requirement in poultry (McDowell, 2000). According to NRC (1994) reports, biotin requirement for broilers has been determined as 0.15 mg/kg/DM. There are sufficient and insufficient biotin sources among the raw materials commonly included in broiler diets. Corn, wheat and other cereals contain relatively insufficient levels of biotin. Oilseed meals are richer in biotin than cereal grains (Frigg and Volker, 1994).

The symptoms observed in its deficiency can be listed as growth retardation, mathematical increase in feed conversion ratio, breakage in feathers and feathers, dermatitis and leg and beak deformities. If the symptoms are to be listed; The first symptoms start with growth retardation and a decrease in the density of feathers is noticeable, followed by dermatitis findings. Then, perosis in the leg, bending of the legs (Bain, Newbrey and Watkins., 1988) and beak deformity are seen. Hardness and bleeding cracks can be seen on the soles of the feet. The picture can worsen with secondary bacterial infection caused by bleeding cracks and can progress to necrosis and organ loss. Dry scaling can also be seen on the upper parts of the feet and legs. Swelling and exudate discharge and adhesions can be seen on the eyelids. Since the development of symptoms can occur from the chick period to the 25th day, it is possible to see all of these symptoms when the commercial life of the broilers is considered. Even in cases where the symptoms are mild, the meat market value of the broiler can be negatively affected since there will be muscle development retardation. It should not be forgotten that biotin application cannot be curative when perosis deformities occur, therefore, it would be a more appropriate practice to apply preventive measures (McDowell, 2000). Biotin is commercially available in pure, 1%, 2% and 10% levels. The common physical properties of the products are white or almost white in color, powder and ready to spray. Its durability in feed and premixes is at least 6 months, while it can extend up to 2 years in production form (Blum et al., 2014).

Vitamin C (C₆H₈O₆)

In general, since ascorbic acid can be biosynthesized in many animal species (poultry, ruminants, pigs, horses, dogs and cats), there is no clear information regarding the required level of vitamin C in their diets (McDowell, 2000). However, in addition to this, studies reporting the positive effects of vitamin C added to the diet, especially on the reproductive potential of breeding animals, show the importance of this vitamin. In a study by Monsi and Onitchi (1991) in which 0, 125, 250 or 500 ppm ascorbic acid was added to the diets of broilers subjected to heat stress, it was reported that reproductive parameters (semen volume, sperm count and motility) improved in parallel with the increasing level.

In another study conducted with broilers subjected to heat stress (Njoku 1986), it was reported that 200 ppm ascorbic acid supplementation had a positive effect on live weight and feed conversion ratio. Pardue, Thaxton and Brake, (1985) showed that vitamin C reduced plasma adrenal corticosteroid concentration under heat stress. It is generally recommended to include it in rations, especially in broiler breeding, due to its immune-strengthening effect against infectious diseases (Newcastle disease, *Mycoplasma gallisepticum*, E. Coli, typhoid infections, etc.). Orban, Roland, Cummins and Lovell, (1993) and Zapata and Gernat (1995) showed in their studies in broilers that vitamin C plays an important role in the metabolic conversion of vitamin D3.

Vitamin C is commercially available as ascorbic acid, in white to slightly yellow crystalline powder form, in phosphorylated beige powder form, and in spray-dried powder form. Its durability in production form can last up to 2 years (Blum et al., 2014)

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CHAPTER 7

STRATEGICALLY IMPORTANT MINERALS FOR THE PERFORMANCE, REPRODUCTION AND HATCHABILITY OF BROILER BREEDERS

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INTRODUCTION

Broiler breeding lines are bred for high egg production , high growth rate and high feed conversion performance. Thus, breeders carry a constant stress due to their heavy metabolic load. Due to this stress, their performance decreases, their immune system weakens and they become more vulnerable to diseases. In order to reduce these negativities, care is taken to be extremely sensitive in the feeding of breeders and to meet their nutrient needs optimally. In this context, micro minerals such as Selenium (Se), Zinc (Zn), Copper (Cu), Iron (Fe) and Manganese (Mn), which are low in amount but have great effects on metabolism, are found in metal-containing enzymes and in extra-embryonic membranes of the embryo and cells. It functions as a catalytic or structural cofactor in proteins. These minerals play unique and comprehensive roles in important biological processes, including protection against oxidative stress, antimicrobial, antimutagenic, immune system regulating, growth, development and reproduction. The use of minerals with high bioavailability produced in breeders with the most modern production technologies not only reduces oxidative stress, but also contributes positively to the embryonic period and post-incubation development of chicks, beyond increasing the reproductive performance of breeders. On the other hand, due to limited information on the trace mineral requirements of breeders, the old literature continues to be used as a reference. In this review, current studies on the performance of broiler parents and the effects of some minerals (Se, Zn, Cu, Fe, Mn) that are thought to be strategically important for breeders on embryo development and later life periods of chicks are discussed.

Meat and egg production in poultry depends on the availability of good quality hatching eggs. Egg production in commercial flocks is largely determined by the hen's genotype, while egg quality and hatchability depend on various factors such as the age of the animal, feeding and housing conditions. Therefore, it is vital for breeders to achieve fertility and for egg producers, maximum egg yield and maximum income per hen. Vitamins and minerals are very important nutrients for metabolic and physiological processes and therefore fertility and reproductive performance of the animal (Yaripour et al., 2018).

The great importance in parent flocks of Factors such as survivability, flock uniformity, number of hatching eggs, high hatchability and healthy

chicks from these eggs. In this sense, the basic nutritional needs of breeding animals are met in the most ideal way and maximum benefit from their genetic potential is aimed. Existing lines have been genetically improved with a focus on maximum performance based on rapid growth and continuous improvement of feed conversion (Givisiez et al., 2020). It has been reported that body weight gain and feed efficiency increased by 3.30% and 2.55% per year over a 48-year period from 1957 to 2005 (Zuidhof et al., 2014). Thus, over a period of approximately 50 years, the broiler industry has halved the amount of feed required to produce chicken meat, while increasing breast meat yield by 67%. However, brood chickens that lay eggs for approximately 64 weeks are under a very high stress due to their metabolism being overloaded (Min et al., 2018; Liu et al., 2019; Umar et al., 2020). Differences in bone properties between embryos of fast-growing and slow-growing lines incubated under the same conditions may be due to embryo genotype and maternal influences (egg size, egg content, and male-female genetic differences). Understanding the importance of these two factors can help focus on more effective ways to reduce the incidence of leg problems in broilers (Yair et al., 2017). At this point, the content of eggs obtained from breeders gains importance. Stress caused by both metabolic load and other environmental factors negatively affects many performance parameters such as reproduction, egg production and quality, hatchability of broiler breeders (Sharideh et al., 2019). In addition, the aging of animals adversely affects all production parameters, especially reproductive efficiency, and ultimately the profitability of the enterprise (Surai et al., 2019).

A balanced diet plays an important role in the strength of the breeder's immune system, hatching egg production and the quality life of their offspring. Minerals play a very important metabolic role in maintaining body homeostasis, bone formation, and being present in intracellular and intercellular fluids. Unlike macronutrients, they come to the forefront by causing significant metabolic disorders even with a deficiency or excess of ten parts per million. Trace minerals such as Iron (Fe), Selenium (Se), Copper (Cu), zinc (Zn), Manganese (Mn) are involved in the activity of many enzymes associated with the metabolism of carbohydrates, lipids, proteins, nucleic acids, hormone secretion pathways and immune defense systems. As it is a cofactor of various liver enzymes, Zn deficiency causes various liver

diseases, including cirrhosis and hepatitis. They are necessary for the antioxidant defense system, are structural components of superoxide dismutase, glutathione peroxidase, and increase the synthesis of different metallothioneines that act as free radical scavengers. Thus, these minerals have a unique and comprehensive role in important biological processes, including immune function, growth, development and reproduction (Yaripour et al., 2018; Fouad et al., 2020).

Recently, it has been determined that the use of trace minerals in the feed of broiler breeders strengthens the immune system, regulates metabolism, increases egg and progeny yield, and positively affects embryo development and yield and viability in the following periods (Uni and Ferket, 2003; De Olivera et al., 2008; Bozkurt. et al., 2011; Sobczak and Kozłowski, 2015; Sahin and Öztürk, 2018; Zhao et al., 2019; Givisiez et al., 2020). There is a need to develop research-based strategies for reducing the pathogen load to improve growth rate, feed efficiency, health status and meet increasing demand. For this purpose, vitamins and minerals are recently added to rations to support health, growth and feed efficiency (Roy et al., 2020).

Embryo development largely depends on the nutrients that hens accumulate in their eggs (Konanc and Ozturk, 2016). Along with other nutrients, amino acids such as myoglobin and hemoglobin, as well as micro minerals such as Fe, Cu, Zn, which are found in the structure of many enzymes, play a key role in the development of the intestinal system (Uni and Ferket, 2003; De Olivera et al., 2008). Raising breeding chickens with competent immune systems contributes significantly to the prevention of diseases, optimal growth and improvement in performance, as well as the health and productivity of the next generation. In this article, current studies on the performance, reproduction, fertility, immune system and embryo development of their offspring and their effects on later life periods of broiler breeders are evaluated.

1. THE EFFECTS OF MATERNAL TRACE MINERAL NUTRITION DURING AND AFTER EMBRYO DEVELOPMENT

Today, the chick skeleton may not be sufficiently calcified to support body growth before and after hatching. In recent years, there has been

increased focus on understanding factors that influence skeletal mineralization early in life and its impact on leg problems in broilers post-hatch. Chicks with healthier skeletons have easier access to food and water after being placed on the farm, resulting in earlier access to nutrients and therefore increased post-hatch growth. Also, newly hatched chicks with a strong, well-formed skeleton are less likely to be reluctant to move, and therefore, carcass defects such as breast inflammations and knee inflammations may be reduced later in life (Torres et al., 2018).

Adequate mineral intake also positively affects egg production, fertility, hatchability, development and performance of the chick during and after the embryonic period. Since there is no external source of nutrients during incubation, the nutrition of embryos is completely dependent on the nutritional content of the egg. During incubation, metabolism varies according to the type of substrate and oxygen source available (Uni et al., 2003). For this reason, the egg must contain all the necessary nutrients in a balanced manner along with macro and micro minerals for the development and survival of the chick. There are current findings that the nutrition of the parent chicken has very important effects on the health and development of their offspring, and that minerals play an indispensable role along with other nutrients (El-Husseyin et al., 2018; Wang et al., 2018; Umar et al., 2020; Foued et al., 2020; Hassan et al., 2020; Noetzold et al., 2020). It is known that maternal nutrient transfer is effective on embryonic bone development (Uni and Ferket, 2003; De Oliveira et al., 2008; Givisiez et al., 2020). However, it has been determined that egg yolk mineral concentration and bone growth in progeny are not affected when chickens are fed with different trace mineral forms, and embryonic bone formation stops in case of insufficient nutrient supply (Torres et al., 2018). Moreover, disturbances during embryonic development can affect the entire production cycle and cause irreversible losses in broiler chicken production (Konanc and Ozturk, 2016).

Torres et al., (2018) embryos from young chickens had moderate bone calcification at day 17 during incubation, while embryos from older chickens had lower bone calcification at day 20 during incubation. As a result, the bones of chicks from 45- and 59-week-old broilers were stronger at hatch than those from 32-week-old hens. Embryos from very young chickens may have sufficient yolk mineral to support the highest rate of embryonic bone

development until day 17 of incubation, but after day 17 this begins to decline. It is known that the bone development of chicks from old chickens is better than that of young chickens (Torres et al., 2018). Micro-minerals such as Zinc (Zn), Copper (Cu), Iron (Fe), Manganese (Mn) and Selenium (Se) act as catalytic or structural cofactors in metal-containing enzymes and proteins in the extra-embryonic membranes of the embryo and cells. These compounds are factors that contribute to embryo survival (Londero et al., 2020).

The observed differences in the gastrointestinal development of the hatched chicks may partly explain the effects of transporting nutrients in the diet to the brood. There are several studies on the transitional effects of treatments on breeders, such as feeding schedule, feed area per bird, and source of trace minerals, on broiler chicks after hatching. According to these studies, these transition effects should be observed according to the development of certain organs of the chicks after hatching, since hatching of the chick causes very small changes in live weight (Moraes et al., 2011). Moraes et al., (2011) evaluated the hypothesis that breeder feeding practices and trace mineral source during the rearing and laying period may affect brood development at the final stage of brood incubation. Embryos evaluated accordingly were confirmed to come from eggs of similar initial weight with a maximum variation of 2 grams between eggs. Egg weight wasn't considered as a possible cause of observed variations in embryo organ development. The total nutrient availability during the rearing period and the amount of feeder area from the rearing period to the laying period can affect the competition for feed within the flock and change the eggshell characteristics. Eggshell conductivity is important for embryo development, especially at the final stage of incubation.

