ALTERNATIVE SOURCES OF FAT AND ZINC IN BROILER BREEDER DIETS

AND THEIR INFLUENCES ON PERFORMANCE

by

BENTON PATRICK HUDSON

(Under the Direction of Jeanna L. Wilson)

ABSTRACT

Experiments were conducted to evaluate the influences of dietary fat and zinc sources on broiler breeder or progeny performance. The first experiment indicated that providing Menhaden oil rather than poultry fat in broiler breeder male diets maintained higher fertility of eggs laid during the second week following single inseminations at different ages. However, sperm quality measurements did not reveal differences between semen from the two male groups. A series of experiments were conducted to determine the effects of zinc source on performance and immune status of broiler breeder hens and their progeny. From 0 through 65 weeks of age, broiler breeder hens were given diets supplemented with 160 ppm zinc from $ZnSO_4$, Availa®Zn zinc-amino acid complex, or a mixture of $ZnSO_4$ and Availa®Zn. Eggshell quality was enhanced when a mixture of the two zinc sources was provided. As a result, fewer cracked eggs were produced and hatching egg production and estimated chick production were increased. There was a direct relationship between Availa®Zn intake and humoral and cellular immune responses. Two different experiments were conducted with progeny from these hens. On both occasions, chicks were provided 140 ppm supplemental zinc as $ZnSO_4$ or a mixture of $ZnSO_4$ (100 ppm) and Availa®Zn (40 ppm). In experiment 1, the chicks were subjected to either low or normal brooding temperatures. Humoral immune status of broilers was increased when hens were provided diets with $ZnSO_4$, but dietary zinc source did not influence broiler performance. Mortality, feed intake and body weight were increased when broilers were subjected to cold temperatures. In experiment 2, body weight gain and feed conversion were enhanced when broilers were provided a mixture of $ZnSO_4$ and Availa®Zn. These data suggest that $ZnSO_4$ and Availa®Zn may have synergistic effects, improving bird performance. Finally, influences of hen age and zinc source in hen diets on physiological development of chicks were evaluated. Results indicated that yolk sacs and hearts were less developed in progeny from young hens and may limit broiler performance.

INDEX WORDS: Broiler Breeder, Menhaden Oil, Sperm Quality, Zinc, Fertility, Chick Production

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BENTON PATRICK HUDSON

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BENTON PATRICK HUDSON

Major Professor: Jeanna Wilson

Committee: Jeff Buhr
Nick Dale
Adam Davis
Jean Sander

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
May 2003

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# CHAPTER 1

# LITERATURE REVIEW

## INTRODUCTION

Nutrient intake in broiler breeders may have drastic influences on reproductive performance. Deficient nutrient status of males will ultimately lead to poor semen quality and reduced mating activity. Simultaneously, females require proper nutrition to produce quality eggs containing adequate nutrients for normal embryonic and progeny development. This dissertation primarily discusses the effects of various fat and zinc sources on reproductive performance of broiler breeder males and females, respectively.

Declining fertility in broiler breeder flocks is expected as birds surpass 45 to 50 weeks of age. While introducing younger males to middle-aged flocks improves fertility, manipulations to ingredient or nutrient levels may improve semen quality. Higher quality semen would then prolong duration of fertility, especially if mating activity is infrequent. Since dietary fatty acids can be incorporated into body tissues (Huang *et al.*, 1990), the fatty acid profile of the diet may alter sperm membrane characteristics. Researchers have previously reported that feeding relatively high concentrations of dietary n3 polyunsaturated fatty acids (PUFA) to broiler breeder males improves fertility (Kelso *et al.*, 1997b; Blesbois *et al.*, 1997; Cassanovas, 1999). These authors suggested that decreasing the n6/n3 ratio of PUFA in the sperm membrane increases membrane

flexibility and reduces peroxidation. Marine fish oils contain high concentrations of n3 PUFA (Chanmugan *et al.*, 1992), and these fat sources may be used as a supplemental fat source in poultry feeds. Prior research has not evaluated the effects of relatively high n3 PUFA intake during the entire rearing and breeding periods. To further examine the relationship between high n3 fatty acid intake and increased fertility, broiler breeder males were provided dietary Menhaden oil from placement until 65 weeks of age.

Zinc is another nutrient that may alter reproductive performance of broiler breeders. Research conducted over four decades ago indicated that hens with inadequate zinc intake produced embryos with numerous abnormalities (Turk *et al.*, 1959; Blamberg *et al.*, 1960; Kienholz *et al.*, 1961). Chicks that hatched were often of low quality and had poor feathering. Although most practical corn - soybean meal diets provide approximately 30 ppm dietary zinc or more, supplemental zinc is often provided to ensure maximum performance of both breeders and broilers. There is limited evidence that supplemental zinc in corn-soybean meal diets increases fertility and hatchability (Anshan, 1990). In addition, supplemental zinc has enhanced immune status (Kidd *et al.*, 1992; Kidd *et al.*, 1993; Kidd *et al.*, 2000; Virden *et al.*, 2002a) and livability of progeny (Virden *et al.*, 2002b). Researchers have also reported that more zinc from organic sources is absorbed compared to inorganic zinc sources (Wedekind *et al.*, 1992), and that zinc from organic sources is more readily retained (Spears, 1989). With this in mind, organic zinc products may be used in poultry diets to enhance performance and immune status. Previous research in this area has evaluated the influences of zinc source on performance and immune status of breeders and/or their progeny (Flinchum *et al.*, 1989; Kidd *et al.*, 1992; Kidd *et al.*, 1993; Kidd *et al.*, 2000; Barber *et al.*, 2002; Khajarern *et*

*al.*, 2002; Virden *et al.*, 2002a). However, many of these projects were conducted for short durations during the breeding period or source comparisons were confounded by dietary zinc concentration. To address these concerns, broiler breeder hens were provided different supplemental zinc sources at the same concentration from placement until 65 weeks of age.

In many cases, nutritional or immune status of hens may influence chick quality. Inadequate incorporation of nutrients into the hatching egg by the hen may result in marginal quality and performance of progeny. In addition, hen age may influence broiler performance. McNaughton *et al.* (1978) reported that mortality of broilers from 29 wk-old breeders was approximately 3% greater than that of broilers from 58 wk-old breeders. Chick quality is often assessed subjectively, but efforts have been made to find quantitative measurements that correlate with chick performance. Some of these quantitative measurements will be discussed.

## MEASURING REPRODUCTIVE PERFORMANCE OF BROILER BREEDER MALES

There are four basic elements that must be present in broiler breeder flocks to produce fertile eggs: 1) ability and willingness of males to mate, 2) hen receptivity to males, 3) adequate sperm/semen quality and 4) proper conditions in the oviduct. Mating activity likely influences fertility to a greater degree than sperm quality, because fertility can be maintained in aging flocks by artificial insemination (Brillard and McDaniel, 1986) or by replacement of old males with young males in a commercial flock. When considering mating activity, more emphasis is placed on males because fertility level depends greatly

on their ability and willingness to mate. Hen receptivity is not often a problem with proper flock management. Fertility can be increased with improved semen quality (Bramwell *et al.*, 1995; Donoghue *et al.*, 1998), primarily because more sperm are selected for transport to the uterovaginal junction. The commercial turkey industry relies on artificial insemination, and use of samples from males with superior semen quality improves labor efficiency and fertility. The use of artificial insemination with caged broiler breeders presents an opportunity to increase broiler performance by increased selection pressure and the use of fewer males while still maintaining excellent chick yield (Wineland, 1995). Artificial insemination of chickens would likely require more frequent inseminations and be more labor intensive, because chickens release sperm from their storage tubules seven times faster than turkeys (Brillard, 1993). Selecting males with high sperm quality and efficient mating activity will reduce the need for artificial insemination in broiler breeder flocks.

Once birds have mated, semen quality must be adequate for fertilization of the egg in the oviduct. Less than 1% of inseminated sperm is selected for transport and enters the sperm storage tubules (Brillard, 1993). Only motile sperm are able to reach primary sperm storage site (Allen and Grigg, 1957), but there are some factors that may prevent motile sperm from fertilizing an egg. For example, Froman and Engel (1989) altered the sperm glycocalyx by treatment with neuraminidase. The 19% reduction in fertility was attributed to low levels of spermatozoal sialic acid reducing sperm release from the utero-vaginal glands. Additionally, conditions within the hen's oviduct may also reduce chances for fertilization. Evaluating such factors is not practical at a commercial level, because they require a great deal of time, resources and knowledge.

When mating frequency is low, semen quality likely becomes a factor that influences duration of fertility. Therefore, superior semen quality is a desirable trait in broiler breeder males. Evaluating and selecting males for improved sperm quality will improve flock fertility, but there is still no single method that is widely accepted and used. Detailed descriptions of sperm quality assays are given in the following paragraphs.

*Sperm Viability (Bilgili and Renden, 1984)*

Assay tubes are filled with 4mL of phosphate buffered saline containing 12.5µg Ethidium Bromide (EtBr)/mL. The mixture is placed in a fluorometer and fluorescence is adjusted to zero. An aliquot (10µL) of semen is mixed with the EtBr solution and a first fluorescence reading is taken, which corresponds to the number of dead or damaged sperm cells. EtBr binds with nucleic acids of sperm and induces an intense fluorescence. Then 25 µL digitonin (125 µg/25 mL ethyl alcohol) is added to rupture the membranes of remaining sperm cells. Fluorescence is measured again, and it is proportionate to total sperm concentration. This technique was modified by Bakst *et al.* (1991) to evaluate sperm membrane integrity and fragility. In the latter assay, sperm were added to hypotonic solutions containing EtBr. This test may be useful in detecting an unfit population of stored sperm.

*Metabolic Activity of Sperm (Chaudhuri and Wishart, 1988)*

In this assay, tetrazolium salts are used. Oxidoreductase enzyme activity reduces the tetrazolium salt to a strongly colored formazan, which is used to estimate the reducing

capacity, and thus metabolic activity of the sperm. Upon reduction, the colorless dye is reduced to a red formazan pigment by fowl sperm.

An aliquot of semen (constant concentration) is mixed thoroughly with 150 m*M* NaCl with 20 m*M* TES, pH 7.4; 100 m*M* glucose; 100 m*M* $CaCl_2$; and 100 m*M* KCN. The mixture is equilibrated at 20ºC in a water bath and tetrazolium dye (160 μ*M*) and phenazine methosulphate (7.7 μ*M*) solutions are added and mixed. After incubating at 20ºC for 20 min, 200 μL of Triton X-100 (50 mL/L) in 0.1 *M* HCl is mixed and allowed to stand at room temperature for 5 min. Samples are then centrifuged at 700 g for 10 min. The absorbance of the supernatant is then measured in a spectrophotometer at 520 nm.

*Sperm-Egg Binding Assay (Cramer et al., 1994)*

Heat-solubilized egg membranes (perivitelline membranes) are dried on flat-bottomed microtiter well plates. A 100 μL dose of semen is added to each well and incubated for 2-3 hr. Providing the same number of sperm in each well will remove confounding effects of sperm concentration. Unbound sperm are then washed away. Bound sperm are enumerated by DNA staining (DAPI) and epifluorescence microscopy.

*Sperm-hole Penetration of the Inner Perivitelline Layer (Bramwell et al., 1995)*

The number of sperm that penetrate the inner perivitelline layer of the ovum can also be counted to assess sperm quality, when a given number of sperm are inseminated in a controlled setting. To conduct the assay, the ovum is taken from an oviposited egg and placed into a 1% NaCl solution for 30-60 seconds to remove albumen. A small section

(0.5-1.0 $cm^2$) of the inner perivitelline membrane is removed from the area overlying the germinal disc. Adhering material is removed by stirring the layer in saline solution, then the layer is placed onto a microscope slide. After a brief suspension with 20% formalin, Schiff's reagent is then placed onto the membrane for approximately 1 min. The excess solution is then removed with a Pasteur pipette and saline. The excess formalin – Schiff's solution should be placed in a waste container for safe disposal. A coverslip is applied, and sperm-holes are quantified by microscopic observations at 100 x magnification.

An advantage of this assay is that it takes all fertility-affecting factors into account and gives an objective result. In addition, it is a useful tool for predicting fertility. According to Bramwell (2000), a flock averaging 30 holes per ovum may not currently have fertility problems, but declines in fertility should be expected in the near future. This assay may be used to test female influences on fertility by pooling semen from a group of males and artificially inseminating numerous hens with the same dose of sperm.

*Trapped Sperm in the Outer Perivitelline Layer (Wishart, 1987)*

A technique similar to the sperm-hole penetration assay was developed in which the number of sperm that become trapped in the outer perivitelline layer can be counted. To conduct this assay, a section of the perivitelline membrane is removed from the ovum of an oviposited egg and rinsed vigorously in $Ca^{2+}$-, $Mg^{2+}$- free Dulbecco's phosphate buffered saline to remove adhering yolk material. The membrane is stained at room temperature by adding 1 μg/mL solution of 4,6 diamidino-2 phenylindole in PBS. A coverslip is applied and excess water removed. A Photomicroscope II is used to

observed blue fluorescing sperm using a wavelength of 350 nm and an emission cutoff of 450-500 nm. The disadvantage of this assay compared to the sperm-hole penetration assay is the expensive equipment required for observing sperm.

*Sperm Motility using Accudenz® Solution (Froman and Feltman, 1998)*

Semen is collected and diluted to a concentration of $5x10^8$ sperm/mL in motility buffer (120 m*M* NaCl, 10 m*M* glucose, and 2 m*M* $CaCl_2$ in 50 m*M* TES, pH 7.4). A 60 µL volume of the diluted semen is overlaid on 600 µL of the 6% (wt/vol) Accudenz® solution in a cuvette, then placed into the hot water bath (41C) for 5 min. Prior to collecting semen samples, motility buffer and Accudenz® solutions are allowed to reach 41°C in a hot water bath. Using a spectrophotometer, absorbance at 550 nm is recorded. Absorbance is directly related to the net movement of a sperm cell population in a specific direction.

*Sperm Motility using the Sperm Quality Analyzer® (Bartoov et al., 1991)*

The Sperm Quality Analyzer (SQA) was developed so that semen could be analyzed quickly and easily. Semen is taken into a small glass capillary. A field in the capillary is examine for 40 seconds by the photoelectric cell in the SQA to detect variations in optical density caused by sperm motility. The SQA then displays the sperm motility index value. If a constant sperm concentration is not used, then the index value is representative of both concentration and motility.

As noted earlier, sperm quality may influence flock fertility, especially in older flocks when matings become less frequent. There are numerous approaches to improving

semen quality in broiler breeder males. Selecting males with superior sperm quality may become a popular technique in the future, but using alternative feed ingredients and altering the nutrient content of the diet may be a more practical method to enhance sperm quality.

## MENHADEN OIL IN POULTRY DIETS

### ROLES OF FATTY ACIDS IN SPERM FUNCTION

Chicken semen contains high proportions of polyunsaturated fatty acids (PUFA), making it more susceptible to lipid peroxidation. In turn, this could lead to sperm deterioration during storage (Surai *et al.*, 1998). Cerolini *et al.* (1997b) evaluated the lipid and fatty acid composition of semen. This report stated that C18:0, C18:1 n9 and C22:4 n6 were the major saturated, monounsaturated and polyunsaturated (PUFA) fatty acids found in sperm phospholipid, respectively. Fatty acid composition of sperm phospholid has been researched previously (Table 1.1).

Cerolini *et al.* (1997a) reported a direct correlation between sperm motility and C22:6 n3 in sperm. In addition, fertility was positively correlated with phospholipid-bound C20:4 n6 and C22:4 n6 in sperm. Cerolini *et al.* (1997b) stated that the PUFA C22:4 n6 is essential for sperm production.

Enrichment of diets with n3 fatty acids evokes a small but significant change in the fatty acid composition of semen (Blesbois *et al.*, 1997; Kelso *et al.*, 1997a; 1997b). For example, n6:n3 ratios of maize oil and fish oil supplemented (3% Tuna Orbital Oil) diets were 13.9 and 0.9, respectively (Kelso *et al.*, 1997a). After consuming the test diets from

Table 1.1. Phospholipid-bound fatty acids in avian (*Gallus domesticus*) sperm

| % Total fatty acid | Cerolini *et al.* (1997b) | Surai *et al.* (1998) | Kelso *et al.* (1997a) | Kelso *et al.* (1997b) | Darin-Bennett *et al.* (1974) |
|---|---|---|---|---|---|
| *Saturates* | | | | | |
| C16:0 | 13.2 | 14.8 | 13.7 | 14.1 | 13.6 |
| C18:0 | 21.5 | 21.1 | 20.9 | 19.5 | 20.3 |
| C20:0 | nd | 0.8 | nd | nd | 2.9 |
| *Monounsaturates* | | | | | |
| C17:1 n7 | <1 | nd | nd | nd | |
| C18:1 n9 | 13.0 | 14.8 | 14.4 | 12.2 | 19.2 |
| C18:1 n7 | 1.7 | 1.8 | nd | nd | nd |
| C20:1 n9 | 3.4 | 3.7 | nd | 3.1 | nd |
| C22:1 n9 | <1 | nd | nd | nd | nd |
| *N6 Polyunsaturates* | | | | | |
| C18:2 | 3.6 | 2.3 | 2.5 | 3.4 | 1.6 |
| C20:2 | 1.1 | nd | nd | nd | nd |
| C20:3 | 2.0 | 1.8 | nd | nd | nd |
| C20:4 | 12.6 | 10.7 | 11.4 | 11.8 | 10.2 |
| C22:4 | 22.3 | 18.7 | 21.7 | 22.9 | 24.6 |
| *N9 Polyunsaturates* | | | | | |
| C22:3 | <1 | nd | nd | nd | 3.8 |
| *N3 Polyunsaturates* | | | | | |
| C22:5 | <1 | 1.5 | nd | 1.0 | nd |
| C22:6 | 2.8 | 4.2 | 3.8 | 2.5 | 2.3 |

nd, nondetectable

10 to 40 weeks of age, the resulting n6:n3 ratios in sperm phospholipids were 9.4 and 2.1 for males fed maize oil or fish oil, respectively. Fish oil diets increase the proportion of 22:6 n3 in the sperm phospholipids while decreasing the proportions of 20:4 n6 and 22:4 n6. Furthermore, changing the fatty acid composition of sperm by dietary means alters the sperm membrane and fertilizing ability of sperm.

Lipid composition is a major determinant of the membrane flexibility required for flagella movement of spermatozoa and normal sperm mobility (Cerolini *et al.*, 1997a). Unsaturated fatty acids cause phospholipid structure to be bent and less compact.

Therefore, membranes made of unsaturated fatty acids are more fluid and flexible than rigid membranes composed mostly of saturated fatty acids (Stryer, 1995). Data supports the importance of both n3 and n6 PUFA in sperm, but specific mechanisms by which these fatty acids affect sperm function are not fully understood.

## MENHADEN OIL EFFECTS ON SEMEN QUALITY AND FERTILITY

Menhaden oil contains relatively high proportions of n3 PUFA, especially eicosapentaenoic (C20:5 n3) and docosahexaenoic (C22:6 n3) acids (Chanmugan *et al.*, 1992). Kelso *et al.* (1997b) reported that a small increase in the proportion of n3 fatty acids in the sperm phospholipids induced by enriching the diet with α-linolenic acid improved fertility at 39 wk of age by 14%. However, fertility differences were nonsignificant at other ages. Measurements of semen volume, sperm concentration and sperm motility were not influenced by supplemental α-linolenic acid. Previous research has reported that replacing corn oil (Blesbois *et al.*, 1997) or poultry fat (Casanovas, 1999) in rooster diets, with fish oil enhances subsequent fertility of hatching eggs. Blesbois *et al.* (1997) found that fertility was improved by 4% when broiler breeder male diets were supplemented with n3-rich salmon oil rather than corn oil. Unfortunately, these researchers only measured fertility from 44 through 47 wk of age. Sperm concentration and volume were not influenced by supplemental fat source. Cassanovas *et al.* (1999) reported that providing Menhaden oil to broiler breeder males after 33 wk of age resulted in a 6% increase in fertility at 49 wk of age. However, fertility levels of males being fed Menhaden oil or poultry fat were similar at 63 wk of age. In a second study, an older group of males were first given experimental diets containing Menhaden

oil or poultry fat at 57 wk of age. By 67 wk, fertility in males given Menhaden oil and poultry fat had dropped by 12% and 4%, respectively. The authors concluded that males may require an acclimation period when altering the fatty acid profile of the diet and sperm.

## INCORPORATION OF N3 FATTY ACIDS INTO TISSUES

Animal performance, human health and other factors may be positively impacted when alternative feed ingredients are provided to animals. However, consumer acceptability is often the deciding factor when determining whether or not to use an alternative. For example, n3-rich fish oils have cardiovascular benefits (Sinclair, 1953) but yield an off flavor in meat and egg products (Lipstein and Bornstein, 1973; Adams *et al.*, 1989; Van Elswyk, 1997). Nutritionists must be careful when providing dietary fish oil so that poultry products do not become tainted with a fishy flavor. Hen diets with 6% Menhaden oil or 6% omega-3 oil® resulted in eggs with a slight off flavor (Adams *et al.*, 1989). Broilers that consumed 2 or 4% fish oil soapstock, in addition to 3% fish meal were unpalatable (Lipstein and Bornstein, 1973). Research by Van Elswyk (1997) indicated that panelists accepted eggs, and shelf life of eggs was unaffected when hens were fed 1.5% Menhaden oil. However, eggs were unacceptable to panelists when 3.0% Menhaden oil was fed to hens. Research by Huang *et al.* (1990) reported that eggs and thigh meat from laying hens were acceptable with up to 3% dietary fish oil stabilized with ethoxyquin. The n6/n3 ratio of egg yolk was reduced six-fold when laying hens were provided 3% Menhaden oil rather than an isocaloric diet with no added fat (Hargis *et al.*, 1991). Chanmugan *et al.* (1992) stated that linolenic acid (C18:3 n3) produces less

of an off flavor in muscle and is less susceptible to auto-oxidation compared to other n3 fatty acids. Hence, if it is desirable to increase n3/n6 ratio in poultry without producing off flavors, then linseed meal or oil could be used in the feed. If n3 PUFA can be economically incorporated into food products without producing off flavors, then such foods may appeal to consumers due to their health benefits.

## MENHADEN OIL EFFECTS ON GENERAL HEALTH AND IMMUNITY

Research regarding dietary Menhaden oil has not been limited to fertility and other effects on animal performance. Menhaden oil consumption in poultry has improved immune status through enhanced antibody production (Fritsche *et al.*, 1991) and increased resistance to cecal coccidiosis (Allen *et al.*, 1996a; Allen *et al.*, 1996b). Korver and Klasing (1997) supplemented broiler diets with corn oil, Menhaden oil or linseed oil. Feeding Menhaden oil enhanced phytohemagglutin-induced wattle swelling and ameliorated growth depression induced by *S. aureus* or *Salmonella typhimurium*.

An undesirable effect of dietary Menhaden oil is increased incidence of hepatic lipidosis. Van Elswyk *et al.* (1994) reported this occurrence in reproductively active laying hens after three months of feeding 3% Menhaden oil. The authors suggested that a combination of estradiol and Menhaden oil might decrease lipid transport from the liver to the developing ovaries.

Sinclair (1953) was the first to display high intake of fish by humans was a possible means of reducing coronary heart disease. Positive effects of fish intake on the cardiovascular system were attributed to the fish oils. Dietary fish oil in laying hen diets has resulted in eggs with lower cholesterol (Prakash *et al.*, 1996). Therefore, human

consumption of meat and eggs from animals consuming fish oil may reduce health risks. Other than cardiovascular effects, increased n3 PUFA consumption may alleviate symptoms of cancer, rheumatoid arthritis, psoriasis and multiple sclerosis (Simopoulos, 1989). In a review article by Leskanich and Noble (1997), they suggest that high intake of n3 PUFA, particularly docosahexaenoic acid, aids in neural development of children.

## MENHADEN OIL EFFECTS ON HEN PERFORMANCE

Supplemental fish oil in hen diets has been associated with decreased egg weights, possibly by reducing lipid transport to the ovary (Van Elswyk *et al.*, 1994; Herstad *et al.*, 2000; Miles *et al.*, 2001). Herstad *et al.* (2000) compared the effects of supplemental fish oil (Pescomar® 30 TG) and rendered fat at various inclusion rates. Compared to hens fed 3% rendered animal fat, hens fed 3% fish oil had lower egg weights by 2.5 g, higher percentage of cracked eggs by 3.3%, lower fertility by 8.2%, and lower hatchability of fertile eggs by 23.3%. Van Elswyk *et al.* (1994) suggested that the combination of Menhaden oil and estradiol might alter lipogenic activity in the liver, decreasing serum lipid and available lipid for yolk formation. Scheideler and Froning (1996) have reported a reduction in the ratio of yolk:albumen in eggs laid by hens consuming Menhaden oil or flaxseed oil. In turn, there is a reduction in yolk lipid, yolk size and egg size. Research by Washburn (1990) indicated that there is a direct relationship between egg cholesterol level and hatchability. Therefore, the cholesterol reducing effects of Menhaden oil (Prakash *et al.*, 1996) may be more beneficial in table egg production than in hatching egg production. Menhaden oil research with male and female broiler breeders indicate that the dietary supplement should be provided exclusively to males.

## MEASURING REPRODUCTIVE PERFORMANCE OF BROILER BREEDER FEMALES

### EGG PRODUCTION

Due to selection for rapid growth in broiler breeder flocks, reproductive performance has deteriorated. Feed restriction is commonly practiced to achieve target body weights, because performance suffers greatly when breeders become obese. Feed restriction during rearing and breeding reduces the incidence of erratic ovipositions, defective eggs and multiple ovulations (Yu *et al.*, 1992). Overfeeding poultry during reproductive development results in the formation of excess large yellow ovarian follicles, which are likely to be arranged in multiple hierarchies (Renema *et al*., 1999). According to these authors, this increases the production of unsettable eggs.

The number of large yellow follicles may be counted to estimate reproductive status of hens. Hocking *et al.* (1987) indicated that restricting feed intake of dwarf broiler breeders reduced the number of yellow follicles from 9.0 to 6.8. Research by Hocking (1993) suggests that 6 to 7 large yellow follicles (> 8mm) are optimal for an actively reproducing hen. The impact of cumulative feed intake on egg production has been researched in detail, but the effects of specific nutrients (especially micronutrients) present a new area of research in broiler breeder production.

### FERTILITY

Although low fertility is often attributed to problems with the broiler breeder male, hen activity and physiology may influence fertility. Hen receptivity to mating may be reduced if males are overly aggressive, but is typically not a problem if body weights are

managed well. Physiological changes occur as hens age, altering the concentration or activity of sperm in the oviduct. Reduced fertility in older hens has been attributed to the increased number of empty sperm storage tubules (Van Krey *et al.*, 1967; Pierson *et al.*, 1988). Increased deposition of adipose tissue around the uterovaginal junction may deter normal functioning of the sperm storage tubules. In addition, oviducal secretions may contain antisperm antibodies, causing fertility to be reduced (Burke and Reiser, 1972; McCorkle *et al.*, 1983). These authors reported that antisperm antibody titers increase as turkey hens age, and injuries to the oviduct will result in high titers and poor fertility. Currently, there are many aspects of sperm activity in the oviduct that are not fully understood.

## EGGSHELL QUALITY

If quality of an eggshell is poor, then the chances for that egg to result in a quality chick are reduced. Low eggshell quality reduces production of settable eggs and causes excessive moisture loss during incubation. Disease, nutrition, rate of lay, age, environment, husbandry and egg handling are factors that can influence shell quality (Petersen, 1965; Wolford and Tanaka, 1970). Roland *et al.* (1975) suggested that the age-related decline in shell quality is due to increasing egg size as hens age, while a constant amount of calcium is deposited for shell formation. Measurements proposed for shell quality assessment have included shell weight, shell thickness, percentage shell, shell weight per unit of surface area, egg specific gravity, extent of eggshell deformation and resistance to crushing by compression (Strong, 1989). Strong (1989) suggested that specific gravity and percentage shell were the most reliable indicators of shell quality.

McDaniel *et al.* (1979) reported that eggs with specific gravities lower than 1.080 had the increased early embryonic mortality and decreased hatchability.

