

## TRACE MINERALS AND AVIAN EMBRYO DEVELOPMENT<sup>1</sup>

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### RESUMO

#### MINERAIS-TRAÇO E DESENVOLVIMENTO EMBRIONÁRIO EM AVES

É essencial que a dieta da reprodutora seja adequada em todos os nutrientes para que o embrião dentro do ovo possa se desenvolver normalmente. Quando uma deficiência ou toxidez de minerais-traço ocorrem e a incubabilidade do ovo é afetada, o problema pode, na maioria das vezes, ser atribuído à dieta das reprodutoras. Amostras de rações usadas em fazendas de reprodutoras devem ser mantidas por vários meses no caso da necessidade de um ajuste para

**PALAVRAS-CHAVE:** reprodução, dieta, toxidez, incubação, produtividade

resolver problemas à incubação e a qualidade do pinto de um dia. Deve ser uma prática-padrão para as granjas de reprodutores a contínua avaliação dos suplementos minerais e vitamínicos usados para assegurar a sua qualidade. Estes suplementos geralmente constituem apenas um por cento da dieta, mas as vitaminas e os minerais fornecidos aos embriões em desenvolvimento são responsáveis por 100% de seu metabolismo.

### SUMMARY

It is essential that breeder diet be adequate in all nutrients so that the embryo inside the egg can develop normally. When a deficiency or toxicity of trace mineral occurs and egg hatchability is affected the problem can, in the majority of instances, be traced back to the diet of the breeder hen. Samples of all batches of feed used on breeder farms should be kept in inventory for several months in case a trace back needs to be done to help solve hatchability

**KEY WORDS:** reproduction, diets, toxicity, incubation, performance

and chick quality problems. It should be standard practice for breeder companies to continuously check the vitamin and mineral premixes used to ensure that they are of high quality. These premixes usually comprise only one percent of the diet, but the vitamins and minerals they furnish to the parent birds and developing embryos are responsible for 100% of their metabolism.

### INTRODUCTION

The mammalian embryo or fetus is continuously supplied with nutrients from its mother's circulation via the placenta during development in her uterus. The avian embryo must also rely on its mother to supply it with the needed nutrients required for development. However, the avian embryo develops in a porous package

outside of the hen's body. The supply of nutrients in the egg originates in the diet of the hen and from her metabolism (Wilson, 1997). All of the minerals required by the developing avian embryo are supplied by specific storage pools in the egg and each of these elements had to be supplied in the diet of the hen (White, 1991). Nutritionists know very well the consequences of providing their breeder birds with a diet that is not adequate in nutrients. This is the reason that the vitamin and

mineral premix should be reviewed frequently. When breeder diets are formulated to contain an adequate nutrient supply the birds will produce eggs of relatively constant composition and physical condition when hens are housed in a disease-free thermoneutral environment (Reddy, 1993). Optimal embryogenesis requires adequate micronutrients supplied to the breeder parents. The breeder feed not only dramatically influences the total production of eggs and health of the breeders, but also the production of high quality, fertile eggs able to provide the essential nutrients to the developing chick for 21 days.

Fifteen trace elements are considered to be essential in animals. Among these, the physiological role of cobalt, chromium, copper, iodine, iron, manganese, molybdenum, selenium, zinc, and fluorine are well recognized. Although deficiencies of nickel, vanadium, silicon, lead and arsenic have been demonstrated in an ultraclean environment, with exception of silicon, the physiological function of these trace elements has not been clearly demonstrated (Lall, 1989). The trace elements are required in the diet in very low concentrations (milligrams or micrograms per kg of diet). Using today's modern analytical techniques, researchers are able to determine the quantity of each element in the diet. Also, not only can the mineral in the animal's diet be quantified, but the compound within the cell with which the mineral functions can also be identified and its quantity or activity determined. This has allowed scientists to identify the unique functional role that each trace mineral plays in many metabolic processes. Books have been written about the metabolic role of the trace elements in cellular metabolism. This paper is not going to present and discuss these well-understood functions. The information in this paper centers around the location of the trace minerals in the egg, how certain ones are handled by the developing embryo, and a brief description of what can be expected when there is a deficiency or toxicity of certain elements in the egg.

When a deficient or toxic concentration of trace mineral(s) exists in the egg, the diet of the parent should be evaluated. Simply stated, if the hen does not have the required quantity of a specific trace mineral in her diet it will not be in the egg in a

sufficient quantity for the embryo to develop normally or if there is too much of a specific mineral(s) in the diet, then the possibility exists that the egg will also contain high levels. In this case a toxicosis may result.