2. GENERAL EFFECTS OF MINERALS IN THE NUTRITION OF BREEDERS

Minerals are needed for the formation of the skeletal system, general health, as components of general metabolic activity and for maintaining the body's acid-base balance. It is important to determine the correct mineral concentration of feed ingredients. Because incorrect assumptions about the mineral composition of feed ingredients can lead to deficiencies, poor growth and production losses. The symptoms of nutritional deficiencies can

sometimes interact with each other, leading to difficulties in interpreting these symptoms. On the contrary, if more minerals are added to the ration, it may cause increased environmental pollution due to toxicities, slowed growth, increased excretion with faeces (Fouad et al., 2018; Wang et al., 2018). Unlike other nutrients, there is limited information on trace mineral requirements in poultry production environments. Given the growing knowledge base, the NRC's recommendations from 25 years ago may not be optimal to meet the growth and other yield potential of high yield lines developed in recent years. Therefore, it makes sense to re-evaluate the use of trace mineral supplements at recommended levels in commercial settings. Studies have shown that breeder nutrition plays a vital role in fetal growth and development. It has been determined that essential minerals (Cu, Fe, Mn, Se) that affect the lipid metabolism of breeders reduce the lipid peroxidation products and thus protect the offspring from oxidative damage. Moreover, some trace minerals are cofactors of many enzymes that regulate eggshell formation and gonadal hormone synthesis. However, inorganic trace minerals can be added above the requirements in commercial poultry feed to prevent common problems caused by mineral deficiency such as skeletal problems, poor growth and fertility. However, high inorganic mineral levels cause antagonism, affect mineral bioavailability, and increase excretion, resulting in environmental problems (Wang et al., 2018).

Most mineral sources used in feeds for breeder chickens are derived from inorganic compounds such as oxides, sulfates, carbonates and phosphates. Organic trace minerals do not decompose in the acidic gastric pH environment, remain electron neutral and are protected from chemical reactions with other molecules in the intestinal lumen. It is a chelated or complex mineral form with organic compounds such as organic trace minerals, amino acids, protein or organic acids. These minerals are more stable due to their organically bound structure with better digestion and absorption in the gut. This, in turn, increases their bioavailability and assimilation, and consequently reduces fecal and urinary excretion (Świątkiewicz et al., 2014). Umar et al., (2020) reported that glycinate complex trace minerals positively affect some egg quality characteristics, while decreasing trace mineral levels negatively affects the fertilization rate. In other words, a positive correlation was found between fertility and trace

mineral levels, and the lowest fertilization rate occurred when trace minerals in the form of glycinate complexes were reduced by 50%. However, hatchability and reproductive hormones (E2 and P4) were not affected. Thus, it has been stated that higher SOD activities in broilers fed with glycinate mineral complexes show protective roles in oxidative stress or that glycinate mineral complexes can increase the availability of trace minerals that reduce the accumulation of reactive oxygen species (Umar et al., 2020).

Wang et al., (2018) observed the highest level of mineral excretion in feces in animals with the highest inorganic mineral supplementation. There were no significant differences in storage and fecal excretion between the ration with low organic mineral levels and the ration with high inorganic mineral levels given to animals. Therefore, organic mineral sources have been proposed to control environmental pollution from intensive poultry farms. Trace minerals (TM) in poultry feeds are necessary for maintaining poultry health, stimulating the immune system, revealing production potential and improving eggshell quality. TM is typically introduced into poultry diets as inorganic salts such as carbonates, sulfates and oxides. However, such inorganic forms tend to decompose when exposed to the low pH of the upper digestive tract, making them susceptible to antagonism of several feedstuffs and nutrients, thereby reducing their availability for use and ultimately increasing their excretion in the environment (Araújo et al., 2019).

In industrial poultry production, one male is involved in the production of more than 1,000 fertilized eggs per year. Various factors such as genetic background, stressors, aflatoxicosis and aging can impair sperm characteristics and reduce fertility in male birds. On the other hand, nutritional factors may alter the deleterious effects on semen quality and characteristics and fertility in aging poultry males. can be counted as factors that increase fertility and progeny quality (Fouad et al., 2020). In addition to vitamins, minerals such as Se can help protect semen against oxidative damage and increase fertility, maintain membrane integrity and sperm fluidity (Fouad et al., 2020). As a matter of fact, Surai et al., (2019) reported that stress factors associated with oxidative stress and damage to important biological molecules adversely affect fertility and reproductive performance in poultry.

Insufficient trace minerals in the broiler breeder diet can reduce egg production and shell quality and increase embryonic mortality (Araújo et al.,

2019). It has been determined that animals fed with organic micro minerals tend to increase egg production, and organic trace minerals increase the fertility rates of eggs. An increase in sperm viability of males was observed with organic trace mineral supplementation. One of the major challenges facing the broiler breeder industry is the decline in eggshell quality as breeders age. However, it has been reported that higher egg production, egg shell thickness and breaking strength increased in breeders fed with organic trace mineral until 53 weeks of age compared to those fed with inorganic trace mineral (Araújo et al., 2019). In the same study, it was stated that the feed conversion ratio of the offspring of the breeders fed with organic trace mineral, except the offspring of the 35-week-old breeders, was better, and it increased the performance of the broiler breeders and their offspring. Important minerals that affect breeder performance, embryo development and life-long performance of the chicks are discussed below.

3. SELENIUM

Selenium is chemical element 34, a metalloid with six stable isotopes 76, 77, 78, 79, 80 and 82. These elements have many common chemical properties. Selenium and S have similar electro negativities, atomic radius, and the same oxidation states (-2, 0, +4, +6). Selenium is a S analog and substitutes S in a number of compounds such as sulfate (selenate), sulfite (selenite), sulfenic acid (selenic acid). Selenium and S easily react with each other to form selenylsulfide bonds. Despite these similar properties, there are some important differences between S and Se, and substitution of each other results in different chemical properties (Brandt-Kjelsen et al., 2017).

Oxidative stress is a serious detrimental factor for cellular integrity through the sustained release of reactive oxygen species mediated by various biotic (bacteria, virus, fungi, etc.) and abiotic stressors. Trace elements such as selenium (Se) with strong antioxidant potential have wide applicability as feed additives to reduce oxidative stress in living systems. Se can contribute to protecting semen against oxidative damage and increasing fertility, maintaining membrane integrity and sperm fluidity. Increases in semen production and quality may be due to the fact that male reproductive organ development requires adequate dietary Se to increase the number and survival of Sertoli cells, as well as reduce the induction of apoptosis in germ cells

during spermatogenesis, reducing the expression of related genes and upregulating expression of the GSH-Px2 and GSH-Px4 genes. (Fouad et al., 2020).

Selenium (discovered by Jacob Berzelius in 1818) is widely found in organic and inorganic compounds in nature and has a variety of multiple functions (Figure 1). Selenium often acts as a co-factor in the animal's body and is an essential part of many enzymatic chemical structures (selenoproteins). Glutathione peroxidase was the first selenoprotein identified with antioxidant activities in physiological systems. Selenium is mainly known for its antioxidant activities. Selenium plays an important role in the anti-oxidative mechanism, male reproduction, thyroid metabolism, muscle development and anti-carcinogenesis. Se supplementation in both compound and nanoform has been used in poultry diets to observe the response on growth performance (Hassan et al. 2020).

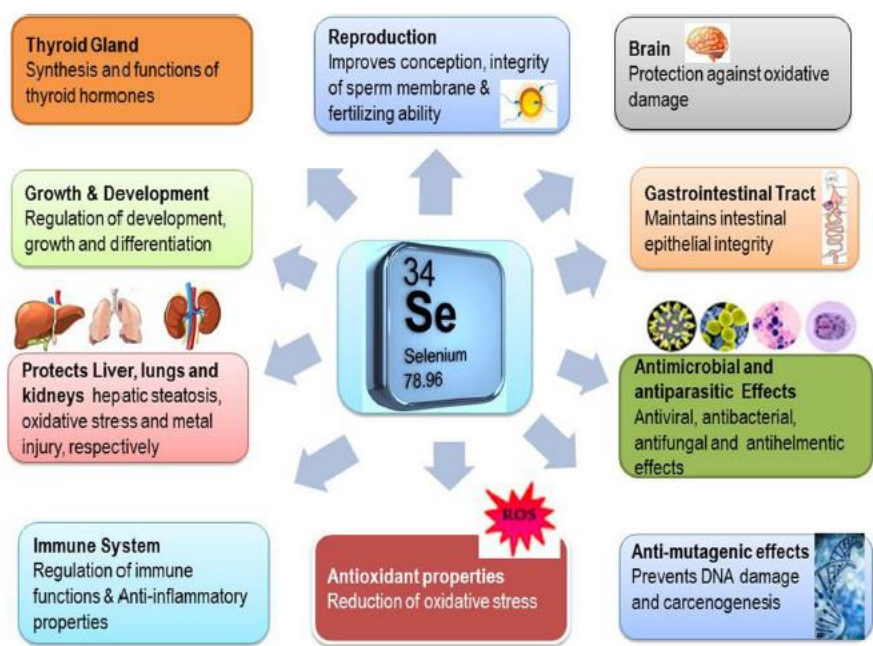


Figure 1. Functions of Selenium in Poultry (Hassan et al. 2020)

Selenate is more accessible to plants than organic Se such as selenite and SeMet. Selenate and SeMet uptake follow active transporters of the analogous S species and are metabolized by sulfate assimilation. However,

selenite is not mediated by membrane sulfide transporters. Selenite is rapidly converted to SeMet or SeCys after ingestion and accumulates in the proteins in the roots, while selenate is highly mobile in the xylem and displaces to the above ground plant parts. Sequencing in plants is related to the overall protein content of plants and their different ability to accumulate Se. Grasses, as well as feed sources used in feeds, are arenon-accumulators and therefore have low Se concentrations. Studies on Se concentrations have revealed that crop producing areas with very low Se concentrations (<0.1 mg Se kg^{-1}) to meet animal requirements are more common than areas producing Se at toxic levels (>2 mg Se kg^{-1}) in crops. Therefore, dietary supplementation is necessary to meet the Se requirements of animals. There is a growing interest in investigating the beneficial use of medicinal plants and phytogetic compounds in poultry diets, some of these plants are Se hyperaccumulators and future research will prove whether these plants can replace the traditional supplement used today (Brandt-Kjelsen et al., 2017).

4. ZINC

Zinc is one of the most essential trace minerals required for numerous metabolic and synthetic pathways and different physiological functions. It is involved in the activity of more than 200 enzymes of six enzyme types associated with the metabolism of carbohydrates, lipids, proteins and nucleic acids, hormone secretion pathways and immune defense systems. Because it is a cofactor of several liver enzymes, including alanine aminotransferase, aspartate aminotransferase, and gamma glutamyl transferase, Zn deficiency causes a variety of liver diseases, including cirrhosis and hepatitis. Zn is essential for the antioxidant defense system because it is one of the structural components of superoxide dismutase and enhances the synthesis of different metallothionines that act as free radical scavengers. Zinc (Zn) has a unique and comprehensive role in important biological processes, including immune function, growth, development and reproduction. Providing chickens with additional zinc in their corn-soybean meal based diet results in improvements in progeny, cellular immunity status, humoral immunity status and viability. Insufficient Zn uptake by breeder chickens results in low hatching yield and poor chick quality. In addition, Zn plays a synergistic role on vitamin A. Vitamin A and zinc affect the transport, absorption and accumulation of Zn.

Thus, the absorption, transport and use of vitamin A are affected by the Zn state (El-Husseyin et al., 2018). El-Husseyin et al., (2018) stated in a study they conducted on breeding chickens that high level of zinc in the ration (264 mg/kg ration) increased the weight of 0-day-old chicks, but did not affect hatchability and fertility.

Zinc is a very important trace mineral found in type-2 nutrients essential for overall metabolism. Zinc functions as a cofactor in many enzyme systems. It acts as a co-factor for more than 300 metalloenzymes and plays an important role in the metabolism of fats, carbohydrates, proteins, nucleic acids and membranes. The distribution of zinc in tissues parallels the distribution of zinc-containing enzyme systems in the body. The high level of zinc concentration in the pancreas is associated with zinc-containing digestive enzymes and zinc linked to the hormone insulin. It is essential for optimum feathering, growth, skeletal development, skin quality and reproduction in birds and improves immunological functions and disease resistance. Zinc also plays a role in maintaining appropriate eggshell thickness and strength in laying birds. In its deficiency, eggshell thickness decreases and causes an increased number of broken eggs in older birds, but can increase carbonic anhydrase activity, which catalyzes the conversion of $\text{ZnCO}_2 + \text{H}_2\text{O}$ to HCO_3^- , the main component of eggshells (Hassan et al. 2020).

Zinc (0.12–0.18 g/kg) is commonly added to poultry diets for optimal growth, bone development, feathering and immunity. Inorganic feed quality Zn sources—zinc oxide (ZnO) and zinc sulfate (ZnSO_4)—are used commercially in poultry feed. 80-90% of additional Zn is used as ZnO , which is less bioavailable and reactive than Zn sulfate. On the other hand, sulfate is highly reactive, promoting free radical formation of reactive metal ions, which reduces the nutritional value of diets by facilitating reactions that degrade vitamins, fats and oils (Patra et al., 2020).

Intestinal health is a key element in maintaining nutrient absorption and immune function in poultry. Zinc has the potential to regulate poultry gut health, but studies on this vital topic are limited. A recent study found that the use of a combination of ZnO and probiotic (*Bacillus coagulans*) nanoparticles in broilers improved performance, immune function (antibody titer against sheep red blood cells), and gut morphology (larger villus height, length,

width, and villus length to crypt depth ratio).) was observed to be quite effective (Hassan et al. 2020).

