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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorijps@gmail.com

Effects of Zinc Supplementation on Laying Performance, Serum Chemistry and Zn Residue in Tibia Bone, Liver, Excreta and Egg Shell of Laying Hens

O.M.O. Idowu¹, R.O. Ajuwon², A.O. Oso¹ and O.A. Akinloye³

¹Department of Animal Nutrition, College of Animal Science and Livestock Production, University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria, West Africa

²Department of Biomedical Sciences, Faculty of Health and Wellness Sciences, Cape Peninsula University of Technology, Bellville Campus, Bellville 7535, South Africa

³Department of Biochemistry, University of Agriculture, P.M.B. 2240, Abeokuta, Nigeria, West Africa

Abstract: The effect of different sources of Zinc (Zn) in the diets of laying birds was investigated in a 10 weeks trial. Diets were formulated to include Zinc Oxide (ZnO), Zinc Sulphate (ZnSO₄), Zinc Carbonate (ZnCO₃) and Zinc Proteinates (ZnP) in which Zn in each diet was supplied at 140 mgKg⁻¹ diet. Responses measured included performance, some serum biochemistry, Zn residues in tibia bone, liver, excreta and egg shell. Significant (p<0.05) differences were recorded among the treatment means in final body weight, feed intake, egg production and feed conversion ratio. Birds fed control diet significantly (p<0.05) consumed more feed than the birds on ZnP and other inorganic Zn sources. Birds on ZnP supplemented diet recorded significantly (p<0.05) higher egg production, this was however similar to the group fed ZnSO₄ supplemented diet. Egg qualities were not significantly (p>0.05) different due to Zn sources except HU values. Serum glucose, SGPT and creatinine concentrations did not vary (p>0.05) due to Zn sources while serum protein, uric acid, SGOT and serum Zn concentrations were consistently lowered (p<0.05) in the control group. Birds on ZnP supplemented diet showed marked significant (p<0.05) difference in the value of Zn residue in tibia bone, liver and excreta of the chickens. The values of the stress indicators were consistently more pronounced in the control group than birds on Zn supplemented diets. For better laying performance, higher Zn retention and alleviation of stress, 140 ppm of Zn in bioplex form (Zn proteinates) was recommended for laying chickens in the tropics.

Key words: Laying hens, zinc salts, residues

INTRODUCTION

Zinc is a trace element that is necessary for normal growth, maintenance, bone development, feathering, enzyme structure and function and appetite regulation for all avian species (Batal *et al.*, 2001). Zinc (Zn) also plays an important role in egg shell and shell membrane formation because they are co-factor and (or) structural products of enzyme systems responsible for carbonate formation and mucopolysaccharide synthesis, respectively. NRC (1994) recommended between 29-44 mgZn/kg for laying strains of leghorn type chickens, which appeared to be based on the results that considered laying performance as the only criterion. Higher Zn supplementation was found to be beneficial (Bartlett and Smith, 2003; Hudson *et al.*, 2004). Zinc at 0.012-0.018% on a total weight basis is commonly added as a supplement to all formulated poultry diets (Leeson and Summers, 1997; Batal *et al.*, 2001). Currently there are two inorganic feed-grade zinc sources commercially used by the poultry feed industry (Wedekind and Baker, 1990; Batal *et al.*, 2001): zinc oxide (ZnO: 72% Zn) and zinc sulfate monohydrate

(ZnSO₄•H₂O: 36% Zn). Of the supplemental zinc fed, 80 to 90% is ZnO, which is less bioavailable for poultry than reagent-grade or feed-grade Zn sulfate (Wedekind and Baker, 1990; Sandoval *et al.*, 1997; Edwards and Baker, 2000). Zinc amino acid complex has also been shown to have increased bioavailability when compared to inorganic sources of zinc. High levels of Zn can accentuate deficiencies of Fe and Cu. Broiler and laying hens showed a tolerance to Zn at 1200-1400ppm of diet and appetite depression at 3000 ppm (Underwood, 1981). Gill (1997) reported that chelated trace mineral (organic) sources are more biologically available in an animal's digestive system than inorganic minerals and that perhaps resulted in less mineral excretion and pollution of the environment. Clear information on Zn requirements for growth, mineral bioavailability and immune response in commercial laying hens is scanty. Therefore, the present study was undertaken to study the effect of different source of higher Zn supplementation on laying performance, serum chemistry and mineral availability in tibia bone, liver, excreta and egg shell of laying hens at early laying age without molting.