### MINERAL LOCATION IN THE EGG

The avian egg is essentially a chemical and nutrient store for a potential new life and is composed of three main parts: shell, albumen, and yolk. Each of these parts contain minerals. The egg's mineral stores are deposited at the time of its formation within the hen's reproductive tract. These stored minerals are obtained by the embryo during development through the coordinated actions of extraembryonic membranes (Richards and Packard, 1996; Grau et al., 1979). Most of the minerals in the egg are in conjugated form and only a small portion is present as inorganic compounds or ions (Romanoff and Romanoff, 1949). The majority of the minerals found in the egg are concentrated in the yolk with phosphorus being its most abundant mineral component. More than 61% of the phosphorus in the yolk is contained in phospholipids (Sugino et al., 1997). Sodium and potassium are concentrated in the albumen. The largest concentration of calcium is found in the egg shell. The shell and its associated membranes contain varying amounts of zinc, copper, iron, and manganese (Mas and Arola, 1985; Burley and Vadehra, 1989). The shell membrane of the chicken and turkey egg contains a high concentration of copper (Richards, 1991a; Baumgartner et al., 1978). Richards and Packard (1996) identified the location of an egg's minerals. These authors reported that in an egg weighing 58 g (19 g yolk, 34 g albumen and 5 g egg shell) the yolk contained 112, 27, 20, 12 and 3 mg of phosphorus, calcium, potassium, sodium and magnesium, respectively. The albumen contained 5, 3, 49, 57 and 4 mg of the same minerals, respectively. The egg shell also contained 1526 mg calcium.

Richards and Packard (1996) reported that in the 58-g egg the yolk's concentration of iron, zinc, copper, iodine, manganese and selenium was 1017, 571, 37, 32, 10, and 9 micrograms, respectively. The concentration of these same trace

minerals in the albumen was 53, 5, 10, 2, 0.05 and 2 micrograms, respectively. Richards (1997) also reported that the yolk contained the highest concentration of iron, zinc, copper, iodine, manganese and selenium, but also reported the concentration of chromium, nickel and cobalt were greatest in the yolk of the chicken egg.

### *IN OVO* TRACE MINERAL METABOLISM

*Note from Author:* Two excellent reviews were cited during the writing of this text. The review by Dr. Mark Richards (USDA, Beltsville, MD; Richards, 1997) is recommended to anyone with an interest in learning more about how the hen, as well as the developing embryo, utilizes trace minerals. Also, Dr. Henry Wilson (University of FL; Wilson, 1998) prepared a review on the maternal nutrition effects on hatchability.

In general, the trace minerals which are in the egg and available to the developing embryo function either as catalytic or structural cofactors in enzymes and proteins. These enzymes and proteins are located in the cells of the embryo and its extraembryonic membranes. Richards and Steele (1987a) induced a trace mineral deficiency in developing embryos by feeding laying hens a diet deficient in the elements. These authors also described impaired growth, abnormal development of all the major organ systems and other signs which were associated with these deficiencies.

The concentration of the trace minerals deposited in the hen's egg is dependent on the form of the mineral and the amount of the mineral in the diet of the hen (Stadelman and Pratt, 1989; Naber, 1979). Cantor (1997) was able to increase the selenium concentration of eggs from commercial egg-type laying hens by feeding either an inorganic selenium source (sodium selenite,  $\text{Na}_2\text{SeO}_3$  or two organic sources (selenium yeast as Sel-Plex 50, and high-selenium soybean meal). It is known that organically-bound and natural forms of selenium, such as selenomethionine and selenized yeast promoted deposition of this mineral into egg albumen to a greater concentration than that of the yolk (Latshaw, 1975; Latshaw and Osman, 1975; Latshaw and Biggert, 1981; Swanson, 1987). In ge-

neral, increasing the dietary concentrations of zinc, copper, and iron did not result in higher egg concentrations of these minerals (Kienholz et al., 1964; Stahl et al., 1988). The age of the hen and environmental conditions also exert an influence on the mineral content of the egg (Conningham et al., 1960).

The yolk contains the greatest concentration of trace minerals in the egg. Unlike albumen, egg yolk is a homogeneously emulsified fluid. The major portion of egg yolk exists as lipoproteins which are separated into plasma and granule fractions. There are two very important components of the egg yolk that are responsible for binding trace minerals, especially zinc, copper, and iron. One of these components, lipovitellin, is a HDL and the other is phosvitin, a phosphoprotein containing about 10% phosphorus (Sugino et al., 1997). Phosvitin is known to avidly bind iron because it contains a high concentration of phosphorylated serine residues (Toborsky, 1980). Both lipovitellin and phosvitin are packaged together in the granule subfraction of the yolk and constitute the major store of zinc, copper, iron and manganese (Bellairs et al., 1972; Grau et al., 1979; Richards, 1991a).