Zinc toxicity is mediated by oxidative stress, lipid peroxidation, cell membrane damage and oxidative DNA damage (Hassan et al. 2020). It is present in large amounts in animal foods, especially milk and fish meal. The main sources of zinc are: Zn-acetate dihydrate, Zn-lactate-trihydrate, Zn-carbonate, Zn-chloruremonohydrate, Zn-oxide, Zn-sulphate-monohydrate. Animal feeds should contain 20-80 ppm of zinc. Calcium and phytic acid increase the need for zinc. Interacts with many trace elements; Iron and copper reduce the absorption of zinc. Zinc is one of the essential trace elements needed in the body; It enters the structure of many enzymes such as carbonic anhydrase, alkaline phosphatase. It is required for RNA synthesis. Zinc is needed for the normal development and repair of the body. In zinc deficiency, growth retardation is seen in animals. The metabolism of epithelial cells is disturbed. Deformations occur in other keratinous structures such as horns, hairs, and feathers. Healing of wounds is delayed and reproduction is impaired (Filazi, 2017). Zinc, which is included in the structures of RNA and DNA polymerase enzymes, is also associated with protein biosynthesis. The enzyme carbonic anhydrase in erythrocytes catalyzes the synthesis and breakdown of carbonic acid. One of the most important ways to evaluate the efficacy of different mineral sources in poultry is to test their relative bioavailability in relation to standard or inorganic forms such as sulfates of microelements. Most experimental results carried out in recent years have demonstrated the high relative bioavailability of organic Zn sources. However, this is not always reflected in the improved performance parameters (Świątkiewicz et al., 2014).

5. COPPER

Copper (Cu) is an essential trace element that plays a vital role in the physiology of animals such as hemoglobin synthesis, connective tissue maturation, bone development and inflammatory processes, especially in the cardiovascular system and bones, for proper neural function, fetal growth and early post-incubation development. Copper is added to poultry diets at prophylactic concentrations for its growth promoting effects (El-Husseiny et al., 2018). Pearce et al., (1983) suggested that it might affect pharmacological

levels of copper (> 250 mg/kg ration) caused changes in 17 beta-estradiol and enzymes involved in carbohydrate, lipid and amino acid metabolism in laying adult chickens, and Cu supplements improved reproductive physiology and lipid metabolism.

Copper is an important microelement for animals, found in many enzyme systems in the body, superoxide dismutase, cytochrome oxidase and lysyl oxidase, or ceruloplasmin, where it acts as a cofactor (Świątkiewicz et al., 2014). Copper is an important trace element in poultry nutrition due to its various biological activities. It functions as a component of various metalloenzymes and proteins, including cytochrome oxidase, superoxide dismutase, ascorbate oxidase, and tyrosinase. It plays a key role in hemoglobin synthesis, iron metabolism and erythrocyte formation. It is also involved in the biosynthesis and cross-linking of elastin fibers and collagen and the synthesis of keratin and melanin. Birds fed a diet supplemented with 100 ppm Cu nano-chitosan (CNP-Cu) had higher body weight gain, immunoglobulins (IgA, IgG, IgM), and complement C3 and C4 (Hassan et al. 2020).

A primary function of Cu is related to its role in Fe oxidation as part of ceruloplasmin (Cp), an essential step in Fe absorption and hemoglobin (Hb) synthesis. Cu, which is necessary for the adequate functioning of reproductive functions, is a precursor of β -monooxygenase, which catalyzes the hydroxylation of dopamine to norepinephrine, which is necessary for the production of gonadotropin-releasing hormone. Important to support successful chick production is the maturity of the eggshell membrane. The maturity of this membrane is dependent on adequate Cu uptake, due to its role in forming collagen cross-links as part of lysyl oxidase. Due to this role of Cu in collagen synthesis, an adequate supply of this mineral is also essential for embryo bone development and therefore for the chick's robust independent forage seeking soon after hatching. A significant number of studies have been published on Cu supplementation for broilers and layers. However, few studies have been conducted with broiler breeders (Berwanger et al., 2018).

In a study conducted by Berwanger (2018) et al. in broiler breeders, they added different amounts of copper (5.82 -20.19 ppm) to their rations at different periods of yield periods. According to the results of the study, there was no difference between the breeding periods of the breeders and the

amount of copper used in terms of the characteristics discussed. As the amount of copper given to the breeders increased, egg yield, total egg yield per hen and hatching egg yield per hen ($P < 0.05$) increased at the end of the 44th week (hen/day). Again, according to the same study, as the amount of Cu in the ration reached 12.92 ppm, Hematocrit, serum Cu amount, hatching chick Hematocrit, day 0 body weight and hatched chick length increased in breeders ($P < 0.05$). In this study, the observed symptoms related to the lowest Cu content in the diets used in the feed were a decrease in the egg yolk Cu concentration as well as the hematocrit of the hatched chicks. Copper deficiency impairs eggshell quality, as observed by scanning with electronic microscopy in this study. In conclusion, data from this study indicate a Cu requirement range of 6.2 to 16.3 ppm (0.89 to 2.33 mg/chicken/day) depending on production targets. The average of all Cu requirement estimates obtained in the current trial is 12.5 ppm copper.

Traditionally, requirement tables for poultry are not sufficient to provide Cu requirements for broiler breeders (Berwanger et al., 2018). The European Commission (EC) has recently recommended a maximum of 25 ppm /kg of total Cu in poultry feed (EFSA, 2016). On the other hand, intensive agriculture has led to a decrease in Cu contents in plant feeds in the last century. Therefore, ration contents for poultry are uncertain as a reliable source of Cu supply. Deficiency is rare in poultry, as Cu requirements for poultry are low and supplementation is common (Berwanger et al., 2018).

6. MANGANESE

Manganese is a metal activator of enzymes involved in the synthesis of mucopolysaccharides and glycoproteins that contribute to the formation of the organic matrix of the shell (Londero et al., 2020). Manganese (Mn) is an essential trace element found in all tissues and plays a vital physiological role in the metabolism of proteins, amino acids, carbohydrates and lipids. Mn-dependent enzymes mainly include transferases, lyases, hydrolysis, oxidoreductases and isomerases, as well as ligases. These metalloenzyme families include arginase, glutamine synthetase, and Mn-superoxide dismutase (Mn-SOD) and phosphoenolpyruvate decarboxylase. Mn is also essential for regulating blood sugar, immune function, cellular energy, reproduction, digestion and bone growth. It also supports cellular defense

against free radicals. Manganese and vitamin K are vital for coagulation and blood hemostasis (Figure 2). Inadequate nutritional consumption leads to Mn deficiency, which ultimately causes growth retardation, skeletal defects and reduced bone development, birth changes and reduced fertility, defects in carbohydrate and lipid metabolism and a typical abnormal glucose tolerance (Hassan et al. 2020).

In feed raw materials, Mn varies according to soil composition. Therefore, the Mn content in broiler breeder feeds is expected to vary with the dietary source. The Mn content in wheat bran, which is largely used in broiler breeder feeds, ranges from 88 to 163.9 mg/kg. It has been reported that it ranges from 5 to 15 mg / kg in maize and 36 to 48 mg / kg in soybean meal (Noetzold et al., 2020). The availability of Mn in feedstuffs, as with other positively charged minerals, depends on its total chelation by phytic acid. Unlike Fe, Cu and Zn, the total Mn content in routinely used broiler breeder feeds is low (An exception is wheat bran, which has a high phytate content and thus potentially reducing Mn availability for poultry) (Noetzold et al., 2020).

Mn is often supplemented in broiler breeder feeds as part of a micromineral premix, usually as a sulfate salt, but sometimes combined with various organic minerals. Over-mineralization of commercial livestock has been reported to become an environmental problem that has been shown to contaminate groundwater. Recent regulations have set 150 ppm as the maximum Mn content in poultry feeds in the European Union (Noetzold et al., 2020).

Xie et al. (2014) reported that dietary Mn supplementation affects gene expression of GnRH-I in the brain and FSH in the pituitary, as well as eggshell quality. These results suggest that there may be a central mechanism for dietary Mn influencing egg laying performance of broiler breeders and it may take longer to show these effects in laying performance behavior.

Noetzold et al., (2020) Mn increases in the ration increased the total and hatching eggs and the total and hatching eggs per hen. While most broiler breeder chickens fed an inadequate manganese diet showed deficiency symptoms, positive improvements were observed as Mn increased. However, it should be taken into account that the metabolic response to the Mn amount will be different due to the different biology of each animal. In fact, Mn is a

component of metalloenzymes. Superoxide dismutase plays a role in the control of oxidative stress in mitochondria by converting superoxide to peroxide and then reducing it to water (Londero et al., 2020). Comparisons between inorganic and organic Mn supplementation showed that supplementation of manganese as MnSO_4 had a deeper stimulatory effect on gene expression of GnRH-I than supplemental Mn proteinate (Xie et al., 2014).



Figure 2. Functions of Manganese in Poultry (Hassan et al. 2020)

Mn metallo enzymes include arginase, phosphoenol pyruvate decarboxylase, glutamine synthetase, as well as Mn-superoxide dismutase (Mn-SOD). In particular, MnSOD is significantly expressed in organs including high mitochondria counts such as the heart, liver, and kidneys. Among tissues expressing MnSOD in human, mouse, and chicken, the heart has the highest level of steady-state mRNA expression. In previous trials using chickens, he noted that even though the Mn level was significantly lower in the heart compared to other tissues, MnSOD activity was higher and was very sensitive to supplemental Mn levels in corn-soybean diets. In addition, MnSOD activity in the liver and pancreas of birds is not affected by dietary supplement Mn, and the level of MnSOD mRNA in the heart is consistently sensitive to supplemental Mn concentrations in diets containing corn and soybean meal (Tufarelli et al., 2017).

One of the most popular commercial sources of organic manganese is Mn proteinate. In a study evaluating the bioavailability of organic Mn proteinate in association with inorganic Mn sulfate for chickens fed a conventional corn-soybean meal diet, based on multiple linear regressions of indices such as Mn concentration in heart tissue, Mn superoxide dismutase activity (MnSOD) and MnSOD mRNA level at the added Mn level, no significant difference in bioavailability was found between the Mn sources tested (Świątkiewicz et al., 2014).

It has been reported that Mn absorption is associated with high dietary Ca consumption. The possible relationship between Ca, Mn and bone health may be important in the development of osteoporosis. Given that Fe and Mn participate in binding sites in the gut, it is not unexpected that diet can inhibit Mg using Iode. Manganese is important for mucopolysaccharide synthesis and a lack of these trace elements in the poultry diet can determine a perose state, a decrease in egg production and poor eggshell development. The inclusion of Mn in diets for poultry is usually achieved using inorganic mineral salts; however, the bioavailability of inorganic mineral salts was very low, indicating high levels of these minerals in poultry feces. In addition, high Ca and P levels have been evaluated to reduce the bioavailability of Mn in poultry. However, it has been reported that excess dietary Ca has little or no effect on Mn absorption and any effect can be attributed to diet P alone (Tufarelli et al., 2017).

Due to the beneficial functions of Mn, poultry diets are often enriched with this element by the addition of various inorganic or organic forms, for example, Mn-oxide, Mn-sulphate, Mn-amino acid chelate or Mn-Bioplex. However, there are no studies showing the physiological properties of Mn nanoparticles (Mn-NPs), including antioxidant and immune effects (Jankowski et al., 2019).

7. IRON

Fe, a vital component of hemoglobin in erythrocytes, is essential for the transport of oxygen throughout the body. In addition, both hemoglobin and myoglobin are used to deliver, store and use oxygen to the muscles. Hemoglobin and myoglobin play an important role in maintaining normal

meat color, which is the most visual indicator of meat quality. There are few studies on the effects of dietary Fe on meat quality, especially in broilers.

Currently, some research data focus on Fe feeding in poultry, and current recommendations for dietary Fe for broilers range from 45 to 136 mg/kg (Lin et al., 2020). Iron is stored in the body in large quantities, primarily in the reticuloendothelial cells of the liver, spleen, and bone marrow. Dietary demands of Fe can be altered to increase or decrease the rate of absorption in different known ways according to the Fe status of the body (Taschetto et al., 2017). The absorption of Fe is associated with receptors on the surface of enterocytes. For example, a carrier protein 1 (HCP1) is responsible for the absorption of both Fe from the gut, whereas divalent metal transporter 1 (DMT1) can take up inorganic Fe + 2 and release it directly into the cytoplasm. DMT1 expression is increased when body Fe levels are low. The absorption of the Fe-AA complex is based on the principle that this compound can be internalized as an AA and then hydrolyzed to release the Fe ion. On the other hand, Fe dissociates from the AA complex before absorption passes through the inorganic route (Ebbing et al., 2019). Iron (Fe) is an essential element for body homeostasis and is normally added to poultry diets. It is an integral part of various enzymes and protein, which not only aids in the transport of oxygen, but also regulates the growth and differentiation of healthy and cells. It is also involved in the synthesis of hemoglobin, myoglobin, and red blood cell components, which play a key role in oxygen transport throughout the body. However, in animals it is mainly found as part of hemoglobin (60% to 70%), myoglobin, cytochromes and other Fe-containing enzymes (10%), as well as ferritin and hemosiderin (20% to 30%) (Taschetto et al., 2017). By assisting enzymes, it plays a leading role in almost every step of the tricarboxylic acid cycle and mediates the removal of dangerous metabolic products via iron-containing peroxidases and catalase (Figure 3). In addition, the development of connective tissue is affected by iron-activated hydroxylases. In poultry, nano-Fe by injection has been used to elucidate its effects on performance. It has been observed that intramuscular injection of agglomerates of elemental, nano and micro iron particles significantly affects body weight gain in chickens. It has been found that iron injections are associated with higher protein accumulation in tissues, resulting in improved daily weight gain of birds (Hassan et al. 2020).