## CHICK QUALITY

Desirable characteristics of a chick have been described previously (Cervantes, 1993; Raghavan, 1999; Decuypere *et al.*, 2001). Good quality chicks should be clean, dry and free from dirt and contamination, with clear and bright eyes, free from deformities, with a completely sealed and clean navel. Yolk sacs or dried membrane should not protrude from the navel area. The body should be firm to the touch and properly hydrated, and there should be no signs of respiratory distress. The chick should be alert and responding to sounds. Leg conformation should be normal, with no swollen hocks or skin lesions. The beak should be well formed and the toes firm and straight (Decuypere *et al.*, 2001). The chick should be free of pathogenic bacteria and fungi; and it should have protective levels of maternal antibodies to combat common viral diseases (Cervantes, 1993). However, untimely management of high quality chicks may result in poor performance. Although there are numerous factors that influence chick quality, only a few physiological aspects are discussed here.

## EARLY NUTRITION AND DIGESTIVE PHYSIOLOGY OF CHICKS

Yolk lipids provide the primary source of nutrients to the developing embryo and hatchling (Noy and Sklan, 1997). Noy and Sklan (1997) reviewed the role of the yolk in newly hatched chicks. They reported that deutectomy (surgical removal of the yolk) at hatch resulted in little or no growth for 2 to 3 days following the procedure, but growth

rate was similar to untreated chicks 9 to 10 days later. Research by Noy *et al.* (1996) noted that yolk is utilized more rapidly in fed than in fasted chicks. Slow yolk absorption may result in yolk nutrients that are never utilized by the chick.

Rapid development of the intestines is associated with superior nutrient absorption and rapid growth of young broilers (Nir *et al.*, 1993). Relative weights of digestive organs in chicks are greatest at 3 to 5 days of age (Noy and Sklan, 1997). The development and morphological traits of chick intestines have been investigated (Uni *et al.*, 1998; Pinchasov and Noy, 1993). Data have indicated that withholding feed after hatch delays mucosal development and retards growth of chicks. Delayed access to feed caused clumping of microvilli at day 1 and abnormal crypt structure at 8 days of age (Uni *et al.*, 1998). The authors suggested that presence of feed in the intestine is the stimulus for intestinal maturation. Therefore, prolonged processing and delayed placement of chicks will reduce the its growth potential. To deter this effect, hatching supplements have been provided to chicks prior to placement. Oasis® (Novus International, Inc., St. Louis, MO.) is a semi-solid supplement that contains 70% water, 10% protein, 20% carbohydrate and less than 1% fat (Dibner *et al.*, 1996). Research has indicated that giving Oasis to chicks will enhance growth performance when processing and shipping times are lengthy (Noy and Sklan, 1999; Batal and Parson, 2002).

## GLUCOSE METABOLISM IN CHICKS

Because oxygen level is relatively low in the air cell during the final states of embryonic development (Rahn, 1981), the embryo relies greatly on anaerobic metabolism for energy (Christensen *et al.*, 1999). This is accompanied by increases in

blood glucose and decreases in tissue glycogen stores (Freeman, 1965). Since the egg contains little glucose, glycogen is synthesized and stored earlier in incubation as a source of glucose in preparation for the anoxia of hatching (Freeman, 1965). The liver plays an important role in gluconeogenesis and glycogenesis, converting lactate into glucose-6-phosphate when blood glucose levels decline (Watford *et al.*, 1981). Lactate, produced anaerobically in muscle, is transported to the liver where it is resynthesized into glucose in a pathway involving phosphoenolpyruvate carboxykinase and returned to muscle for reuse (Donaldson *et al.* 1994). Heart and skeletal muscle tissues lack the capability to recycle lactate. Therefore, the liver is a supply organ, and both heart and pipping muscle may be viewed as demand organs (Christensen *et al.*, 1999).

Measuring glycogen, lactate and glucose concentrations in tissue or blood are good indicators of metabolic status of chicks or poults. High glycogen:lactate ratio in tissues suggest that the hatchling still has a large glycogen pool and it would be able to endure nutrient deprivation for longer periods than chicks with low glycogen:lactate ratios. Blood glucose level is also a good indicator of the size of the glycogen pool (Donaldson and Christensen, 1991). Low plasma glucose concentrations in combination with low lactate and glycogen levels are good indicators of a hatchling in dire need of immediate carbohydrate intake. Research has indicated that as poult holding time increases, liver (Christensen and Donaldson, 1992) and carcass glycogen levels (Donaldson *et al.*, 1994) are reduced. In both cases, blood glucose levels were increased as a result of glycogen catabolism.

## SUPPLEMENTAL ZINC IN POULTRY DIETS

Among different species, zinc-containing proteins are present in more than 160 enzymes (Sigel, 1983) and zinc is the only metal to be essential for one or more enzymes in all six enzyme classes (Vallee and Auld, 1990). Zinc has both structural and catalytic roles in metalloenzymes (O'Dell, 1992). Though the relationship between zinc and many physiological activities are not fully understood as of yet, the previous statements reveal the importance of adequate zinc status in animals.

### ZINC AND THE REPRODUCTIVE SYSTEM

Reproductive performance of animals may be altered by zinc status. Male rats subjected to a zinc deficiency showed reduced weight and abnormal histology of testes, as well as malformed sperm, reduced sperm numbers and motility (Apgar, 1985). Zinc deficient female rats have expressed failure to mate, defective ova, failure to maintain pregnancy and excessive bleeding at parturition (Apgar, 1985). Zinc effects on broiler breeder males have not been investigated in detail.

According to Richards (1997), the majority of zinc and other trace elements in the egg are deposited in the yolk, and much smaller quantities are deposited into the albumen. Vitellogenin, which is produced in the hen's liver, transports zinc and other minerals to the developing follicle. It is then cleaved into lipovitellin and phosphitin proteins (Richards, 1989). More than 90% of zinc in the yolk is bound to lipovitellin (Richards and Steele, 1987). Turkey egg yolk and albumen were found to contain 91.8 and 0.7% of total egg zinc, respectively (Richards, 1991). The remainder of the zinc was found in the shell and shell membranes. Sandrock *et al.* (1983) indicated that approximately 86% of

the zinc originally present in the fertilized egg is transferred to the chick. Badawy *et al.* (1987) reported that 69.7% of zinc in yolk was transferred to the chick embryo just prior to hatching, and that zinc concentrations decline in eggs from very old breeders (17 months). Badawy *et al.* (1987) indicated that as the Zn concentration in egg contents increased, hatchability increased. This increased hatchability was primarily due to decreased incidence of late embryonic mortality when zinc level was increased in the egg.

Supplemental zinc is essential in poultry diets to achieve normal reproductive performance. Zinc status has been related to low activity of carbonic anhydrase (Underwood, 1962), an essential enzyme for eggshell formation (Pearson *et al.*, 1977). Inadequate zinc status of hens may reduce eggshell quality. As a result, settable egg production and hatchability may decrease. Zinc studies with actively reproducing hens indicate that an adequate level of zinc must be present in eggs for normal embryo and chick development. Feeding purified hen diets containing low concentrations of zinc (<10 ppm zinc) results in high incidence of embryonic mortality and poor chick quality (Supplee *et al.*, 1958; Turk *et al.*, 1959; Blamberg *et al.*, 1960; Kienholz *et al.*, 1961). A more detailed discussion on supplemental zinc and reproductive performance is provided in a later section.

## ZINC AND THE IMMUNE SYSTEM

Zinc has many influences on the immune system, primarily affecting production of T lymphocytes in the thymus (Dardenne and Bach, 1993; Kidd *et al.*, 1996). Helper T lymphocytes are responsible for B cell growth and differentiation, and cytolytic T

lymphocytes are involved with lysis of virus-infected cells and tumor cells. Both types of T lymphocytes are involved in macrophage activation (NIH, 1991). According to Fraker *et al.* (1986), zinc is an essential cofactor for thymulin, a thymic hormone that regulates T lymphocyte maturation. The negative effects of zinc deficiency on cellular immunity have been well documented, although its effects on humoral immunity have not been as consistent. Research regarding zinc effects on cellular and humoral immune responses is discussed in the following paragraph.

Supplementing broiler breeder hen and turkey hen diets with zinc has increased cellular immune response of progeny as measured by cutaneous basophil hypersensity tests (Kidd *et al.*, 1992; Kidd *et al.*, 1993; Kidd *et al.*, 2000; Virden *et al.*, 2002a). Research by Pimental *et al.* (1991) indicated that dietary zinc concentration (8-58 ppm) or source (zinc-methionine or ZnO) did not influence antibody titers to human gammaglobulin or delayed-type hypersensitivity to phytohemagglutinin in broilers. Guo *et al.* (2002) reported that anti-BSA antibody production was increased in a dose-response manner as supplemental zinc increased (0-160 ppm). In research by Khajarern *et al.* (2002), greater zinc supplementation (75 vs 175 ppm) resulted in higher antibody titers to Newcastle disease, infectious bursal disease and infectious bronchitis. Contrasting data has indicated that dietary zinc concentration did not influence antibody titers of broilers in response to sheep red blood cell injections (Stahl *et al.*, 1989; Mohanna and Nys, 1999). Rats given a zinc deficient diet (1 ppm) had impaired lymphocyte response to phytohemagglutinin stimulation as compared to pair-fed rats given supplemental zinc in the drinking water (50 ppm). However, humoral immune responses (antibody titers to *Francisella tularensis*) were similar (Pekarek *et al.*, 1977).

Zinc deficiencies have caused thymic atrophy (Chandra and Au, 1980) and lymphopenia in mice (Fraker *et al.*, 1986). In human research conducted by Duchateau *et al.* (1981), elderly men given daily supplements of 100 mg of zinc for over a month had increased numbers of both circulating T-cells and IgG antibodies. Although results have been highly variable, they generally suggest that supplemental zinc enhances cellular and humoral immune status of animals.

## ZINC AND THE SKELETAL SYSTEM

Fresh bone contains approximately 40-60 ppm zinc (Haumont and McLean, 1966). A review by Brandeo-Neto *et al.* (1995) describes many of the roles of zinc in skeletal development and maintenance. Zinc stimulates differentiation of chondrocytes, osteoblasts and fibroblasts essential for bone maturation. Zinc deficiency interferes with DNA synthesis affecting calcium and fibrous collagen deposition in bone connective tissue. In addition, zinc is involved in synthesis of somatomedin-C, a hormone that stimulates cartilage proliferation and linear growth of the skeleton. Alkaline phosphatase, which aids in calcium deposition in the bone diaphysis, becomes sparse in zinc-deficient animals. The enzyme levels are reduced because zinc is required for RNA synthesis and zinc is a structural component of alkaline phosphatase.

Effects of various zinc intakes on skeletal development in animals have been researched. Increased zinc concentrations in pig diets have been associated with increased bone ash (Hill *et al.*, 1986). Supplemental zinc (0, 9 or 12 ppm) in swine diets increased breaking strength of tibiae. Research by Odutuga (1982) indicated that zinc deficient rats had reduced bone length, bone weight and body weight. Calcium contents

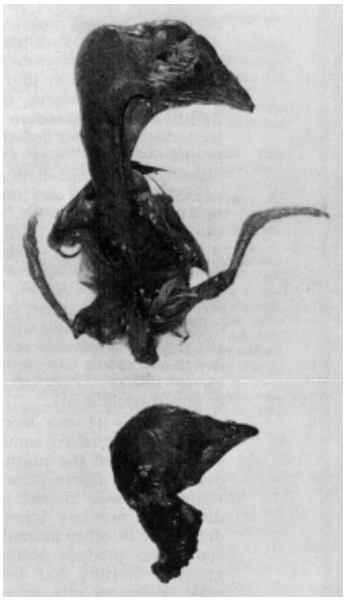

Figure 1.1. Abnormal embryos from hens fed a zinc-deficient diet (Kienholz *et al.*, 1961)

of dried femur, tibia and humerus were respectively reduced by 5.4, 4.8 and 3.7% when rats were given no supplemental zinc (6 vs. 100 ppm zinc). Embryonic malformations associated with zinc deficient hens are shown here (Figure 1.1). Blamberg *et al.* (1960) researched the effects of zinc deficiency in laying hens on embryonic development. While possible effects on the hens' skeleton or performance were not addressed, the authors discussed in detail the embryonic skeletal malformations that occurred when hens consumed diets with low concentrations of zinc (4-28 ppm):

> "The characterizing feature of these malformations was evidence of some degree of faulty trunk and limb development. The caudal-most part of the vertebrae was always absent (anouria) and, in some instances, a larger portion of the trunk appeared to be missing (ectrosomia) or shortened. Dorsal curvature of the spine (lordosis) was also found. In some cases, portions of the limbs or the limbs in their

entirety (ectromelia) were missing. A common occurrence was a short leg with only 2 toes (ectrodactyly). Occasionally, an apparent fusion of the lower limbs was observed (symmelia)."

## ZINC ABSORPTION AND TRANSPORT

Although there are many theories, there is no single method of zinc absorption that is universally accepted. Evans (1976) proposed the following mechanism for zinc absorption and transport from the intestine: the pancreas secretes a ligand into the duodenum, where zinc complexes with the molecule. Complexed with a ligand, zinc is transported through the microvillus and into the epithelial cell where the metal is transferred to binding sites on the basolateral plasma membrane. A review of zinc metabolism was written by Cousins (1979). He proposed that zinc in the lumen may enter the intestinal cell as free zinc, zinc metalloproteins or low molecular weight zinc chelates. Increasing concentrations of zinc in the intestinal cell stimulate production of metallothionein, which binds to zinc and acts as a zinc reserve. Metal-free albumin interacts with the plasma membrane and removes zinc from the receptor sites. The quantity of metal-free albumin available at the basolateral membrane probably determines the amount of zinc removed from the intestinal epithelial cell and thus regulates the absorption of zinc (Evans, 1976). Emmert and Baker (1995) stated that zinc can accumulate in bone, liver and intestine, and can subsequently be released for use during a period of zinc deficiency (Figure 1.2). They also reported that chicks fed a diet containing 10.6 ppm zinc cannot accumulate zinc reserves that become available during periods of zinc depletion.

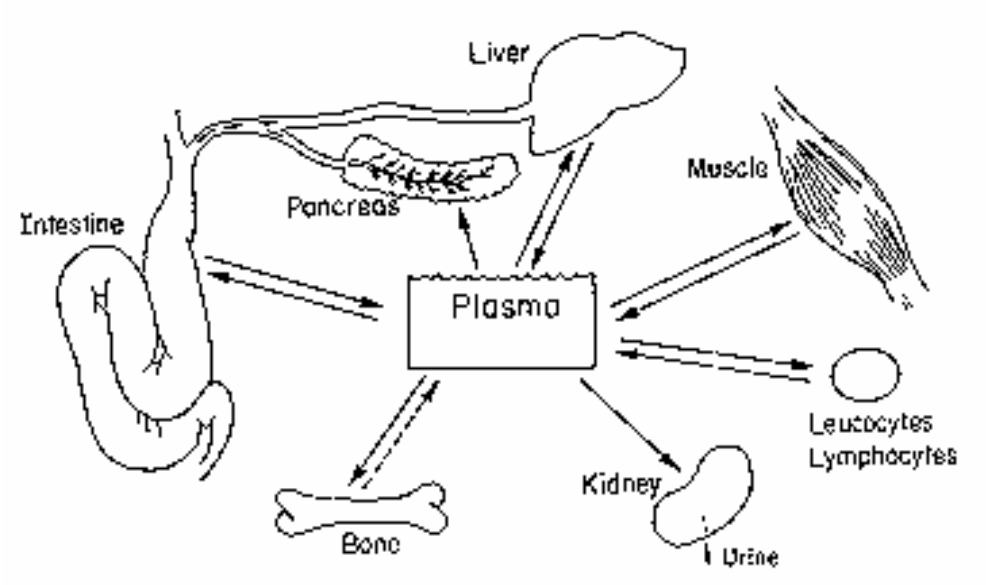


Figure 1.2. The major tissues contributing to the regulation of systemic zinc (Cousins, 1989)

The site of zinc absorption in the small intestine has been evaluated in rats. Methfessel and Spencer (1973) reported that absorption of $^{65}Zn$ was higher in the duodenum than from other segments when an *in vivo* ligated sac model was used. However, studies by Antonson *et al.* (1979) suggest that zinc absorption is much higher in the ileum (60%), than in the duodenum (19%) or jejunum (20%). These authors emphasized that the ligated sac model does not allow normal peristalsis or allow for normal variations in intestinal contents. *In vitro* research by Pearson *et al.* (1966) also found that zinc absorption was greatest in the distal third of the small intestine of rats.

Organic chelating agents may positively or negatively influence zinc absorption. Vohra and Kratzer (1964) showed that the efficacy of chelators was related to their stability constants. Maximal growth of turkey poults was obtained by using compounds with stability constants (-log $K_d$) near 15. Amino acids, particularly histidine, are thought to be involved in the passage of zinc from the intestinal lumen to the blood

stream (Gurd and Goodman, 1952; Smith *et al.*, 1978). Nielsen *et al.* (1966) researched the effects of supplemental histadine on zinc deficient chicks. They indicated that histadine alleviated bone defects and, to some extent, feather defects associated with zinc deficiency. Smith *et al.* (1978) showed increased $^{65}Zn$ absorption in rat intestines when histadine was introduced into the lumen. Supplementing diets with ethylenediamine-tetraacetic acid (EDTA) has also been associated with improved utilization of zinc. Zinc was found to bind to EDTA in the intestinal digesta and remained bound as it passed through the intestinal mucosa and was transported in the blood plasma (Suso and Edwards, 1971; Suso and Edwards, 1972).

Some dietary components may decrease zinc absorption. The primary zinc antagonists are phytic acid (O'Dell and Savage, 1960; Oberleas *et al.*, 1962), fiber (Baker and Ammerman, 1995) and calcium (Forbes, 1964). The dietary concentrations of these nutrients should be considered when determining zinc concentrations in poultry diets. Dietary zinc concentration has been shown to influence zinc retention. Mohanna and Nys (1999) reported that decreasing zinc levels in broiler diets from 190 to 65 ppm, increased zinc body retention from 8 to 20%, and reduced zinc concentration in manure by 75%. Burrell *et al.* (2002) reported that zinc excretion could be reduced by 53% in 17 d old broilers by excluding supplemental zinc (80 mg/kg) from the diet.

## SUPPLEMENTAL ZINC SOURCES IN ANIMAL FEEDS

Practical corn-soy diets contain approximately 30 ppm zinc without supplemental zinc. Soybean meal and corn contain approximately 55 and 18 ppm zinc, respectively (NRC, 1994). However, it is common practice to provide supplemental zinc to practical

poultry diets to ensure that marginal zinc intake does not limit bird performance or immunocompetence. Various forms of supplemental zinc are discussed in the following paragraphs.

The two most commonly used sources of supplemental zinc in practical diets are ZnO (72% Zn) and $ZnSO_4 \cdot H_20$ (Batal *et al.*, 2001). Relative bioavailability of the zinc sources have been estimated by broken line, slope ratio analyses. At low supplementation levels, ZnO is 44% (Wedekind and Baker, 1990) to 61% (Wedekind *et al.*, 1992) less bioavailable than $ZnSO_4$ when using bone zinc as the response criteria. However, the sulfate form is highly water soluble, allowing reactive metal ions to promote free radical formation, possibly leading to the breakdown of vitamins and degradation of fats and oils (Batal *et al.*, 2001).

Supplemental zinc in the form of organic zinc chelates or complexes have been of interest in recent years, because they have been associated with increased bioavailability of zinc when compared to inorganic sources (Wedekind *et al.*, 1992). Wedekind *et al.* (1992) used slope ratio analyses to estimate the bioavailability of zinc-methionine, $ZnSO_4$ and ZnO in corn-soybean meal diets. Tibial zinc was used as the response criteria. They reported that the ratio of slopes (zinc-methionine:$ZnSO_4$) below the inflection points were 206%, indicating that zinc-methionine was more bioavailable than $ZnSO_4$ at low supplementation levels. However, Cao *et al.* (2000) reported that most organic zinc compounds and $ZnSO_4$ yield similar results when provided to domestic animals. Researchers have reported that zinc bioavailability from zinc-methionine and ZnO is similar in chickens (Pimental *et al.*, 1991), rats (Hempe and Cousins, 1989) and

pigs (Hill *et al.*, 1986). Various methods of analysis and response criterion may cause variation in such results.

Numerous dietary zinc sources have been evaluated for their effects on immunity and progeny performance. Feeding zinc to breeding hens in the form of zinc-amino acid complexes improved cellular immunity and increased thymus weights (Virden *et al.*, 2002a), improved livability (Flinchum *et al.*, 1989; Virden *et al.*, 2002b) and increased total and left ventricular mass of progeny (Virden *et al.*, 2002c). It was suggested that increases in ventricular mass may decrease occurrence of ascites. Kidd *et al.* (1992, 1993) reported that supplemental zinc-methionine in broiler breeder hen diets resulted in an increased cellular immune response to phytohemagglutinin when compared to dietary ZnO. Kidd *et al.* (2000) evaluated the influences of zinc sources in turkey hen diets (40 ppm, zinc-methionine or $ZnSO_4$) on progeny. They found that poults from hens consuming zinc-methionine rather than $ZnSO_4$ had higher bursa weights and had enhanced cellular immune response to phytohemagglutinin. Spears (1989) suggested that zinc-methionine and ZnO have similar bioavailability in ruminants, but that zinc-methionine may be metabolized differently after absorption. Sheep given zinc-methionine had greater plasma zinc concentrations and lower urinary zinc concentrations than sheep provided ZnO. Suso and Edwards (1972) speculated that zinc may be transferred to an enzyme as a metallo-ligand complex and the ligand may have a major influence on enzyme activity. These data suggest that dietary zinc source may influence zinc metabolism and enzyme activity.

Research has compared the influence of dietary zinc source on hen performance. Guo *et al.* (2002) reported that providing Availa®Zn zinc amino acid complex to laying hens

rather than $ZnSO_4$ increased yolk zinc concentration, and these researchers stated that less Availa®Zn (120 vs 160 ppm) was required to reduce the incidence of cracked eggs at 59 wk of age. At a supplementation level of 80 ppm, they found that providing a mixture of Availa®Zn (40 ppm) and $ZnSO_4$ (40 ppm) rather than 100% of either, resulted in fewer cracked eggs.

Effects of dietary zinc source on animal growth and feed utilization have been evaluated. Supplementing practical broiler diets (75 ppm) with 40 ppm zinc from three different zinc amino acid complexes decreased feed conversion of female broilers exposed to high temperatures by about seven points at 35 and 42 d of age (Hess *et al.*, 2001). However, positive effects in this research may have been confounded by dietary zinc concentration. Feed utilization of broilers fed supplemental zinc from zinc-methionine rather than ZnO was enhanced (Sanford and Kawchumnong, 1972). Unfortunately, zinc concentrations in the diets were not mentioned in the abstract. Research by Pimental *et al.* (1991) comparing dietary zinc concentrations ranging from 8 to 58 ppm and zinc sources (zinc-methionine or ZnO) indicated that increasing dietary zinc concentration increased pancreas and liver zinc levels in broilers. They also reported that pancreatic zinc was elevated when 28 and 58 ppm of zinc was provided as zinc-methionine rather than ZnO. However, zinc source had no effect on broiler growth rate. Using a zinc-methionine complex increased carcass quality grade, marbling score and backfat in cattle, when compared to ZnO (Greene *et al.*, 1988). Research by Cheng *et al.* (1998) indicated that pigs performed equally well when provided supplemental $ZnSO_4$ or a zinc-lysine complex. In general, zinc-amino acid complexes improve or have no effect on animal performance.

## ZINC REQUIREMENTS IN POULTRY DIETS

Symptoms of zinc deficiency in young chicks include retarded growth, shortening and thickening of leg bones, enlargement of the hock joint, scaling of the skin, poor feathering, poor feed efficiency, loss of appetite, mortality in severe cases, rigid joints and reduced bone ash (O'Dell and Savage, 1957; Young *et al.*, 1958). Less severe deficiency symptoms observed by Edwards *et al.* (1958) were attributed to presence of zinc in the galvanized steel brooders used in the experiment. According to Young *et al.* (1958), no more than 55 ppm zinc in a purified diet is required for maximum growth rate of Single Comb White Leghorn chicks. Edwards *et al.* (1959) reported that supplementing practical rations with 20 ppm zinc (total of 57 ppm zinc) improved growth rate of White Rock chicks. Using tibia zinc as their response criteria, Wedekind *et al.* (1992) estimated zinc requirements of 60 and 54 ppm for New Hampshire x Columbian male chicks, when $ZnSO_4$ and zinc-methionine were used, respectively. Zinc requirement for chicks 1 to 3 weeks of age fed a soy concentrate diet was estimated at 22.4 ppm; however, the zinc requirement of phytate-containing diets, such as corn-soybean meal diets, would be expected to be higher (Batal *et al.*, 2001). Research by Mohanna and Nys (1999) indicated that whole body zinc of broilers was saturated when dietary zinc content was 90 ppm. Pimental *et al.* (1991) suggested that acceptable growth rates could be achieved for broilers when providing 28 to 38 ppm dietary zinc. O'Dell and Savage (1957) provided purified Drackett protein diets containing 55 ppm zinc to broilers, and broilers displayed growth retardation, shortening and thickening of long bones, and reduced tibia ash. Burrell *et al.* (2002) supplemented corn-soybean meal diets with graded levels of $ZnSO_4$ or Availa®Zn zinc amino acid complex. These researchers

reported that progressive dietary concentrations of zinc increased final body weight of broilers.

Sunde (1972) evaluated the effects of dietary zinc level on feather condition in Leghorn pullets. The data indicated that feeding diets with 52 ppm zinc for 3 weeks did not prevent feather fraying. However, normal feathering occurred with 78 ppm zinc during the first week. Hegazy and Adachi (2000) reported that diets with higher zinc concentrations (40 vs 100 ppm zinc) were associated with improved body weight gain and feed efficiency when White Leghorn chicks were given a *Salmonella* inoculation or a combination of *Salmonella* and aflatoxin. The authors stated that the effect may be attributed to an increased plasma concentration of insulin-like growth factor-1. Heindl *et al.* (1993) has discussed the relationship between zinc deficiency and reduced plasma insulin-like growth factor-1 in calves, and its association with retarded growth. Overall, these data have reported a wide range of zinc requirements. The variations in zinc requirements lead commercial poultry producers to provide supplemental zinc in excess of requirements to further prevent deficiency situations.

A majority of zinc research with hens has been conducted with Single Combed White Leghorns. Savage (1968) suggests that the zinc requirement of hens for egg production is appreciably lower than the amount required for hatchability or for optimum growth and feathering in chicks and poults. High concentrations of calcium in hen diets have antagonistic effects on zinc absorption (Forbes, 1964), resulting in high zinc requirements of hens relative to growing birds. Research by Anshan (1990) indicated that breeder layers provided 40 ppm supplemental zinc had improved fertility (87.5 vs 75.5%) compared to hens given a basal diet containing approximately 30 ppm zinc.

When high concentrations of dietary calcium (4.7%) were provided, 40 ppm supplemental zinc also improved hatchability by 11%. These data suggest that a total of 70 ppm zinc may be required for breeder layers to achieve acceptable fertility and hatchability. Research by Abdallah *et al.* (1994) indicated that 35 ppm supplemental zinc (51 vs 86 ppm) in practical laying hens diets had no influence on egg production, egg mass, feed efficiency, egg weight, specific gravity, percent shell or hatchability. Guo *et al.* (2002) had similar findings, reporting that egg production and egg weight were not influenced by supplemental zinc concentration (0 to 160 ppm). However, Guo *et al.* stated that at least 80 ppm supplemental zinc was required to maintain eggshell strength at 59 wk of age. Effects of zinc supplementation on hen performance are highly dependent on the dietary zinc concentration prior to supplementation. Zinc antagonists in the diet may also influence supplementation effects on performance.

While zinc requirements have been established for both broilers and egg-type hens, no research has documented the requirement for broiler breeders. Since zinc is needed by the progeny of breeding hens, supplementation of zinc in breeding hen diets usually exceeds that of laying hens. Research by Khajarern *et al.* (2002) comparing dietary zinc concentration (75 and 175 ppm) indicated that zinc supplementation in broiler breeder diets improved egg production, decreased downgrade eggs and increased chick production. As discussed earlier, concentration of zinc in broiler breeder hen diets usually has little or no influence on progeny growth rate, but often influences immune response to antigens. A summary of zinc requirement data is presented here (Table 1.2).