MATERIALS AND METHODS

Experimental diets and design: A total 360 (Point of lay) Brown Yaffa strain hens were allotted to 6 dietary treatment groups replicated thrice. The control group was fed basal diet without Zn supplement. Basal diet (control) was formulated to meet NRC (1994) requirements and was supplemented with Zinc oxide, Zinc sulphate, Zinc carbonate, Zinc chloride (Sigma Chemical, St! Louis, M.O.) and Zinc proteinate (Bioplex Zinc, Alltech, Nicholasville, KY) respectively to supply 140 mgZn/kg of diets. Each of the six dietary treatments was fed to triplicate groups of chickens for ten weeks. The composition of the basal diet in percentage are: Maize 49.00, Soyabean 11.00, Wheat offal 12.00, Groundnut cake 9.64, Palm kernel cake 5.00, Fish meal 1.50, Bone meal 2.50, oyster shell 8.5, Salt 0.30, Lysine 0.15, Methionine, 0.16 and Vitamin/mineral premix without zinc 2.5 respectively. The basal diet supplied 2598 kcal ME per kg, 17.5% crude protein, 7.5% fibre, 3.8% Ca, 0.9% P and 35 mg Zn per kg of diet. Experimental birds were kept in a standard battery cage with automatic nipple drinkers and standard feeding trough. All recommended health and management practices were strictly observed.

Egg quality determination: For egg internal and external quality assessments thirty eggs per replicate from each treatment group were sampled. Weight of each egg sample and the albumen, yolk and shell weights were measured, respectively with a sensitive weighing scale (Mettler - Toledo® PB3002) to the nearest 0.01 g. Egg albumen quality (Haugh units) was evaluated by a P6085 Spherometer (tripod micrometer) having an accuracy of 0.01 mm. Egg shells weight and thickness were individually measured; shell thickness was measured by a 25M-micrometer gauge (Ames, Waltham, M.A., USA). Egg Shape Index (ESI) and Egg Shell Index (I) were calculated according to Sauveur (1998) and Iposu *et al.* (1994) using the formula:

$$ESI = EB/EL$$

$$I = 100 SW/S$$

Where, EB is the egg breadth (mm) and EL is the egg length (mm) measured with a vernier caliper with accuracy of 0.01 mm. SW is the shell weight (g) and S the surface area (cm²); S was calculated from Egg Weight (EW) from the equation:

$$S = K. EW^{2/3}$$

Where, K has a value of 4.67 for egg weight less than 60 g, 4.68 for egg weight between 60 g and 70 g and 4.69 for egg weight greater than 70 g respectively. Formula for estimating the Egg Specific Gravity (ESG) was based on

weight of egg and shell as used by Poultry Adviser (1992):

$$ESG = EW/[0.9680 (EW - SW) + (0.4921 SW)]$$

Serum analyses: At the termination of the 10-week experiment, 5 ml of blood was drawn from the brachial vein of 18 birds per treatment (6 per replicate) into heparinized tubes. The serum was obtained by centrifugation of non-coagulated blood at 700 g for 5 min. The serum were frozen at -20°C until needed for analysis. Serum biochemical indices determined were serum glucose, urea, Alkaline Phosphatase (ALP), Glutamic-Oxaloacetic Transaminase (SGOT or AST), Glutamic-Pyruvic Transaminase (SGPT or ALT) and total protein.