The albumen contains very low concentrations of trace minerals. The elements iron, zinc, copper, selenium, and manganese found there are known to be bound by specific proteins such as ovalbumin and conalbumin (Magat and Sell, 1979; Goux and Venkatasubramanian, 1986; Burley and Vadehra, 1989; Palmer and Guillet, 1991). It has been widely accepted by many researchers that the egg albumen proteins may function as anti-microbial agents because of their ability to bind and limit the availability of iron and the other trace elements. Even though there is a low concentration of trace minerals in the albumen it must not be forgotten that the albumen mixes with the yolk and amniotic fluid during embryonic development. A specific role for these minerals bound in the albumen in early embryonic development may exist (Richards, 1997). As stated earlier, the egg shell membrane contains high concentrations of trace minerals, especially copper. It is not yet known whether the elements located in

the membrane of the shell are utilized and play a major role in embryonic development, but their nutritional significance should not be discounted (Richards, 1997).

During embryogenesis the developing embryo requires the trace minerals stored in the yolk to be transferred to developing tissues. The yolk sac membrane has been extensively studied and plays a very important role in mobilization of yolk's nutrient stores. The endodermal cells that line the inner surface of this membrane are in direct contact with the yolk. The mobilization of the yolk's trace minerals involves the uptake and processing of yolk granules by the endodermal cells of the yolk sac's epithelial lining (Richards, 1997). In fact, the yolk sac of the turkey embryo has the ability to concentrate and store the trace minerals obtained from the yolk during incubation (Richards 1991c). In this same reference, the author also proposed that the yolk sac membrane is not simply a passive barrier between the embryo and yolk nutrient stores, but that it has the ability to absorb yolk trace minerals, store them for short periods of time, and subsequently release them to embryonic tissues via the vitelline circulation. It seems as though the yolk sac membrane is coordinating the mobilization of yolk-granule-derived trace minerals with their incorporation into storage proteins and the synthesis of plasma trace mineral transporting proteins and, thus, the yolk sac can effectively regulate the supply of trace minerals provided to the embryo according to specific tissue requirements dictated by the rate of growth or the stage of development (Richards, 1997).

It is not uncommon in the broiler industry to have differences in chick performance related to breeder age. Eggs from older breeder flocks are larger than eggs from younger breeder flocks. At times, the smaller chicks from very young broiler breeder flocks have increased mortality and reduced performance (McNaughton et al., 1978) compared to the larger chicks from older breeder flocks. Vieira and Moran (1998) reported that eggs from older breeders (62 weeks of age) were heavier and had a greater proportion of yolk and smaller proportions of albumen and shell than those

from younger birds (27 weeks of age). Chick and yolk sac weights from old breeders were greater, but yolk sac percentages were not different. Analyses for crude protein and amino acids, as well as ash and minerals of eggs, chicks, and yolk sacs, did not reveal obvious differences in concentrations.

Iodine is an essential trace mineral required for maintaining thyroid function. The major supplier of iodine, in the form of iodide, to the developing embryo is the yolk (Daugeras-Bernard et al., 1993). These same authors also reported that allantoic fluid contains iodine levels in excess of those found in plasma and a constant ratio between iodine levels in these two embryonic fluid compartments appeared to be maintained throughout development. Thus, iodine excretion via the chorioallantoic membrane into allantoic fluid seemed to be an important mechanism for regulating plasma iodine concentrations.

During the first few days of incubation the developing chick embryo has the ability to transfer and concentrate trace minerals from the egg. Dewar et al. (1974) reported that whole chick embryo concentrations of zinc, copper, iron, and manganese were initially high on Day 5 of incubation and that they had declined sharply by Day 10. Romanoff (1967) proposed that in the early stages of development of the chick embryo higher trace mineral concentrations are required to support the very rapid expansion of embryo mass. The yolk sac membrane has the ability to accumulate and store iron. Ramsay (1951) studied changes in heme and nonheme iron in the embryo and the yolk sac membrane and related the changes in yolk sac iron levels with growth and development of the embryo. Toward the end of incubation, there is a sharp decline in yolk sac heme iron due to diminished erythropoietic activity and a subsequent accumulation of nonheme iron in the embryo. The nonheme iron store was proposed to be utilized by the chick during the early posthatching period (Richards, 1997).