Table 1.2. Zinc requirements of poultry

| Zinc in basal diet, ppm | Supplemental Zinc, ppm | Supplemental Zinc Source | Total Zinc, ppm | Age period | Response Criteria | Breed | Reference |
|---|---|---|---|---|---|---|---|
| 15 | 20 | Distillers dried solubles | 35 | 1-42 d | Growth, feathering, bone development | White Rock | O'Dell *et al.*, 1958 |
| 15 | 40 | ZnCl | 55 | 1-10 d | Growth, bone development | White Leghorn | Young *et al.*, 1958 |
| 30 | 5 | ZnCl | 35 | 12-26 d | Growth, feed efficiency | White Plymouth Rock | Morrison and Sarett, 1958 |
| 10 | 20 | ZnCl | 30 | 1-28 d | Growth | White Meteor x White Rock | Roberson and Shaible, 1958 |
| 37-41 | 20 | $ZnSO_4$ | 57-66 | 1-42 d | Growth | White Rock | Edwards *et al.*, 1959 |
| 37 | 10-20 | $ZnSO_4$ | 47-57 | 1-14 d | Growth, tibia ash | White Rock | Edwards *et al.*, 1959 |
| 36-43 | 0, 100 | ZnCl | >43 | 1-28 d | Growth, hock enlargement | White Plymouth Rock | Zeigler *et al.*, 1961 |
| 14 | 20 | ZnO | 34 | To 1st egg | Growth, feed efficiency | White Leghorn | Rahman *et al.*, 1961 |
| 52 | 26 | $ZnCO_3$ | 78 | 1-7 d | Growth, feathering | White Leghorn | Sunde, 1972 |
| 52 | 0 | $ZnCO_3$ | >52 | 1-21 d | Growth, feathering | White Leghorn | Sunde, 1972 |
| 28 | 0 | $ZnCO_3$ | 28 | 22wk-4yr | Egg yield, hatchability | White Leghorn | Stahl *et al.*, 1986 |
| 0 | 14 | $ZnCO_3$ | 14 | 8-22 d | Growth | New Hampshire x Columbian | Southern and Baker, 1983 |
| 7 | 12 | ZnO | 19 | 1-21 d | Growth | Broiler strain | Dewar and Downie, 1984 |
| 28 | 10 | $ZnCO_3$ | 38 | Not specified | Progeny feather condition | White Leghorn | Stahl *et al.*, 1986 |
| 0 | 28 | ZnO, Zn-methionine | 28 | 1-42 d | growth | Broiler strain | Pimental *et al.*, 1991 |
| 20 | 25 | Zn-methionine, $ZnSO_4$ | 45 | 5-21 d | Growth, tibia ash | Broiler strain | Mohanna and Nys, 1999 |

Caine *et al.* (2001) reported that greater zinc supplementation (120 vs 370 ppm) in sow diets increased villus height, lower crypt depth, increased epithelial lymphocyte and goblet cell counts in the small intestine of progeny. High concentrations of dietary zinc (2,500-3,000 ppm) have decreased incidence of post-weaning diarrhea of weanling pigs (Holm, 1988; Hahn and Baker, 1993). Although such high concentrations would not be practical in poultry diets, the relationship between zinc intake and intestinal morphology

in poultry have not been researched in detail. High concentrations of dietary zinc cause rapid declines in feed intake and cessation of egg production in laying hens (Shippee *et al.*, 1979). Because of these effects on egg production, high concentrations of dietary zinc are an alternative means of initiating a molt in actively reproducing flocks. Research by Creger and Scott (1977) indicated that providing 2,000 ppm dietary zinc for less than 12 days was an effective method of molting laying hens. However, this report stated that feeding high zinc more than 16 days resulted in poor egg production relative to conventionally molted hens.

## MEASURING ZINC STATUS

Zinc status of poultry is typically not a concern, because dietary zinc concentration often exceeds the requirement of the bird. However, measuring zinc status of animals in a research setting is a useful tool in determining zinc requirements.

Metallothionein levels are sometimes used as an indicator of zinc status, because its concentration is directly related to zinc status. Metallothionein is a small, cysteine-rich, metal binding protein that is thought to have a role in normal zinc homeostasis (Fleet and McCormick, 1988). Metallothionein may store zinc for use in periods of high demand during development (Ridlington *et al.*, 1983). Fleet and McCormick (1988) showed that the initial zinc concentration in the yolk has a significant impact on the final metallothionein concentration in the neonatal liver.

Growth performance is a less sensitive measurement of zinc status. Although, Southern and Baker (1983) have stated that growth is a more reliable indicator of zinc status than blood or bone observations. Edwards *et al.* (1959) stated that the first sign of

a deficiency that hens show upon receiving a zinc deficient diet is poor rate of growth of progeny on zinc deficient diets. However, research has shown that cellular immune status of chicks is often more sensitive than growth (Kidd *et al.*, 1992; Kidd *et al.*, 1993; Virden *et al.*, 2002a). Cutaneous basophil hypersensivity tests are often used to measure cellular immune status. To conduct this test, an antigen (eg., phytohemagglutinin, pokeweed mitogen) may be intradermally injected into wattles, comb or toe web. Width of the injected area is measured before the injection and 18-24 hr after injection, and the degree of swelling is considered the cellular immune response (Corrier and DeLoach, 1990).

Because human hair can be analyzed for mineral content (Bos *et al.*, 1985), measuring zinc content of feathers may be a simple method of determining zinc status. However, analysis of hair, plasma zinc and zinc-dependent enzymes in humans have not been well received because of insensitivity and imprecision (Baer and King, 1984; Wood, 2000). Sunde (1972) has subjectively measured zinc status by observing feather condition. Zinc concentrations in whole carcass (Mohanna and Nys, 1999), bone ash (Wedekind *et al.*, 1992), pancreas (Pimental *et al.*, 1991) and eggs (Badawy *et al.*, 1987) have also been used as indicators of zinc status.

## SUMMARY

Attempts are constantly being made to enhance reproductive performance of broiler breeders. This dissertation primarily addresses two topics: 1) the influence of dietary fat source on broiler breeder male performance and 2) the influence of dietary zinc source on broiler breeder hen and progeny performance. Dietary fat source in broiler breeder male

diets alters the sperm membrane, influencing flock fertility; and zinc source in broiler breeder hen diets has influenced performance and immune status of progeny. Unfortunately, previous studies compared various sources of zinc and fat during only a portion of the reproductive period. Providing alternative feed ingredients for extended periods may be a practical and simple means of enhancing reproductive performance of broiler breeders.

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# CHAPTER 2

# EFFECTS OF DIETARY MENHADEN OIL ON FERTILITY AND SPERM QUALITY OF CAGED BROILER BREEDER MALES[1]

[1] Hudson, B.P. and J.L. Wilson. Submitted to *Journal of Applied Poultry Research*, 3/5/03.

**SUMMARY**

To assess the effects of dietary fat source on semen quality and fertility, either poultry fat or Menhaden oil was included in broiler breeder male diets. Males received diets containing either poultry fat or Menhaden oil from 0 to 65 wk of age. When males were 47 wk of age, hens were inseminated every third week through 65 wk with $7.5x10^7$ sperm. Fertility and hatch data were collected by incubating eggs laid during each 2 wk post-insemination (WPI) period. The approximate number of sperm that penetrated the perivitelline membrane of the ovum was determined from eggs laid on the eighth day post-insemination (DPI). Sperm mobility index (SMI) was measured at 60 and 63 wk of age from all males producing semen.

When males were provided Menhaden oil, fertility and hatchability of eggs set from the second WPI were greater by 6.4 and 6.9%, respectively. Testis weight, SMI, sperm mobility and the number of males producing semen were similar between treatments. Although most reproductive characteristics were similar, fertility during the second WPI was significantly enhanced by providing dietary Menhaden oil. This suggests that roosters fed Menhaden oil produced sperm that were better able to maintain fertility over the 2 wk periods following each insemination.

**DESCRIPTION OF PROBLEM**

Chicken semen contains high proportions of polyunsaturated fatty acids (PUFA) making the semen susceptible to lipid peroxidation, which could lead to deterioration of sperm within sperm storage tubules [1]. Docosahexaenoic acid (C22:6 n3) is the predominant PUFA found in mammalian sperm [2,3,4,5], but docosatetraenoic acid

(C22:4 n6) is the primary PUFA in chicken sperm [6,7]. Lipid composition is a major determinant of the membrane flexibility required for flagella movement of sperm and also of the fusing properties of the membranes associated with the acrosome reaction and fertilization [8]. Prior research has revealed that high contents of n6 PUFA in the chicken spermatozoa membrane influence sperm function, and that modifications to dietary fatty acids can influence sperm characteristics [9,10,11].

Metabolizable energy values of Menhaden oil and poultry fat are similar [12,13], but their fatty acid profiles differ greatly. Menhaden oil contains relatively high proportions of n3 PUFA, especially eicosapentaenoic (C20:5 n3) and docosahexaenoic (C22:6 n3) acids [14]. Kelso *et al.* [15] reported that a small increase in the proportion of n3 fatty acids in the sperm phospholipids induced by supplementing the diet with α-linolenic acid resulted in higher fertility at 39 wk of age. Blesbois *et al.* [10] initially provided experimental diets to broiler breeder males at 30 wk of age, and they reported that fertility was improved by 4.4% when diets were supplemented with n3-rich salmon oil rather than corn oil. Unfortunately, fertility was only measured from 44 through 47 wk of age. Casanovas [16] reported that providing Menhaden oil to broiler breeder males after 33 wk of age, resulted in a 6.4% increase in fertility at 49 wk of age. However, fertility levels of males being fed Menhaden oil or poultry fat were similar at 63 wk of age. Improvements in fertility were attributed to a reduction in the n6:n3 ratio of fatty acids in the spermatozoa membrane, which may alter the physical properties of the membrane or its resistance to peroxidative damage. In turn, fertilizing capacity of the sperm is increased. Prior research suggests that positive effects of n-3 rich ingredients on fertility may require an acclimation period and effects may be transient. If Menhaden oil

is provided to cockerels during rearing, then improvements in fertility may be more pronounced and persist for longer durations.

Research regarding dietary Menhaden oil has not been limited to fertility. Dietary Menhaden oil has been reported to cause hepatic lipidosis in reproductively active laying hens after 3 months of feeding 3% Menhaden oil [17]. Supplemental fish oil in hen diets has been associated with decreased egg weights [17,18,19]. Herstad *et al.* [18] compared the effects of supplemental fish oil and rendered fat at various inclusion levels. They reported that feeding fish oil diets to hens increased cracked eggs, reduced fertility, and reduced hatchability of fertile eggs. Van Elswyk *et al.* [17] suggested that the combination of Menhaden oil and estradiol may alter lipogenic activity in the liver, causing decreases in serum lipid and available lipid for yolk formation. A reduction in yolk lipid would then cause decreased yolk and egg size. Although dietary supplements with high n3 fatty acids may adversely affect hen performance, providing these nutrients exclusively to males may give positive results.

The objectives of this experiment were to further investigate the improvement in fertility and sperm quality when broiler breeder males consume Menhaden oil as the dietary fat source instead of poultry fat, and to determine if previously observed positive effects on fertility are more pronounced when birds consume Menhaden oil from hatch.

## MATERIALS AND METHODS

### BIRD MANAGEMENT

Two hundred-fifty broiler breeder males (Cobb) and four hundred broiler breeder females (Cobb 500) were reared by primary breeder recommendations in single-sex floor

pens. Due to an outbreak of Avian Leukosis Virus, Subgroup J (ALV - J), all infected males (n = 40) were removed from the flock at 44 wk, and all original hens were replaced by 26 wk-old hens (n = 299) simultaneously. Results obtained prior to males reaching 44 wk are not discussed due to possible confounding effects of ALV-J. A total of 96 males were used for experimental observations, with six replicate groups containing eight males per treatment.

The birds were housed in a light tight, evaporatively cooled and forced-air heated facility. Half of the males received poultry fat (PF), while the remaining half received Menhaden oil (MO) as the primary dietary fat source in all diets (starter, developer and breeder; Table 2.1). Inclusion level of both fat supplements in starter and developer diets was 3%, yielding 5.58 and 5.77% total fat, respectively. Both fat sources contained 500 ppm of ethoxyquin as a stabilizer. For males, starter and developer diets were provided from 0 to 4 wk and from 5 to 24 wk, respectively. A breeder diet was provided after 24 wk and contained 2% supplemental fat (PF or MO), yielding 4.58% total dietary fat. All females received a common diet containing MO as the supplemental fat source. In order to determine feed allotments and achieve target body weights, 20 to 30% of males were weighed weekly from 1 to 20 wk, and all males were weighed weekly from 21 to 30 wk and biweekly thereafter. At 21 wk of age, 201 males and 409 females were housed in cages and provided 15 hr light/day.

INSEMINATION AND INCUBATION PROCEDURES

When males reached 47 wk of age, and every 3 wk thereafter through 65 wk of age, hens were artificially inseminated once with semen taken from roosters receiving either dietary PF or MO. If ejaculates were less than 0.1 mL, the sample was discarded.

Otherwise, 0.1 mL of semen from each male was pooled within each group of 8 males, then diluted accordingly. Each hen was inseminated with 0.5 mL of semen containing $7.5x10^7$ sperm diluted with Poultry Buffer® [20]. Sperm concentration was determined at each insemination using a Micro Reader® [21] spectrophotometer. Semen from each replicate was used to inseminate a maximum of 24 hens. Each group of hens was paired with a replicate group of males and this pairing was maintained so that hens received semen from the same group of roosters at each consecutive insemination.

Eggs laid from 2 to 14 days post-insemination (DPI) were sorted and incubated every week by replicate group, with the exception of those laid at 8 DPI. Eggs laid at 8 DPI were saved for sperm-hole penetration analysis to quantify the number of sperm that penetrated the perivitelline layer of the ovum [22]. Eggs were set weekly to accommodate scheduling of hathery procedures (sorting, candling, transferring and hatching) at the same time of day and same day of the week.

Total incubation time was 21.5 d. Temperature settings from 0-18 and 19-21 d of incubation were 37.8 and 37.2°C, respectively. Relative humidity settings at 0-19 and 20-21 d of incubation were 53 and 70%, respectively. All incubated eggs were candled after 11 d of incubation, and clear eggs were removed, opened and classified as early dead embryos (0-7 d) or infertile eggs. Clear eggs were classified as early dead embryos if the areas opaca and pellucida could be differentiated or if positive development was observed. All incubated eggs that were cracked prior to incubation or at transfer were removed from the data set. Residue analysis was conducted after each hatch to determine percentages of middle deads (8-14 d), late deads (15-21 d) and pips. Percent fertility, hatchability of fertile and total eggs set were calculated.

## SEMEN PRODUCTION AND SPERM QUALITY

Beginning at 25 wk of age, all roosters were trained to the semen collection process using the abdominal massage method [23]. This was done twice each week throughout the experiment. The percentage of males producing semen was calculated as a percentage of live males at each collection period. At both 60 and 63 wk of age, one ejaculate per male was evaluated for sperm mobility phenotype using procedures described by Holsberger *et al.* [24] with Accudenz® [25] solution and a densimeter [26]. At 65 wk of age, males were euthanized and testes were weighed.

## FATTY ACID ANALYSIS OF FEED AND SPERMATOZOA

Semen was collected from all males and pooled within each treatment at 61 wk of age. The semen was stored at -4°C until analyzed. Semen was washed 1:1 with 0.9% NaCl, centrifuged and supernatant was removed. Methods described by Folch *et al.* [27] were used for lipid extraction of both feed and spermatozoa samples. Methyl esters of fatty acids were formed, then identification of fatty acids was conducted by gas chromatography [28].

## STATISTICAL ANALYSIS

Analysis was conducted using the General Linear Models procedure of SAS® [29]. Each group of eight males served as the experimental unit. To separate means, the pdiff function of SAS® [29] was used. Independent variables included diet and age, although only diet effects are discussed in detail. Diet effects were tested using group(diet) as the error term, and a significance level of $P \leq 0.05$ was used in all cases. All percentage data were subjected to arc sine transformation to obtain homogenous variances. While conclusions were drawn from the transformed data, only untransformed data are

presented for relevance. All fertility and hatch data were summarized by wk post-insemination (WPI).

## RESULTS AND DISCUSSION

Cumulative fertility was similar between treatments during the first WPI (MO, 79.8%; PF, 76.3%), but the MO treatment expressed significantly higher fertility for eggs collected during the second WPI (MO, 53.5%; PF, 47.1%) (Table 2.2). These data suggest that sperm from males provided dietary MO may survive for longer periods in the oviduct of the hen following a single insemination. Altering the fatty acid profile of the semen may have made it less susceptible to peroxidation, resulting in decreased sperm deterioration during storage within the hen. If this implication is indeed true, then MO may be used to maintain levels of fertility. This would be most beneficial in situations when male to female ratios or mating frequencies are low. When fertility data from the first and second WPI were combined, diet effects on fertility were observed at 47, 50 and 56 wk of age (Table 2.2). In agreement, Cassanovas [16] previously reported a transient elevation in fertility when feeding MO to broiler breeder males.

Low levels of fertility in this experiment were likely a result of the marginal dose of sperm that was inseminated. However, our goal was not to maximize fertility, but to increase the sensitivity of the test comparing the fertilizing ability of semen from two groups of males.

Differences in hatchability of eggs set during the first WPI were nonsignificant, but MO males expressed significantly higher hatchability of eggs set during the second WPI as a result of higher fertility (Table 2.3). Dietary fat source had no effect on hatchability

of fertile eggs at either 1 or 2 WPI. Percentages of early deads, middle deads, late deads and pips were also unaffected by dietary fat source.

The percentage of males producing semen was similar between treatments throughout the trial (MO, 74.6%; PF, 73.2%). In addition, fat source had no significant effect on sperm hole penetration, SMI or testis weights (Table 2.4).

Feed containing MO expressed a markedly lower n6/n3 fatty acid ratio than feed containing PF (Table 2.5). Compared to previous research [6,10,16], sperm from both groups contained relatively high levels of palmitic acid (C16:0), and low levels of arachidonic (C20:4 n6), docosatetraenoic (C22:4 n6) and docosahexaenoic acids (C22:6 n3). Reasons for this occurrence are unknown. Nonetheless, contrasting levels of dietary fatty acids influenced fatty acid profile of the spermatozoa as expected (Table 2.6). The n6/n3 ratios of the spermatozoa reflected the fatty acid profile of the diet. Previous reports have also shown similar occurrences when males consumed different levels of n3 and n6 polyunsaturated fatty acids [9,10,15,16]. Differences in fertility during the second WPI suggest that sperm from males consuming MO may be better able to maintain fertility. This may be related to previous suggestions that sperm with increased n3 fatty acids concentrations are less prone to peroxidation [10,16].

## CONCLUSIONS AND APPLICATIONS

1. The fatty acid profile of sperm was influenced by dietary fat source; however, fat source did not markedly affect sperm quality, as measured by sperm hole penetration and sperm mobility index.

2. Hatchability of total eggs set and fertility were improved in eggs laid during the second wk post-insemination in hens artificially inseminated with semen from males provided diets containing Menhaden oil rather than poultry fat. These data suggest that roosters fed Menhaden oil produced sperm that were better able to maintain fertility over the 2 wk periods following each insemination.
3. Providing Menhaden oil to broiler breeder males throughout their life may be a simple means to maintain fertility in aging flocks.

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Table 2.1. Ingredient and nutrient composition of diets provided to males from 0 to 65 wk of age

| | Diet [A] | | |
|---|---|---|---|
| Ingredient, % as is | Starter | Developer | Breeder |
| Corn | 59.14 | 63.04 | 63.15 |
| Soybean meal | 25.41 | 19.25 | 22.05 |
| Wheat middlings | 8.46 | 10.88 | 3.47 |
| Dicalcium phosphate | 1.80 | 1.69 | 1.54 |
| Limestone | 1.25 | 1.20 | 6.84 |
| Salt | 0.45 | 0.45 | 0.47 |
| Vitamin premix [B] | 0.30 | 0.30 | 0.30 |
| DL methionine | 0.11 | 0.11 | 0.10 |
| Mineral premix [C] | 0.08 | 0.08 | 0.08 |
| Poultry fat or Menhaden oil | 3.00 | 3.00 | 2.00 |
| | | | |
| Calculated analysis | | | |
| Crude protein (%) | 18.34 | 16.09 | 16.25 |
| Energy (kcal ME/kg) | 3024.3 | 3052.5 | 2905.5 |
| Total fat (%) | 5.58 | 5.77 | 4.58 |

[A] Males received diets that contained either supplemental Menhaden oil or poultry fat from 0 to 65 wk of age.
[B] Vitamin mineral premix provided the following per kilogram of diet: vitamin A, 6,600 IU; vitamin D3, 1,320 IU; vitamin E, 13 IU; vitamin K, 1.3 mg; vitamin B12, 0.01 mg; thiamine 2.6 mg; riboflavin, 5.3 mg; vitamin B6, 2.6 mg; niacin, 53 mg; pantothenic acid, 13 mg; folic acid 0.7 mg; biotin, 0.13 mg; choline, 230 mg.
[C] Trace mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 107; iron, 21; iodine, 0.8; zinc, 86; copper, 3.2; magnesium, 21; calcium, 3.

Table 2.2. Influence of dietary fat source on fertility[A]

| | Diet[B] | | |
|---|---|---|---|
| | PF | MO | pooled SEM |
| Fertility | ----------%---------- | | |
| 1 WPI[C] | 76.3 | 79.8 | 1.5 |
| 2 WPI | 47.1[b] | 53.5[a] | 1.5 |
| Fertility | | | |
| 47 wk | 57.1[b] | 65.3[a] | 0.9 |
| 50 wk | 59.7[b] | 72.7[a] | 1.2 |
| 53 wk | 71.3 | 71.9 | 1.3 |
| 56 wk | 62.7[b] | 73.2[a] | 1.7 |
| 59 wk | 52.9 | 55.9 | 1.6 |
| 62 wk | 66.0 | 62.3 | 1.2 |
| 65 wk | 62.0 | 65.3 | 1.3 |

[A] Eggs were collected during 2 wk periods following inseminations at 47, 50, 53, 56, 59, 62 and 65 wk of age (MO, n = 4,267 eggs; PF, n = 3,791 eggs).
[B] Male diets were supplemented with either Menhaden oil (MO) or poultry fat (PF) from 0 to 65 wk of age.
[C] WPI, weeks post-insemination.
[a-b] Means with different superscripts within a row are significantly different ($P \leq 0.05$).

Table 2.3. Influence of dietary fat source on viability of embryos[A]

| | Diet[B] | | |
|---|---|---|---|
| | PF | MO | pooled SEM |
| Hatchability of fertile | ----------%[C]---------- | | |
| 1 WPI[D] | 86.5 | 86.8 | 1.0 |
| 2 WPI | 77.2 | 81.0 | 1.8 |
| Hatchability of eggs set | | | |
| 1 WPI | 66.0 | 69.3 | 1.7 |
| 2 WPI | 36.4[b] | 43.3[a] | 1.4 |
| Early dead | | | |
| 1 WPI | 7.5 | 7.8 | 0.8 |
| 2 WPI | 14.2 | 12.7 | 1.6 |
| Middle dead | | | |
| 1 WPI | 0.5 | 0.3 | 0.1 |
| 2 WPI | 0.9 | 0.2 | 0.4 |
| Late dead | | | |
| 1 WPI | 3.3 | 3.0 | 0.5 |
| 2 WPI | 5.4 | 4.2 | 0.8 |
| Pips | | | |
| 1 WPI | 2.2 | 2.0 | 0.4 |
| 2 WPI | 2.3 | 1.9 | 0.5 |

[A] Eggs were collected during 2 wk periods following inseminations at 47, 50, 53, 56, 59, 62 and 65 wk of age (MO, n = 4,267 eggs; PF, n = 3,791 eggs).
[B] Male diets were supplemented with either Menhaden oil (MO) or poultry fat (PF) from 0 to 65 wk of age.
[C] All values are presented as percentages of fertile eggs with the exception of hatchability (% of eggs set).
[D] WPI, weeks post-insemination.
[a-b] Means with different superscripts within a row are significantly different ($P \leq 0.05$).

Table 2.4. Influence of dietary fat source on sperm quality and reproductive traits of broiler breeder males

| Treatment[A] | Sperm Holes[B] | | | | SMI[C] | Testis Weight (g)[D] | Males Producing Semen (%)[E] |
|---|---|---|---|---|---|---|---|
| | 0-10 | 11-30 | 31-60 | >61 | | | |
| PF | 82.2 | 11.5 | 5.2 | 1.0 | 41.2 | 24.8 | 73.2 |
| MO | 78.9 | 16.1 | 4.6 | 0.4 | 40.3 | 24.8 | 74.6 |
| Pooled SEM | 2.6 | 1.8 | 1.7 | 0.7 | 3.3 | 1.7 | 5.8 |

[A] Males received diets that contained either supplemental Menhaden oil (MO) or poultry fat (PF) from 0 to 65 wk of age. There were no treatment effects on any of the variables presented ($P > 0.05$).

[B] Eggs were collected 8 d after artificial inseminations at 47, 50, 53, 56, 59, 62 and 65 wk of age. The number of holes penetrating the perivitelline layer were determined [22]. Values are percentages of eggs that fall within each range (MO, n = 299 eggs; PF, n = 281 eggs).

[C] Sperm mobility index (SMI) was measured using Accudenz solution [25] and a densimeter [26] as described by Holsberger *et al.* [24].

[D] Testes were removed from all males at 65 wk of age and weighed.

[E] Calculated as a percentage of males evaluated at each collection period from 44 to 65 wk of age. Males were abdominally massaged twice each week.

Table 2.5. Fatty acid composition of breeder feeds containing either 2% supplemental Menhaden oil or poultry fat

| | Diet [A] | |
|---|---|---|
| Fatty Acid | PF | MO |
| Saturates | ----------%---------- | |
| C14:0 | 0.33[B] | 1.60 |
| C16:0 | 18.72 | 15.60 |
| C18:0 | 3.76 | 3.21 |
| Monounsaturates | | |
| C16:1 n7 | 3.07 | 3.27 |
| C18:1 n9 | 33.51 | 23.55 |
| PUFA[C] n3 | | |
| C18:3 n3 | 2.16 | 2.63 |
| C20:5 n3 | ND[D] | 3.01 |
| C22:5 n3 | ND | 0.62 |
| C22:6 n3 | ND | 3.70 |
| PUFA n6 | | |
| C18:2 n6 | 34.64 | 36.61 |
| C22:4 n6 | 0.09 | 0.35 |
| Total PUFA | 36.89 | 46.92 |
| n6/n3 ratio | 16.08 | 3.71 |

[A] Males received diets that contained either supplemental Menhaden oil (MO) or poultry fat (PF) from 0 to 65 wk of age.
[B] All values are given as a percentage of total fatty acids.
[C] PUFA, polyunsaturated fatty acids.
[D] ND, not detected.

Table 2.6. Fatty acid profiles of sperm collected at 61 wk of age, from males consuming diets supplemented with either Menhaden oil (MO) or poultry fat (PF)[A]

| | Diet | |
|---|---|---|
| Fatty acid | PF | MO |
| | ----------%[B]---------- | |
| C14:0 | 1.28 | 1.48 |
| C16:0 | 40.75 | 40.88 |
| C16:1 n7 | 2.90 | 2.56 |
| C16:4 n1 | 3.00 | 4.12 |
| C18:0 | 9.83 | 11.65 |
| C18:1 n9 | 21.58 | 14.72 |
| C18:2 n6 | 4.11 | 1.14 |
| C20:1 n9 | 1.07 | 1.30 |
| C20:4 n6 | 0.91 | 1.45 |
| C21:0 | 1.01 | 1.17 |
| C22:4 n6 | 3.28 | 4.50 |
| C22:5 n3 | ND[C] | 0.37 |
| C22:6 n3 | 0.49 | 1.03 |
| Total PUFA[D] | 11.79 | 12.61 |
| n6/n3 ratio | 16.94 | 5.06 |

[A] Semen was collected and pooled by treatment at 61 wk of age.
[B] All values are given as a percentage of total fatty acids.
[C] ND, not detected.
[D] PUFA, polyunsaturated fatty acids.

# CHAPTER 3

# REPRODUCTIVE PERFORMANCE AND IMMUNE STATUS OF CAGED BROILER BREEDER HENS PROVIDED DIETS SUPPLEMENTED WITH EITHER INORGANIC OR ORGANIC SOURCES OF ZINC DURING A 65 WEEK PRODUCTION PERIOD[1]

[1] Hudson, B.P., W. A. Dozier, III, J. L. Wilson, J.E. Sander, and T.L. Ward. To be submitted to *Journal of Applied Poultry Research*

## SUMMARY

Zinc participates in several reproductive functions that include sperm storage, eggshell formation and embryonic development. Zinc from organic complexes has been reported to be more bioavailable than inorganic sources. Thus, providing organic zinc complexes to broiler breeder hens may enhance reproductive performance. This study examined the responses of caged broiler breeder hens fed diets supplemented with 160 ppm zinc from either $ZnSO_4$ (control), Availa®Zn zinc-amino acid complex (ZnAA) or a mixture of ZnAA and $ZnSO_4$ (80 ppm zinc from each). Settable egg production and estimated chick production were optimized when a mixture of ZnAA and $ZnSO_4$ was provided. Specific gravity, cracked egg production and settable egg production data indicated that eggshell quality was enhanced when hens were fed ZnAA. Immune response to phytohemagglutinin-P and antibody titers to Newcastle disease virus were increased with birds given diets supplemented with 160 ppm zinc from ZnAA. Generally, supplementing diets with a combination of ZnAA and $ZnSO_4$ or ZnAA resulted in superior performance of caged broiler breeder hens compared to hens provided diets with added zinc from $ZnSO_4$ exclusively.