Zn residue in the bone, liver and faeces: At the end of the experiment (10 wks) six hens per treatment were randomly selected and slaughtered, eviscerated and tibia bone and the liver from the hens were collected. The right and left tibiae from each bird sampled were pooled group-wise and pressure cooked for 1 h. Attached muscle and cartilage were removed and washed with distilled water and oven dried. The tibiae were then weighed and ashed in a muffle furnace at 600±5°C for 4 h. Approximately 0.2 g of ash sample from each replicate was solubilized in 5 mL of 50% HCl and the mineral extract was filtered into a volumetric flask. The extract was then diluted using deionized water to the required volume and Zn, Ca and P contents were determined by atomic spectrophotometry (Perkin- Elmer, Atomic Absorption Spectrophotometer) at wavelength of 324.7 nm (Chiou *et al.*, 1998). Also liver, feed and faeces were oven dried at 100°C for 24 h and finely ground for mineral analysis as described above.

Lymphoid organs, white blood cell count and heterophil-lymphocyte ratio: Six birds from each replicate group were killed by cervical dislocation and the weight of spleen was recorded and expressed as grams per kilograms of live weight. Anti-coagulated blood samples were collected prior the killing of the birds. White blood cell count, heterophils and lymphocytes were counted according to methods described by Edington and Gilles (1981) and heterophil-to-lymphocyte ratio was calculated to indicate the relative stress in birds due to dietary Zn levels (Shyam Sunder *et al.*, 2008).

Statistical analyses: Statistical analyses (ANOVA) were performed using General Linear Model (GLM) procedure of SAS (1985). Significant differences between treatment means were determined at p<0.05 using Duncan's new multiple range tests.

Table 1: Effects of zinc salts supplementation on the performance of commercial laying hens

Parameters	Supplemental form of zinc					
	Control	ZnCl ₂	ZnSO ₄	ZnO	ZnCO	ZnP
Initial weight (kg/hen)	1.76±0.09	1.76±0.09	1.73±0.19	1.76±0.12	1.87±0.13	1.93±0.07
Final weight (kg/hen)	1.85±0.07 ^b	1.90±0.09 ^a	1.92±0.19 ^a	1.85±0.07 ^b	1.91±0.14 ^a	1.95±0.09 ^a
Feed intake (g/day)	145.00±0.00 ^a	135.00±0.00 ^b	130.00±0.06 ^b	139.00±0.05 ^b	137.00±0.00 ^b	129.00±0.00 ^c
Zn intake (mg/bird)	355.25±7.24 ^b	1653.75±12.7 ^a	1592.50±8.11 ^a	1702.55±16.15 ^a	1677.45±13.37 ^a	1580.05±7.22 ^a
Hen day (%)	66.29±11.45 ^b	68.15±4.07 ^b	71.59±5.55 ^a	67.12±4.96 ^b	66.49±6.48 ^b	74.19±9.97 ^a
Feed/kg egg (kg)	4.30±0.99	4.21±0.62	3.77±0.421	4.11±0.61	3.97±0.45	3.31±0.28
Feed/dozen egg (kg)	3.45±0.75 ^a	3.34±0.23 ^{ab}	3.10±0.35 ^b	3.20±0.35 ^b	3.20±0.36 ^b	3.00±0.34 ^c

^{abc}Means on the same row with the same or without superscript are not significantly different (p<0.05)