The most important organ for the storage and regulation of trace mineral metabolism in the developing avian embryo is the liver (Richards and Steele, 1987a, b). The liver's concentration of the

trace minerals will increase and decline depending on the stage of embryo development (Sandrock et al., 1983; Fleet and McCormick, 1988). Richards (1991b, c) suggested that these hepatic trace mineral fluctuations may correlate with the mobilization of these minerals from egg stores. Ferritin is a protein in liver that is known to bind iron. The zinc and copper binding protein, metallothionein, is also present in the liver. The concentration of metallothionein in the livers from developing chick embryos was investigated by Sandrock et al. (1983) and Fleet and McCormick (1988). These authors found the metallothionein concentration to be elevated during periods of increased zinc and later in incubation due to a rise in copper concentration. Wei and Andrews (1988) studied developmental changes in hepatic metallothionein gene expression and reported that metallothionein mRNA levels are higher at midincubation and then decline toward hatching as copper levels in the liver increase. The greatest levels of hepatic metallothionein protein bound zinc and copper occur during the first week posthatching and presumably reflect the stress of emerging from the egg (Fleet and McCormick, 1988; Chakraborty and Biswas, 1987).

Even when the trace minerals are present in the egg in sufficient quantities to provide for normal embryonic development, the possibility exists that incubation conditions will be such that the embryo has to make adaptive changes in how it metabolizes the trace minerals. It is well documented that changes in the environmental conditions during incubation can impact trace mineral metabolism. A discussion of how incubation conditions affect the embryo and its metabolism of trace minerals is presented in the review by Richards (1997) and will not be discussed here because it is beyond the theme of this paper.

#### TRACE MINERAL DEFICIENCY AND TOXICITY

Caskey and Norris (1940) and Landauer (1967) were able to clearly describe the effects of a manganese-deficient breeder diet. These authors reported that reduced hatchability and embryonic abnormalities were a direct result of a manganese

deficiency. The embryonic abnormalities which were described by these authors are similar, if not almost identical, to those observed in a biotin deficiency. Signs of a biotin-deficient embryo include increased mortality peaks prior to 5 days or after 17 days of incubation, a chondrodystrophy or micromelia condition with shortened bones and "parrot beak" (Cravens, 1944; Couch et al., 1947). Mortality of embryos from biotin-deficient eggs increased during the first and third weeks of incubation (Cravens, 1944; Couch et al., 1948; Leeson et al., 1979). Caskey et al. (1944) reported that chicks hatched from hens fed a manganese-deficient diet may possess a tetanic spasm which resembles that observed in chicks with a condition referred to a "stargazer". Abdallah et al. (1994) reported that if a laying hen diet suddenly becomes deficient in manganese, several weeks may pass with no apparent adverse effects on hatchability. It is important to remember when troubleshooting hatchability problems that a considerable amount of time may have passed before a problem in hatchability, which results from feeding a deficient diet, will be detected. Many times, it is often forgotten that a hatchability problem observed "today" really began 4 or more weeks ago in the breeder house due to a deficient or adulterated diet.

The concentrations of zinc which are required in the breeder diet to maintain normal hatchability are low (Supplee et al., 1958). Zinc-deficient embryos have low hatchability, increased mortality, and impaired development of the skeleton and feathers (Kienholz et al., 1961) as well as a variety of other abnormalities (Blamberg et al., 1960; Romanoff and Romanoff, 1972). In some cases a zinc deficiency is associated with embryos having no wings or legs and tufted down. It has been reported that it may not be necessary to supplement Single Comb White Leghorn breeder diets based on corn and soybean meal with zinc in order to obtain normal egg production and hatchability (Stahl et al., 1986; Abdallah et al., 1994). Stahl et al. (1986) did report that the chicks hatched from eggs produced from hens fed no supplemental zinc exhibited abnormal feather development at hatch. Abdallah et al. (1994) conducted a 10-week study in which the trace minerals zinc, manganese, copper, and iron were

omitted from the diet either alone or all in combination. The only negative effect was in shell quality when manganese was not present in the diet. This may imply that diets of breeder hens need not be supplemented with one or more trace elements. Also, relying on such a false sense of security may result in costly consequences if the trace mineral premix is accidentally left out of the diet for any length of time. A word of caution is appropriate to those thinking about using this “no zinc or other trace mineral supplementation” strategy in their breeder flocks. There are many stressors in chicken houses, feed ingredient nutrient content (especially trace minerals) varies, management practices differ, etc. and to deliberately omit certain essential trace minerals from the diets of breeders as well as those of commercial egg production-type hens simply to save on costs may result in “saving dimes and losing lots of dollars”.