## DESCRIPTION OF PROBLEM

Supplemental zinc is essential in broiler breeder diets to achieve optimum reproductive performance. Zinc status has been directly related to activity of carbonic anhydrase [1], an essential enzyme for eggshell formation [2]. Eggshell quality has been maintained with increased supplementation of zinc in laying hen diets [3]. As a result of poor zinc status in breeding hens, settable egg production and hatchability may decline.

It will also result in inadequate zinc supply for developing embryos. The relationship between low intake of zinc by hens and poor hatchability was reported over four decades ago [4,5,6,7]. Although normal hatchability may be achieved without dietary zinc supplementation, additional zinc in hen diets (corn-soybean meal) has enhanced egg production and immune function of hens [8], and has improved immune status of progeny [9,10,11,12].

Research by Wedekind *et al.* [13] indicated that complexed zinc is more available to the chick than inorganic zinc sources. Organic zinc sources can be in the form of metal-amino acid chelate, metal-proteinate, metal-specific amino acid complexes and metal-amino acid complexes. Metal-amino acid chelates and metal-proteinates are the chelations of a soluble metal salt with amino acids or hydrolyzed protein, respectively. In metal-amino acid chelates, the molar ratio is one mole of a soluble salt with one to three moles of an amino acid. However, metal-specific amino acid complexes and metal-amino acid complexes consist of a specific or nonspecific amino acid complexed with a soluble metal salt in a molar ratio of 1:1. The complex of one metal ion bound to a single amino acid may increase utilization of the mineral by the bird. Availa®Zn (ZnAA) [14] is a metal specific amino acid complex that consists of zinc bound by the amino and carboxyl groups of an amino acid. Antagonistic effects of phytic acid [15], fiber [16] and calcium [17] on zinc absorption may be reduced when this zinc-amino acid complex is provided to hens.

Barber *et al.* [18] supplemented broiler breeder diets with 150 ppm zinc from $ZnSO_4$ alone or a mixture of ZnAA and $ZnSO_4$ (75 ppm zinc from each). These authors reported that supplemental zinc source had little effect on reproductive performance of broiler

breeders. However, the experimental diets were only provided from 21 to 43 wk of age. Given the increased bioavailability of zinc from zinc-amino acid complexes that has been documented with poultry, the responses of reproduction and immune status may be enhanced with hens provided diets supplemented with zinc from zinc-amino acid complexes during a 65-wk production cycle. This study examined reproductive and immune responses of broiler breeder hens given diets supplemented with ZnAA or an inorganic zinc source at concentrations being representative of industry practice. To address concerns regarding previously conducted research, experimental diets were provided throughout the rearing and breeding periods.

## MATERIALS AND METHODS

### EXPERIMENTAL DIETS AND BIRD MANAGEMENT

Slow-feathering, Cobb 500 broiler breeder hens were given one of three diets from 0 to 65 wk of age (Table 3.1). Experimental diets consisted of 160 ppm supplemental Zn from $ZnSO_4$ (control), ZnAA, or a mixture of ZnAA and $ZnSO_4$ (ZnAA + $ZnSO_4$, 80 ppm zinc from each). The diet supplemented with $ZnSO_4$ was considered the control since a majority of trace premixes for breeders contain zinc in the inorganic form as $ZnSO_4$ and/or ZnO. Personal communication with nutritionists in the U.S. poultry industry has revealed that broiler breeder diets typically contain supplemental zinc in the range of 120 to 180 ppm. Thus, the experimental diets were formulated to contain 160 ppm supplemental zinc. This concentration of zinc may exceed the actual requirement for maximum hen performance, although the dietary zinc requirement for broiler breeder hens has not been established. Diets were manufactured every three to four wk

throughout the experiment, and two representative samples were collected from basal and experimental diets in each batch. Inductively Coupled Plasma (ICP) analyses were conducted to determine the zinc content of the basal and experimental diets [19]. Determined concentrations were comparable to anticipated concentrations throughout the experiment (Basal: calculated value = 12 ppm; determined value = 39±8 ppm; Supplemented: calculated value = 172 ppm; determined value = 185±36; n = 18). Differences between calculated and determined concentrations were likely due to an underestimation of zinc in the natural feedstuffs.

Pullets were reared in an environmentally-controlled facility on pine shavings with 8 hr daylengths prior to being moved to triple-deck cages at 20 wk of age. A standard vaccination program was used during the rearing period. At 20 wk of age, all hens were caged (2 hens per cage, 1.32 $ft^2$/bird) and 16 hr light/d was provided. Twelve cages, each containing 2 hens, served as the experimental unit. Each treatment was represented by eight experimental units. The same sample of 24 females/trt was weighed weekly through 40 wk and biweekly thereafter, to determine feed allotments. All dietary treatments were provided the same amount of feed on a per bird basis. Management was provided according to primary breeder recommendations. The feeding program was based on recommendations provided by a broiler integrator and was adapted for production in cages.

HEN PERFORMANCE MEASUREMENTS

Every four weeks, egg weights and specific gravity of all settable eggs laid on a single day were measured beginning at 26 wk of age. To evaluate eggshell quality, the flotation method [20] was used to determine specific gravity. Solution concentrations ranged from

1.065 to 1.095 in intervals of 0.005 specific gravity units. At 50, 58 and 66 wk, breaking strength of eggshells was measured on all settable eggs laid on a single day using an Instron machine [21].

At 32 and 62 wk of age, contents from six randomly selected eggs per replicate group were pooled and homogenized. Egg contents were stored at -4°C until analyzed for zinc concentration by ICP [19].

Eggs were collected three times daily and classified as settable, cracked, shell-less, double-yolked, abnormally-shaped or no-yolk eggs. Total and settable egg production were summarized weekly on a hen-housed and hen-day basis.

From 23 to 45 wk of age, hens were artificially inseminated with 50 μL of pooled, neat semen from broiler breeder males. Thereafter, hens were artificially inseminated twice at 48, 52, 56, 60 and 64 wk of age with a 6 d period between each successive insemination at each age. Timing of all inseminations ensured presence of sperm in the hens' reproductive tract while eggs were being collected for incubation. To make certain there were no male effects on fertility and hatchability measurements, semen was collected on a rotating basis from a population of 209 males then pooled. Semen was inseminated within 20 min of collection.

A maximum of 90 eggs per experimental unit were incubated and hatched weekly (25–35 wk), biweekly (37–45 wk) and every fourth wk (49-65 wk) [18]. Eggs were transferred to hatchers after 18 d of incubation. Chicks were counted and graded as high quality or cull chicks after a total incubation time of 21.5 d. Temperature settings from 0 to 18 and 19 to 21 d of incubation were 37.8 and 37.2°C, respectively. Relative humidity settings at 0 to 19 and 20 to 21 d of incubation were 53 and 70%, respectively. At 10 d

of incubation, eggs were candled and clear eggs broken out to determine early dead (0 to 7 d) embryos, middle dead embryos (> 8 d) and infertile eggs. Clear eggs were classified as early dead embryos if the areas opaca and pellucida could be differentiated or if positive development was observed. All incubated eggs that were cracked prior to incubation or at transfer were removed from the data set. Residue analysis was conducted on all unhatched eggs to determine the number of middle dead (< 14 d), late dead (15 to 21 d) and pipped embryos. These values were then calculated as a percentage of fertile eggs in each replicate group. Fertility, hatchability of fertile eggs and hatchability of total eggs set were calculated after each incubation period. Mortality was recorded daily and calculated at the termination of the experiment.

IMMUNE STATUS

A standard vaccination schedule was used for Newcastle disease virus (NDV) during the rearing period. Live vaccines [23] were provided through water at 2, 6 and 16 wk of age. To assess humoral immune status, antibody titers to NDV were measured by hemagglutination inhibition testing. Serum was collected from the same hens (16/treatment) at 6 wk intervals from 20 through 62 wk of age to measure antibody titers to NDV. To measure cellular immune response, 24 hens from each dietary treatment were given a 0.1 mL intradermal injection of phosphate-buffered saline (PBS) and phytohemagglutinin-P (0.1mg PHA-P in PBS) in the left and right wattle, respectively. Cellular immune response was determined using this method at 26, 44 and 62 wk of age. Digital calipers [24] were used to measure wattle thickness to the nearest 0.01 mm prior to injection and 18 hr post-injection. Post-injection width of the left (saline-injected)

wattle was subtracted from the post-injection width of the right (PHA-injected wattle), and the difference was deemed as a cellular immune response [25].

STATISTICAL ANALYSIS

Statistical analysis was conducted using the General Linear Models procedure of SAS [26], and group (12 cages, 24 hens) served as the experimental unit. Sources of variation included zinc source, age and zinc x age. Age effects are discussed by separating data into three 14 wk periods (early = 24 to 37 wk, middle = 38 to 51 wk, late = 52 to 65 wk) unless noted otherwise. Because zinc effects are the basis of this experiment, age effects are not discussed in detail. Means were separated by the PDIFF function when significance ($P \leq 0.05$) occurred. All percentage data were subjected to arc sine transformation. While conclusions were drawn from the transformed data, only nontransformed data are presented for relevance.

## RESULTS AND DISCUSSION

Feeding ZnAA + $ZnSO_4$ resulted in the lowest incidence of cracked eggs, while hens fed $ZnSO_4$ had the highest incidence of cracked eggs (Table 3.2). Increased incidence of cracked eggs negatively influences the financial income of both poultry producers and contracting poultry companies. As expected, percentage of cracked eggs was inversely related to percentage of settable eggs. Housing hens in cages likely caused the incidence of cracked eggs to be relatively high in all treatments. Eggs from hens fed ZnAA and ZnAA + $ZnSO_4$ had improved shell quality as reflected by higher specific gravity than those from the $ZnSO_4$ treatment. Differences in eggshell quality were apparent with breeder flock age as indicated by decreased specific gravity (early = 1.083; late = 1.077),

higher percentage of cracked eggs (middle = 5.7 %; late = 8.1 %) and reduced eggshell strength (50 wk = 3.18 kg max load; 66 wk = 2.74 kg max load) as the hens progressed in age. Similar findings by Guo *et al.* [3] previously reported that providing diets with supplemental zinc (80 ppm) from a mixture of ZnAA and $ZnSO_4$ rather than solely $ZnSO_4$ reduced the incidence of cracked eggs in laying hens. Synergistic effects of ZnAA and $ZnSO_4$ may enhance mechanisms involved in calcification of eggshells.

As zinc source effects on specific gravity were observed during the experiment, the more sensitive measurement of eggshell breaking strength was measured. Unfortunately, this variable was measured only during the last weeks of the experiment. Significant zinc source x age effects on eggshell breaking strength revealed that hens consuming diets supplemented with a combination of ZnAA + $ZnSO_4$ had the greatest eggshell strength at 58 wk ($ZnSO_4$ = 2.71, ZnAA = 2.61, ZnAA + $ZnSO_4$ = 2.92 kg max load, $P \leq 0.05$), but had the lowest eggshell strength at 66 wk of age ($ZnSO_4$ = 2.91, ZnAA = 2.70, ZnAA + $ZnSO_4$ = 2.61 kg max load, $P \leq 0.05$). Reasons for inconsistent zinc source effects on eggshell breaking strength at 58 and 66 wk of age are not fully understood. Correlations between shell breaking strength and specific gravity are confirmed only at 58 wk of age ($r = 0.469$, $P < 0.05$). Failure to show a conistent relationship between these two measurements may be due to large differences between test sensitivities.

Zinc concentration of egg contents was elevated when ZnAA was provided to hens (Table 3.2). Similar findings by Guo *et al.* [3] previously reported increased yolk zinc concentration when laying hens were provided diets supplemented with ZnAA rather than $ZnSO_4$. Badawy *et al.* [27] reported a positive relationship between Zn concentration of egg contents and hatchability as shown by a lower incidence of late

embryonic mortality with increased zinc concentration in egg contents. Inadequate transmission of zinc from the hen to the hatching egg was likely responsible for low hatchability and poor chick quality observed in previous studies when dietary zinc intake was extremely low [4,5,6,7].

As expected, egg weight was directly related to breeder age (early, 56.0 g; middle, 65.9 g; late, 68.4 g). Zinc concentration of egg contents declined with age (32 wk, 65 ppm; 62 wk, 55 ppm). Research has indicated that zinc absorption of 10 month-old rats is higher than absorption in 30 month-old rats [28]. The effect was attributed to reduced ileal transport capacity and increased cholesterol content of the brush-border membrane in the ileum. Age effects on zinc absorption in poultry have not been investigated in detail.

Hens fed diets supplemented with ZnAA + $ZnSO_4$ had the highest hen-day egg production and settable eggs per hen housed (Table 3.3). Total hen-day egg production and settable egg production were decreased when hens were provided $ZnSO_4$ exclusively. A significant zinc x age effect indicated that zinc source affected hen-day egg production primarily during the early production period (Figure 3.1). However, hen-day egg production was similar for all groups during the middle and late stages of egg production. Hen-housed egg production and total eggs per hen-housed were not affected by zinc source. Egg production was relatively low in all treatments, because slow-feathering hens traditionally have lower egg production than fast-feathering hens [29]. Slow-feathering Cobb hens typically produce an average of 144 settable eggs per hen-housed and have a total mortality of 17.1% under commercial practice in the U.S. during the breeding period [29]. High incidence of mortality (21.9%) and cracked eggs (7.3%) in

our experiment caused hen-housed egg production (139 settable eggs/hen-housed) to be lower than the industry average. Post-mortem observations such as bacterial infections or hepatic lipidosis were observed in many hens, but necropsies did not elucidate a single cause for high mortality. In addition, breeding facilities (cages *vs* floor and slats) may influence the incidence of cracked eggs and settable egg production.

Early embryonic death (0-7 d) was elevated in the ZnAA group, but pipped embryos and embryonic mortality during the middle (8-14 d) and late (15-21 d) stages of incubation were not affected by zinc source (Table 3.4). Significant zinc source x age effects revealed that hens provided $ZnSO_4$ had the lowest early embryonic mortality during the early production period ($ZnSO_4$ = 5.8, ZnAA = 8.1, ZnAA + $ZnSO_4$ = 7.4 %, $P \leq 0.05$). However, early embryonic mortality was lowest when hens were provided ZnAA + $ZnSO_4$ late in production ($ZnSO_4$ = 7.9, ZnAA = 8.7, ZnAA + $ZnSO_4$ = 5.9 %, $P \leq 0.05$).

Chick production was influenced by dietary zinc source, but cumulative fertility, hatchability of eggs set and hatchability of fertile eggs were not affected by dietary zinc source (Table 3.4). Data presented here indicate that dietary zinc source influences chick production by altering the number of settable eggs produced without affecting hatchability. In accordance with the egg production data, estimated chick production per hen-housed was highest in the ZnAA + $ZnSO_4$ treatment.

Significant zinc x age effects revealed that fertility was highest for hens consuming $ZnSO_4$ at 25 wk of age, but subsequent fertility was not affected by zinc source. In contrast to results reported by Badawy *et al.* [27], egg zinc content was not related to hatchability in this experiment. This may have occurred because zinc status of all hens

was sufficient for adequate hatchability. If hens had been fed marginal concentrations of zinc, then the positive effects of ZnAA on egg zinc content and resulting influence on hatchability may have been more apparent.

Fertility declined with age (early, 93.5%; middle, 92.0%, late, 81.1%) and hatchability of fertile eggs was depressed during the early phase of production (early, 83.4%; middle, 86.1%; late, 86.9%). Hatchability of total eggs set was similar during the early (78.1%) and middle (79.2%) stages of production, but decreased significantly in the late period (70.5%) due to reduced fertility. Less frequent inseminations after 45 wk of age may be partially responsible for age-related declines in fertility and hatchability.

Cellular immune response to PHA-P injection was enhanced as dietary ZnAA increased, but the incidence of mortality was not influenced by dietary zinc source (Table 3.5). Other research has demonstrated that supplementing broiler breeder hen diets with zinc-methionine rather than inorganic zinc sources increased the cellular immune response of progeny to phytohemagglutinin [9,10,11,12]. In addition, providing zinc from amino acid complexes significantly increased thymus weights [12] and livability of progeny [30,31]. Previous research has indicated that NDV, infectious bursal disease and infectious bronchitis titers were increased when hen diets were supplemented with ZnAA [8]. In agreement, antibody titers to NDV were higher when supplemental Zn was provided by ZnAA in this experiment. Although enhanced immune status of hens consuming ZnAA did not decrease the occurrence of mortality, parental immunity of progeny from hens consuming ZnAA may be improved.

Age also influenced immune responses of broiler breeder hens. Antibody titers to NDV declined after 50 wk of age, and cellular immune response to phytohemagglutinin

injection (degree of swelling) was reduced at 26 wk (0.90 mm) compared to 42 wk (1.13 mm) and 58 wk of age (1.11 mm).

## CONCLUSIONS AND APPLICATIONS

1. Eggshell quality and settable egg production were enhanced when caged hens consumed diets containing a mixture of ZnAA and $ZnSO_4$ rather than diets with $ZnSO_4$ as the sole supplemental zinc source. As a result, hens consuming diets with a mixture of ZnAA and $ZnSO_4$ produced 2.3 more chicks per hen housed than the hens consuming the $ZnSO_4$ supplemented diet.
2. Immune responses to phytohemagglutinin-P and antibody titers to Newcastle disease virus were elevated when hens consumed more ZnAA. Therefore, disease resistance or transmission of antibodies to progeny may be enhanced when broiler breeder hens consume ZnAA.

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Table 3.1. Ingredient composition and calculated nutrient analysis of the basal diets provided to the breeder pullets and hens[A]

| Ingredients, % "as-is" | Starter[B] | Developer[C] | Breeder 1[D] | Breeder 2[D] |
|---|---|---|---|---|
| Corn | 62.95 | 58.81 | 68.15 | 68.43 |
| Soybean meal (48% CP) | 22.24 | 16.90 | 20.71 | 18.68 |
| Poultry oil | … | … | 1.00 | 1.67 |
| Wheat middlings | 10.53 | 20.10 | … | … |
| Limestone | 1.15 | 1.16 | 7.41 | 8.37 |
| Dicalcium phosphate | 1.75 | 1.76 | 1.46 | 1.50 |
| Salt | 0.54 | 0.58 | 0.54 | 0.54 |
| Lysine | 0.10 | … | … | … |
| Methionine | 0.15 | 0.06 | 0.14 | 0.11 |
| Trace mineral premix[F] | 0.08 | 0.08 | 0.08 | 0.08 |
| Vitamin premix[G] | 0.50 | 0.50 | 0.50 | 0.50 |
| Copper sulfate | 0.01 | 0.05 | 0.01 | 0.01 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| | | | | |
| Calculated Analyses | | | | |
| | | | | |
| Crude protein (%) | 18.00 | 15.50 | 15.90 | 15.00 |
| Metabolizable energy (kcal/kg) | 2865 | 2819 | 2900 | 2900 |
| Calcium (%) | 0.91 | 0.90 | 3.22 | 3.60 |
| Available phosphorus (%) | 0.45 | 0.42 | 0.38 | 0.36 |
| Lysine (%) | 1.00 | 0.78 | 0.83 | 0.82 |
| Methionine (%) | 0.43 | 0.32 | 0.40 | 0.33 |
| TSAA (%) | 0.73 | 0.60 | 0.66 | 0.66 |
| Sodium (%) | 0.21 | 0.22 | 0.21 | 0.20 |
| Copper (ppm) | 16 | 16 | 16 | 16 |
| Zinc (ppm) | 13 | 12 | 12 | 12 |

[A] Experimental diets consisted of 160 ppm supplemental zinc from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA) or a mixture of ZnAA and $ZnSO_4$ (80 ppm zinc from each). Natural feed ingredients provided 40 ppm zinc.

[B] Diet was provided from 0 to 19 d of age to all females.

[C] Diet was provided from 20 d to 22 wk of age to all females.

[D] Diet was provided from 23 to 47 wk of age to all females.

[E] Diet was provided from 48 to 66 wk of age to all females.

[F] Trace mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8.

[G] Vitamin premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

Table 3.2. Influences of dietary zinc source on egg quality parameters from 24 through 65 wk of age[A]

| Treatment[B] | Specific Gravity | Eggshell Breaking Strength, kg max load | Egg Weight, g | Cracked eggs, % | Settable eggs, % | Zn in egg contents[C], ppm |
|---|---|---|---|---|---|---|
| $ZnSO_4$ (control) | 1.0791[b] | 2.93 | 64.2 | 8.03[a] | 90.27[c] | 56.0[b] |
| ZnAA | 1.0797[a] | 2.85 | 64.1 | 7.27[b] | 91.25[b] | 62.8[a] |
| ZnAA + $ZnSO_4$ | 1.0802[a] | 2.88 | 64.2 | 6.59[c] | 92.10[a] | 61.4[ab] |
| SEM | 0.0002 | 0.045 | 0.15 | 0.200 | 0.218 | 1.96 |
| | | | | | | |
| Source of Variation | | | | | | |
| Zinc | *** | NS | NS | *** | *** | * |
| Age | *** | *** | *** | *** | *** | *** |
| Zinc x Age | NS | * | NS | NS | NS | NS |

[A] Values are least-squares means involving 24 hen groups, each with 24 hens at housing.
[B] Broiler breeder hens were given one of three diets. Diets consisted of 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), or a mixture of ZnAA and $ZnSO_4$ (ZnAA + $ZnSO_4$, 80 ppm zinc from each).
[C] Zinc analysis was conducted on five eggs per replicate group at 32 and 62 wk of age. Values are presented on a dry-matter basis.
[a-c] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).
NS, $P > 0.05$.
*, $0.01 < P \leq 0.05$.
***, $P \leq 0.001$.

Table 3.3. Influences of dietary zinc source on egg production from 24 through 65 wk of age[A]

| Treatment[B] | HDEP[C], % | HHEP[C], % | Total eggs/hen-housed | Settable eggs/hen-housed |
|---|---|---|---|---|
| $ZnSO_4$ (control) | 56.56[b] | 51.63 | 151.79 | 137.05[b] |
| ZnAA | 56.71[ab] | 51.55 | 151.56 | 138.39[ab] |
| ZnAA + $ZnSO_4$ | 57.33[a] | 51.85 | 152.45 | 140.42[a] |
| SEM | 0.292 | 0.325 | 0.955 | 0.971 |
| | | | | |
| Source of Variation | | | | |
| Zinc | * | NS | NS | * |
| Age | *** | *** | *** | *** |
| Zinc x Age | NS | NS | NS | NS |

[A] Values are least-squares means involving 24 hen groups, each with 24 hens at housing.
[B] Broiler breeder hens were given one of three diets. Diets consisted of 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), or a mixture of ZnAA and $ZnSO_4$ (ZnAA + $ZnSO_4$, 80 ppm zinc from each).
[C] HDEP, hen-day egg production; HHEP, hen-housed egg production.
[a-b] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).
NS, $P > 0.05$.
*, $0.01 < P \leq 0.05$.
***, $P \leq 0.001$.

Table 3.4. Influences of dietary zinc source on fertility, embryonic viability and chick production from 25 through 65 wk of age[A]

| Treatment[B] | Fertility, % | Hatchability of Eggs Set, % | Hatchability of Fertile, % | Chicks per hen housed[C] | Early Dead[D], % | Middle Dead[D], % | Late Dead[D], % | Pips[D], % |
|---|---|---|---|---|---|---|---|---|
| $ZnSO_4$ (control) | 91.44 | 77.64 | 84.91 | 106.41[ab] | 6.11[b] | 0.15 | 5.61 | 3.03 |
| ZnAA | 90.40 | 75.93 | 83.99 | 105.08[b] | 7.95[a] | 0.23 | 5.33 | 2.39 |
| ZnAA + $ZnSO_4$ | 90.93 | 77.38 | 85.10 | 108.66[a] | 6.49[b] | 0.19 | 5.46 | 2.57 |
| SEM | 0.400 | 0.556 | 0.485 | 0.803 | 0.311 | 0.052 | 0.283 | 0.228 |
| | | | | | | | | |
| Source of variation | | | | | | | | |
| Zinc | NS | 0.082 | NS | * | *** | NS | NS | NS |
| Age | *** | *** | *** | *** | *** | NS | *** | *** |
| Zinc x Age | * | NS | NS | NS | *** | NS | NS | NS |

[A] Values are least-squares means involving 24 hen groups, each with 24 hens at housing. Hens were artificially inseminated, and eggs were incubated and hatched weekly (25–35 wk), biweekly (37–45 wk) or every fourth wk (49-65 wk). Total eggs incubated, $ZnSO_4$ = 8,494; ZnAA = 8,375; ZnAA + $ZnSO_4$ = 8,773

[B] Broiler breeder hens were given one of three diets. Diets consisted of 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), or a mixture of ZnAA and $ZnSO_4$ (ZnAA + $ZnSO_4$, 80 ppm zinc from each).

[C] Estimation by multiplication of weekly settable eggs per hen-housed and hatchability of total eggs set per hatch.

[D] Calculated as a percentage of fertile eggs.

[a-b] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).

NS, $P > 0.05$.

*, $0.01 < P \leq 0.05$.

***, $P \leq 0.001$.

Table 3.5. Influences of dietary zinc source on immune status and mortality

| Treatment[A] | NDV titer, 2.5 x $\log_2$ [B] | PHA[C], mm | Mortality [D], % |
|---|---|---|---|
| $ZnSO_4$ (control) | 4.47[b] | 0.97[b] | 21.88 |
| ZnAA | 5.09[a] | 1.12[a] | 21.35 |
| ZnAA + $ZnSO_4$ | 4.64[b] | 1.04[ab] | 22.40 |
| SEM | 0.085 | 0.04 | 3.500 |
| | | | |
| Source of Variation | | | |
| Zinc | *** | * | NS |
| Age | *** | *** | NS |
| Zinc x Age | NS | NS | NS |

[A] Broiler breeder hens were given one of three diets. Diets consisted of 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), or a mixture of ZnAA and $ZnSO_4$ (ZnAA + $ZnSO_4$, 80 ppm zinc from each).
[B] n = 16.
[C] Calculated by subtracting the postinjection width of left (control, PBS-injected) wattle from postinjection width of right (PHA-injected) wattle; n = 24.
[D] Total mortality from 20 through 65 wk of age.
[a-b] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).
NS, $P > 0.05$.
NA, not applicable.
*, $0.01 < P \leq 0.05$.
***, $P \leq 0.001$.

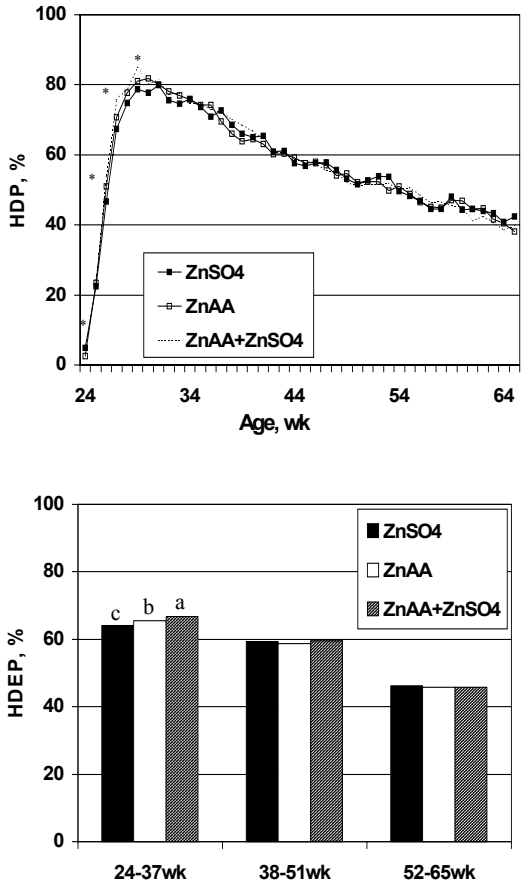


Figure 3.1. Influence of dietary zinc source on hen-day egg production. Broiler breeder hens were given one of three experimental diets consisting of 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), or a mixture of ZnAA and $ZnSO_4$ ($ZnSO_4$+ZnAA, 80 ppm zinc from each). Means with different superscripts within an age group signifies significant differences ($P \leq 0.05$, * $P \leq 0.05$).