There has been limited research on Fe supplementation for poultry, particularly broiler breeders. Also, the relative bioavailability of Fe from important mineral sources such as limestone and natural phosphates has not been well studied (Taschetto et al., 2017). The recommended amount of Fe currently available in the literature is based on statistical analysis and the derivation of estimates using various models. Current nutrient requirements, recommendations, and guide tables have several recommendations for Fe as a dietary supplement. Current dietary Fe recommendations of broiler breeders vary widely from 20 to 140 mg/kg. Laying performance, hematocrit and hemoglobin of hens, and egg quality and hatchability of eggs are affected by the Fe content in the diet of highly selected breeders (Gou et al., 2019, Taschetto et al., 2017). According to the study by Taschetto et al., 2017, the average of all requirement estimates obtained is 97 - 106 ppm Fe. Adequate Fe levels are needed to maintain brood egg production as well as egg yolk Fe content and the corresponding hematological parameters of chicks (Taschetto et al., 2017).

According to the data obtained from Chinese Yellow broiler breeder chickens in the laying period, Gou et al., (2019) showed that the trace element Fe deficiency in the diet was reflected by the lowest blood hematocrit in the blood, a negative effect on the egg shape index and yolk color score, especially in the tibial fracture of live embryos. shows that it leads to the lowest level in vigor and hatchability. Similarly, excess dietary Fe has been reported to be reflected in the highest plasma content of the lipid peroxidative product MDA, resulting in shell strength with poor hematocrit, impaired egg shape index, and lower hatchability of viable embryos.

According to Ebbing et al., (2019), it increased the fertility and hatchability of eggs of broiler breeder chickens fed with Fe-Amino Acid (Fe-AA) complex. Chicks from dark brown eggs and breeders fed Fe-AA had increased body weight gain. Diets supplemented with Fe-Sulphate or Fe-AA did not affect eggshell color and Hematocrit and Hemoglobin levels of 0-day-old chicks.

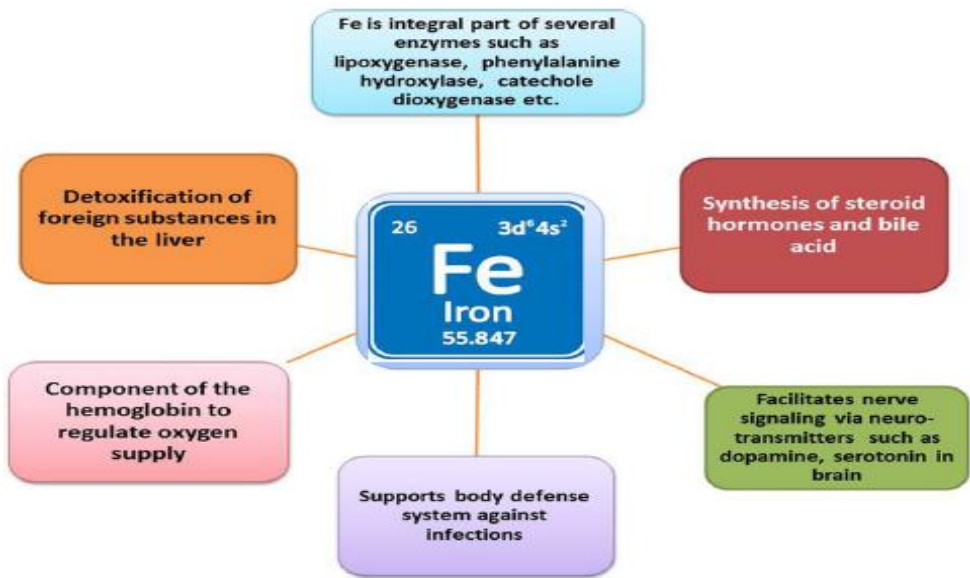


Figure 3. Functions of Iron in Poultry (Hassan et al. 2020)

8. RESULT AND DISCUSSION

High yield hybrid genotypes were produced in poultry by optimizing both genetic breeding methods and maintenance, feeding and all environmental factors. Due to the high productivity, the nutritional needs of the poultry, especially the breeder parents, whose metabolic loads increase, increase. However, the levels recommended by the NRC, which is accepted as a reference all over the world, are now considered to be controversial. Already these levels are created to prevent deficiencies in the animal, disease, high temperature, humidity, vaccination etc. It should be taken into account that it will not compensate for any stress factors or activity losses due to feed processing and storage (Barroeat et al., 2012). Optimum regulation of the digestive system microbiota under stress conditions also requires the development of strategies to reduce the stress of breeders and to manage their current metabolic loads (Roy et al., 2020). Se, Zn, Fe, which play an important role in metabolism for the prevention of low fertility (Yaripour et al., 2018; Surai et al., 2019; Fouad et al., 2020) observed in breeders, and to ensure a healthy and high performance in the next generation. Beyond the adequate and balanced supply of Cu and Mn minerals, their digestibility and antagonism should also be considered.

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CHAPTER 8

STRATEGICALLY IMPORTANT VITAMINS FOR THE PERFORMANCE, REPRODUCTION AND HATCHABILITY OF BROILER BREEDERS

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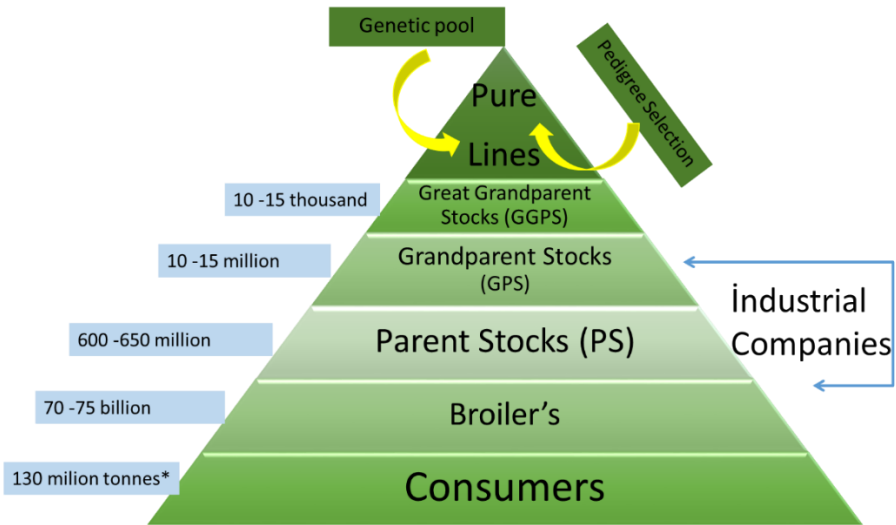
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INTRODUCTION

Existing commercial lines have been genetically enhanced with a focus on maximum performance based on rapid growth and continuous improvement of feed conversion. Thus, since the 1950s, the slaughter age has decreased by about 40%, while the slaughter weight has more than doubled. In addition to their weight, their high productivity has maximized the metabolic load and therefore the stress of the breeders, suppressing the immune system and reducing the resistance against diseases. This stress has reached its peak in the breeding females producing the eggs that form the chicks of the next generation. The resulting stress adversely affects all performance values such as egg production, fertility, hatchability and survivability in animals. Various additives have been added to the rations in recent years to reduce these negative effects. Among the additives, vitamins that are rich in phenolic substances, have radical scavenging properties, reduce stress and have cell renewal properties come to the fore. More functional and effective use of vitamins has come to the fore in order to reduce stress due to significant advances in vitamin premix technologies to increase stability and digestibility. As a matter of fact, giving vitamins, the quantity and quality of which are increased during this period, not only affects the performance and hatchability of the breeder, but also positively affects the embryonic period and post-hatching development of the chick, quality of life and business economy. In this review, current studies on the performance of broiler parents and the effects of some vitamins (Vitamin A, D, E, B2, choline, folic acid) which are thought to be strategically important for breeders, on embryo development and later life periods of chicks are discussed.

Breeding commercial broiler flocks; These are the flocks in which the roosters of the paternal line and the hens of the parent line coexist for the production of fertile hybrid eggs. Currently, broilers used in production are based on double, triple or quadruple crosses between closed-bred lines. There are four generations between pure line production and broilers, which are the final production material. The changes from the first step in poultry meat production to the broiler production stage are summarized in the drawing below (Figure 1).



* Estimated Value of World Poultry Production in 2020 (FAO, 2020)

Figure 1. World Poultry Production Status

Hatching eggs obtained from parent flocks (breeding establishments) are used in broiler production. Therefore, factors such as survivability, flock uniformity, number of hatching eggs to be obtained, high hatchability in these eggs and obtaining healthy chicks are of great importance in these flocks. In this sense, the care, feeding, management and management program to be implemented from the acceptance of the breeding animals to the farm until the end of the 64-week yield period should aim to maximize the genetic potential of the herd along with its basic needs. Existing lines have been genetically improved with a focus on maximum performance based on rapid growth and continuous improvement of feed conversion (Givisiez et al., 2020). Thus, since the 1950s, the slaughter age has decreased by about 40%, while the slaughter weight has more than doubled. Body weight control is one of the most critical goals in order to get the desired level of yield from breeders that gain weight quickly and have high genetic capacity. In this process, the nutrition of broiler breeders not only affects the health and yield performance of the animal, but also affects the development and health of the offspring (Konanc and Ozturk, 2016).

Embryo development largely depends on the nutrients that chickens accumulate in their eggs. Specific nutrients such as some vitamins, micro-minerals, fatty acids, carotenoids play a key role in the development of the immune and intestinal system (Uni and Ferket, 2003; De Olivera et al., 2008; Konanc and Ozturk, 2016). Embryonic development currently accounts for more than 33% of the entire lifespan of commercial broiler lines (Givisiez et al., 2020). For this reason, the transfer of nutrients necessary for the normal development of the embryo to the egg is closely related to the adequate and balanced nutrition of the breeding animals. In general, the composition of macronutrients in eggs is more stable than elements usually found in lower amounts in diets. On the other hand, the interrelationships of some micronutrients with other nutrients may affect the fertility and reproductive performance of animals, especially in the last period of the production period (El-Husseyin et al., 2018). Therefore, a balanced diet plays a strategic role in keeping the breeders healthy, producing quality eggs and developing the body, including during the growth period. As a matter of fact, recently, the use of diets fortified with feed additives that are supposed to strengthen the immune system, regulate the digestive system and increase comfort in broiler breeder feeding has been investigated (Hameed, 2021; Konac and Öztürk, 2016; Şahin and Öztürk, 2018; Bozkurt et al., 2009). As a matter of fact, these rations are also known as nutraceutical ethyl, which has nutritional and pharmaceutical importance by preventing various diseases, having immunomodulatory potential, providing health benefits and thus increasing productivity (Dogan and Öztürk, 2019; Mahmoud et al., 2020). Nutrient or non-nutritive substances such as amino acids, minerals, vitamins, fatty acids, enzymes, prebiotics, probiotics, synbiotics, pigments, herbs, herbal extracts, antioxidants, organic acids can be used for this purpose. The nutritional and healthier properties of feed ingredients have recently been the focus of poultry science due to the negative effects of chemical pharmaceuticals such as antibiotic resistance and drug residues (Ozturk et al., 2010; 2012; 2014; Erener et al., 2010; 2011; Sahin and Ozturk., 2018; Erener et al., 2020). For poultry, vitamins have a privileged place because of their role in healing tissue and oxidative damage and reducing animal stress (Min et al., 2018).

To produce antibiotic-free broilers, it is crucial to improve gut integrity, gut health, and acquired immunity so that diseases such as coccidiosis have

minimal adverse effects on nutrient digestion, absorption and growth performance of the birds (Poudel et al., 2021). Because of the high costs of disease treatment and their potential negative health consequences, health prevention has always been preferred over treatment. Given the key role of the immune system in disease prevention and optimal growth, producing chickens with competent immune systems is an important goal in poultry production, thereby helping to defend against pathogens and providing a stronger response to vaccines. In this article, the effects of some strategically important vitamins (Vitamin A, D, E, Choline, folic acid and Vitamin B2) on the performance, reproduction, fertility, immune system and their offspring of breeders were investigated.

1. EFFECTS OF NUTRITIONAL FACTORS ON BREEDERS

Broilers are genetically selected for rapid growth and broiler breeders have lower egg production and more abnormalities in lay performance behavior than layers. In order to maintain and improve the production of broiler breeder chickens, the effects of nutrition need to be better understood (Xie et al., 2014). It is known that the vitamin requirement required for hatching egg production is higher than table eggs. In other words, the vitamin supply in the male or female breeder bird rations must be higher than the feed produced for the production of infertile eggs. Adequate vitamin intake not only has positive effects on the laying of eggs, but also on their fertility and hatchability, and the embryonic period and post-hatching development of the chick. It has been noted that the level of vitamin provided in the diet has an inverse correlation with the incidence of embryo death during the intermediate stage of incubation (between 7 and 14 days). It should not be forgotten that a fertile egg must contain all the nutrients necessary for the development and viability of the chick. There is no external food source during the incubation process; The nutrition of the embryo depends only on the composition of the egg produced. It is clear that the nutrition of the parent chicken has very important effects on the health and development of their offspring, and vitamins, apart from various nutrients, play a fundamental role (Barroeate et al., 2012). In poultry farming, several recently published studies show that a diet high in B group vitamins can improve the performance and reproductive

capabilities of broilers. Numerous studies in turkeys show that high biotin levels improve feed efficiency and weight gain, while reducing mortality. Similarly, in trials with increased biotin levels in wheat-based diets, broilers showed an improvement in body weight and feed conversion (Barroeate et al., 2012).