A deficiency of selenium in breeder diets will result in decreased hatchability (Jensen, 1968; Arnold et al., 1974). Signs of a deficiency were weak chicks that had gizzard muscle myopathy and many were prostrate with their legs extended backward and curved upward. Toxicosis due to excess selenium has been shown with natural diets (Carlson et al., 1951; Kinder et al., 1994). Abnormal signs due to excess selenium include dwarfing, shortened bones of the legs and wings, and short or missing lower beak.

An iodine deficiency results in a decline in hatchability (Landauer, 1967). It is not common for naturally iodine deficiencies to occur. Strain differences in hatchability responses have been reported in turkeys following iodine supplementation of the breeder diet (Christensen et al., 1991). If a breeder hen is fed excess iodine a hatchability problem may arise. Increased embryonic mortality, unhatched pips, and extended incubation periods are some of the signs often reported during toxicosis (Arrington et al., 1967). During iodine intoxication the embryo is dependent more on glycogenolysis than gluconeogenesis during the pipping and hatching phases (Christensen et al., 1991; Christensen and Ort, 1991).

A copper-deficient breeder diet is associated with a severe decline in hatchability (Savage, 1968). The interrelationship between copper and

zinc has proven to affect hatchability, in that an excess of zinc will aggravate a copper deficiency. Signs of a copper deficiency in the embryo include early mortality with anemia and a high incidence of hemorrhage following 3 to 4 days of incubation. Savage (1968) also reported that the need for molybdenum in breeder diets is inconclusive. Lepore and Miller (1965) reported that excess amounts of molybdenum (500 ppm) in breeder diets fed for 2 weeks or longer resulted in 100% mortality when egg molybdenum concentration reached 17 ppm. These authors reported that approximately 95% of the embryonic deaths occurred from 5 to 12 days of incubation.

Even though a dietary requirement for boron has not been established, breeder birds maintained on litter that has been treated with boron-based insecticides may consume high levels of boron (Lee, 1989). Rossi et al. (1993) fed high levels of boron to broiler breeder hens in the form of borax or boric acid and reported decreased hatchability, but no detrimental effect on egg production. Feeding high levels of boron to caged males caused an increased incidence of abnormal spermatozoa, but did not affect fertility or hatchability of eggs from inseminated hens (Rossi et al., 1993). Infection of boric acid into the egg has been reported to cause many skeletal abnormalities (Romanoff and Romanoff, 1972).

Silicon is one of the most recent trace elements to be established as “essential”, participating in the normal metabolism of higher animals. A series of experiments has contributed to the establishment of silicon as an essential element. Several of these have shown that silicon plays a physiologic role in the bone calcification process. Silicon has been shown to be required in the normal growth and skeletal development in the chick (Carisle, 1972). Silicon has a metabolic role in bone formation and is involved in both collagen and glycosaminoglycan formation. Silicon’s primary effect in bone and cartilage appears to be on formation of the matrix, although silicon appears also to participate in the mineralization process itself. Studies that involved skull bones from 14-day-old chick embryos grown in culture have demonstrated the dependence of bone growth on the presence of silicon and the majority of the growth due to silicon’s

role in collagen synthesis (Carlisle and Alpenfels, 1978). In 12-day cultures, supplemented and unsupplemented with silicon, a difference was reported in proline synthesis. Large differences resulting at 4 and 8 days between deficient and silicon-supplemented culture media, suggested the possibility of a role for silicon in the proline synthetic pathway (Carlisle and Alpenfels, 1984). The literature are void of data which show a benefit of supplementing a source of silicon to commercial breeder diets. However, since it has been shown that silicon is essential for normal chick embryo metabolism, future research may provide more needed information in this area.

Although vanadium can cause toxicosis at relatively low concentrations, it is also an essential element recommended to be in animal diets at very low levels (NRC, 1994). Miles et al. (1997) overcame the reduced egg interior quality associated with feeding 10 ppm vanadium, as ammonium metavanadate, in the diet of commercial egg-type laying hens. Supplementing the diet containing vanadium with any one of the antioxidants ascorbic acid (100 ppm), vitamin E (200 IU/kg) or beta-carotene (500 ppm) restored egg interior quality. Bressman et al. (1999, unpublished data) fed increasing concentrations of vanadium to commercial egg-type laying hens inseminated with pooled semen and incubated the eggs. A significant ( $P < 0.05$ ) decline in hatchability of fertile eggs was observed at 60 ppm vanadium and the number of unpipped eggs increased. No embryonic abnormalities or malpositions were observed.

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