# CHAPTER 4

# INFLUENCES OF ZINC SOURCE IN CAGED BROILER BREEDER HEN AND PROGENY DIETS AND TEMPERATURE DURING THE BROILER PRODUCTION PERIOD ON LIVE PERFORMANCE OF STRAIGHT-RUN BROILERS[1]

[1] Hudson, B.P., W.A. Dozier, III, B.D. Fairchild, J.L. Wilson, J.E. Sander, and T.L. Ward. To be submitted to *Journal of Applied Poultry Research*

**SUMMARY**

Zinc-amino acid complexes have been reported to be more bioavailable to the chick than inorganic zinc sources. Previous work with broiler breeder and turkey hens has indicated that supplementing diets with zinc in the form of a zinc-amino acid complex enhances immune status and livability of progeny. However, possible interactions between dietary zinc and common environmental stressors have not been evaluated. This study examined the effects of supplemental zinc source ($ZnSO_4$ or Availa®Zn 100[2] zinc-amino acid complexes) in the diets of broiler breeder hens and their progeny on performance and immune status of broilers. In addition, the broilers were subjected to either optimum or low temperatures during the first weeks of life to determine environmental effects on the same variables. A commercial light-restriction program was used to maximize nutrient efficiency.

Humoral immune response of progeny was enhanced when hens consumed $ZnSO_4$ or when broilers were exposed to normal brooding temperatures. However, zinc source in broiler breeder hen and progeny diets did not influence livability or cellular immune response of broilers. Broilers subjected to cold brooding temperatures had increased feed intake, body weight ($P = 0.055$) and mortality, but brooding temperature had no effect on adjusted feed conversion. These data revealed that low brooding temperatures during the first weeks of life adversely affect livability and humoral immune response of broilers.

[2] Zinpro Corp., Eden Prairie, MN 55344

## DESCRIPTION OF PROBLEM

Classic signs of zinc deficiency produced by feeding purified diets to hens are low hatchability and underdevelopment of the skeletal and integumentary systems in progeny [1,2,3,4]. Hens receiving zinc from only natural feedstuffs may exhibit adverse hatchability and chick quality, while supplementing hen diets with zinc has enhanced fertility and hatchability [5], eggshell quality [6], egg production [7], progeny livability [8,9] and progeny immune status [10,11,12,13].

Bioavailability differences of zinc in various zinc sources may alter the reproductive performance of broiler breeder hens. Wedekind *et al.* [14] used slope ratio analyses to estimate the bioavailabilities of organic zinc-methionine, inorganic $ZnSO_4$ and inorganic ZnO in corn-soybean meal diets when fed to broiler chicks. Tibia zinc concentration was used as the response criterion. They reported that the ratios of slopes below the inflection points were 206% (zinc-methionine:$ZnSO_4$) and 61% (ZnO: $ZnSO_4$), indicating that zinc-methionine was more bioavailable than $ZnSO_4$ and ZnO at low concentrations. In addition, Pimental *et al.* [15] reported that pancreatic zinc concentrations were elevated by 33% when chicks were provided zinc-methionine rather than ZnO.

Diets supplemented with zinc from zinc-amino acid complexes have improved feed conversion (FC) in broilers. Hess *et al.* [16] supplemented practical broiler diets (55 ppm zinc from $ZnSO_4$) with 40 ppm zinc from three different zinc-amino acid complexes. Feed conversion was improved from 0 to 35 d and from 0 to 42 d of age when supplemental zinc-amino acid complexes were provided to female broilers. Increased dietary zinc concentration may have been responsible for the positive effects on broiler performance in the previously mentioned research. Sanford and Kawchumnong [17]

reported improved feed utilization of broilers when dietary zinc was supplemented as zinc-methionine rather than ZnO. These combined data suggest that both zinc concentration and zinc source influence feed efficiency in broilers.

Providing zinc-amino acid complexes in broiler breeder and turkey hen diets rather than inorganic zinc has enhanced cellular immune response of progeny as measured by cutaneous basophil hypersensitivity tests [10,11,12,13]. Livability of progeny has improved when providing supplemental zinc as a zinc-amino acid complex to hens [8,9]. This enhanced cellular immune response with zinc-amino acid complexes may relate to increased bioavailability compared to ZnO or $ZnSO_4$ [14].

The inability of young chicks to regulate their body temperature during the first days of life requires the use of supplemental heat in rearing facilities [18,19,20,21]. In practice, it is a challenge with some contract growers to maintain proper brooding temperatures, especially when fuel costs are elevated. Broiler chicks subjected to cold brooding temperatures are often found huddling together, which results in reduced feed and water intake. This behavior is known to increase seven-day mortality.

Previous research has evaluated the influences of delayed feed intake and low brooding temperature on broiler performance. According to Uni *et al.* [22], withholding feed for 36 hr slows development of the intestinal tract in chicks. Nutrients and antibodies from the yolk sac are poorly absorbed and immune status may be depressed [23] when chicks are exposed to sub-optimum ambient temperatures during the first weeks of life. Low brooding temperatures have increased mortality of broilers [24,25,26]. Cold stress increases the metabolic demand for oxygen [8], and insufficient oxygen increases occurrence of ascites in broilers [27]. These data reveal many

processes by which low brooding temperatures can negatively impact broiler performance.

Zinc from zinc-amino acid complexes may be more bioavailable to poultry [14]. Therefore, the chick may retain additional zinc during embryonic development when broiler breeder hens are fed zinc-amino acid complexes. Thus, performance and immune function of the chick may be adequate even during exposure to cold brooding temperatures. The objectives of this research were to determine the influences of zinc source in hen and progeny diets as well as low brooding temperature on performance and immune status of broilers.

## MATERIALS & METHODS

### BREEDER HUSBANDRY AND DIETARY ZINC

Two hundred-fifty Cobb[3] broiler breeder males and 750 broiler breeder females (slow-feathering Cobb 500) were obtained from a local hatchery and reared by primary breeder recommendations in single-sex floor pens. In order to determine feed allotments and achieve target BW, a sample of 24 hens per dietary treatment was weighed weekly. At 21 wk of age, all birds were housed in cages and provided 16 hr light/d. The facility was evaporatively cooled and forced-air heated. Supplemental zinc concentrations used in this experiment were based on average zinc level in commercial broiler breeder diets. Throughout the experiment, females were provided one of two experimental diets containing 160 ppm supplemental Zn from either $ZnSO_4$ or Availa®Zn 100 zinc-amino acid complex (ZnAA) (Table 4.1). Basal and zinc-supplemented breeder feeds were

[3] Cobb-Vantress, Inc., Siloam Springs, AR 72761-1030

formulated to contain 12 and 172 ppm zinc, respectively. After conducting Inductively Coupled Plasma (ICP) analyses [28] on duplicate feed samples throughout the experiment, zinc concentrations for basal and zinc-supplemented diets for the broiler breeder females were found to have 37±8 and 192±37 ppm zinc, respectively (n=8). Slight contrasts between formulated and determined values were likely due to an underestimation of actual zinc concentration in natural feedstuffs.

All males received a common diet containing $ZnSO_4$. Semen was collected from males by the abdominal massage technique [29]. Hens were artificially inseminated for four consecutive weeks with neat semen, and hatching eggs laid during 29 and 30 wk of age were incubated. Temperature settings from 0 to 18 and 19 to 21 d of incubation were 37.8 and 37.2°C, respectively. Relative humidity settings at 0 to 19 and 20 to 21 d of incubation were 53 and 70%, respectively. Chicks were removed from the hatcher and counted after 21.5 d of incubation.

BROILER HUSBANDRY AND DIETARY ZINC

A total of 2,016 day-old straight-run chicks were vent sexed and 21 of each sex were commingled and randomly assigned to one of 48 floor pens (0.084 $m^2$/bird). Broilers were provided diets containing 140 ppm supplemental zinc from either $ZnSO_4$ (140 ppm) or a mixture of $ZnSO_4$ (100 ppm) and ZnAA (40 ppm) (Table 4.2). Broiler diets were formulated to contain a total of 160 ppm zinc (20 ppm in basal diet). Actual zinc concentrations as determined by ICP analyses [28] revealed that basal and zinc-supplemented broiler diets contained 30±7 and 151±14 ppm zinc, respectively (n=3).

Normal (N) or low (L) brooding temperatures were provided from 0 to 20 d of age, and initial temperatures were 33.3 or 28.9°C for the N and L treatments, respectively.

Temperatures were reduced on five-day intervals and were the same (26.7°C) for both treatments by 20 d of age (Table 4.3). Each brooding room contained two forced air furnaces. Daily temperatures were determined from a central location in each brooding room at bird height. An average of the high and low temperatures was calculated daily. Feed and water were offered *ad libitum*, and the lighting program provided 23 hr light (20 Lux) from 0 to 3 d, 16 hr light (5 Lux) from 4 to 22 d, and 23 hr light (20 Lux) from 23 to 42 d. The lighting program was modified from those described by Classen and Riddell [30]. All birds were weighed individually at 17 d and by pen at 30 and 42 d. Feed intake was determined during the starter (0 – 17 d), grower (18 – 30 d), finisher (31 – 40 d) and cumulative (0 – 42 d) periods. Mortality was recorded daily and calculated during each phase of production. Adjusted FC during each phase of the growout were calculated and adjusted for mortality. The adjustment was made by altering the actual feed intake of each pen according to daily mortality [31].

IMMUNE MEASUREMENTS

To assess humoral immune status, two randomly selected birds per pen were intravenously injected with a 0.1 mL suspension of sheep red blood cells in phosphate-buffered saline (0.5% SRBC in PBS) at 10 d of age. Birds used for humoral status and other immune tests were identified by applying a small amount of paint to the wing feathers. At 15 d, blood was collected from the brachial vein and centrifuged at 700 *g* for 15 min. After obtaining plasma, it was stored at –4ºC until used to determine antibody titers to SRBC by hemagglutination testing. At 10 d of age, two additional birds per pen were given a 0.1 mL intradermal toe web injection of PBS and phytohemagglutinin-P (0.1 mg PHA-P in PBS) in the left and right feet, respectively. Digital calipers [32] were

used to measure toe web thickness prior to and 18 hr post-injection. To calculate cellular immune response, the postinjection width of the left (PBS injected) toe web was subtracted from the postinjection width of the right (PHA-P injected) toe web [33]. After measuring BW of two birds per pen at 17 d of age, the spleen, thymus and bursa of fabricius weights were determined to evaluate development of immune organs. Organ weights are presented as a percentage of body weight. No birds were used for more than one immune test.

STATISTICAL ANALYSIS

Statistical analysis was conducted using the General Linear Models procedure of SAS [34]. Pen served as the experimental unit. When the main factors or interactions were significant, the least squares means procedure was used to separate means. Within each brooding temperature split-plot treatment, birds were subjected to a randomized complete block design arrangement of 2 hen diets and 2 progeny diets. Experimental hen and progeny diets were each supplemented with two different zinc sources. All possible two-way and three-way interactions were included in the model and a significance level of $P \leq 0.05$ was used. Body weight uniformity was calculated as the coefficient of variation, and mortality data were subjected to arc sine transformation prior to statistical analysis. While conclusions were drawn from the transformed data, only nontransformed data are presented for relevance.

## RESULTS AND DISCUSSION

Differences in growth rate and nutrient utilization of broilers that could be attributable to dietary zinc were not obvious from placement to 17 d of age (Table 4.4). However, a

significant progeny zinc x temperature interaction occurred for feed intake, indicating that broilers provided supplemental $ZnSO_4$ consumed more starter feed than broilers provided ZnAA+$ZnSO_4$ when subjected to cold temperatures. Brooding temperature did not alter feed intake, BW gain, FC or BW uniformity of surviving broilers. Birds subjected to L temperatures had a higher incidence of mortality than birds exposed to N temperatures at 7 (Figure 4.1) and 17 d of age (Table 4.4).

From 18 to 30 d of age, zinc source in hen and progeny diets did not influence broiler performance (Table 4.5). Low brooding temperatures stimulated feed intake and growth rate during this period. In agreement, the indirect relationship between feed intake and temperature has been previously reported [35]. By optimizing brooding temperature, the maintenance needs of the broiler are reduced and more nutrients should be available for growth [36]. However, FC and the incidence of mortality in this study were not affected by brooding temperature during this period.

During the final phase of growout, supplemental zinc in the hen and progeny diets did not improve broiler performance with the exception of FC (Table 4.6). A significant hen zinc x progeny zinc source interaction indicated that FC was optimized when both hens and their progeny consumed ZnAA and ZnAA+$ZnSO_4$, respectively. Feed intake and FC during the finisher period were not influenced by brooding temperature, but mortality was significantly increased for broilers brooded at low temperatures.

Zinc source in hen or progeny diets did not alter final BW or cumulative feed consumption (Table 4.7). Although previous research has shown that feeding zinc-amino acid complexes to broiler breeder [9] or breeder layer hens [8] reduced early mortality of progeny, zinc source in hen and progeny diets did not influence mortality in this

experiment. A significant hen zinc source x progeny zinc source interaction indicated that cumulative FC was lowest when both hens and their progeny consumed ZnAA and ZnAA+$ZnSO_4$, respectively. However, the main contrasts of hen zinc source and progeny zinc source did not significantly affect FC.

In the present study, brooding temperature did not influence cumulative FC, but broilers subjected to L temperatures consumed more feed and had a higher incidence of total mortality throughout the 42-d growout period. Stimulatory effects of low brooding temperatures on final BW approached significance (P=0.055). Previous research has reported that final BW was unaffected by brooding temperature [37,38], but feed intake was increased with low ambient temperatures [35]. Other research has demonstrated poor nutrient utilization when broilers were subjected to low ambient temperatures [26,35,37,38,39]. In most cases, previous researchers used temperatures considerably lower (7.2 – 26.7°C) than those used in this experiment. Data presented here indicate that moderately low temperatures stimulate feed consumption and growth, but decrease broiler livability.

Thymus weight, bursa weight and cellular immune response of broilers were unaffected by zinc source in hen or progeny diets (Table 4.8). Spleen weights were increased when hens and their progeny consumed different zinc sources. Since the spleen is the site where B-cells proliferate into antibody-producing plasma cells [40], it was expected that spleen weights would be directly related to antibody production. However, there was no correlation between spleen weights and antibody titers to SRBC ($r = 0.017$, $P = 0.907$). Broilers expressed higher antibody titers to SRBC when their parent hen consumed $ZnSO_4$. Reasons for this occurrence are unclear, because previous

research has reported that humoral immune response of progeny are not affected when breeder hens consume zinc-amino acid complexes rather than inorganic zinc [10,11,13]. Prior research has reported enhanced cellular immune response of progeny when turkey or broiler breeder hens consume zinc complexes rather than inorganic zinc [10,11,12,13]. However, zinc source in hen or progeny diets had no influence on cellular immune status of progeny, possibly due to higher concentrations of dietary zinc used in this experiment compared to previous reports.

Brooding temperature did not influence organ weights or cellular immune response, but antibody titers to SRBC were greater for broilers kept at N temperatures. Previous research has not observed reduced antibody titers to SRBC in cold-stressed poultry [41,42,43], but such a relationship has been reported in heat-stressed broilers [44]. Thaxton and Siegel [44] suggested that elevated concentrations of plasma ACTH or glucocorticoids were responsible for immunodepression in heat-stressed chicks. A similar phenomenon may have occurred in chicks subjected to low temperatures in this experiment, decreasing humoral immune response. Further research is needed to evaluate the relationships between dietary zinc source, brooding temperature, and broiler performance.

## CONCLUSIONS AND APPLICATIONS

1. Humoral immune response of progeny was lower when hens were fed zinc in the form of Availa®Zn compared to zinc sulfate or when chicks were exposed to low brooding temperatures. These effects may limit the disease resistance of broilers.

2. Initial brooding temperature near 29°C increased feed intake and final BW (P = 0.055) of broilers, but increased incidence of mortality by 3.2%.

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Table 4.1. Ingredient composition and calculated nutrient analysis of the basal diets provided to broiler breeder females[A]

| Ingredients, % "as-is" | Starter[B] | Developer[C] | Breeder[D] |
|---|---|---|---|
| Corn | 62.96 | 58.81 | 68.15 |
| Soybean meal (48% CP) | 22.24 | 16.90 | 20.71 |
| Poultry oil | - | - | 1.00 |
| Wheat middlings | 10.53 | 20.10 | - |
| Limestone | 1.15 | 1.16 | 7.41 |
| Dicalcium phosphate | 1.75 | 1.76 | 1.46 |
| Salt | 0.54 | 0.58 | 0.54 |
| Lysine | 0.10 | - | - |
| Methionine | 0.15 | 0.06 | 0.14 |
| Trace mineral premix[E] | 0.08 | 0.08 | 0.08 |
| Vitamin premix[F] | 0.50 | 0.50 | 0.50 |
| Copper sulfate | 0.005 | 0.05 | 0.01 |
| Total | 100.00 | 100.00 | 100.00 |
| | | | |
| Calculated Analyses | | | |
| Crude protein (%) | 18.00 | 15.50 | 15.90 |
| Metabolizable energy (kcal/kg) | 2865 | 2819 | 2900 |
| Calcium (%) | 0.91 | 0.90 | 3.22 |
| Available phosphorus (%) | 0.45 | 0.42 | 0.38 |
| Lysine (%) | 1.00 | 0.78 | 0.83 |
| Methionine (%) | 0.43 | 0.32 | 0.40 |
| TSAA (%) | 0.73 | 0.60 | 0.66 |
| Sodium (%) | 0.21 | 0.22 | 0.21 |
| Copper (ppm) | 16 | 16 | 16 |
| Zinc (ppm) | 13 | 12 | 12 |

[A] Either zinc sulfate or Availa®Zn was added to provide 160 ppm supplemental zinc. Actual zinc concentrations were determined by inductively coupled plasma analysis. Analyzed zinc concentrations in basal and finished diets were 37 and 203 ppm.
[B] Diet was provided from 0 to 19 d of age to all females.
[C] Diet was provided from 20 days to 22 wk of age to all females.
[D] Diet was provided from 23 to 45 weeks of age to all females.
[E] Trace mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8.
[F] Vitamin premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

Table 4.2. Ingredient composition and calculated nutrient analysis of the basal diets fed to straight-run broilers during a 42 d production period[A]

| Ingredient, % "as-is" | Starter[B] | Grower[C] | Finisher[D] |
|---|---|---|---|
| Corn | 58.51 | 61.99 | 66.57 |
| Soybean meal (48% CP) | 34.00 | 27.95 | 25.55 |
| Poultry meal (60% CP) | - | 2.20 | - |
| Poultry oil | 3.17 | 4.22 | 4.31 |
| Limestone | 1.10 | 0.96 | 0.84 |
| Dicalcium phosphate | 1.73 | 1.25 | 1.31 |
| Lysine | 0.01 | - | - |
| Methionine | 0.24 | 0.21 | 0.20 |
| Salt | 0.53 | 0.51 | 0.51 |
| Copper sulfate | 0.05 | 0.05 | 0.05 |
| Coban | 0.08 | 0.08 | 0.08 |
| Mineral premix[E] | 0.08 | 0.08 | 0.08 |
| Vitamin premix[F] | 0.50 | 0.50 | 0.50 |
| Total | 100.00 | 100.00 | 100.00 |
| | | | |
| Calculated Analyses | | | |
| Crude protein (%) | 21.57 | 20.20 | 18.16 |
| Metabolizable energy (kcal/kg) | 3075 | 3165 | 3240 |
| Calcium (%) | 0.91 | 0.82 | 0.70 |
| Av. Phosphorus (%) | 0.45 | 0.40 | 0.36 |
| Lysine (%) | 1.21 | 1.08 | 0.96 |
| Methionine (%) | 0.58 | 0.53 | 0.50 |
| TSAA (%) | 0.92 | 0.85 | 0.79 |
| Sodium (%) | 0.21 | 0.21 | 0.21 |
| Copper (ppm) | 130 | 130 | 130 |
| Zinc (ppm) | 20 | 20 | 20 |

[A] Supplemental zinc was provided by either zinc sulfate (140 ppm) or a combination of both Availa®Zn (40 ppm) and zinc sulfate (100 ppm). Actual zinc concentrations were determined by inductively coupled plasma analysis. Analyzed zinc concentrations in basal and finished diets were 30 and 143 ppm, respectively.

[B] Diet was provided from 0 to 17 d of age.

[C] Diet was provided from 18 to 30 d of age.

[D] Diet was provided from 31 to 42 d of age.

[E] Trace mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8.

[F] Vitamin mineral premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

Table 4.3. Temperature set points and actual temperatures (°C) provided to straight-run broilers subjected to two different brooding temperatures during a 42 d production period[A]

| | Temperature Setpoint | | Actual Temperature[B] ± SD | |
|---|---|---|---|---|
| Age, days | Normal | Low | Normal | Low |
| 0-4 | 33.3 | 28.9 | 32.7±0.5 | 28.6±0.2 |
| 5-9 | 32.2 | 28.3 | 32.1±0.5 | 28.2±0.5 |
| 10-14 | 28.9 | 27.8 | 29.6±1.5 | 27.3±0.3 |
| 15-19 | 27.8 | 27.2 | 27.8±0.5 | 26.9±0.2 |
| 20-24 | 26.7 | 26.7 | 26.6±0.5 | 26.3±0.3 |
| 25-29 | 25.6 | 25.6 | 25.6±0.4 | 25.4±0.4 |
| 30-34 | 23.9 | 23.9 | 24.0±0.5 | 24.3±0.6 |
| 35-39 | 22.2 | 22.2 | 23.2±0.5 | 23.3±0.7 |
| 40-42 | 21.1 | 21.1 | 24.3±0.8 | 24.0±0.6 |

[A] Broilers were exposed to normal or low brooding temperatures prior to 20 d of age.
[B] Calculated as an average of the average daily temperatures during the periods indicated.

Table 4.4. Live performance responses of broilers originating from hens fed diets with two different zinc sources, subjected to two different brooding temperatures and fed two different zinc sources from placement until 17 d of age[A]

| Treatments | | | Feed Intake (kg) | BW (kg) gain 0-17 d | FC[C] 0-17 d | Total Mortality (%) 0-17 d | CV of BW at 17 d (%) |
|---|---|---|---|---|---|---|---|
| Hen Zn | Progeny Zn | Temp.[B] | | | | | |
| $ZnSO_4$ | $ZnSO_4$ | N | 0.681 | 0.520 | 1.311 | 1.19 | 10.9 |
| $ZnSO_4$ | $ZnSO_4$ | L | 0.697 | 0.528 | 1.320 | 1.59 | 12.9 |
| $ZnSO_4$ | ZnAA | N | 0.691 | 0.524 | 1.321 | 0.40 | 11.2 |
| $ZnSO_4$ | ZnAA | L | 0.685 | 0.518 | 1.323 | 4.37 | 13.8 |
| ZnAA | $ZnSO_4$ | N | 0.690 | 0.517 | 1.336 | 1.98 | 13.2 |
| ZnAA | $ZnSO_4$ | L | 0.703 | 0.533 | 1.318 | 2.78 | 10.5 |
| ZnAA | ZnAA | N | 0.693 | 0.525 | 1.321 | 3.57 | 11.3 |
| ZnAA | ZnAA | L | 0.680 | 0.518 | 1.314 | 6.35 | 13.9 |
| SEM | | | 0.008 | 0.007 | 0.011 | 1.35 | 1.1 |
| Sources of Variation | | | | | | | |
| Hen Zn | | | NS | NS | NS | NS | NS |
| Progeny Zn | | | NS | NS | NS | NS | NS |
| Temperature | | | NS | NS | NS | * | NS |
| Hen Zn x Progeny Zn | | | NS | NS | NS | NS | NS |
| Hen Zn x Temperature | | | NS | NS | NS | NS | NS |
| Progeny Zn x Temperature | | | * | NS | NS | NS | NS |
| Hen Zn x Progeny Zn x Temp. | | | NS | NS | NS | NS | NS |

[A] Values are least-squares means involving 48 pens, each with 42 chicks at placement. Chicks resulted from eggs laid during 29 and 30 wk of age. Average chick weight with standard error was 37.5 $\pm$ 0.2 g at hatch.

[B] Broilers were exposed to normal (N) or low (L) brooding temperatures prior to 20 d of age.

[C] Feed conversion values are corrected for mortality.

NS, $P \geq 0.05$.

*, $0.01 < P \leq 0.05$.

Table 4.5. Live performance responses of broilers originating from hens fed diets with two different zinc sources, subjected to two different brooding temperatures and fed two different zinc sources from 18 to 30 d of age[A]

| Treatments | | | Feed Intake (kg) 18-30 d | BW (kg) gain 17-30 d | FC[C] 18-30 d | Total Mortality (%) 18-30 d |
|---|---|---|---|---|---|---|
| Hen Zn | Progeny Zn | Temperature[B] | | | | |
| $ZnSO_4$ | $ZnSO_4$ | N | 1.407 | 0.996 | 1.412 | 0.83 |
| $ZnSO_4$ | $ZnSO_4$ | L | 1.477 | 1.053 | 1.403 | 1.20 |
| $ZnSO_4$ | ZnAA | N | 1.417 | 0.999 | 1.419 | 1.20 |
| $ZnSO_4$ | ZnAA | L | 1.463 | 1.038 | 1.410 | 0.83 |
| ZnAA | $ZnSO_4$ | N | 1.392 | 0.997 | 1.396 | 0.79 |
| ZnAA | $ZnSO_4$ | L | 1.500 | 1.059 | 1.417 | 0.41 |
| ZnAA | ZnAA | N | 1.413 | 1.001 | 1.412 | 1.26 |
| ZnAA | ZnAA | L | 1.472 | 1.053 | 1.398 | 2.04 |
| SEM | | | 0.0176 | 0.0100 | 0.0084 | 0.644 |
| | | | | | | |
| Sources of Variation | | | | | | |
| Hen Zn | | | NS | NS | NS | NS |
| Progeny Zn | | | NS | NS | NS | NS |
| Temperature | | | * | *** | NS | NS |
| Hen Zn x Progeny Zn | | | NS | NS | NS | NS |
| Hen Zn x Temperature | | | NS | NS | NS | NS |
| Progeny Zn x Temperature | | | NS | NS | NS | NS |
| Hen Zn x Progeny Zn x Temp. | | | NS | NS | NS | NS |

[A] Values are least-squares means involving 48 pens, each with 42 chicks at placement.
[B] Broilers were exposed to normal (N) or low (L) brooding temperatures prior to 20 d of age.
[C] Feed conversion values are corrected for mortality.
NS, $P \geq 0.05$.
*, $0.01 < P \leq 0.05$.
***, $P \leq 0.001$.

Table 4.6. Live performance responses of broilers originating from hens fed diets with two different zinc sources, subjected to two different brooding temperatures and fed two different zinc sources from 31 to 42 d of age[A]

| Treatments | | | Feed Intake (kg) 31-42 d | BW (kg) gain 31-42 d | FC 31-42 d[C] | Total Mortality (%) 31-42 d |
|---|---|---|---|---|---|---|
| Hen Zn | Progeny Zn | Temperature[B] | | | | |
| $ZnSO_4$ | $ZnSO_4$ | N | 1.905 | 0.998 | 1.908 | 0.83 |
| $ZnSO_4$ | $ZnSO_4$ | L | 1.930 | 1.012 | 1.908 | 2.58 |
| $ZnSO_4$ | ZnAA | N | 1.875 | 0.987 | 1.901 | 0.84 |
| $ZnSO_4$ | ZnAA | L | 1.863 | 0.960 | 1.945 | 1.70 |
| ZnAA | $ZnSO_4$ | N | 1.908 | 0.995 | 1.919 | 0.00 |
| ZnAA | $ZnSO_4$ | L | 1.908 | 0.988 | 1.931 | 1.70 |
| ZnAA | ZnAA | N | 1.878 | 1.012 | 1.858 | 0.45 |
| ZnAA | ZnAA | L | 1.905 | 1.002 | 1.902 | 0.85 |
| SEM | | | 0.0228 | 0.0143 | 0.0189 | 0.581 |
| | | | | | | |
| Sources of Variation | | | | | | |
| Hen Zn | | | NS | NS | NS | NS |
| Progeny Zn | | | NS | NS | NS | NS |
| Temperature | | | NS | NS | NS | * |
| Hen Zn x Progeny Zn | | | NS | NS | * | NS |
| Hen Zn x Temperature | | | NS | NS | NS | NS |
| Progeny Zn x Temperature | | | NS | NS | NS | NS |
| Hen Zn x Progeny Zn xTemp. | | | NS | NS | NS | NS |

[A] Values are least-squares means involving 48 pens, each with 42 chicks at placement.
[B] Broilers were exposed to normal (N) or low (L) brooding temperatures prior to 20 d of age.
[C] Feed conversion values are corrected for mortality.
NS, $P \geq 0.05$.
*, $0.01 < P \leq 0.05$.