In industrial poultry production, one male plays a role in producing a large number of eggs, which can exceed more than 1,000 fertilized eggs per year. Semen volume, sperm count (total count), viable sperm, dead sperm and abnormal sperm count, and advanced motility as characteristics of a rooster semen are often tested to evaluate and predict male fertility in poultry. Various factors such as genetic background, stressors, aflatoxicosis and aging can impair sperm characteristics and reduce male bird fertility. Nutritional factors can alter semen quality and characteristics and deleterious effects on fertility in aging bird males. Nutritional factors that can successfully increase fertility in roosters and their mechanisms of action are summarized in Figure 2. (Fouad et al., 2020).

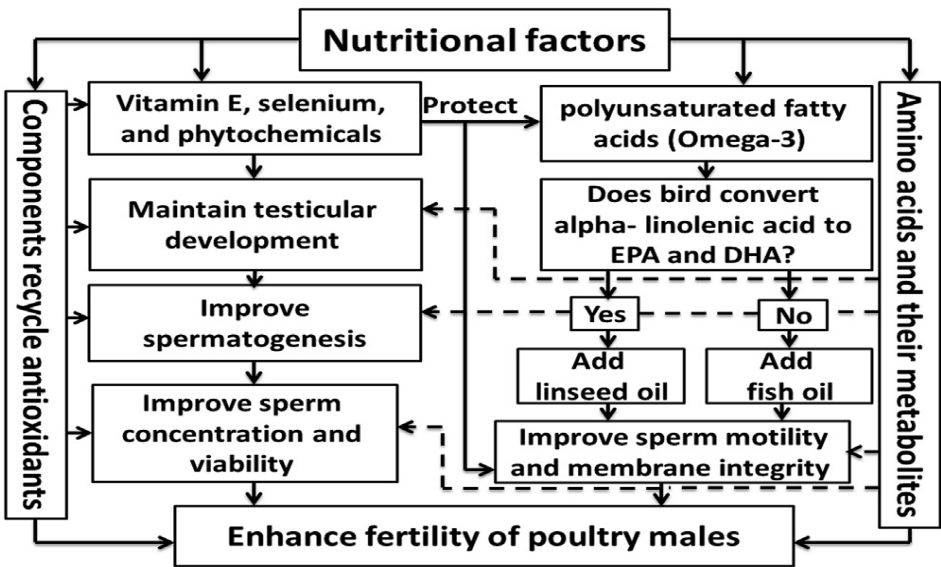


Figure 2. Effects of nutritional factors on fertility in male birds (Fouad et al., 2020), *
DHA, docosahexaenoic acid, EPA, eicosapentaenoic acid

Amino acids and their metabolites, n-3 polyunsaturated fatty acids, vitamin E, selenium, oils rich in phytochemicals and natural antioxidants

regenerative components can sustain testicular development and improve spermatogenesis, progeny quality and fertility. It has been shown in breeders that high levels of the vitamin are needed for the production of live chicks. The embryo is very sensitive not only to changes in the environment but also to deficiencies of most vitamins, and embryonic malformations and death are some of the main visible symptoms of deficiency in one or more of the vitamins (Barroeate et al., 2012).

In particular, vitamin supplements with antioxidant properties and the capacity to recycle and regenerate antioxidants can help neutralize reactive oxygen species, protect semen against oxidative damage, and maintain membrane integrity and sperm fluidity to positively increase fertility. Increases in semen production and quality may be due to the fact that male reproductive organ development requires adequate dietary Se to increase the number and survival of Sertoli cells, as well as reduce the induction of apoptosis in germ cells during spermatogenesis, reducing the expression of related genes and upregulating expression of the GSH-Px2 and GSH-Px4 genes. (Fouad et al., 2020).

Commercial poultry production is associated with a range of stress conditions, including environmental, technological, nutritional and metabolic. These stress factors are believed to be responsible for decreased fertility and reproductive performance of poultry, and at the molecular level most stresses are associated with oxidative stress and damage to important biological molecules (Surai et al., 2019).

Meat and egg production in poultry depends on the availability of good quality hatching eggs. Egg production of a hen is largely determined by its genetic makeup, while egg quality and hatchability depend on various factors such as the age of the animal, feeding and housing conditions. Daily egg production is an important feature in commercial flocks, whether for food or hatching. Therefore, fertility for breeders is vital for egg producers and breeding companies to achieve maximum number of eggs per hen and maximum return per expense incurred. Vitamins and minerals are essential nutrients for metabolic and physiological processes and therefore for fertility and reproductive performance of the animal. Vitamins also improve immune status and promote normal metabolism in poultry to defend against diseases and stress (Yaripour et al., 2018).

In this review, current studies on the performance of broiler parents and the effects of some vitamins (Vitamins A, D, E, B2, choline, folic acid) that are thought to be strategically important for breeders and their effects on embryo development and later life periods of chicks were evaluated.

2. VITAMINS

Vitamins are essential micronutrients that play a central role in most metabolic processes. They are necessary for normal physiological functions such as optimum health, growth and development, reproduction. They are also an integral part of fetal development and rations for adult breeder chickens probably contain the highest level of supplemental vitamins of any feed produced in a commercial feed mill. Feeds with substantially insufficient vitamin levels are not commonly seen in commercial applications. They are more likely to encounter marginal deficiencies from low vitamin supplementation, feed ingredients of questionable quality, their availability in feedstuffs, and breeders consuming less than the calculated average feed intake. Significant deficiency of any vitamin is known to result in adverse reactions in egg production, fertilization, hatchability and brood performance because there is significantly increasing evidence of transfer of vitamins to chicks. With a marginal vitamin supplement, offspring will not exhibit classical deficiency syndromes but will not need to fulfill their potential. Unfortunately, what we consider to be the optimum vitamin needs of breeders is often questioned as too high and too expensive in terms of cost per kg of feed. In reality, however, the ultimately most expensive scenario for breeder or integrated broiler operations is low vitamin incorporation levels in the feed. Vitamins account for about 4% of the cost of a breeder's diet, so saving on vitamin inclusion rates is rarely a cost-effective option (Hubbard, 2011).

The impact of increased vitamin levels on the progeny performance of breeders is an area of considerable commercial interest recently. Increasing the vitamin levels in the diet above what is considered adequate will increase the vitamin content in the egg. However, this may not automatically lead to higher brood concentrations and a positive effect on chick growth and viability. Although fertility and chick quality are critical at the beginning of the production period, nutrients are not efficiently transferred to the egg at this stage. In commercial conditions, progeny from flocks of young parents fed

high levels of vitamins showed improved early growth and reduced mortality. It has been reported that broilers from breeders fed with high vitamin and mineral levels have an increase in daily leukocyte counts, indicating stimulation of the immune system (Hubbard, 2011).

Breeders are usually satisfied with the addition of all vitamins as a synthetic source. Normal feed ingredients such as corn, wheat and soybean meal all contain natural sources of vitamins and in some cases can theoretically contain enough to meet the needs of growers. However, the concentration of vitamins in feed ingredients will vary due to crop location, fertilizer use, plant genetics, plant diseases and weather conditions. Harvest conditions often play an important role in the vitamin content of most feedstuffs. When the harvest months are not conducive to full ripening, the vitamin content of maize is greatly reduced. Adding to this natural variability is the effect that factors such as natural plant toxins and mycotoxins can have on vitamin availability. Given these constraints, it is important not to rely on normal feed ingredients to provide vitamins, and therefore vitamin premixes are designed to provide all the vitamins needed by the breeder. However, it is very difficult to determine the vitamin needs of breeders. Breeding chicken research takes a long time to complete and is expensive. The purpose of practical nutrition of breeders is not only to prevent the symptoms of vitamin deficiency, but also to support optimum health and ensure good egg production and hatchability. While the genetics and breeding of breeders have changed significantly in recent years, there have been several studies focused on the application of vitamins in optimizing the hatching and post-hatching viability of chicks in the first week of life. As a result, there is a wide variety in vitamin supplementation worldwide (Hubbard, 2011).

The vitamin needs of animals are largely dependent on their physiological structure, age, health and nutritional status, and functions such as meat or egg production. Stud chickens have higher vitamin requirements for optimum hatchability because the vitamin requirements for egg production are often less than those required for hatchability. Breeder chicken feeds require higher levels of vitamins A, D3, and E than the feed of fast-growing chickens. Selection for faster growth can allow animals to reach much higher weights by consuming less feed at a much younger age. Vitamin requirements established a few decades ago may not be applicable to today's poultry, as

genetic potential increases feed conversion rate by 0.8% per year and most NRC vitamin requirement data are 20–40 years old. Vitamin nutrition must now be seen as important not only to prevent deficiency symptoms, but also to optimize animal health, productivity and product quality. Therefore, vitamin supplementation limits should be adjusted for different management systems, taking into account environmental temperatures, fluctuations in feed energy content, and factors that may affect feed composition or vitamin requirements (e.g., infectious diseases, stress, parasites, biological variations, dietary composition, bioavailability, and nutrient interactions). and Ward, 2017).

Different maternal vitamin combinations have important effects on tibiae of broilers, broiler performance, immune functions and mechanisms of calcium and phosphorus (Peng, 2011).

The critical roles of vitamins in the normal function of the immune system have been extensively studied and well understood. Insufficient vitamin levels can also cause dysfunction of the immune system, resulting in increased rates of infection or inflammation and ultimately reduced growth. Of all the vitamins, vitamins A, D, E, and C have been shown to have the greatest effects on immune system function through various mechanisms (Shojadoost et al., 2021).

These effects include potentiation of innate responses against microorganisms, more effective adaptive immune responses in response to infection and vaccination, and regulation of inflammatory responses. However, many questions remain regarding the rationing of vitamins in poultry. For example, research has shown inconsistent results regarding dose-response relationships for some vitamins. However, as more data are produced regarding the immune system and vitamin-related effects of poultry, the health and production of poultry has a chance to increase further as dietary supplementation of vitamins is further optimized (Shojadoost et al., 2021).

Although the nutritional needs of the breeder parents are increasing, the recommended levels of the NRC, which is accepted as a reference all over the world, are now considered to be controversial. It should be taken into account that these levels are created to prevent deficiencies in the animal, and will not cover the activity losses caused by disease, high temperature, humidity, vaccination, etc., any stress factors or feed processing and storage (Barroeat et al., 2012).

3. VITAMIN A

Vitamin A is an unsaturated monoalcohol, retinol, found naturally only in animal tissues. Plants contain carotene precursors, of which β -carotene has the greatest biological potential. In the intestinal epithelium of birds, carotenes are converted to vitamin A. Pure synthetic vitamin A is also available and sold in stabilized form. When exposed to air, light or high temperatures, it oxidizes rapidly and loses its activity (Barroeat et al., 2012).

Vitamin A acts at different levels in the animal's body and fulfills many functions. However, the main ones can be summarized as follows (Barroeat et al., 2012):

- It affects the eyesight, prevents night blindness.
- Skeletal development. Retinoic acid is necessary to maintain tissue growth during growth.
- Reproductive performance.
- Development and maintenance of epithelial tissue. Vitamin A is necessary for the regeneration of epithelial tissue. Apart from its structural function, the epithelium serves as a line of defense against the invasion of pathogenic organisms. Loss of the integrity of the cell membrane allows invasion and impairs the bird's immune system, particularly antibody production and proliferation of T cells.
- Vitamin A supplementation has been associated with an anti-stress effect, which may be explained by its involvement in the adrenal response. Vitamin A is necessary for the production of corticosteroids in the adrenal gland and is necessary to support gluconeogenesis in situations of stress.

Vitamin A is essential for many critical vital activities, including reproduction, metabolism, differentiation, hematopoiesis, bone development, and pattern formation during embryogenesis. Vitamin A has an important role in vision, bone and muscle growth, reproduction and maintenance of healthy epithelial tissue. While the vitamin A content in the egg yolk remained constant throughout egg production when chickens were supplemented with 9,000 IU/kg of vitamin A feed, the levels decreased and were unable to sustain egg production in unfortified hens. Therefore, vitamin A or any of its precursors should be provided in the diet (El-Husseiny et al., 2018).

Optimal Vitamin A level needs of hatching eggs were evident in chicks hatching from eggs with insufficient carotenoids exhibiting impaired immune responses, with increased signs of systemic inflammation (decreased body weight, increased copper and haptoglobin, and decreased serum zinc levels and increased weight of Bursa fabricius, thymus, and spleen) (Shojadoost et al., 2021).

High levels of vitamin A (15,000-20,000-25,000-30,000 IU/kg, etc.) added to feeds do not affect egg production in broiler parents in some studies, while in some studies, for example, vitamin A added to 10,800 IU/kg feed in native broiler parents of China. and egg mass, and in another study, it was stated that it significantly increased egg production in layers (El-Husseiny et al., 2018).