RESULTS AND DISCUSSION

No significant difference was noticed in the initial body weight and the feed per dozen egg laid (Table 1). Zn supplementation significantly influenced the values of final body weight, feed intake, hen day egg production and feed per kilogram egg laid (Table 1). The mean final body weight was significantly lower in the control and ZnCl₂ group than the rest of other treatment groups. However there was no weight loss but weight gain across treatment groups as body weight loss in hens has been directly associated with decreased muscle weight, decreased liver weight, decreased use of adipose tissue, involution of the reproductive tissue and greater reproductive regression (Brake and Thaxton, 1979; Berry and Brake, 1985). The body weight loss has also been identified as a major factor contributing to the induced molting because body weight loss has influence on the successful results of an induced molting procedure. Hens fed the control diet had significantly (p<0.05) greater feed intake (145.0 g/bird/day) than did hens fed diet supplemented with 140 mg/kg zinc in the form of Zn proteinate or inorganic Zn salts. However, there was no significant (p>0.05) difference in feed intake (129-139 g/bird/day) between hens fed Zn supplemented feeds (Table 1). There were approximately 5-11% reduction in feed intake of Zn supplemented fed hens when compared to the feed intake of hens fed non-supplemented control layer ration. Similar feed intake reduction was seen by Shippee *et al.* (1979). The reduced feed intake could be due to appetite depression (Brink *et al.*, 1950) or low palatability of high levels of zinc (Fox, 1989). It has also been reported that the reduced feed intake could be due to the ability of zinc cation (Zn²⁺) to induce follicular atresia and halt egg laying (Scott and Creger, 1976; Shippee *et al.*, 1979; Berry and Brake, 1985; McCormick and Cunningham, 1987; Johnson and Brake, 1992). It is likely that the efficiency of dietary zinc treatments was directly related to the suppression of feed intake.

Hen day egg production was significantly highest in the Zn Proteinate group which was statistically similar with ZnSO₄ group. Lowest hen day production was noticed in the control group and was statistically the same with those obtained for ZnO, ZnCl₂ and ZnCO₃ groups. The result suggest that Zn from Proteinate group and ZnSO₄ have a different effect on reproduction organs that was

distinct from that of ZnO, ZnCl₂ and ZnCO₃. Feed conversion efficiency in terms of feed per dozen egg laid was optimum in the ZnP treatment group. However Scott and Creger (1976) and Creger and Scott (1980) reported that the 20,000 ppm (2%) of zinc as zinc oxide stopped a egg production completely within 5 days. Shippee *et al.* (1979) found that the addition of 10,000 ppm (1%) zinc as either zinc oxide or zinc acetate to the layer ration for 14 days caused hen day egg production to decline form 60 to 0% in 6 days. Berry and Brake (1985) noticed cessation of ovulation when hens were fed high concentrations of zinc which was also linked to effectiveness of zinc at high concentrations to a depress feed intake. However, Breeding *et al.* (1992) reported that the moderate dietary concentrations of zinc (≤2,800 ppm) were sufficient to suppress hen reproduction systems.

All egg quality parameters measured were not significantly affected by Zn supplementation except Haugh unit (Table 2). The Haugh unit values does not follow a definite trend however Zn treatment groups had the highest value (58.12) compared to the control and other groups. However, the addition of Zn has been reported to increase the utilization of Ca in hens and to improve the qualitative parameters of the eggshell (Klecker *et al.*, 2002) (Table 4). Zinc supplementation also has been reported to improve eggshell quality because it is a component of the carbonic anhydrase enzyme, which supplies the carbonate ions during eggshell formation (Innocenti *et al.*, 2004). Sahin *et al.* (2002) reported that supplementation of Zn sulfate increased egg weight, eggshell thickness, egg specific gravity and Haugh units when layers were subjected to low ambient temperatures. Kita *et al.* (1997) in another study reported that the addition of dietary Zn-Met did not improve egg quality in layers subjected to high ambient temperatures. The findings in this study corroborated the findings of Mabe *et al.* (2003) who reported that dietary Zn supplementation did not affect percentage eggshell and eggshell index. The trend noticed in this study agreed with the findings of Klecker *et al.* (2002), who reported that the substitution of 20-40% supplemental inorganic Zn with their chelates resulted in an increase in laying hen performance, eggshell weight and eggshell thickness. On contrary Lim and Paik (2003) noticed a decreased in hen day egg production.