Table 4.7. Final live performance responses of broilers originating from hens fed diets with two different zinc sources, subjected to two different brooding temperatures and fed two different zinc sources from placement to 42 d of age[A]

| Treatments | | | Total Feed Intake (kg) | BW (kg) 42 d | FC 0-42 d[C] | Total Mortality (%) 0-42 d |
|---|---|---|---|---|---|---|
| Hen Zn | Progeny Zn | Temperature[B] | | | | |
| $ZnSO_4$ | $ZnSO_4$ | N | 3.992 | 2.552 | 1.588 | 2.81 |
| $ZnSO_4$ | $ZnSO_4$ | L | 4.104 | 2.630 | 1.583 | 5.30 |
| $ZnSO_4$ | ZnAA | N | 3.983 | 2.547 | 1.587 | 2.42 |
| $ZnSO_4$ | ZnAA | L | 4.012 | 2.553 | 1.595 | 6.83 |
| ZnAA | $ZnSO_4$ | N | 3.990 | 2.547 | 1.590 | 2.78 |
| ZnAA | $ZnSO_4$ | L | 4.111 | 2.618 | 1.593 | 4.84 |
| ZnAA | ZnAA | N | 3.985 | 2.575 | 1.571 | 5.18 |
| ZnAA | ZnAA | L | 4.057 | 2.610 | 1.577 | 9.17 |
| SEM | | | 0.0399 | 0.0253 | 0.0077 | 1.252 |
| | | | | | | |
| Sources of Variation | | | | | | |
| Hen Zn | | | NS | NS | NS | NS |
| Progeny Zn | | | NS | NS | NS | NS |
| Temperature | | | * | NS | NS | ** |
| Hen Zn x Progeny Zn | | | NS | NS | * | NS |
| Hen Zn x Temperature | | | NS | NS | NS | NS |
| Progeny Zn x Temperature | | | NS | NS | NS | NS |
| Hen Zn x Progeny Zn x Temp. | | | NS | NS | NS | NS |

[A] Values are least-squares means involving 48 pens, each with 42 chicks at placement. Chicks resulted from eggs laid during 29 and 30 wk of age. Average chick weight with standard error was 37.5 $\pm$ 0.2 g at hatch.

[B] Broilers were exposed to normal (N) or low (L) brooding temperatures prior to 20 d of age.

[C] Feed conversion values are corrected for mortality.

NS, $P \geq 0.05$.

*, $0.01 < P \leq 0.05$.

**, $P \leq 0.01$

Table 4.8. Immune responses of broilers originating from hens fed diets with two different zinc sources, subjected to two different brooding temperatures and fed two different zinc sources[A]

| Treatments | | | Bursa (% of BW) | Thymus (% of BW) | Spleen (% of BW) | SRBC titer[C] (log 2) | PHA-P[D] |
|---|---|---|---|---|---|---|---|
| Hen Zn | Progeny Zn | Temp.[B] | | | | | |
| $ZnSO_4$ | $ZnSO_4$ | N | 0.183 | 0.528 | 0.071 | 3.83 | 0.88 |
| $ZnSO_4$ | $ZnSO_4$ | L | 0.197 | 0.563 | 0.068 | 3.13 | 0.86 |
| $ZnSO_4$ | ZnAA | N | 0.181 | 0.492 | 0.086 | 3.13 | 0.93 |
| $ZnSO_4$ | ZnAA | L | 0.172 | 0.532 | 0.087 | 2.79 | 0.88 |
| ZnAA | $ZnSO_4$ | N | 0.190 | 0.541 | 0.085 | 2.75 | 0.75 |
| ZnAA | $ZnSO_4$ | L | 0.192 | 0.569 | 0.089 | 2.54 | 0.83 |
| ZnAA | ZnAA | N | 0.185 | 0.506 | 0.078 | 3.17 | 0.77 |
| ZnAA | ZnAA | L | 0.195 | 0.548 | 0.074 | 2.08 | 0.91 |
| SEM | | | 0.0124 | 0.0264 | 0.0042 | 0.373 | 0.063 |
| Sources of Variation | | | | | | | |
| Hen Zn | | | NS | NS | NS | * | NS |
| Progeny Zn | | | NS | NS | NS | NS | NS |
| Temperature | | | NS | NS | NS | * | NS |
| Hen Zn x Progeny Zn | | | NS | NS | *** | NS | NS |
| Hen Zn x Temperature | | | NS | NS | NS | NS | NS |
| Progeny Zn x Temperature | | | NS | NS | NS | NS | NS |
| Hen Zn x Progeny Zn x Temperature | | | NS | NS | NS | NS | NS |

[A] Spleen, thymus and bursa of Fabricius were harvested from 17 d old broilers. There were no treatment effects (P>0.05) on BW of the sampled birds. Values are least-squares means involving 48 pens, each with 42 chicks at placement. Immune measurements were conducted on two randomly selected birds from each pen.

[B] These broilers were exposed to normal (N) or low (L) brooding temperatures prior to being sampled.

[C] Hemagglutination tests were done to assess antibody titers to sheep red blood cells (SRBC) after intravenously injecting 0.1mL of a suspension (0.5% SRBC) at 10 d of age, then collecting plasma at 15 d of age.

[D] At 10 d of age, birds were given a 0.1 mL intradermal injection of saline and phytohemagglutinin-P (0.1mg PHA-P in suspension) between the second and third digits of the left and right feet, respectively. At 18 hr post-injection, the width of the left (saline-injected) toe web was subtracted from the postinjection width of the right (PHA-injected) toe web.

NS, $P \geq 0.05$.

*, $0.01 < P \leq 0.05$.

***, $P \leq 0.001$.

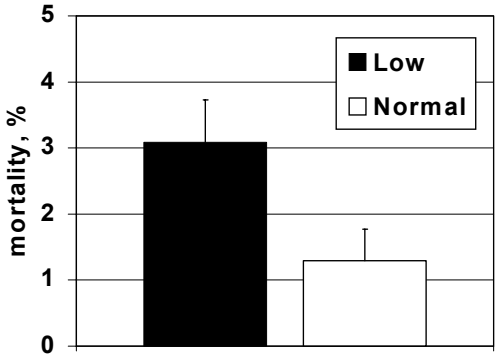


Figure 4.1. Seven-day mortality of broilers exposed to normal or low brooding temperatures (mean $\pm$ SEM). The difference between the two groups was significant ($P < 0.05$).

# CHAPTER 5

# EFFECTS OF SUPPLEMENTAL ZINC SOURCE IN BROILER BREEDER AND PROGENY DIETS ON BROILER LIVE PERFORMANCE[1]

[1] Hudson, B.P., W. A. Dozier, III, J. L. Wilson and T. L. Ward. To be submitted to *Poultry Science*

**ABSTRACT**

An experiment was conducted to examine the effects of zinc source or zinc concentrations in caged broiler breeder hen diets on progeny performance. In addition, progeny were provided diets supplemented with inorganic zinc or a mixture of the inorganic source and a zinc-amino acid complex. Slow-feathering, Cobb 500 broiler breeder hens were given one of four experimental diets containing 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn[2] zinc-amino acid complex (ZnAA), a mixture of $ZnSO_4$ and ZnAA ($ZnSO_4$ + ZnAA, 80 ppm Zn from each), or no supplemental zinc after 20 wk (BASAL). Prior to 20 wk, hens in the BASAL treatment were fed diets containing 160 ppm supplemental zinc. Hatching eggs laid during 43 wk were incubated and hatched. Male chicks were given feeds containing 140 ppm supplemental zinc from either $ZnSO_4$ (140 ppm) or $ZnSO_4$ + ZnAA ($ZnSO_4$, 100 ppm Zn; ZnAA, 40 ppm Zn) during a 17 d growth assay. Providing supplemental zinc to hens in the form of $ZnSO_4$ or ZnAA did not improve progeny performance. At 17 d, broilers fed $ZnSO_4$ + ZnAA had lower feed conversion and higher BW than broilers fed $ZnSO_4$. These data indicate that broiler performance may be improved when replacing 40 ppm dietary zinc from $ZnSO_4$ with 40 ppm zinc from Availa®Zn zinc-amino acid complex.

(*Key words*: broiler breeder, broiler performance, dietary zinc, $ZnSO_4$, Availa®Zn zinc-amino acid complex)

[2] Zinpro Corp., Eden Prairie, MN 55344

## INTRODUCTION

For optimum development of the embryo, it is essential that the hen deposit an adequate amount of the nutrients into the hatching egg. Classic signs of embryos or chicks resulting from hens provided zinc-deficient diets are low hatchability, embryonic malformations, weak chicks with poor feathering, and a high incidence of mortality (Supplee *et al*., 1958; Turk *et al.*, 1959; Blamberg *et al.*, 1960; Kienholz *et al.*, 1961). Reduced weight gain, shortening and thickening of leg bones, enlarged hocks, reduced bone ash and poor feather development may also occur when zinc intake by chicks is insufficient (O'Dell and Savage, 1957; Edwards *et al.*, 1958; Young *et al.*, 1958). In contrast, research with layer breeders has indicated that egg production, fertility and hatchability are not improved by zinc supplementation of corn-soy diets (Stahl *et al.*, 1986; Abdallah *et al.*, 1994). However, providing corn-soybean meal diets with supplemental zinc to broiler breeder hens enhances cellular (Kidd *et al.*, 1992; Kidd *et al.*, 1993) and humoral immune status (Stahl *et al.*, 1989; Kidd *et al.*, 1993), increases thymus weight (Virden *et al.*, 2002a) and improves livability of progeny (Virden *et al.*, 2002b; Flinchum *et al.*, 1989).

Zinc from amino acid complexes has been reported to be more bioavailable than zinc from inorganic sources (Wedekind *et al.*, 1992). In addition, Spears (1989) reported that zinc retention in ruminants was increased when providing zinc from a complexed zinc source rather than an inorganic source. Supplementing breeder hen diets with zinc-amino acid complexes improved livability and/or immune status of progeny (Flinchum *et al.*, 1989; Kidd *et al.*, 1992; Virden *et al.*, 2002a; Virden *et al.*, 2002b). Sanford and

Kawchumnong (1972) indicated that diets supplemented with zinc-methionine rather than ZnO improved feed efficiency of broilers.

This study examined the performance of chicks provided diets supplemented with zinc from a combination of $ZnSO_4$ (100 ppm) and ZnAA (40 ppm) or solely $ZnSO_4$ (140 ppm). Furthermore, the chicks originated from broiler breeder hens that were given a diet without supplemental zinc or diets supplemented with different sources of zinc.

## MATERIALS AND METHODS

Two hundred-fifty broiler breeder males (Cobb[3]) and 750 slow-feathering broiler breeder females (Cobb 500) were reared by primary breeder recommendations in single-sex floor pens. In order to determine feed allotments and achieve target BW, the same sample of 24 females/trt was weighed weekly through 40 wk and biweekly thereafter. A standard vaccination program was used. At 21 wk of age, all birds were housed in cages and provided 16 hr light/d. The facility was evaporatively cooled and forced air heated.

Caged broiler breeder hens were fed corn-soybean meal based diets formulated to contain nutrient concentrations similar to industry specifications (Agri Stats, 2002) with the exception of zinc (Table 5.1). Four experimental diets were prepared by adding 160 ppm of zinc from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), a mixture of $ZnSO_4$ and Availa®Zn ($ZnSO_4$ + ZnAA, 80 ppm zinc from each), or were given no supplemental zinc (BASAL) (Table 5.1). Prior to 20 wk of age, hens in the BASAL treatment were given diets containing 160 ppm supplemental zinc. Determined zinc

[3] Cobb-Vantress, Inc., Siloam Springs, AR 72761-1030

concentrations[4] in zinc-supplemented (n=14) and basal broiler breeder diets (n=14) were 185 and 37 ppm, respectively, which were higher than formulated values (zinc-supplemented = 172 ppm; basal = 12 ppm). Differences between formulated and determined concentrations were due to an underestimation of zinc content of natural feedstuffs.

Hens were fertilized by artificial insemination, and hatching eggs laid during 43 wk of age were incubated and hatched. A total of 336 d-old broiler male chicks was feather-sexed and seven chicks were randomly assigned to one of 48 stainless steel battery cages[5]. Broilers were provided feeds containing 140 ppm supplemental zinc from either $ZnSO_4$ (140 ppm) or a mixture of $ZnSO_4$ (100 ppm) and ZnAA (40 ppm) (Table 5.2). Determined zinc concentration in broiler diets averaged 214 ppm zinc, which was higher than the calculated value (160 ppm). Too few observations (n=2) may have caused the discrepancy between determined and calculated zinc concentrations in broiler diets. Feed and water were offered *ad libitum*, and constant lighting was provided. At 17 d of age, all birds were weighed and feed intake was determined. Feed conversion was calculated after adjusting for daily mortality. Since many birds showed signs of chondrodystrophy, the the number of birds with any leg abnormality was recorded upon completion of the experiment.

Data were analyzed using the General Linear Model procedure of SAS (1996) and battery cage served as the experimental unit. The treatment structure was a 4 x 2 factorial arrangement with zinc source in the breeder hen diets and zinc source in the

[4] Inductively Coupled Plasma analysis, Agricultural Services Laboratory, Athens, GA 30602
[5] Petersime Incubator Co., Gettysburg, OH 45328

broiler diets as the main contrasts. The results are presented as main contrasts since no significant interactions were apparent for the variables tested in this study. A significance level of $P \leq 0.05$ was used. All percentage data were subjected to arc sine transformation to obtain homogenous variances. While conclusions were drawn from the transformed data, only untransformed data are presented for relevance.

## RESULTS AND DISCUSSION

Hen zinc treatment had no influence on chick performance or incidence of mortality (Table 5.3). Although the detrimental effects of zinc deficient hens on hatchability and chick quality have been well documented (Supplee *et al*., 1958; Turk *et al.*, 1959; Blamberg *et al.*, 1960; Kienholz *et al.*, 1961), the BASAL zinc concentrations in this study did not yield such negative results on chick performance. Providing a diet with adequate zinc to broilers may have negated adverse effects of low zinc intake by breeder hens. Emmert and Baker (1995) reported that sufficient BW gains were achieved in broilers consuming diets with only 10.6 ppm zinc. However, 10.6 ppm dietary zinc did not allow the birds to accumulate zinc reserves in tissues for subsequent periods of zinc depletion.

Previous research conducted in our laboratory (B. Hudson *et al*., unpublished data) revealed no effects of supplemental zinc source in broiler diets on feed conversion or BW at 17 d of age. In addition, Kidd *et al.* (1992) previously reported that broiler performance was not affected by supplementing diets with either inorganic ZnO or zinc-methionine complex. However, providing broilers with ZnAA+$ZnSO_4$ in this experiment

improved feed conversion and BW at 17 d of age, but did not affect feed intake, the occurrence of mortality or the incidence of leg abnormalities (Table 5.3). Davis *et al.* (2003) determined that tom turkeys provided diets supplemented with 40 ppm of zinc from ZnAA had a lower incidence of leg abnormalities than birds given diets with added zinc from an inorganic source. However, dietary zinc did not influence the incidence of leg abnormalities in the present study. Rapid growth or use of wire flooring may have caused relatively high incidence of leg abnormalities for all treatments.

Wedekind *et al.* (1992) suggested that antagonistic effects of phytic acid on zinc absorption are reduced when chicks are fed zinc-methionine rather than ZnO or $ZnSO_4$. Methionine-bound zinc is devoid of divalent cations, which may reduce phytic acid chelation in the lumen (Kidd *et al.*, 1996). It is possible that such an effect occurred when ZnAA+$ZnSO_4$ was provided to broilers, increasing zinc absorption and enhancing zinc-requiring functions of the body. Spears (1989) reported that ruminants absorbed zinc from zinc-methionine and ZnO similarly, but those consuming zinc-methionine retained more zinc. The author suggested that zinc from zinc-methionine was metabolized differently once absorbed. Zinc sulfate and ZnAA may complement each other in broiler diets during the absorption process, enhancing nutrient utilization and growth rate.

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Table 5.1. Ingredient composition and calculated nutrient analysis of the basal feeds provided to the broiler breeder females[A]

| Ingredients, % "as-is" | Starter[B] | Developer[C] | Breeder[D] |
|---|---|---|---|
| Corn | 62.95 | 58.81 | 68.15 |
| Soybean meal (48% CP) | 22.24 | 16.90 | 20.71 |
| Poultry oil | ... | ... | 1.00 |
| Wheat middlings | 10.53 | 20.10 | ... |
| Limestone | 1.15 | 1.16 | 7.41 |
| Dicalcium phosphate | 1.75 | 1.76 | 1.46 |
| Salt | 0.54 | 0.58 | 0.54 |
| Lysine | 0.10 | ... | ... |
| Methionine | 0.15 | 0.06 | 0.14 |
| Trace mineral premix[E] | 0.08 | 0.08 | 0.08 |
| Vitamin premix[F] | 0.50 | 0.50 | 0.50 |
| Copper sulfate | 0.01 | 0.05 | 0.01 |
| Total | 100.00 | 100.00 | 100.00 |
| | | | |
| Calculated Analyses | | | |
| Crude protein (%) | 18.00 | 15.50 | 15.90 |
| Metabolizable energy (kcal/kg) | 2865 | 2819 | 2900 |
| Calcium (%) | 0.91 | 0.90 | 3.22 |
| Available phosphorus (%) | 0.45 | 0.42 | 0.38 |
| Lysine (%) | 1.00 | 0.78 | 0.83 |
| Methionine (%) | 0.43 | 0.32 | 0.40 |
| TSAA (%) | 0.73 | 0.60 | 0.66 |
| Sodium (%) | 0.21 | 0.22 | 0.21 |
| Copper (ppm) | 16 | 16 | 16 |
| Zinc (ppm) | 13 | 12 | 12 |

[A] Experimental diets consisted of 160 ppm supplemental zinc from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), a mixture of $ZnSO_4$ and ZnAA (80 ppm zinc from each), or no supplemental zinc after 20 wk of age (BASAL). Analyzed zinc concentration in basal and supplemented diets was 37 and 195 ppm, respectively.

[B] Feed was provided from 0 to 19 d of age to all females.

[C] Feed was provided from 20 d to 22 wk of age to all females.

[D] Feed was provided from 23 to 45 wk of age to all females.

[E] Trace mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8.

[F] Vitamin premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

Table 5.2. Ingredient composition and calculated nutrient analysis of the basal diets fed to male broilers during a 17 d production period[A]

| Ingredient, % "as-is" | Starter |
|---|---|
| Corn | 58.51 |
| Soybean meal (48% CP) | 34.00 |
| Poultry oil | 3.17 |
| Limestone | 1.10 |
| Dicalcium phosphate | 1.73 |
| Lysine | 0.01 |
| Methionine | 0.24 |
| Salt | 0.53 |
| Copper sulfate | 0.05 |
| Coban | 0.08 |
| Mineral premix[B] | 0.08 |
| Vitamin premix[C] | 0.50 |
| Total | 100.00 |
| | |
| Calculated Analyses | |
| Crude protein (%) | 21.57 |
| Metabolizable energy (kcal/kg) | 3075 |
| Calcium (%) | 0.91 |
| Available phosphorus (%) | 0.45 |
| Lysine (%) | 1.21 |
| Methionine (%) | 0.58 |
| TSAA (%) | 0.92 |
| Sodium (%) | 0.21 |
| Copper (ppm) | 130 |
| Zinc (ppm) | 20 |

[A] Supplemental zinc was provided by either $ZnSO_4$ (140 ppm) or a combination of both Availa®Zn (40 ppm) and $ZnSO_4$ (100 ppm). Analyzed zinc concentration in broiler diets averaged 214 ppm, which included zinc content in the basal diet.

[B] Trace mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8.

[C] Vitamin mineral premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

Table 5.3. Live performance responses of male broilers originating from hens provided two different zinc sources and levels of supplementation, and fed two different zinc sources from placement until 17 d of age[A]

| Contrast | Feed Intake (kg) | BW gain (kg) 0 - 17 d | Feed Conversion[B] 0 - 17 d | Total Mortality (%) 0 - 17 d | Incidence of Leg Abnormalities (%) |
|---|---|---|---|---|---|
| Hen Zn[C] | NS | NS | NS | NS | NS |
| $ZnSO_4$ | 0.707 | 0.530 | 1.335 | 0.00 | 4.76 |
| ZnAA | 0.708 | 0.526 | 1.347 | 1.19 | 3.57 |
| | 0.716 | 0.533 | 1.343 | 3.57 | 1.19 |
| $ZnSO_4$+ZnAA | | | | | |
| BASAL | 0.713 | 0.532 | 1.339 | 2.38 | 6.15 |
| SEM | 0.010 | 0.009 | 0.009 | 1.30 | 2.12 |
| | | | | | |
| Progeny Zn[D] | NS | * | *** | NS | NS |
| $ZnSO_4$ | 0.705 | 0.519 [b] | 1.359 [a] | 0.60 | 2.38 |
| | 0.717 | 0.542 [a] | 1.323 [b] | 2.98 | 5.46 |
| $ZnSO_4$+ZnAA | | | | | |
| SEM | 0.007 | 0.006 | 0.007 | 0.92 | 1.50 |

[A] Values are least-squares means involving 48 battery cages, each with seven chicks at placement. Chicks resulted from eggs laid at 43 wk of age. Average chick weight with standard deviation was 42.6 $\pm$ 0.9 g at hatch.

[B] Values are corrected for mortality.

[C] Hens were given one of four diets. Diets consisted of 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), a mixture of $ZnSO_4$ and ZnAA ($ZnSO_4$ + ZnAA, 80 ppm zinc from each), or no supplemental zinc after 20 wk of age (BASAL).

[D] Experimental diets consisted of 140 ppm supplemental zinc from either $ZnSO_4$ (140 ppm) or a combination of both Availa®Zn (40 ppm) and $ZnSO_4$ (100 ppm).

[a-b] Means with different superscripts within a column signify significant differences.

NS, $P \geq 0.05$.

*, $0.01 < P \leq 0.05$.

***, $P \leq 0.001$.

# CHAPTER 6

# EFFECT OF BROILER BREEDER AGE AND ZINC SOURCE IN BROILER BREEDER HEN DIETS ON PROGENY CHARACTERISTICS AT HATCHING[1]

[1] Hudson, B.P., B. D. Fairchild, J. L. Wilson and W. A. Dozier, III. To be submitted to *Journal of Applied Poultry Research*

**SUMMARY**

Status of day-old chicks can impact the incidence of morbidity and mortality of broilers during the first weeks of production. Chick quality assessments have been made primarily by subjective observations with few quantitative measurements. Given a better understanding of how broiler breeder hen age and nutrient intake influence physiology of their progeny, certain actions can be taken to improve chick quality. This experiment evaluated the effects of broiler breeder age and dietary zinc source on physiological characteristics of chicks. Caged broiler breeder hens were provided one of three diets from hatch through 65 wk of age. All experimental diets consisted of 160 ppm supplemental zinc from inorganic $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA) or a mixture of ZnAA and $ZnSO_4$ (ZnAA + $ZnSO_4$, 80 ppm zinc from each). Incubation time, selected organ development and carbohydrate metabolism were measured in chicks from hens at 29, 41, 53 and 65 wk of age. Variation of hatching time was not influenced by hen age, but mean incubation time decreased from 29 to 53 wk of age and subsequently increased from 53 to 65 wk. Relative yolk sac weight and relative heart weight were decreased in progeny from 29 wk-old hens. Chick heart glycogen declined as hens aged, and liver lactate was lowest in progeny from 65 wk-old hens. These data indicate that underdevelopment of some supply organs may limit the performance of chicks from young hens. Supplemental zinc source in breeder hen diets did not influence chick physiology at hatching.

## DESCRIPTION OF PROBLEM

Progeny from young broiler breeder hens are typically small and may perform poorly, especially when intermingled with larger chicks. McNaughton *et al.* [1] reported that mortality of broilers from 29 wk-old breeders was approximately 3% greater than that of broilers from 58 wk-old breeders. Evaluating chicks from broiler breeders at various stages of the reproductive period may help expose problems causing inadequate performance in chicks. Sequentially, these problems may be addressed to improve quality of progeny throughout the reproductive period.

There is no universally accepted method to evaluate chick quality. Traditionally, subjective assessments have been made by observing physical characteristics. Cervantes [2] developed a system in which chicks were scored based on physical (50%) and microbiological (50%) status. Physical parameters evaluated were chick weight, overall appearance, hydration, alertness, and appearance of eyes, navel, vent, hocks, legs, toes and beak. Microbiological score was based upon presence of *Salmonella* (yolk sac, ileocecal junction), *Coliforms* (yolk sac), *Aspergillus* (lung) and *Staphylococci* (yolk sac). In addition to parameters observed by Cervantes [2], physiological development of the embryo and chick will alter hatching and subsequent performance.

The yolk is the primary provider of nutrients to the embryo and newly hatched chick. Just prior to hatching, the remaining yolk is internalized and comprises 20 to 25% of BW for chicks [3]. At this time, the yolk contains approximately 50% lipids and supplies energy for maintenance during the initial days after hatching [3]. The embryo's oxygen demand increases with age and its oxygen supply decreases near the end of embryonic development [4], requiring the embryo to use anaerobic metabolism for energy

production [5]. Glycogen is formed during embryonic development by gluconeogenesis and utilized as an energy source during the hypoxic conditions of pipping and hatching [6]. The liver plays a critical role of converting lactate into glucose-6-phosphate when blood glucose concentrations decline [7], but cardiac and skeletal muscles lack the ability to recycle lactate [5]. Ultimately, poor glucose status or abnormal glucose regulation may adversely affect the hatching process and subsequent chick performance.

Intake of exogenous feed in the days following hatch is accompanied by rapid development of the gastrointestinal tract [8]. Early maturation of the intestines is associated with superior nutrient absorption and rapid growth of young broilers [9]. Delayed access to feed and water results in depressed mucosal development, clumping of microvilli and abnormal crypt structure [10]. If variablility of hatching time is reduced, then the interval between hatching and placement can be decreased. Minimizing holding time and enhancing gastrointestinal development may stimulate early growth and overall chick performance.

Although quantitative measurements have previously been made with poults, objective data regarding physiological development of chicks is limiting. The purpose of this experiment was to determine the effects of broiler breeder age and dietary zinc source in broiler breeder hen diets on chick characteristics at hatch.

## MATERIALS AND METHODS

### EXPERIMENTAL DIETS AND BIRD MANAGEMENT

Slow-feathering, Cobb 500 broiler breeder hens were given one of three diets from 0 to 65 wk of age (Table 6.1). Supplemental zinc concentrations used in this experiment

were based on average zinc level in commercial broiler breeder diets. Experimental diets consisted of 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn zinc-amino acid complex (ZnAA), or a combination of $ZnSO_4$ and Availa®Zn ($ZnSO_4$+ZnAA, 80 ppm zinc from each). Inductively Coupled Plasma (ICP) analyses were conducted in duplicate to determine the zinc content of the basal and experimental diets [11]. Determined concentrations were comparable to anticipated concentrations throughout the experiment (Basal: calculated value = 12 ppm; determined value = 39 $\pm$ 8 ppm; Supplemented: calculated value = 172 ppm; determined value = 185 $\pm$ 36; n = 18). Differences between calculated and determined concentrations were likely due to an underestimation of zinc in the natural feedstuffs.

Pullets were raised in an environmentally-controlled facility with 8 hr daylengths on pine shavings. At 20 wk of age, all hens were caged (2 hens per cage) and 16 hr light/d was provided. There were eight replicate groups of 24 caged hens for each zinc treatment. A sample of females was weighed weekly through 40 wk and biweekly thereafter, to determine feed allotments. Management was provided according to primary breeder recommendations, and feed allotments adjusted for cage management coincided with those suggested by a broiler integrator.

INCUBATION PROCEDURES

Hens were artificially inseminated with 50 μL neat semen on two occasions for each of the four incubation periods. Eggs that were laid at 29, 41, 53 and 65 wk of age were incubated and hatched in Natureform [12] incubators and hatchers. Temperature settings from 0 to 18 and 19 to 21 d of incubation were 37.8 and 37.2°C, respectively. Relative humidity settings at 0 to 19 and 20 to 21 d of incubation were 53 and 70%, respectively.