In a study by El-Husseiny et al. in broiler breeders, it was reported that vitamin A levels (12,500 and 25,000 IU/kg) did not have a significant effect on fertility and day old chick weight, while its low level significantly ($p < 0.05$) provided better hatching.

In addition, there are studies indicating that dietary vitamin A supplementation does not affect the antibody titer of Newcastle disease virus in broiler breeders (El-Husseiny et al., 2018).

Vitamin A is a very effective antioxidant and can slow down the lipid peroxidation process by breaking the chain reactions involved in this process and control the membrane degradation process caused by heat stress. It is suggested that vitamin A supplementation higher than the recommendations of the NRC plays a crucial role in restoring membrane integrity and normal development of reproductive organs in laying hens under heat stress. It has been reported that high vitamin A in diets improves performance indices and egg quality criteria in laying hens experiencing heat stress. In addition, there are studies indicating that the highest egg mass value ($P < 0.05$) was recorded in chickens fed diet supplemented with 10,000 IU of vitamin A per kilogram of diet, but all other egg characteristics were not affected by vitamin A supplementation (El-Hack et al, 2016). Recent research from this laboratory has revealed that 10,800 IU/kg of maternal vitamin A (VA), insulin-like growth factor 1-receptor transcripts in the walls of yellow follicles, follicle stimulating hormone receptor expression, luteinizing hormone receptor and growth hormone receptor transcripts and egg production, egg-feed ratio and

hatching weight of chicks from these breeders also increased hepatic retinol and retinyl palmitate concentrations.

According to the results of a study by Yibing (2020) and his team, it was found that broilers whose parents' diets were supplemented with 10,800 IU/kg of vitamin A significantly increased on Day 0 (Incubation) body weight for 21-day periods compared to those who did not receive maternal vitamin A supplementation ($P < 0.05$). has been reported. In the same study, the mortality rate of broilers from the groups that received 0 or 21,600 IU/kg maternal vitamin A (VA) supplementation was significantly lower than that of 5,400 IU/kg maternal VA supplementation. According to the author, there was an interaction between maternal and broiler vitamin A supplementation on serum activity of GSH-Px ($P < 0.05$), and serum activity of GSH-Px was at levels of 10,800 IU/kg of maternal vitamin A and 5,000 IU/kg of broiler vitamin A. was highest ($P < 0.05$). As a result of the present study, it was reported that 5,000 IU/kg of vitamin A supplementation in the ration throughout the broiler rearing period reduced end-of-period body weight, mean daily body weight gain, and mean daily feed consumption, but reduced feed conversion and mortality in broilers compared to non-fortified diets.

According to Yibing (2020), supplementation of vitamin A to both the breeders and the diets of broilers obtained from these breeders had an effect on meat quality and immune function in broilers. In addition, it was stated that the growth performance of vitamin A supplementation with the diet increased, while the vitamin A supplement applied to the breeders increased the hatching weight. However, both must be taken into account in broiler nutrition to achieve good meat quality and immune status in broilers.

Blood WBC (White Blood Cell) counts play an important role in immunity, phagocytosis, and thus in defense against diseases and infections. Vitamin A is one of the most common micronutrients in relation to immunity. Vitamin A deficiency has been associated with impaired immune response. In addition, it has been stated that vitamin A plays an important role in improving immune function (El-Hack et al, 2016).

Vitamin A is important in mucosal immune regulation and epithelial cell differentiation. One of 600 naturally occurring carotenoids known to date is lutein, which acts as an antioxidant in tissues. Dietary supplementation of lutein (at 50 mg/kg) has been shown to affect oxidative and inflammatory

parameters. Compared to some other vitamins, many biological properties of vitamin A, including the mechanisms by which it affects the immune system, are well defined and are mediated through the interactions of retinoic acid with nuclear hormone receptors in immune system cells. These findings suggest that vitamin A supplementation in chicken diets not only increases T cell proliferation but also alters effector functions against infectious agents. Vitamin A has also been reported to play a critical role in immunoglobulin synthesis. Studies suggest that dietary vitamin A deficiency is associated with impaired IgA and IgG synthesis, but not IgM. These observations and studies highlight the importance of vitamin A in chicken diets for producing optimum IgA levels to facilitate antimicrobial mucosal defense. Other types of viral infections have also been shown to affect vitamin A levels in chickens, including infectious bronchitis virus (IBV) and reovirus (RV), which primarily affect the respiratory and intestinal tract, respectively. IBV and RV infection during normal or marginal vitamin A intake in chickens resulted in decreased plasma retinol levels. Collectively, the above studies suggest that supplementing poultry diets with adequate or possibly increased amounts of vitamin A can contribute to improved health status of chickens (Shojadoost et al., 2021).

In a study by El-Hack (2016) and colleagues, they looked at the effect of vitamin A supplementation on spawning performance during the trial under heat stress conditions. The results indicated that supplementation of up to 16,000 IU/kg of vitamin A to the ration improved all egg laying characteristics (FCR, egg count and egg mass) compared to the control group (8000 IU/kg). In the same study, it was stated that all egg quality criteria were not significantly ($P < 0.05$) affected by dietary vitamin A, except for albumin percentage and Haugh unit score, and the Haugh unit gradually increased as the vitamin A level increased. In this study, the experimental groups that received vitamin A supplementation showed significant ($P < 0.05$ and 0.01) decreases in plasma albumen, total lipids and total cholesterol compared to the control group, whereas the amount of plasma globulin increased gradually with increasing vitamin A levels. In conclusion, El-Hack(2016) et al. stated that their findings confirm the effectiveness of vitamin A (16,000 IU / kg diet) in improving the parameters of ovulatory performance.

Vitamin A deficiency causes decreased egg production, blindness and keratinization of the epithelium. However, as with other fat-soluble vitamins, deficiencies are not often found in animals because animals can store these vitamins in their adipose tissues, releasing them into the bloodstream at times of increased demand or when there is little or no need for them (Barroeat et al., 2012).

4. VITAMIN D

Vitamin D is a fat-soluble vitamin and is obtained by being produced in the skin when exposed to sunlight or supplemented with feed. Dietary vitamin D is absorbed by the small intestine by binding to the blood vitamin D-binding protein (DBP) or albumin and then rapidly taken up by the liver. It should also be noted that the absorption of vitamin D₃ depends on the levels of vitamins A and E in the diet. When formulating poultry rations, it is necessary to take into account the factors affecting vitamin D uptake from the gut. Intensive livestock production puts poultry under great performance stress, which leads to weakened immune function, resulting in increased susceptibility to infectious agents and inflammation. Current evidence suggests that vitamin D may benefit the host by exerting immunomodulatory effects that are accentuated by anti-inflammatory abilities. The critical role that vitamin D plays in regulating T and B cells is evident as VDR expression is significantly increased in active T and B cells. These observations suggest that 1,25(OH)₂D₃ can regulate cellular responses through cytokine production, but the effects do not come at the expense of impairing the cytotoxic function of these cells. In support of this, others have shown that in chronic disease states (such as immunopathological disorders), vitamin D regulates the proliferation of T cells. In conclusion, it is reasonable to conclude that dietary vitamin D supplementation in chickens may offer beneficial effects, including attenuation of inflammatory responses as well as potentiation of cell and antibody-mediated immune responses, and may contribute significantly to potentiation of antimicrobial defenses (Shojadoost et al., 2021).

All metabolic processes involving vitamin D are regulated by two hormones, calcitonin and parathyroid hormone, which are closely linked to the level of calcium in the blood. Today, with the addition of the enzyme

klotho to these two hormones, calcium metabolism has begun to be explained. Therefore, the main effect of this vitamin is on calcium and phosphorus levels and the balance between them. Basic functions of calcitriol (Barroeat et al., 2012):

- It stimulates the absorption of calcium and phosphorus in the intestine.
- Increased renal reabsorption of calcium and phosphorus.
- Stimulating the mobilization and deposition of existing bone calcium and phosphorus in order to provide and maintain optimum bone mineral content in the chicken and developing egg.

Vitamin D (D3) and 25-hydroxycholecalciferol (25-OHD3) both play a role in calcium and phosphorus absorption and bone mineralization, and have regulatory functions for the immune system and muscle development in broiler chickens. This vitamin is converted to the biologically active D3 (1,25-dihydroxycholecalciferol [1,25-(OH)₂D3]) form in the kidneys by 1- α -hydroxylase before being hydroxylated to 25-OHD3 by 25 hydroxylase in the liver (Fatemi et al., 2020). Breast meat yield and performance of broilers have also been reported to increase when serum 25-OHD3 levels are increased in response to dietary supplementation with 25-OHD3 (Fatemi et al., 2020).

When laying hens are fed a diet deficient in vitamin D, the first sign of deficiency is thinning of the eggshells. Animals will continue to lay reduced shell eggs for weeks. If the diet does not contain vitamin D3 completely, egg production decreases rapidly and eggs with very thin shells or without shells appear. Vitamin D3 has traditionally been used by the poultry industry as an important source of vitamin D. Since 2006, 25-hydroxyvitamin D3 (25-OHD3) has been permitted and is widely used in the poultry industry as another source of vitamin D (Adhikari et al., 2019).

Since both forms of vitamin D share a similar pathway, it may be possible that one form of vitamin D aids another form of vitamin D in the absorption of Ca and P from the intestines of chickens. We hypothesize that there may be a pathway of interaction between vitamin D2 and vitamin D3, which can increase P absorption in the gut of laying hens. Further research is required to examine the effect of vitamin D2 when used in combination with other potentially effective vitamins (Adhikari et al., 2019).

According to the results of the study by Adhikari (2019) et al., it is stated that rations supplemented with vitamin D3 and vitamin D2 may cause an increase in Ca and P use in laying hens at the end of the laying period, although it is stated that vitamin D3 in the use of Ca and P in the late laying period (57th week) in laying hens. There was no difference between 25-OHD3 and 25-OHD3. According to the researcher, the use of Ca and P can be improved by increasing the combined isoforms of vitamin D in the diets. Again, according to this study, it was reported that sufficient Ca, P and D vitamin supplementation in the ration may be sufficient to protect the bone health of the laying hen in the late laying period.

According to the findings of a study by Fatemi (2020) and his team, when D3 and 25-OHD3 were injected into eggs in-ovo, treatments at doses between 0.60 and 4.8 mg, alone or in combination, were observed in broilers compared to non-injected and diluent-injected control groups. showed that embryos increased serum 25-OHD3 concentrations. However, D3 at these dosages did not affect the hatchability of live-injected eggs and the live weights of broilers. It may then have the potential to improve broiler performance with increased circulating 25-OHD3 levels. It has been reported that further studies are required to determine possible synergistic effects of egg-injected D3 and 25-OHD3 and possible synergistic effects between them on neonatal performance of broilers. Vitamin D deficiency in human subjects is associated with obesity, metabolic syndrome, hypertension, arterial remodeling and dysfunction, and cardiomyopathy. In various animal models and clinical cases, vitamin D has been reported to improve heart health and ameliorate functional failure by alleviating hypoxia, arterial biology and systemic signs such as vascular impedance and type 2 diabetes mellitus (Yeh et al., 2020).

In the study of Yeh (2020) et al., it was stated that 25-OH-D3 vitamin supplemented to the ration (69 µg/kg) improved the heart health of chickens. It has been reported to provide vascular remodeling and vasodilation regulation to relieve arterial pressure, thus having various ameliorating effects, including improving cardiac function for better survival in broiler breeder chickens. On the other hand, according to the results of a study by Lin (2019) et al, it was reported that the inclusion of additional 25-OH-D3 in the basal diet effectively improves the viability of chickens over a yield period.

Of the vitamin D3 metabolites 25-OHD3 and 1,25-OHD3, 25-OHD3 is biologically more active than vitamin D3 and less toxic than the 1,25OHD3 metabolite. This makes it safer to include in commercial poultry rations (Araújo et al., 2018). In a study by Mattila (2011) et al., it was stated that 25-OHD3 supplementation in broiler breeder feeds increased hatchability and chick quality by increasing egg accumulation. Again, in a study comparing vitamin D3 and 25-OHD3 supplementation to the diet, Saunders-Blades and Korver (2015), a decrease in embryo mortality was observed throughout the entire incubation period. According to the authors, the transfer of 25-OHD3 from parent to egg was more efficient than vitamin D3, potentially increasing the efficiency of vitamin D-dependent functions such as vitamin D status, calcium metabolism and homeostasis, and improving incubation efficiency by regulating bone growth. These results demonstrate the importance of sensitive broiler breeder nutrition, as the nutrients provided in the diet are transferred to the eggs and thus used by the embryo (Araújo et al., 2018).

In a study by Araújo (2018) et al., after the use of 25-OHD3 and canthaxanthin in the diet, the feed intake of broilers was not affected by either the breeder's diet or the broiler's diet ($P > 0.05$), regardless of breeder age. However, when the diet of breeders whose diet was fortified with 25-OHD3 cantaxanthin and broilers obtained from these breeders were also supplemented with 25-OHD3 cantaxanthin, there was a significant increase in body weight compared to broilers whose diet was supplemented with 25-OHD3 cantaxanthin only. affected regardless of breeder age. The highest weight gain was obtained in broilers fed canthaxanthin + 25-OHD3 up to 21 days of age from breeders fed this supplement ($P < 0.05$). In the offspring of breeders whose diet was supplemented with canthaxanthin + 25-OHD3, the feed conversion ratio (FCR) was significantly reduced ($P < 0.05$), regardless of breeder age. Likewise, carcass characteristics and breast meat yield were also positively affected by this ration supplement.