Table 2: Effect of Zn salt supplementation on the internal and external qualities of egg

Parameters	Supplemental form of zinc					
	Control	ZnCl	ZnSO ₄	ZnO	ZnCO ₃	ZnP
External qualities						
Egg wt (g)	61.99±1.15	63.45±1.63	67.73±3.14	65.08±2.03	66.20±2.82	67.90±3.07
Egg length	4.50±0.25	4.39±0.34	4.29±0.49	4.45±0.17	4.50±0.17	4.60±0.18
Egg breadth	3.24±0.35	3.22±0.34	3.16±0.31	3.09±0.15	3.13±0.16	2.96±0.11
Albumen properties						
Weight (g)	38.47±2.28	39.31±3.45	38.64±1.97	38.97±3.56	38.07±3.13	36.89±1.89
Height (mm)	4.15±0.17	4.28±0.17	4.11±0.19	4.10±0.2	4.16±0.21	4.24±0.22
Yolk wt (g)	16.76±0.94	17.36±0.23	15.89±0.96	17.32±1.82	16.89±1.17	16.61±1.57
Dry shell properties						
Thickness (mm)	0.11±0.01	0.12±0.01	0.14±0.01	0.14±0.33	0.14±0.03	0.15±0.03
Weight (g)	5.47±0.33	6.50±0.39	6.67±0.72	6.24±0.73	6.31±0.69	6.85±0.90
Shell index (l)	8.28±0.59	8.23±0.57	8.82±0.95	8.07±0.92	8.03±0.80	8.31±0.73
Derived egg qualities						
ESI	0.73±0.11	0.74±0.16	0.75±0.18	0.69±0.04	0.70±0.04	0.65±0.03
ESG	1.06±0.03	1.09±0.04	1.09±0.05	1.08±0.05	1.07±0.03	1.05±0.01
Haugh unit	55.57±2.98 ^{ab}	56.73±2.69 ^{ab}	56.79±2.34 ^{ab}	55.55±3.26 ^{ab}	54.92±2.45 ^b	58.12±2.39 ^a

^{abc}Means on the same row with the same or without superscript are not significantly different (p<0.05)

Table 3: Effect of zinc supplementation on serum metabolites of laying chickens

Parameters	Supplemental form of zinc					
	Control	ZnCl	ZnSO ₄	ZnO	ZnCO ₃	ZnP
Glucose (mg/dl)	135.65±7.85	132.35±6.75	129.10±3.50	131.25±6.07	130.90±0.00	128.90±4.34
Protein (g/dl)	29.81±2.74 ^b	31.70±2.70 ^b	32.35±3.05 ^b	32.05±3.01 ^b	32.10±3.40 ^b	35.20±4.50 ^a
Uric acid (mg/dl)	2.35±0.15 ^c	5.20±1.00 ^a	3.50±0.30 ^b	5.20±0.80 ^a	4.60±0.40 ^{ab}	4.40±0.70 ^{ab}
SGPT/ALT (IU/l)	7.35±1.25	11.10±1.80	17.80±2.02	17.50±2.00	16.80±1.93	18.65±2.65
SGOT/AST (U/l)	119.95±0.45 ^c	135.60±2.98 ^a	128.40±1.90 ^c	132.70±3.40 ^a	132.70±12.80 ^a	125.00±10.30 ^c
ALP (IU/l)	100.70±0.60 ^c	115.35±1.35 ^{ab}	119.05±1.31 ^b	117.90±1.20 ^b	119.80±1.30 ^b	128.15±2.35 ^a
Creatinine (mg/dl)	0.95±0.05	0.95±0.15	0.90±0.01	0.90±0.01	0.90±0.20	0.86±0.01
Zn ⁺ (mg/dl)	9.50±0.50 ^c	15.50±0.66 ^b	20.00±1.43 ^a	17.00±1.84 ^b	16.50±1.06 ^b	27.50±1.97 ^a

^{abc}Means on the same row with the same or without superscript are not significantly different (p<0.05)

Table 3 showed that serum glucose, SGPT and creatinine concentrations did not vary due to dietary treatments imposed (p>0.05). Significant variations were noticed in the serum protein, uric acid, SGOT and serum Zn concentrations and consistently lowest values were obtained in the control group when compared with all the Zn supplemented dietary treatments. Birds on Zn proteinate supplemented diet had highest (p<0.05) values except the uric acid. Statistical similarities were noticed between ZnSO₄ and ZnP groups in terms of serum Zn⁺, protein, uric acid, SGPT, creatinine and glucose. The values of glucose, protein, SGPT and creatinine did not differ among the diets supplemented with Zn. Decline was noticed in the value of alkaline phosphatase. This has been associated with increase or adequacy in dietary Zn (Kirchgebner and Roth, 1981). This was due to Zn binding capacity of serum and because of this capacity alkaline phosphatase has been used as good indicator of Zn status.