Eggs from each group of 24 hens represented the experimental unit. The eggs were sorted by replicate group within the incubator and hatcher. Eggs were transferred to hatchers at 18 d of incubation. Chicks that had dry down except for dampness on the back of the neck were removed and counted on 4 hr intervals from 468 through 528 hr of incubation. Mean incubation time and distribution of hatch time were calculated. All hatching eggs laid on a single day were weighed on four wk intervals from 26 to 66 wk of age.

PHYSIOLOGICAL DEVELOPMENT OF CHICKS

To evaluate physiological development of chicks, two chicks from each replicate group of hens were randomly removed from the hatcher at 492 hr (20.5 d) of incubation. After being weighed, chicks were decapitated and trunk blood samples were collected in tubes containing 72 USP units of sodium heparin. Plasma was stored at –4°C until analyzed for glucose concentration [13]. Yolk sac, heart, liver and pipping muscle were excised from each chick and weighed to the nearest 0.001 g. The portion of the small intestine between the terminal end of the duodenal loop and the cecal branch was removed. The intestinal segment was then separated into jejumun and ileum sections at Meckel's diverticulum [14]. Length and weight of each segment was then measured. Yolk sac weights are presented as a percentage of chick weight, and all other organ (liver, heart, pipping muscle, jejunum and ileum) weights are presented as percentages of yolk-free chick weight.

Left and right liver lobes were separated at the narrowest junction. Right liver lobes were held at –4°C until analyzed for zinc concentration on a dry matter basis by Inductively Coupled Plasma Analysis [15]. Left liver lobes and hearts were immediately

placed in a cold 7% perchloric acid solution. The homogenates were centrifuged at 700 *g* for 10 min at 4°C. Glycogen and lactate concentrations were determined in the supernatant fraction. Glycogen concentrations in heart and liver homogenates were assayed by the technique of Dreiling *et al.* [16]. Lactate concentration was measured in liver and heart homogenates by techniques described by Donaldson and Christensen [17].

STATISTICAL ANALYSIS

The experimental design was a 4 x 3 factorial arrangement of age of the breeder hen (29, 41, 53 and 65 wk) and dietary zinc source in breeder diets ($ZnSO_4$, ZnAA, $ZnSO_4$+ZnAA). Statistical analysis was conducted using the General Linear Models procedure of SAS [18], and hen group (2 chicks at each hatch) served as the experimental unit. Sources of variation included age, zinc source, and age x zinc source. Contrasts were used to test for linear and quadratic trends as hens aged. Means were separated by the least squares means procedure when significance ($P \leq 0.05$) occurred. To determine age of egg weight plateau, the NLIN procedure of SAS was used. All percentage data were subjected to arc sine transformation prior to analysis. While conclusions were drawn from the transformed data, only untransformed data are presented for relevance.

## RESULTS AND DISCUSSION

Dietary zinc source in hen diets had no influence on parameters evaluated here, with the exception of intestinal length of progeny when hens were 53 wk of age (Table 6.2). Significant zinc source x age effects indicated that progeny from 53 wk-old hens had increased jejunum and ileum lengths when hens consumed $ZnSO_4$ rather than ZnAA or ZnAA+$ZnSO_4$. This may suggest that nutrient absorption would be enhanced in these

chicks. Due to limited effects of dietary zinc source on chick physiology, the hen age effects are the primary focus of this paper. However, main effect means for each zinc treatment are presented in the text.

INCUBATION TIME

Incubation period generally lengthens as hen age progresses and egg size increases [19,20,21]. In this experiment, egg weight increased rapidly until approximately 48 wk of age (Figure 6.1). However, the mean incubation time declined consistently from 29 to 53 wk of age, and increased from 53 to 65 wk of age (Table 6.3). Increasing ambient temperatures associated with the changing seasons (December to October) were likely responsible for decreasing incubation times. High temperatures in the breeding facility and hatchery during the late spring and early summer months may have accelerated embryonic development. Based on the conductance constant equation [22,23], the age-related decline in incubation time implies that eggshell conductance increased rapidly from 29 to 51 wk of age. In agreement, Christensen *et al.* [23] reported that incubation time of turkey eggs decreased from 33 (667 hr) to 43 wk (661 hr) and from 43 (661 hr) to 54 wk (656 hr), but possible seasonal effects were not mentioned. Reis *et al.* [24] reported that incubation time was similar in a more narrow age comparison between eggs from broiler breeder hens at 33 and 49 wk of age.

Distribution of hatch time may be influenced by variations in egg size, shell quality, degree of development prior to artifical incubation and environmental conditions within the incubator or hatcher. However, spread of hatch time was not influenced by breeder age (Table 6.3). Although the pattern of hatching was similar as hen age progressed, age influenced the time of hatch initiation (Figure 6.2). These data agree with Reis *et al.*

[24], who reported that hen age (33 *vs* 49 wk) did not alter distribution of incubation time. However, these authors made no mention of influence of hen age on initiation of hatch. Christensen *et al.* [25] stated that poults hatching early or late may perform poorly. The same concept is generally accepted for chicks. However, Casteel *et al.* [26] demonstrated that holding chicks in the hatcher for an additional 24 hr (528 *vs* 552 hr) did not dehydrate chicks or cause poor performance. Although, these authors reported that extended holding time depressed antibody titers to IBD. Based on these data, uniformly hatched groups of chicks are desirable so that chicks are robust during processing and are capable of performing well after being placed in growout facilities. Incubation data indicate that distribution of hatching time can be minimized by incubating and hatching eggs together according to hen age.

Dietary zinc source in hen diets did not influence average incubation time ($ZnSO_4$ = 487.1, ZnAA = 487.2, ZnAA+$ZnSO_4$ = 486.6 hr) or CV of incubation time ($ZnSO_4$ = 1.40, ZnAA = 1.49, ZnAA+$ZnSO_4$ = 1.37 %).

CHICK ORGANS

Chicks from young broiler breeders are typically small and do not perform as well as chicks from older hens [1]. Body weight of chicks from 41-wk-old hens was 8.5 g greater than BW of progeny from the same hens at 29 wk of age (Table 6.4). Chick weight also increased significantly from 41 to 65 wk of age, but to a lesser degree. Age effects on yolk-free chick weight followed a similar trend.

Organ weights in chicks at hatching was influenced by age of the breeders (Table 6.4). Relative liver weights of chicks were inconsistent as hen age progressed. Relative liver weights of progeny were lowest at 41 wk, highest at 53 wk, and intermediate at 29 and 65

wk of age. Relative heart weights in progeny were lowest when hens were 29 wk of age, indicating that chicks from young hens may have a limited ability to supply nutrients to demand organs. Fairchild and Christensen [27] suggested that poults with relatively low heart or liver weights may be at a disadvantage, because of underdevelopment of these supply organs. Relative yolk sac weight at 29 wk of age was significantly lower than at subsequent hen ages. These data agree with previous reports of increasing relative yolk sac weights as broiler breeder hens [28,29] and turkey hens [30] age. Because the yolk sac likely provides less nutrients to chicks from young hens, excessive chick processing and delivery times may be detrimental to their performance.

Chick jejunum length was greatest when hens were 53 wk of age, but ileum length was not affected by hen age (Table 6.5). Relative jejunum weights of progeny were increased when hens were 29 and 53 wk of age, and relative ileum weight was greatest at 29 wk of age. Reasons for inconsistencies in intestine section weights are unclear. Applegate *et al.* [31] reported that poults from hens at 48 wk had longer jejunal villi than poults from 34 wk-old hens. Poults from older hens also showed more advanced enterocyte migration along the crypt-villus axis. In this experiment, chick jejunum density was greatest when hens were 53 wk of age. Since small intestine length [32] and luminal surface area [33] have been directly related to rapid growth of poultry, intestinal data may imply that growth of chicks from 53 wk-old hens may be enhanced. Increased jejunum, liver and heart weights observed in progeny of 53 wk-old hens may be attributed to high ambient temperatures in the hatchery and the resulting shorter incubation period.

Dietary zinc source in hen diets did not affect weight of chick ($ZnSO_4$ = 45.3, ZnAA = 44.5, ZnAA+$ZnSO_4$ = 45.4 g) or relative weights of liver ($ZnSO_4$ = 2.31, ZnAA = 2.42, ZnAA+$ZnSO_4$ = 2.30 %), heart ($ZnSO_4$ = 0.81, ZnAA = 0.81, ZnAA+$ZnSO_4$ = 0.78 %), yolk sac ($ZnSO_4$ = 15.5, ZnAA = 15.4, ZnAA+$ZnSO_4$ = 15.0 %) pipping muscle ($ZnSO_4$ = 1.72, ZnAA = 1.56, ZnAA+$ZnSO_4$ = 1.56 %), ileum ($ZnSO_4$ = 0.80, ZnAA = 0.88, ZnAA+$ZnSO_4$ = 0.82 %) and jejunum ($ZnSO_4$ = 0.91, ZnAA = 0.95, ZnAA+$ZnSO_4$ = 0.92 %). In addition, the main effect of dietary zinc source did not influence hepatic zinc concentration ($ZnSO_4$ = 63.0, ZnAA = 62.8, ZnAA+$ZnSO_4$ = 62.6 ppm), ileum length ($ZnSO_4$ = 12.6, ZnAA = 12.5, ZnAA+$ZnSO_4$ = 12.9 mm) or jejunum length ($ZnSO_4$ = 14.2, ZnAA = 13.9, ZnAA+$ZnSO_4$ = 13.9).

CARBOHYDRATE METABOLISM

Metabolism of carbohydrates was influenced by hen age (Table 6.6). Researchers have reported that plasma glucose is elevated as hepatic glycogen stores are depleted in poults exposed to stressors or held for extended periods [34,35,36]. Therefore, high glycogen concentrations are a desirable trait in chicks, because they will have additional energy reserves which may be utilized during servicing. Applegate and Lilburn [37] reported that poults from young hens had higher fasting plasma glucose concentrations than those from older hens, indicating that progeny from young hens may be less capable of regulating glucose metabolism. Sixty min after a glucose injection, ducklings from younger hens had elevated blood glucose concentrations [38]. Researchers have suggested that the inability of poults to regulate glucose metabolism may increase early mortality [39,40]. In this experiment, plasma glucose concentrations were lowest when hens were 53 wk of age. Conversely, Latour *et al.* [28] reported that chicks from 26 wk-

old hens had lower plasma glucose concentrations than chicks from 36 or 48 wk-old hens. Reasons for inconsistencies between these two research reports are unclear. If glucose status of progeny from 53 wk-old hens was poor, it would be expected that liver glycogen would be depleted. However, this was not the case in these chicks.

Breeder age had no influence on glycogen concentration in chick livers. In contrast, Christensen *et al.* [23] reported that young turkey hens produced embryos with greater amounts of glycogen than did older hens, perhaps due to a limited ability of these chicks to catabolize glycogen. In this experiment, glycogen concentration in the heart decreased consistently as the parents aged. This may indicate that more glycogen is mobilized from the liver to the heart in embryos from young breeders. Liver lactate concentration was decreased in progeny from 65 wk-old hens, suggesting that chicks from older hens are more capable of recycling lactate in the liver or less gluconeogenesis was required in these chicks. It would be expected that decreased liver lactate would coincide with decreased glycogenolysis and increased glycogen concentrations in muscle, but data presented here do not support this suggestion. Lactate concentration in the heart was not influenced by hen age.

Dietary zinc source did not influence plasma glucose ($ZnSO_4$ = 380, ZnAA = 375, ZnAA+$ZnSO_4$ = 358 mg/dL), liver glycogen ($ZnSO_4$ = 8.5, ZnAA = 8.6, ZnAA+$ZnSO_4$ = 8.3 mg/g), heart glycogen ($ZnSO_4$ = 6.2, ZnAA = 6.8, ZnAA+$ZnSO_4$ = 6.2 mg/g), liver lactate ($ZnSO_4$ = 0.51, ZnAA = 0.50, ZnAA+$ZnSO_4$ = 0.51 mg/g) or heart lactate ($ZnSO_4$ = 0.1.51, ZnAA = 1.53, ZnAA+$ZnSO_4$ = 1.50 mg/g).

These data indicate less developed supply organs may limit the performance of chicks from young hens. Additional work is needed to determine breeder and incubation parameters that can improve chick physiology at hatching to enhance chick performance.

## CONCLUSIONS AND APPLICATIONS

1. Chicks from 29 wk old hens had lower relative yolk sac weight and relative heart weight. These may be critical factors limiting the performance of chicks from young broiler breeder flocks.
2. Progeny from 53 wk-old hens had higher relative heart weight, relative jejunum weight and jejunum length. Plasma glucose concentrations were lower in these chicks. High ambient temperatures in the breeding facility and hatchery may have caused these effects.
3. The main effect of zinc source in hen diets did not influence variables that assessed physiological development of their progeny.

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Table 6.1. Ingredient composition and calculated nutrient analysis of the basal diets provided to the breeder pullets and hens[A]

| Ingredients, % "as-is" | Starter[B] | Developer[C] | Breeder 1[D] | Breeder 2[D] |
|---|---|---|---|---|
| Corn | 62.95 | 58.81 | 68.15 | 68.43 |
| Soybean meal (48%CP) | 22.24 | 16.90 | 20.71 | 18.68 |
| Poultry oil | … | … | 1.00 | 1.67 |
| Wheat middlings | 10.53 | 20.10 | … | … |
| Limestone | 1.15 | 1.16 | 7.41 | 8.37 |
| Dicalcium phosphate | 1.75 | 1.76 | 1.46 | 1.50 |
| Salt | 0.54 | 0.58 | 0.54 | 0.54 |
| Lysine | 0.10 | … | … | … |
| Methionine | 0.15 | 0.06 | 0.14 | 0.11 |
| Trace mineral premix[F] | 0.08 | 0.08 | 0.08 | 0.08 |
| Vitamin premix[G] | 0.50 | 0.50 | 0.50 | 0.50 |
| Copper sulfate | 0.01 | 0.05 | 0.01 | 0.01 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 |
| | | | | |
| Calculated Analyses | | | | |
| Crude protein (%) | 18.00 | 15.50 | 15.90 | 15.00 |
| Metabolizable energy (kcal/kg) | 2865 | 2819 | 2900 | 2900 |
| Calcium (%) | 0.91 | 0.90 | 3.22 | 3.60 |
| Available phosphorus (%) | 0.45 | 0.42 | 0.38 | 0.36 |
| Lysine (%) | 1.00 | 0.78 | 0.83 | 0.82 |
| Methionine (%) | 0.43 | 0.32 | 0.40 | 0.33 |
| TSAA (%) | 0.73 | 0.60 | 0.66 | 0.66 |
| Sodium (%) | 0.21 | 0.22 | 0.21 | 0.20 |
| Copper (ppm) | 16 | 16 | 16 | 16 |
| Zinc (ppm) | 30 | 30 | 30 | 30 |

[A] Experimental diets consisted of 160 ppm supplemental zinc from $ZnSO_4$, Availa®Zn zinc amino acid complex (ZnAA) or a mixture of $ZnSO_4$ and ZnAA (80 ppm zinc from each). Natural feed ingredients provided 40 ppm zinc.

[B] Diet was provided from 0 to 19 d of age to all females.

[C] Diet was provided from 20 d to 22 wk of age to all females.

[D] Diet was provided from 23 to 47 wk of age to all females.

[E] Diet was provided from 48 to 66 wk of age to all females.

[F] Trace mineral premix provided the following in milligrams per kilogram of diet: selenium, 0.3; manganese, 121; iron, 75; iodine, 0.8.

[G] Vitamin premix provided the following per kilogram of diet: vitamin A, 11,000 IU; vitamin D3, 2,200 IU; vitamin E, 22 IU; vitamin K, 2.2 mg; vitamin B12, 0.2 mg; thiamine 4.4 mg; riboflavin, 8.8 mg; vitamin B6, 4.4 mg; niacin, 88 mg; pantothenic acid, 22 mg; folic acid 1.1 mg; biotin, 2.2 mg; choline, 380 mg.

Table 6.2. Influences of zinc source in hen diets on jejunum and ileum length of progeny[A]

| | Hen age (wk) | | | |
|---|---|---|---|---|
| | 29 | 41 | 53 | 65 |
| Jejunum length | | | | |
| $ZnSO_4$[B] | 13.8 | 13.2 | 16.4 [a] | 13.5 |
| ZnAA | 13.8 | 13.4 | 14.8 [b] | 13.4 |
| $ZnSO_4$+ZnAA | 14.3 | 14.0 | 14.0 [b] | 13.3 |
| SEM | 0.50 | 0.48 | 0.48 | 0.53 |
| Source of variation | | | | |
| Age | | *** | | |
| Zinc | | NS | | |
| Age x Zinc | | * | | |
| Ileum length | | | | |
| $ZnSO_4$ | 12.0 | 12.2 | 14.0 [a] | 12.1 |
| ZnAA | 12.8 | 13.0 | 12.3 [b] | 12.1 |
| $ZnSO_4$+ZnAA | 13.7 | 13.0 | 12.2 [b] | 12.8 |
| SEM | 0.46 | 0.43 | 0.43 | 0.47 |
| Source of variation | | | | |
| Age | | NS | | |
| Zinc | | NS | | |
| Age x Zinc | | ** | | |

[A] Values (mm) are least-squares means involving 2 chicks from each of 8 hen groups. Chicks were samples at four different breeder ages.
[B] Broiler breeder hens were given one of three diets. Diets consisted of 160 ppm supplemental Zn from $ZnSO_4$, Availa®Zn zinc amino acid complex (ZnAA), or a mixture of $ZnSO_4$ and ZnAA ($ZnSO_4$+ZnAA, 80 ppm zinc from each).
[a-b] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).

Table 6.3. Influences of broiler breeder age on incubation time[A]

| Age, wk | Mean Incubation Time (hr) | Mean Time to 75% hatched (hr) | Distribution of Incubation Time (CV, %) |
|---|---|---|---|
| 29 | 489.3 [a] | 492.3 [a] | 1.44 |
| 41 | 487.5 [b] | 490.9 [b] | 1.33 |
| 53 | 484.4 [c] | 486.1 [c] | 1.46 |
| 65 | 486.8 [b] | 490.4 [b] | 1.45 |
| SEM | 0.31 | 0.45 | 0.056 |
| | ----------Probabilities---------- | | |
| Age | *** | *** | NS |
| Orthogonal polynomials | | | |
| Linear | *** | *** | NS |
| Quadratic | *** | *** | NS |

[A] Values are least-squares means involving 24 hen groups, each with 24 hens at housing. Eggs were incubated at four different hen ages.
[a-c] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).
NS, $P > 0.05$.
***, $P \leq 0.001$.

Table 6.4. Influences of broiler breeder age on chick weight, organ weights and hepatic zinc concentration[A]

| Age, wk | Chick weight (g) | Liver weight (%) [B] | Heart weight (%) [B] | Yolk sac weight (%) [C] | Pipping muscle weight (%) [B] | Liver zinc [D] (ppm) |
|---|---|---|---|---|---|---|
| 29 | 37.9 [c] | 2.41 [ab] | 0.71 [bc] | 12.5 [b] | 1.75 | 63.0 |
| 41 | 46.4 [b] | 2.21 [c] | 0.82 [ab] | 16.2 [a] | 1.59 | 62.4 |
| 53 | 47.1 [ab] | 2.46 [a] | 0.86 [a] | 15.7 [a] | 1.49 | 62.2 |
| 65 | 48.6 [a] | 2.29 [bc] | 0.80 [ab] | 16.6 [a] | 1.62 | 63.6 |
| SEM | 0.66 | 0.048 | 0.019 | 0.48 | 0.083 | 0.45 |
| | ----------Probabilities---------- | | | | | |
| Age | *** | *** | *** | *** | NS | NS |
| Orthogonal polynomials | | | | | | |
| Linear | *** | NS | *** | *** | NS | NS |
| Quadratic | *** | NS | *** | ** | NS | * |

[A] Values are least-squares means involving 2 chicks from each of 24 hen groups. Chicks were sampled at four different breeder ages.
[B] Percentage of yolk-free chick weight.
[C] Percentage of chick weight.
[D] Assayed by inductively coupled plasma analysis. Values are presented on a dry matter basis.
[a-c] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).
NS, $P > 0.05$.
*, $0.01 < P \leq 0.05$.
**, $0.001 < P \leq 0.01$.
***, $P \leq 0.001$.

Table 6.5. Influences of broiler breeder age on intestinal development of progeny[A]

| Age (wk) | Ileum + jejunum weight (%) [B] | Ileum weight (%) [B] | Jejunum weight (%) [B] | Segment length (mm) | | | Segment density [C] | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Ileum + jejunum | Ileum | Jejunum | Ileum + jejunum | Ileum | Jejunum |
| 29 | 1.95 [a] | 0.96 [a] | 0.99 [a] | 26.9 [ab] | 12.9 | 14.0 [b] | 0.024 [b] | 0.025 | 0.024 [b] |
| 41 | 1.65 [b] | 0.80 [b] | 0.85 [b] | 26.2 [b] | 12.7 | 13.5 [b] | 0.025 [b] | 0.024 | 0.025 [b] |
| 53 | 1.90 [a] | 0.84 [b] | 1.06 [a] | 27.9 [a] | 12.8 | 15.1 [a] | 0.027 [a] | 0.026 | 0.028 [a] |
| 65 | 1.54 [b] | 0.74 [b] | 0.81 [b] | 25.7 [b] | 12.3 | 13.4 [b] | 0.024 [b] | 0.024 | 0.024 [b] |
| SEM | 0.052 | 0.035 | 0.029 | 0.44 | 0.25 | 0.29 | 0.0006 | 0.0008 | 0.00077 |
| | ----------Probabilities---------- | | | | | | | | |
| Age | *** | *** | *** | ** | NS | *** | ** | NS | *** |
| Orthogonal polynomials | | | | | | | | | |
| Linear | *** | *** | * | NS | NS | NS | NS | NS | NS |
| Quadratic | NS | NS | NS | NS | NS | * | * | NS | ** |

[A] Values are least-squares means involving 2 chicks from each of 24 hen groups. Chicks were sampled at four different breeder ages.
[B] Percentage of yolk-free chick weight.
[C] Calculated as weight/length.
[a-c] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).
NS, $P > 0.05$.
*, $0.01 < P \leq 0.05$.
**, $0.001 < P \leq 0.01$.
***, $P \leq 0.001$.

Table 6.6. Influences of broiler breeder age on glucose metabolism in progeny[A]

| Age, wk | Plasma glucose (mg/dL) | Liver glycogen (mg/g) | Heart glycogen (mg/g) | Liver lactate (mg/g) | Heart lactate (mg/g) |
|---|---|---|---|---|---|
| 29 | 395 [a] | 7.7 | 8.7 [a] | 0.62 [a] | 1.54 |
| 41 | 383 [a] | 9.0 | 7.0 [b] | 0.58 [a] | 1.51 |
| 53 | 339 [b] | 9.0 | 5.5 [c] | 0.46 [b] | 1.51 |
| 65 | 369 [a] | 8.2 | 4.3 [d] | 0.37 [c] | 1.46 |
| SEM | 9.1 | 0.62 | 0.29 | 0.019 | 0.057 |
| | ----------Probabilities---------- | | | | |
| Age | *** | NS | *** | *** | NS |
| Orthogonal polynomials | | | | | |
| Linear | ** | NS | *** | *** | NS |
| Quadratic | * | NS | NS | ** | NS |

[A] Values are least-squares means involving 2 chicks from each of 24 hen groups. Chicks were samples at four different breeder ages.

[a-c] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).

NS, $P > 0.05$.

*, $0.01 < P \leq 0.05$.

**, $0.001 < P \leq 0.01$.

***, $P \leq 0.001$.

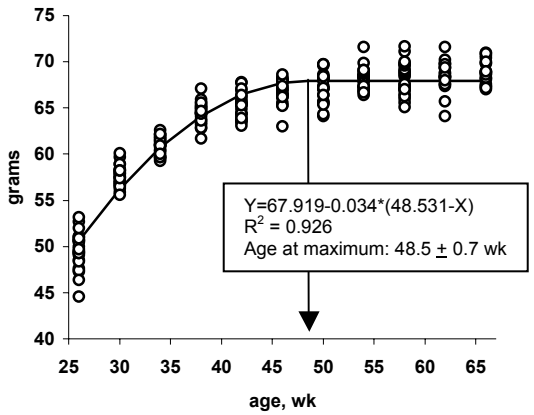


Figure 6.1. Influence of breeder age on egg weight

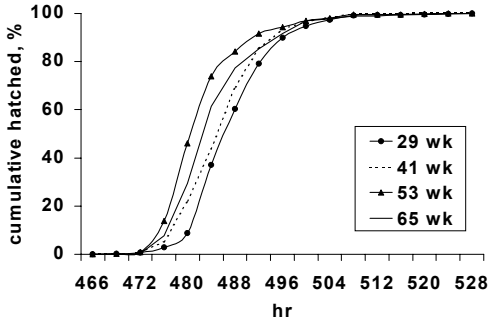


Figure 6.2. Influence of breeder age on incubation time

# CONCLUSIONS

Supplementing broiler breeder male diets with Menhaden oil rather than poultry fat improved fertility of eggs laid during the second week following single inseminations at different ages. Fatty acid composition of sperm was altered by supplemental fat source in breeder diets, but sperm quality measurements did not reveal differences between semen from the two male groups. These data suggest that there was an unidentified factor that enhanced fertilizing capacity of semen when males consumed Menhaden oil.

A series of experiments were conducted to determine the effects of zinc source on performance and immune status of broiler breeder hens and their progeny. Eggshell quality was enhanced when broiler breeder hen diets were supplemented with a mixture of Availa®Zn and $ZnSO_4$. As a result, settable egg production and estimated chick production were increased. Both humoral and cellular immune responses were increased when hens consumed diets with Availa®Zn.

In the first of two experiments with progeny from the previously discussed trial, chicks were subjected to low or normal brooding temperatures and fed two different supplemental zinc sources. Humoral immune status of broilers was increased when hens were fed diets supplemented with $ZnSO_4$ rather than Availa®Zn. Cumulative mortality, feed intake and body weight ($P=0.055$) were increased when broilers were exposed to low brooding temperatures. In the second experiment with progeny, body weight gain and feed conversion were enhanced when broilers were provided a mixture of $ZnSO_4$ and Availa®Zn rather than solely $ZnSO_4$. These data suggest that $ZnSO_4$ and Availa®Zn may

have synergistic effects, improving hen and broiler performance. To validate this suggestion, further research is needed to demonstrate more consistent effects on broiler performance.

Influences of broiler breeder hen age and dietary zinc source in hen diets on physiological development of chicks were evaluated. Results indicated that chicks weights were decreased and some of their supply organs (yolk sac and heart) were less developed in progeny from young hens. This effect may limit the performance of chicks from young hens. The main effect of zinc source in hen diets did not influence progeny characteristics at hatching.

## APPENDIX A. DILUTING POULTRY SEMEN TO ACHIEVE A CERTAIN VOLUME AND CONCENTRATION

1. Determine the total volume of extended semen required to inseminate all hens:

   *Total volume = number of hens x dosage per hen (mL)*

2. Determine the sperm concentration with IMV spectrophotometer:
   a. Fill cuvet with 2mL saline (0.9% NaCL) and press 'zero'.
   b. Pipet 10uL of neat semen into a cuvet containing 2mL saline and press 'read'.
   c. Read absorbance and determine sperm concentration with the standard curve provided by IMV or a standard curve generated by hemocytometer observations.

3. Do calculations to determine the amount of semen included in the final product:

$$\textit{Semen vol. (mL)} = \frac{\textit{[desired total volume (mL) x desired concentration (bil./mL)]}}{\textit{Sperm concentration (bil./mL)}}$$

4. Do calculations to determine extender volume:

*Extender volume (mL) = total volume (mL) – semen volume (mL)*

5. Example:

   Suppose that each of 100 hens needs to be inseminated with 0.05mL of extended semen containing 3.0 billion sperm/mL. After using the spectrophotometer, sperm concentration of neat semen is found to be 5.0 billion sperm/mL. A total volume of 5mL of extended semen will be required to inseminate all hens (100 hens x 0.05mL/hen). Now, use the formulas given above to calculate semen and extender volumes.

$$\textit{Semen volume} = \frac{\textit{[5.0mL x 3.0 billion/mL]}}{\textit{5.0 billion/mL}}$$

***Semen volume = 3.0mL***

*Extender volume = 2.0mL*

*** Add 2.0mL of extender to 3.0mL of neat semen and mix gently.***

## APPENDIX B. USING THE SPERM PENETRATION ASSAY

## TO ASSESS FLOCK FERTILITY

*(adjusted from Bramwell et al., 1995, Poultry Science 74:1875-1883)*

1. Separate the yolk from the albumen with a yolk separator.

2. Place the yolk on a paper towel and roll it around to remove the adhering albumen. Be sure to clean near the germinal disc.