Vignale et al. (2015) fed diets supplemented with standard vitamin D levels (2,760 IU/kg) and high vitamin D or 25-OHD3 levels (5,520 IU/kg) for a cut-off age of 42 days, or only 25-OHD3 for a cut-off age of 21 days. evaluated the chickens. Accordingly, an increase in breast yield and a 3-fold increase in muscle protein fractional synthesis rate were observed in animals with a slaughter age of 42 days fed with ration fortified in terms of 25-OHD3.

5. VITAMIN E

Vitamin E (α -tocopherol), a biological antioxidant, also contributes to the improvement of physiological and immunological status in poultry due to its ability to reduce lipid peroxidation and neutralize free radicals in both skeletal muscle and plasma (Figure 3).

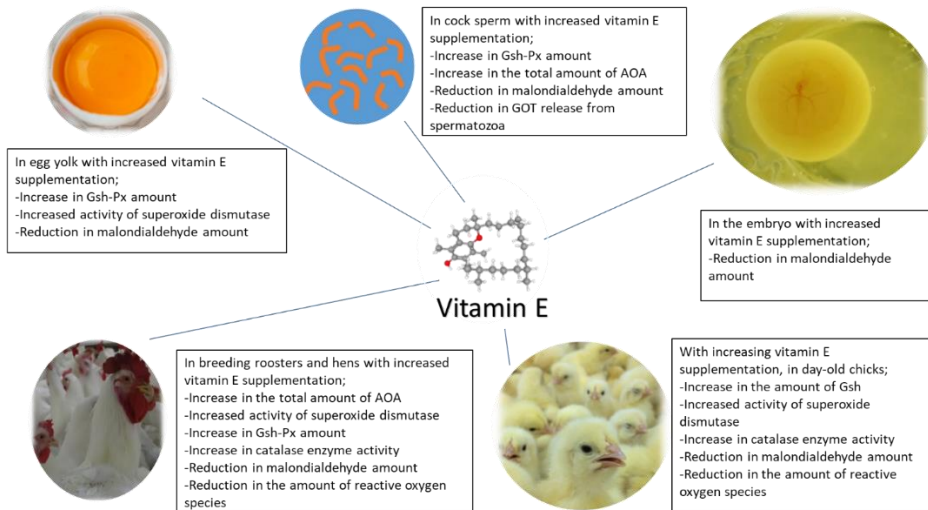


Figure 3. Effect of Dietary Vitamin E on Antioxidant Mechanisms

Vitamin E was discovered in 1922. Since then, vitamin E has come to the present day, gaining more attention and expanding our knowledge of its spectrum of activity. The term vitamin E includes a number of closely related active ingredients. These components are divided into two groups: tocopherols and tocotrienols. Of the tocopherols, the α form exhibits the greatest biological activity, has the widest distribution, and is most commonly found in ingredients used in animal feed. RRR- α -tocopherol or D- α -tocopherol, the natural isomer of α -tocopherol, which is usually the reference compound of vitamin E, is the isomer with the greatest biological activity. While most plants can synthesize vitamin E, animals and humans do not. This means that meeting vitamin E requirements depends solely on food (Barroete et al., 2012).

In most well-controlled studies with a small number of birds and in low-stress “ideal” conditions, breeders find that commercial doses of vitamin

E supplementation (100 mg/kg ration) are also sufficient to maintain antioxidant defense, fertility and yield performance. As a matter of fact, it has been shown that the experimental results obtained by feeding with vitamin E at different dietary levels are affected by the genetic pool, age, and the measurement criteria of vitamin E feeding level in the diet (Surai et al., 2019).

Adequate vitamin E intake is necessary for the following reasons (Barroeat et al., 2012):

- Maintenance of active tissues such as muscle, central nervous system or vascular system.
- In the case of oviducts, oxidative destruction of ovarian follicles reduces egg production and as a result, there is a deterioration in the conversion rate. There is also evidence that vitamin E facilitates the release of vitellogen, the precursor of egg yolk, from the liver, thereby increasing the circulation of compounds necessary for yolk formation.
- Avoiding oxidative destruction or replacement of macrophages, which is the first line of defense against infections. It also increases the production of antibodies by improving the immune response.
- Vitamin E also improves immune function by inhibiting the production of immunosuppressive prostaglandins.
- Vaccination is a routine and frequent practice in current table egg production systems. Vaccination induces immunological stress, which can be aggravated by other environmental conditions such as heat stress.
- Prevention of peroxide production and toxic effects of mycotoxins.

The concentration of vitamin E in plasma, various tissues and egg yolk has been reported to increase significantly with increasing dietary vitamin E supplementation (from 0 to 20,000 mg/kg) (Surai et al., 2019). Increasing vitamin E dietary supplementation improves antioxidant defense in eggs by increasing the concentration of vitamin E in egg yolk, significantly reducing lipid peroxidation as well as decreasing malondialdehyde (MDA) concentration. The concentration of vitamin E in spermatozoa depends on the provision of the diet. For example, the increase in vitamin E supplementation of the turkey diet (from 20 to 80 mg/kg) has been shown to significantly increase the concentration of α -tocopherol in spermatozoa (Surai et al., 2019).

The accumulation of vitamin E in the liver of the developing embryo is considered to be an important adaptive mechanism that ensures optimal antioxidant defense at the critical time of hatching, and the highest concentration of α -tocopherol in embryonic tissues is found at the time of hatching (Surai et al., 2019). Therefore, it can be said that chicken embryos can adapt to various stress factors by upregulating their antioxidant defense mechanisms during development (Surai et al., 2019). There are important interactions between various antioxidants within the antioxidant defense network of the newly hatched chick (Surai et al., 2019).

Lipid manipulation and various stress factors can significantly increase the susceptibility of semen to lipid peroxidation, at which point the protective effect of vitamin E will be much more pronounced. In some studies, it has been reported that the increase in the α -tocopherol concentration of semen with changes in the diet causes significant changes in the susceptibility of semen to lipid peroxidation, and the sensitivity of semen to peroxidation shows a very high negative correlation ($r = -0.998$) with the α -tocopherol content of semen (Surai et al., 2019).

Feeding modulation of antioxidant capacities is an important tool for poultry nutritionists to tackle commercially important problems. Indeed, evidence has recently been presented showing that egg yolk-derived antioxidants effectively protect the chicken embryo from oxidative stress brought on by the environment. Therefore, maternal-derived yolk antioxidants, in which vitamin E plays an important role, can protect the developing chicken embryo against oxidative stress caused by various factors, including hyperoxia (Watson et al., 2018).

There are studies reported that vitamin E plays a key role in balancing cytokine responses, which may be critical in cases of inflammation. It also showed that feeding broiler chickens 200 IU/kg of vitamin E along with a blend of antioxidants (ethoxyquin and propyl gallate) reduced histological inflammatory scores caused by oxidized soybean oil. It is noteworthy that soybean and other vegetable oils are routinely added to chicken feed to increase its energy content. Vitamin E feed supplementation may also be beneficial for chickens raised in stressful environmental conditions. It is clear that vitamin E can benefit the health of chickens through its anti-inflammatory effects. Therefore, vitamin E appears to increase both cell- and antibody-

mediated responses to antigens. Studies in chickens have shown that dietary vitamin E supplementation can increase lymphocyte and monocyte-mediated responses, both quantitatively and qualitatively (Shojadoost et al., 2021).

Efforts to maintain chickens' health and immune balance include the use of dietary supplements, including vitamin E. In addition to its function as an antioxidant and an essential nutrient for reproduction, vitamin E has also been identified as a regulating factor for the immune response. According to the recommendations of both the National Research Council (NRC, 1994) and the China Chicken Nutrition Standard (People's Republic of China Ministry of Agriculture, 2004), diets for healthy laying hens should be supplemented with 4 to 6 IU of vitamin E/kg. However, under practical conditions, both the feed industry and the producer generally prefer to supplement rations with more than 25 IU of vitamin E/kg to improve laying performance or alleviate the adverse effects of heat stress. The results of some studies indicate beneficial effects of supplemental vitamin E supplementation associated with foodborne salmonellosis for broilers or laying hens (Liu et al., 2019).

Liu et al., (2019) determined that supplementation of 30 IU/kg of vitamin E to the diet decreased the mortality rate and increased Superoxide dismutase (SOD) activity in layer hens. In addition to these findings, it was observed that it improved both laying performance and immunity in poultry exposed to *Salmonella Enteridis*, confirming the protective effect of vitamin E on the prevalence of SE in laying hens. Antioxidant systems include enzymes such as superoxide dismutases (SOD), glutathione peroxidases, and catalase, as well as water and fat-soluble antioxidants such as glutathione, ascorbate (vitamin C), α -tocopherol (vitamin E), ubiquinol, and β -carotene to limit oxidative damage in animals. is particularly interesting. Dietary supplements are routinely used to improve and maintain performance in poultry (Min et al., 2018).

The amount of antibodies transferred from breeder chickens to offspring plays an important role in protection against pathogens during the first week of life. In a study to examine the effects of supplemental (more than normal administration) vitamin E supplementation in the rations of breeder flocks on passive antibody transfer, it was determined that the progeny of birds that received more vitamin E had lower rates of low blood consumption when they received 450 IU/kg of vitamin E in the diet before vaccination with

Brucella abortus antigens. It was determined that those who received vitamin E showed higher antibody levels than their progeny of 150 IU / kg. In addition, another study reported that offspring of breeders who received 0.03% total vitamin E supplement in their diet for 3 weeks prior to vaccination against NDV showed higher antibody levels compared to the 1 and 7-day-old control groups. This evidence suggests that supplementation of vitamin E to breeder diets has beneficial effects for chicks in the context of passively transferred antibody-mediated immunity against infectious diseases. These observations suggest that vitamin E enhances antibody responses to vaccine antigens, but the underlying mechanisms are not yet understood. One possible explanation is that the antioxidant property of vitamin E improves cellular function, leading to plasma cells sufficiently programmed for enhanced antibody production. In addition, the immunostimulating properties of vitamin E appear to play a very important role in increasing host resistance in birds against infectious diseases. Based on the available evidence, it can be argued that dietary vitamin E in chickens not only modulates inflammatory responses, but also enhances adaptive immune responses, thereby contributing to antimicrobial immunity. This evidence suggests that supplementation of vitamin E to breeder diets has beneficial effects for chicks in the context of passively transferred antibody-mediated immunity against infectious diseases. These observations suggest that vitamin E enhances antibody responses to vaccine antigens, but the underlying mechanisms are not yet understood. One possible explanation is that the antioxidant property of vitamin E improves cellular functions, leading to plasma cells sufficiently programmed for enhanced antibody production (Shojadoost et al., 2021).

In a study by Yaripour (2018) et al. used an additional 100% vitamin A and 200% vitamin E in the ration, according to a combination of normal ration vitamins A and E (3 mg/kg and 37 mg/kg). It has been shown that this application gives the best results in terms of prolonging the laying period and fertility in old breeding chickens at the end of the laying period (from the 61st week to the 69th week). Therefore, they recommended supplementing the diet with an above-normal combination of vitamins A and E to positively manipulate egg and chick production in older broiler breeders.

In a study by Araújo (2018) et al. reported that in ovo vitamin E supplementation improved the oxidative status of newly hatched chicks, thus

providing better physical quality. In the same study, it was reported that improvement of 0-day-old chick characteristics, better broiler performance with a better start.

According to the findings of Min (2018) et al., it shows that vitamin E can play a role in modulating the expression of endogenous antioxidant enzymes as a gene regulator, as well as preventing oxidative damage as an exogenous antioxidant by scavenging free radicals and superoxide. In summary, the results show that oxidative stress can cause oxidative damage and reduce the antioxidant capacity and immune response of breeding males. They noted that supplementing the diet with vitamin C (300 mg/kg), vitamin E (200 mg/kg) or a combination of these could abolish adverse effects.

Symptoms associated with a deficiency of this vitamin are due to changes in the permeability of cellular membranes. In fact, the main function of vitamin E is to act as an antioxidant at the cellular level, specifically protecting phospholipids in membranes from lipid oxidation. At the cellular level, vitamin E is integrated into cellular membranes, where it neutralizes free radicals and effectively prevents the development of oxidation. The higher the degree of unsaturation of lipids, the higher their susceptibility to oxidation (Barroeatte et al., 2012).

In conclusion, it is clear from the analysis of the above-mentioned data that increased vitamin E supplementation in the diet of the breeder chicken or rooster increases its resistance to various stresses, including high PUFA, mycotoxin or heat stress. Increased vitamin E supplementation in poultry has been shown to be associated with significant increases in the level of α -tocopherol in semen, associated with increased resistance to oxidative stress induced by various external stress factors. Similarly, it has been shown that increased vitamin E concentration in egg yolk due to dietary supplementation is associated with increased α -tocopherol concentration in tissues of developing embryos and newly hatched chicks, resulting in increased antioxidant defenses and decreased lipid peroxidation. Furthermore, increased vitamin E transfer from the feed to the yolk and also to the developing embryo has been shown to be associated with upregulation of antioxidant enzymes, reflecting antioxidant system regulation and adaptation. Undoubtedly, further investigation of vitamin E metabolism and interactions between dietary

vitamin E and other nutrients, including carotenoids in poultry, is needed (Surai et al., 2019).