3. Face the germinal disc upwards and carefully cut the perivitelline layer (PL) overlying the germinal disc. This can be done by placing the yolk on a paper towel or in physiological saline (0.9% NaCl). Try to cut a square with at least 1 cm sides. Place the PL section into a saline solution (1% NaCl) and shake vigorously with forceps to remove all yolk material.

4. Place the PL section on a microscope slide (2-3 will go on a slide) and straighten with needles, probes or fingers. It is important to keep the germinal disc region as smooth as possible to make microscopic observations easier.

5. Add just enough formalin solution (20% formalin) to cover the PL section and let sit for about 15 seconds.

6. Add just enough Schiff's reagent to cover the PL section. After a dark purple color appears, add a few drops of saline to remove excess. Place excess formalin and Schiff's solutions into a waste container for proper disposal. Letting the PL section stain too dark makes observation of the holes difficult. Hold the slide vertically and usa a paper towel to dry the edges of the slide. Place a coverslip over the section and press down with a paper towel to remove bubbles and residual moisture. Do not slide or adjust coverslip, because wrinkling will occur.

7. View the slide with the microscope and locate the germinal disc region on low power, then adjust to a higher power to count holes. For less variation, center the germinal disc in the field of view and count holes without moving the slide horizontally. Grids may also be used for accurate counts.

## APPENDIX C. DETERMINING SPERM MOBILITY USING A DENSIMETER®

1. Turn on the densimeter® (Animal Reproduction Systems, Chino, CA) and select chicken mobility assay. Allow 10-15 minutes for the machine to warm up.

2. Prior to beginning the assay, place 3% NaCl solution, mobility buffer solution[a] and Accudenz® (Animal Reproduction Systems, Chino, CA) in a hot water bath maintained at 41°C.

3. Pipet 3.42mL of 3% NaCl into a cuvet (blank), place into the densimeter and zero the blank.

4. Add 11.4uL of neat semen into the cuvet containing prewarmed NaCl solution, mix by inversion, and place into the machine to determine concentration.

5. Mix the amount of determined prewarmed mobility buffer with 50uL of raw semen.

6. Overlay 300uL of the semen/buffer mixture over 3mL of prewarmed 6% Accudenz® solution.

7. Incubate for 5 minutes at 41°C in a hot water bath.

8. Dry off cuvet and place into densimeter for sperm mobility determination.

[a] 50mM N-Tris-[hydroxymethyl] methyl-2-amino-ethanesulfonic acid, ph 7.4 containing 128mM NaCl and 2mM $CaCl_2$

## APPENDIX D. IODINE BINDING ASSAY FOR MEASURING

## GLYCOGEN IN TISSUES

**Chemicals needed:** 70% Perchloric Acid, catalog no. A229-8lb, Fisher Scientific
Petroleum Ether, catalog no. E139-4, Fisher Scientific
Glycogen, catalog no. G-0885, Sigma Diagnostics
Calcium Chloride Dihydrate, catalog no. C79-500, Fisher Sci..
Iodine, catalog no. I37-500, Fisher Scientific
Potassium Iodine, catalog no. P410-500, Fisher Scientific

**Tissue preparation:** Add a 7% solution of cold Perchloric Acid (PCL) at the rate of 5mL PCL/1g of wet tissue weight. (PCL is prepared by adding 10mL of Perchloric Acid to 90mL of $dH_2O$. Remember to add acid to water and not the reverse.) Store tissues in the refrigerator (4˚C) until they can be homogenized. Homogenize tissue in the PCL until finely grond. After tissues have been homogenized, centrifuge at 2000 rpm at 0-4˚C for 10 minutes. The supernatant must then be immediately decanted into fresh tubes. It cannot stay on the pellet. Clean supernatant by adding 1mL of Petroleum Ether to tube containing supernatant, vortex, centrifuge for 10 minutes at 2000 rpm and aspirate the ether off the top. The cleaning process should be repeated 1-3 times until there is no free-floating material in the supernatant. Never freeze the supernatant. Store covered in the refrigerator (4˚C).

**A. Prepare standards:**
Add the appropriate amount of stock solution and PCL to obtain the standards which will be used to make the standard curve. From this curve the concentration of the glycogen in the samples can be determined. The standard curve should be linear over the range of concentrations of the samples that are being analyzed. There are three different standards which can be used depending on the type and size of tissue. Use the standards in which most of the samples fit that curve.

**Option 1**. Concentration of the stock is 10 mg/mL. Put 0.2g of glycogen in 20mL of 7% PCL. If the dilution rate for tissue collection is 5mL of PCL use these standards:

| Stock solution | Perchloric Acid | Standard value |
|---|---|---|
| 0mL | 5mL | 0mg/mL or 0 mg/5mL |
| 1mL | 4mL | 2mg/mL or 10mg/5mL |
| 2mL | 3mL | 4mg/mL or 20mg/5mL |
| 3mL | 2mL | 6mg/mL or 30mg/5mL |
| 4mL | 1mL | 8mg/mL or 40mg/5mL |
| 5mL | 0mL | 10mg/mL or 50mg/5mL |

**Option 2**. Note: These standards were used on heart and liver tissues from newly hatched chicks in research reported herein. Concentration of the stock is 5 mg/mL. Put 0.1g of glycogen in 20mL of 7% PCL. If the dilution rate for tissue collection is 2.5mL of PCL use these standards:

| Stock solution | Perchloric Acid | Standard value |
|---|---|---|
| 0mL | 5mL | 0mg/mL or 0 mg/2.5mL |
| 0.5mL | 4.5mL | 0.5mg/mL or 1.25mg/2.5mL |
| 1.0mL | 4.0mL | 1.0mg/mL or 2.5mg/2.5mL |
| 1.5mL | 3.5mL | 1.5mg/mL or 3.75mg/2.5mL |
| 2.0mL | 3.0mL | 2.0mg/mL or 5.0mg/2.5mL |
| 5.0mL | 0mL | 5mg/mL or 12.5mg/2.5mL |

**Option 3**. Take the 1.0mg/mL standard from the standard curve above to make the following standards. This standard curve is used if the tissue dilution rate is 2.5mL of PCL and the sample concentrations are less than 1mg/mL of glycogen.

| Stock solution | Perchloric Acid | Standard value |
|---|---|---|
| 0mL | 5mL | 0mg/mL or 0 mg/2.5mL |
| 0.5mL | 4.5mL | 0.1mg/mL or 0.25mg/2.5mL |
| 1.0mL | 4.0mL | 0.2mg/mL or 0.5mg/2.5mL |
| 1.5mL | 3.5mL | 0.3mg/mL or 0.75mg/2.5mL |
| 2.0mL | 3.0mL | 0.4mg/mL or 1.0mg/2.5mL |
| 2.5mL | 2.5mL | 0.5mg/mL or 1.25mg/2.5mL |

**B. Preparation of Solutions used in the Iodine Binding Assay:**
One day before the assay, mix 74g of Calcium Chloride Dihydrate with 100mL of $dH_20$. Mix until solution is clear (let stir gently overnight if possible) and filter using #1 filter paper and an aspiration flask connected to an aspirator. This mixture can then be covered with parafilm and stored at room temperature. Add 0.26g Iodine and 2.6g Potassium Iodide to 10mL $dH_2O$. Mix for 10 minutes. Cover with parafilm and store solution at room temperature. If there are any floating particles, then the mixture must be filtered. To make iodine color reagent, add 1.3mL of the iodine/KI solution for each 100mL of the saturated $CaCl_2$ solution and mix using a stir bar for about 5 minutes. Cover with parafilm to avoid exposure of solution to air. This reagent is stable for a maximum of two hours.

**C.** The microwell plate is kept at room temperature. When setting up the microplate add 2.5µL of reagent to the first three wells, A1, B1 and C1. These will be the blanks. There must be three blanks on each plate. Then add the standards and the samples in triplicates, pipetting 6 µL if option 1 standards were used, 20 µL if option 2 standards were used, and 60 µL if option 3 standards were used. After adding reagent, each well should contain 250µL total volume of fluid. Add 244µL reagent for option 1, 230µL reagent for

option 2 and 190µL reagent for option 3. Mix gently and wait 10 minutes before reading at 460nm. Reaction will be stable for two hours. A yellow/brown color will be evident.

**D.** Use the Softmax® program to change wavelength and templates as needed. Additionally, this program is used for reading plates.

**E. Reference:** Dreiling, C.E., D.E. Brown, L. Casale, and L. Kelly, 1987. Muscle glycogen: Comparison of iodine binding and enzyme digestion assays and application to meat samples. Meat Science 20:167-177.

## APPENDIX E. ENZYMATIC ASSAY FOR MEASURING

## LACTATE IN TISSUES

**Chemicals needed:** 70% Perchloric Acid, catalog no. A229-8lb, Fisher Scientific
Petroleum Ether, catalog no. E139-4, Fisher Scientific
Reagent components (see below)

**Reagent components:**

1. Lactate dehydrogenase, cat. No. 826-6 (Sigma Diagnostics). Store in refrigerator (2-6°C). Mix by gentle inversion until suspension is uniform before removing an aliquot.
2. Glycerine buffer, cat. No. 826-3 (Sigma Diagnostics). Glyycine, 0.6mol/L, and hydrazine, pH 9.2 at 25˚C. Store in refrigerator (2-6˚C). Discard if turbidity is evident.
3. NAD, cat. No. 260-110 (Sigma Diagnostics). Nicotinamide adenine dinucleotide, grade III, 10mg preweighed vial. Store below 0˚C in dessicator box provided. When removed from freezer, package of vials should be allowed to warm to room temperature before opening to avoid moisture pickup. Remove required number of vials, close package quickly and return to freezer.
4. Lactate standard solution, cat. No. 826-10 (Sigma Diagnostics), L(+) lactic acid, 40mg/dL (4.44mmol/L). Sodium azide (0.1%) added as a preservative. Store in refrigerator (2-6˚C).

**PRECAUTIONS:** Wear suitable protective clothing, gloves and eye/face protection. Glycine buffer may cause cancer, heritable genetic damage, and sensitization by inhalation or skin contact. If you feel unwell, seek medical advice. Target organs: liver and kidneys. Lactate standard solution contains sodium azide which is toxic if ingested, and may react with lead and copper plumbing to form highly explosive metal azides. Flush with a large volume of water to prevent azide accumulation. Avoid contact and inhalation of Lactate dehydrogenase solution.

**Tissue preparation:** Add a 7% solution of cold Perchloric Acid (PCL) at the rate of 5mL PCL/1g of wet tissue weight. (PCL is prepared by adding 10mL of Perchloric Acid to 90mL of $dH_2O$. Remember to add acid to water and not the reverse.) Store tissues in the refrigerator (4˚C) until they can be homogenized. Homogenize tissue in the PCL until finely ground. After tissues have been homogenized, centrifuge at 2000 rpm at 0-4˚C for 10 minutes. The supernatant must then be immediately decanted into fresh tubes. It cannot stay on the pellet. Clean supernatant by adding 1mL of Petroleum Ether to tube

containing supernatant, vortex, centrifuge for 10 minutes at 2000 rpm and aspirate the ether off the top. The cleaning process should be repeated 1-3 times until there is no free-floating material in the supernatant. Never freeze the supernatant. Store covered in the refrigerator (4˚C).

**A. Procedure:**

1. Reconstitute the appropriate number of NAD vials required by pipetting into each the following:
   1) 2.0mL Glycine buffer, 2) 4.0mL water and 3) 0.1mL Lactate dehydrogenase. Cap and invert vials several times to dissolve the NAD. Combine contents of vials if more than one is being used. Solution is stable for 4 hours at room temperature or 24 hours refrigerated (2-6˚C).

2. There will be 3 standards: 0, 20 and 40mg/dL. In the first 3 wells (A1, B1 and C1) on the microtiter plate, pipet 10µL PCL (blank). Then pipet 10µL of PCL again in the next 3 wells. This is the 0 standard. In the next 3 wells pipet 5µL of the 40 standard and 5µL of PCL. This is the 20 standard. Then pipet 10µL of the 40 standard in the next 3 wells. Pipet all the standards and 10µL of each sample in triplicates in this order. Add 290µL of the NAD solution. Vortex slowly for 30 seconds on plate shaker. The plate must be read on the microplate reader within 10 minutes at 340nm. Each plate must contain a blank.

3. Use the Softmax® program to change wavelength and templates as needed. Additionally, this program is used for reading plates.

4. The mean result will be multiplied by the dilution factor.

## APPENDIX F. ENZYMATIC DETERMINATION OF GLUCOSE IN SERUM OR PLASMA

**Chemicals needed:** Glucose oxidase reagent set, cat. no. 23666290, Fisher Sci.
Glucose standard, cat. no. 16-300, Fisher Sci.

**Reagent Information:**

1. **Precautions:** Avoid contact with skin. In case of skin contact, flush with copious amounts of water.

2. **Preparation:** It may be necessary to transfer the powder to a larger container. Then reconstitute appropriate number of reagent vials with 100.0mL distilled water and swirl to mix reagent.

3. **Storage and stability:** Reconstituted reagent should be stored in an amber bottle to protect from strong light and is stable for 3 months when refrigerated (2-6˚C) and for 5 days at room temperature.

4. **Deterioration:** The glucose reagent is not suitable for use if the absorbance of freshly reconstituted solution measured in 1cm lightpath at 505nm vs water as reference is greater than 0.2. This must be done on a spectrophotometer.

### Specimen Collection and Storage

Blood collected without anticoagulant should be centrifuged promptly after clot formation. Approximately 5% of the glucose content will be consumed by glycolysis during each hour serum remain with the clot. Serum glucose levels are reported to be stable for 48 hours at room temperature.

If plasma is used, blood should be collected in a tube containing anticoagulant and sodium fluoride to minimize glycolysis. Sodium fluoride may be omitted if plasma is separated from cells within 30 minutes after collection. If samples are not to be tested within 48 hours, they may be preserved by freezing. Turbid and extremely hemolyzed samples may give falsely high values.

**Manual Procedure**

1. Prepare stock standard (500mg/dL). To 20mL of distilled water add 100mg of dextrose which results in a concentration of 500mg/dL. Dilute to prepare standards of lower concentration as shown below:

| Stock solution | Perchloric Acid | Standard value |
|---|---|---|
| 5mL | 0mL | 500mg/dL |
| 4mL | 1mL | 400mg/dL |
| 3mL | 2mL | 300mg/dL |
| 2mL | 3mL | 200mg/dL |
| 1mL | 4mL | 100mg/dL |
| 0mL | 5mL | 0mg/dL |

2. Prepare Glucose reagent according to the instructions. Fibrous floating material is normal. Do not shake, but mix by inversion. Bubbles form easily. Reagent is light sensitive, so mix prior to use in an amber bottle and minimize light exposure.

3. Pipet 5µL of the 0mg/dL standard in wells A1, B1 and C1. These wells are the blanks. There must be 3 blanks on every plate. Proceed with pipeting the same amount of standards or plasma/serum. They will continue in A2, B2 and C2, each triplicate sample following the next. Place a small piece of parafilm on top of each plate after it is completed so samples do not evaporate. Once they have been pipetted, add reagent as soon as possible so evaporation doesn't occur. After all standards and plasma or serum samples have been pipetted, add 200µL of reagent to each well. Shake the plate on a plate shaker for 30 seconds.

4. Incubate each plate for 18 minutes at room temperature. If assay temperature is 30°C, incubate for 15 minutes. If temperature is 37°C, incubate for 10 minutes.

5. Read plate at 505nm on a microplate reader. The Softmax® program may be used.

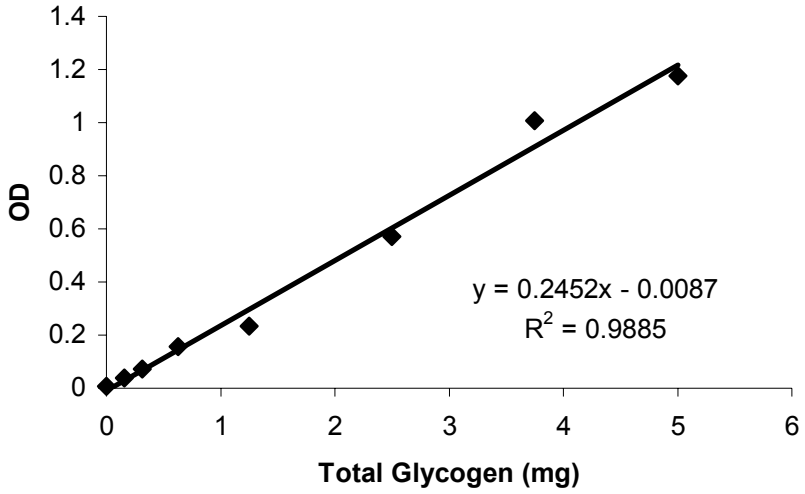


Figure A.1. Glycogen assay validation (linearity and sensitivity)

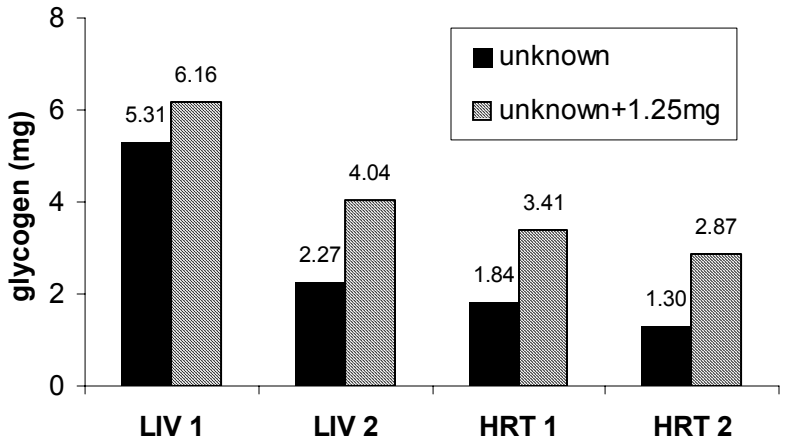


Figure A.2. Glycogen assay validation (accuracy)

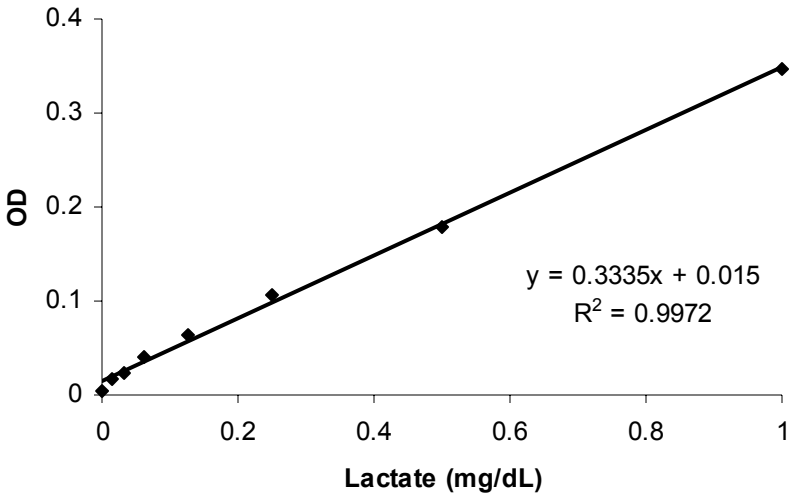


Figure A.3. Lactate assay validation (linearity and sensitivity)

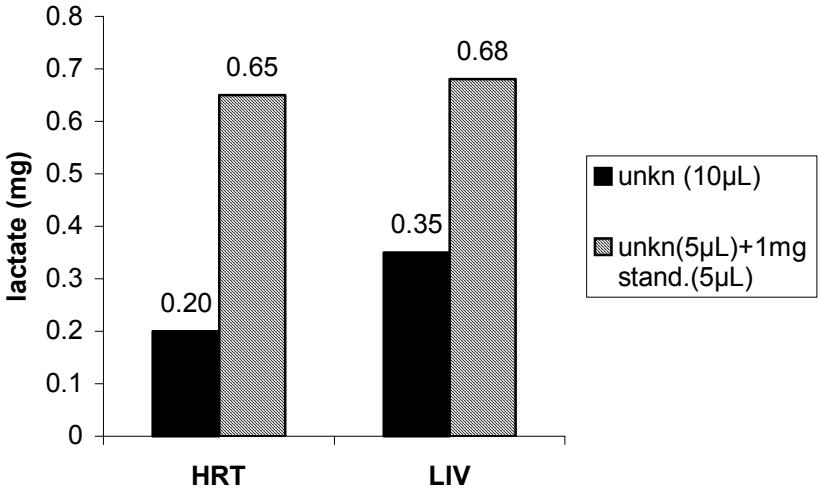


Figure A.4. Lactate assay validation (accuracy)

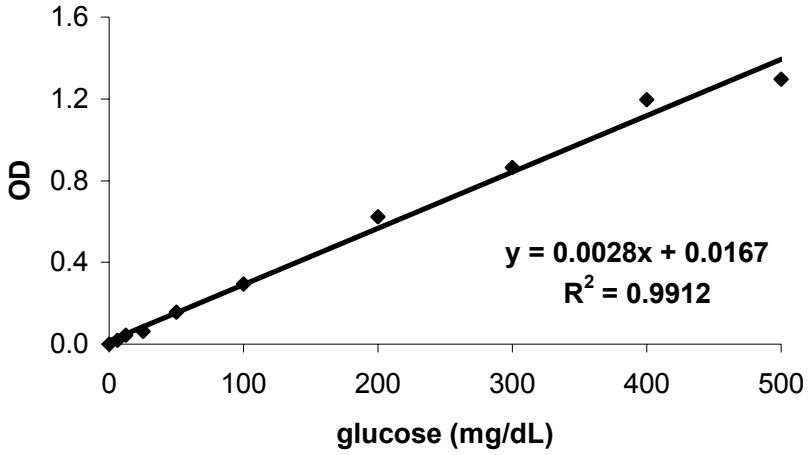


Figure A.5. Glucose assay validation (linearity and sensitivity)

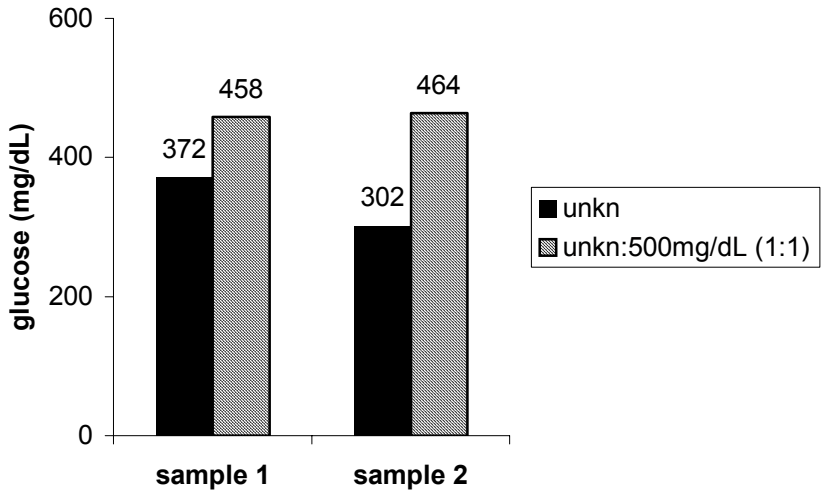


Figure A.6. Glucose assay validation (accuracy)

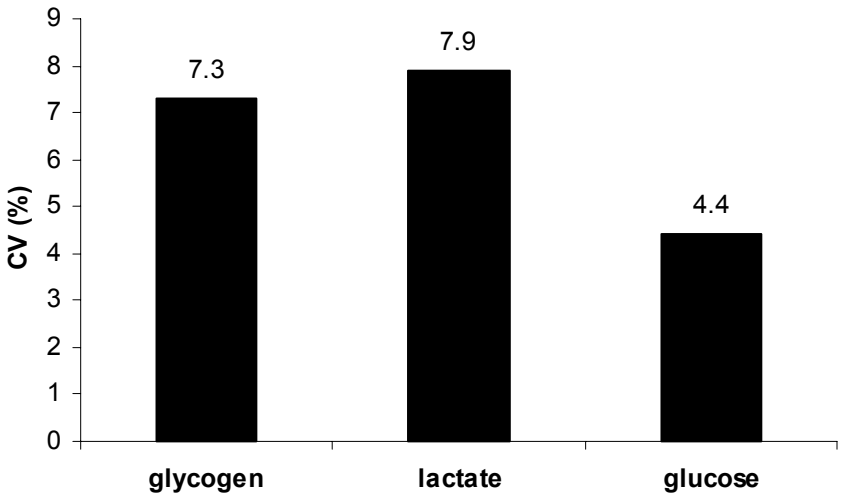


Figure A.7. Precision of glycogen, lactate and glucose assays

Table A.1. Influences of dietary zinc supplementation on egg quality parameters from 24 through 65 wk of age[A]

| Treatment[B] | Specific Gravity | Egg Weight, g | Cracked eggs, % | Zinc in egg contents[C], ppm |
|---|---|---|---|---|
| Basal | 1.081 [a] | 64.0 | 4.7 [b] | 55.4 |
| Zn supplemented | 1.080 [b] | 64.2 | 7.3 [a] | 59.9 |
| SEM | 0.0003 | 0.22 | 0.43 | 2.16 |
| | | | | |
| Source of Variation | | | | |
| Zinc | *** | NS | *** | NS |
| Age | *** | *** | *** | NS |
| Zinc x Age | NS | NS | NS | NS |

[A] Values for Zn supplemented and Basal treatments are least-squares means involving 24 and 9 hen groups, respectively, each with 24 hens at housing.

[B] Broiler breeder hens were given corn-soybean meal diets with or without supplemental zinc (160 ppm).

[C] Zinc analyses were conducted on 5 eggs per replicate group 32 and 62 wk of age.

[a-b] Means with different superscripts within a column signify significant differences ($P \leq 0.05$).

NS, $P > 0.05$.

***, $P \leq 0.001$.

Table A.2. Influences of dietary zinc supplementation on egg production from 24 through 65 wk of age[A]

| Treatment[B] | HDEP[C], % | HHEP[C], % | Total eggs/hen-housed | Hatching eggs/hen-housed |
|---|---|---|---|---|
| Basal | 55.4 | 49.9 | 146.6 | 139.1 |
| Zn supplemented | 56.9 | 51.8 | 152.2 | 138.7 |
| SEM | 0.89 | 0.89 | 2.61 | 1.98 |
| | | | | |
| Source of Variation | | | | |
| Zinc | NS | NS | NS | NS |
| Age | *** | *** | *** | *** |
| Zinc x Age | NS | NS | NS | NS |

[A]Values for Zn supplemented and Basal treatments are least-squares means involving 24 and 9 hen groups, respectively, each with 24 hens at housing.
[B]Broiler breeder hens were given corn-soybean meal diets with or without supplemental zinc (160 ppm).
[C]HDEP, hen-day egg production; HHEP, hen-housed egg production.
[a-b]Means with different superscripts within a column signify significant differences ($P \leq 0.05$).
NS, $P > 0.05$.
***, $P \leq 0.001$.

Table A.3. Influences of dietary zinc supplementation on fertility, hatchability, chick production and mortality from 20 through 65 wk of age[A]

| Treatment[B] | Fertility, % | Hatchability of Eggs Set, % | Hatchability of Fertile, % | Chicks per Hen Housed[C] | Mortality, % |
|---|---|---|---|---|---|
| Basal | 89.3 | 77.1 | 86.3 | 107.3 | 20.8 |
| Zn supplemented | 90.9 | 77.0 | 84.7 | 106.9 | 21.9 |
| SEM | 0.66 | 0.89 | 0.70 | 1.97 | 3.35 |
| | | | | | |
| Source of variation | | | | | |
| Zinc | NS | NS | NS | NS | NS |
| Age | *** | *** | NS | *** | -- |
| Zinc x Age | NS | NS | NS | NS | -- |

[A]Values for Zn supplemented and Basal treatments are least-squares means involving 24 and 9 hen groups, respectively, each with 24 hens at housing. Hens were artificially inseminated, and eggs were incubated and hatched weekly (25–35 wk), biweekly (37–45 wk) or every fourth wk (49-65 wk).
[B]Broiler breeder hens were given corn-soybean meal diets with or without supplemental zinc (160 ppm).
[C]Estimation by multiplication of weekly hatching eggs per hen-housed and hatchability of total eggs set.
[a-b]Means with different superscripts within a column signify significant differences ($P \leq 0.05$).
NS, $P > 0.05$.
***, $P \leq 0.001$.