6. RIBOFLAVIN (B2)

Riboflavin (also known as Vitamin B2) is an essential water-soluble vitamin that acts as a coenzyme in metabolic processes for carbohydrate and protein metabolism. Besides this primary function, riboflavin has other functions as a bile hydrolase salt inhibitor and antioxidant. It can inhibit bile hydrolase salt enzymes produced by various intestinal bacterial organisms, including those used as probiotics, as a result, it can increase feed conversion efficiency and improve the growth performance of chickens, and as an antioxidant, it provides protection to body tissue. Riboflavin can also optimize the functions of probiotics by reducing bile hydrolase salt activity (Poudel et al., 2021).

Vitamin B2 or riboflavin was first isolated from egg albumen in 1933 and later detected in milk and liver. In poultry, riboflavin recommendations rise when the amount of fat or protein in the diet is increased because it acts as a cofactor for numerous enzymes involved in metabolic processes with oxidation processes of nutrients in the form of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). More than 100 enzymes are known to bind FAD or FMN (Barroeatte et al., 2012).

Riboflavin is an odorless, bitter-tasting, heat-resistant orange-yellow compound with a closed formula of $C_{17}H_{20}N_4O_6$ that melts at approximately 280 °C. This vitamin is sensitive to U.V rays and begins to break down immediately upon exposure. It is found in all green plants and fungi. It is synthesized by most bacteria except *Lactobacilli*. Yeast, liver, milk and leafy greens are rich sources. Cereals are poor in this vitamin. Since most of the ration in poultry consists of cereal grains, its deficiency can be seen. It is an essential component of flavoproteins. Riboflavin is found in the body as flavin adenine dinucleotide (FAD) and flavin mononucleotide and takes part in many reduction reactions. Many flavoproteins use FAD as a co-factor. Flavins are also involved in the β -oxidation of fatty acids (Abdurrahman et al., 2012). It is a component of H^+ transporter enzymes and has an important role in respiration (Figure 4.). It is especially related to growth and development during fetal life, reproduction and lactation (Peechakara et al.,

2020). There are also studies showing that the amount of riboflavin in breeder feeds has a clear effect on chick viability and survival capacity (Barroeate et al., 2012).

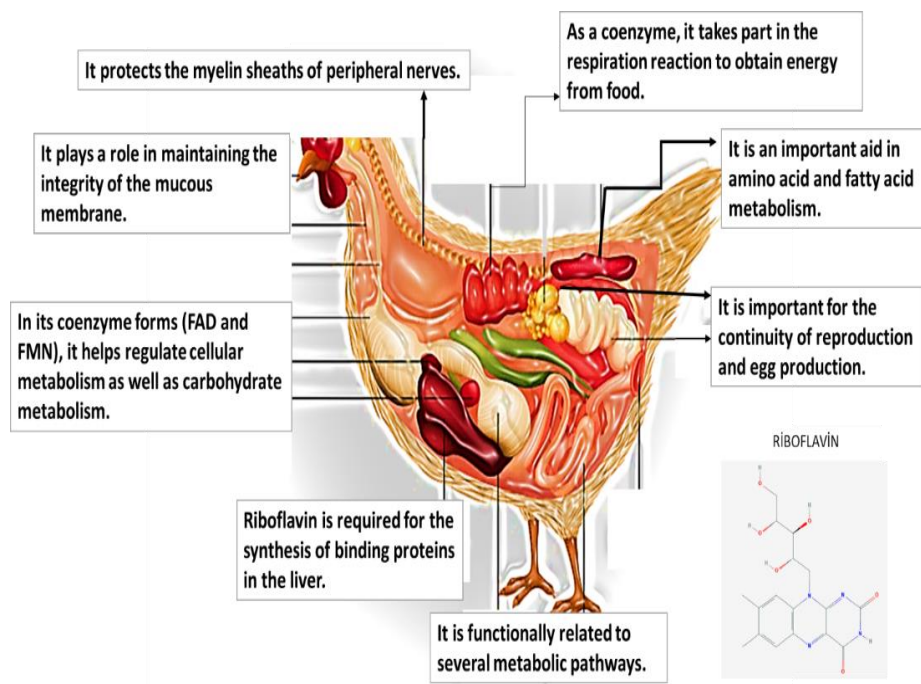


Figure 4. Basic Functions of Riboflavin in Poultry Metabolism

Riboflavin also plays important roles in the antioxidant system. Glutathione reductase is a flavin adenine dinucleotide-dependent enzyme that catalyzes the reduction of glutathione disulfide to reduced glutathione (GSH). It has been previously documented that riboflavin deficiency causes oxidative stress in animals by reducing glutathione reductase activity and GSH concentration (Zhang et al., 2020).

In poultry, there are studies showing that maternal riboflavin deficiency in laying hens results in embryonic death by lowering riboflavin concentrations in egg yolk. There have been reports of hereditary riboflavin deficiency causing chicken embryos to die suddenly mid-incubation, delayed and abnormal feather growth, fatty livers, and extensive skin bleeding. The explanation for sudden death in riboflavin-deficient embryos has been

attributed to inhibition of several critical biological pathways leading to energy depletion, lipid accumulation in the liver, and severe hypoglycemia. Of these, beta-oxidation of lipids was severely impaired in riboflavin-deficient embryos, as evidenced by the decrease in the activity of acyl-CoA dehydrogenase, a flavin-dependent enzyme, and the accumulation of lipids and fatty acid oxidation intermediates in the embryonic liver (Tang et al., 2019).

In a study by Tang (2019) et al. in ducks, it was observed that the hatchability of the hatching eggs of breeders fed with insufficient riboflavin decreased and also caused embryonic deaths.

7. CHOLINE

Choline is considered a member of the B complex vitamin group. Choline is an essential nutrient in chicken diets, necessary for the formation of phosphatidylcholine (PC), the essential phosphorus-containing lipids of chicken liver, blood and eggs. Phosphatidylcholine is the main component of very low density lipoprotein (VLDL); Both choline and phosphatidylcholine are required for VLDL secretion and lipid transport from the liver to the blood and peripheral tissues (Aziza et al., 2019). Choline is an essential nutrient for birds performing structural and neurotransmission functions and transferring methyl groups. Choline is considered a vitamin, although it does not meet some of the prerequisites of this definition. For example, birds need high amounts (less than 1%), amino acids and similar levels of essential fatty acids. It can be synthesized in the liver of birds from the serine and methyl groups, requiring 3 moles of methionine for each mole of choline synthesized. However, for most metabolic processes, the amount and rate of synthesis may be insufficient to meet the requirements, above all when the supply of precursors such as methionine, vitamin B12 or folacin is limited (Barroeat et al., 2012).

Choline is a source of labile methyl groups, part of the neurotransmitter acetylcholine, and a component of the main phospholipids in membranes (phosphatidylcholine and sphingomyelin). The function of choline in improving hepatic lipid accumulation has been widely demonstrated in chickens and humans. Additionally, supplementing the diet with additional choline appears to have a beneficial effect on fatty liver hemorrhagic

syndrome in laying hens and reduce the risk of fatty liver. In addition, betaine, one of the metabolites of choline, participates in the methylation of homocysteine to form methionine, and methionine is an essential amino acid that plays a key role in protein synthesis and is an intermediate in the synthesis of adenosylmethionine. Therefore, it can be assumed that choline has the potential to improve the hepatic redox status of laying hens, thereby protecting liver damage (Dong et al., 2019).

Choline is important for metabolism in maintaining and building cells. In addition, it is required for the maturation of the bone cartilage matrix (Muhammad et al., 2017)

In a study by Rao (2001) et al., the interactions between different energy sources in the ration and supplemental choline were found to be important for liver fat content, offal weight and final body weight. It has been reported that adding choline (760 mg/kg) to the diet significantly reduces fat accumulation in the liver.

In a study by Dong (2019) et al., the total lipid concentration of egg yolk increased at 58 and 68 weeks ($P < 0.05$) in chickens fed diets supplemented with 3,400 mg/kg choline compared to those fed 0 and 425 mg/kg choline supplementation. Egg yolk total lipid concentration increased linearly ($P = 0.014$) and quadratically ($P < 0.001$) with increasing choline levels at week 58, while at week 68 choline increased the egg yolk total lipid concentration of animals quadratically ($P < 0.01$). In the same study, chickens fed diets supplemented with 1,700 and 3,400 mg choline/kg had lower total lipid ($P < 0.05$) and triglyceride concentrations ($P < 0.001$) than those fed diets without choline supplementation. In addition, in the current study, dietary choline supplementation increased GSH-Px activity and total antioxidant capacity and tended to lower liver malondialdehyde levels in laying hens.

In a study by Aziza (2019) et al., it was reported that choline added to the diet increased the antioxidant status and decreased the total lipid content. It has also been stated that choline supplementation can reduce oxidative stress in chickens with flaxseed in their diet.

As a structural component of lecithin, choline plays an important role in the formation of very low-density lipoproteins (VLDL), which are tasked with incorporating and activating triglycerides in the liver. Lecithin deficiency is

associated with the accumulation of fat in the liver and a decrease in the amount of fat deposited in the yolk. In addition, there are studies that show a numerical reduction in abdominal fat storage when animals are fed an increased amount of choline (Barroeat et al., 2012).

According to the results of a study conducted by Wang (2017), it was stated that choline can serve as an inexpensive feed additive and is effective in increasing DHA enrichment in egg yolk in chickens fed a diet rich in DHA.

It has been reported that choline added to the diet increases the antioxidant status and minimizes lipid peroxidation in chickens fed a diet rich in docosahexaenoic acid (DHA). As consumer demands for DHA-rich poultry products such as eggs increase, choline can act as an inexpensive feed additive that can be used in layer diets to reduce oxidative stress while increasing productivity in chickens (Yonke and Cherian, 2019).

Fatty liver is commonly seen in laying hens, for example, as fatty liver syndrome and fatty liver hemorrhagic syndrome. In fatty liver hemorrhagic syndrome, it can cause a decrease in egg production and an increased death rate in laying hens. Chickens with fatty liver hemorrhagic syndrome exhibit hepatic steatosis, while more severe cases develop blood clots and liver rupture (Lin et al., 2020). The pathogenesis of fatty liver diseases in chickens is accompanied by an imbalance in lipid homeostasis such as hepatic lipid accumulation, transport and metabolism. High-energy low-protein diets in chickens can reveal fatty liver. As a methyl donor, choline is important for very low-density lipoprotein (VLDL) secretion, and choline deficiency leads to impaired VLDL secretion in the liver, resulting in fat accumulation (Lin et al., 2020).

8. FOLIC ACID

The terms folacin, folate and folic acid are used interchangeably and refer to a wide variety of compounds that have the biological activity of folic acid. Folacin is a compound that is essential for the transfer of monocarbonate units in metabolic processes, affecting the synthesis of purines and pyrimidines, which form nucleic acids necessary for cell division. It also plays a role in the conversion of serine and glycine, the degradation of histidine, and the addition of methyl groups to compounds such as methionine, choline, and thiamine (Barroeat et al., 2012).

Folic acid is involved in converting homocysteine to methionine via the 5-methyltetrahydrofolate-homocysteine methyltransferase reaction, one of two remethylation pathways. Low folate levels inhibit the synthesis of methionine and S adenosylmethionine, leading to a decrease in methylation (Lu et al., 2021).

High protein levels in the diet raise dietary recommendations for folate. Folic acid is found in most feeds used in chicken feeds, but as it tends to manifest itself in conjugated forms, its absorption efficiency may be reduced (Barroete et al., 2012).

After hatching, chickens begin to consume feed and the lipid accumulated in the liver falls rapidly within the first 6 days after hatching. The utilization and accumulation of lipids is mainly derived either from diets or from de novo lipogenesis in the liver. As the liver is the predominant site for lipogenesis, a higher accumulation of abdominal fat is closely associated with the rate of Triglyceride synthesis and secretion from the liver. These events showed that the period of change in hepatic lipid metabolism (within the first few days after hatching) has a significant effect on the adipose tissue of the chicks (Liu Y. et al., 2019). According to the results of a study by Liu Y. (2019) et al., when the control and folic acid-administered groups were compared, it was reported that folic acid perfusion broilers significantly reduced the abdominal fat percentage ($P < 0.05$).

Folic acid (FA) is medically recommended as it is beneficial for the circulatory system. It is also effective against the formation of congenital and acquired heart defects and is recommended for pregnant women to reduce the risk of fetal malformations. Folic acid also plays an important role in hematopoietic processes and DNA synthesis (Tombarkiewicz et al., 2020). The transition of folic acid from feed to egg is very high. During the breeding phase of turkeys, increased dietary folic acid supplementation increased the availability of this vitamin in eggs, resulting in increases in hatch weight and subsequent growth velocity (Barroete et al., 2012).

9. RESULT AND DISCUSSIONS

Optimum concentrations of vitamins in poultry diets allow birds to realize their genetic potential. Vitamin requirements determined decades ago do not meet the needs of genetically superior animals with today's increased

growth, egg production and improved feed efficiency (Adhikari et al., 2019). The symptoms of nutritional deficiencies can sometimes interact with each other, leading to difficulties in interpreting these symptoms. However, raising the nutrient levels in the ration is not recommended as it can adversely affect the health of animals and environmental pollution. (Fouad et al., 2018).

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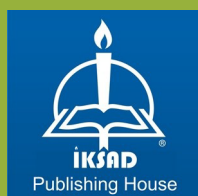
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