Zn residues in the tibia bone, liver and excreta of the laying chickens showed marked significant difference (p<0.05) with Zn Proteinate and the control treatment groups consistently having the lowest and highest values respectively. Similar trend was noticed in the egg shell Zn residues. Shell zinc concentrations were similar (p>0.05) for ZnCl and ZnCO₃ treatment groups. Zn

residue was more in the liver and next to it was the tibia bone while the lowest was in egg shell. Between 80-100% increase in the Zn residue was found in the diets supplemented with Zn salts when compared with control. The shell Zn concentration increased in the following order: control, ZnO, ZnCO₃, ZnCl, ZnSO₄ and ZnP respectively. Twice as much excreted in the control was noticed in the ZnP, ZnSO₄, ZnCl₂ and ZnCO₃ groups. Excretion of Zn was more in the ZnP group. The addition of either inorganic or chelated Zn increased the utilization of Ca and P in hens as shown in Table 4 which has implication on improving the qualitative parameters of the bones and eggshell as indicated by Klecker *et al.* (2002).

The weights of lymphoid organ and the white blood cell count varied significantly across treatment groups (p<0.05) with the birds in the control group having consistently a significantly depressed values while birds fed ZnProteinate supplemented diet had highest values. Similar response was noticed between ZnP, ZnO and ZnSO₄ groups in terms of WBC. Statistical equalities were noticed in weight of spleen among birds on ZnO, ZnSO₄, ZnCl and ZnCO₃ diets. The ratio between heterophils and lymphocytes, an index of stress in birds, was significantly (p<0.05) higher in the control group (p<0.05) than any of the Zn supplemented groups

Table 4: Zn residue and Ca-P concentration in bone, shell liver and kidney of experimental hens

Parameters	Supplemental form of zinc					
	Control	ZnCl ₂	ZnSO ₄	ZnO	ZnCO	ZnP
Ca and P absorption						
Tibia Ca (%)	27.40±1.00 ^c	30.00±1.50 ^c	36.00±1.50 ^b	33.50±1.20 ^b	31.51±1.80 ^c	37.00±1.50 ^a
Tibia P (%)	12.41±6.89 ^c	15.09±7.85 ^b	17.21±8.89 ^a	16.11±7.01 ^b	16.77±7.20 ^b	18.12±9.06 ^a
Shell Ca (%)	30.10±2.00 ^c	39.10±2.20 ^b	41.85±2.70 ^a	40.85±2.40 ^b	39.51±2.30 ^c	42.15±2.80 ^a
Shell P (%)	0.16±0.01 ^c	0.16±0.00 ^c	0.18±0.06 ^a	0.17±0.02 ^b	0.17±0.03 ^b	0.19±0.08 ^a
Zn residue						
Tibia Zn (ppm)	260.10±2.00 ^c	783.50±1.50 ^b	814.50±1.80 ^a	801.00±3.00 ^a	791.00±2.00 ^a	814.50±1.50 ^a
Liver Zn (ppm)	125.50±4.10 ^d	133.50±4.30 ^c	148.10±5.04 ^a	138.50±4.50 ^b	138.02±5.05 ^b	151.50±5.50 ^a
Kidney Zn (ppm)	78.86±0.98 ^c	92.44±1.10 ^b	100.05±1.50 ^a	98.22±1.10 ^b	95.61±1.20 ^b	111.23±1.60 ^a
Excreta Zn (ppm)	104.20±1.16 ^d	207.50±1.50 ^c	244.00±2.00 ^b	239.10±1.22 ^b	226.40±1.70 ^b	251.50±1.50 ^a
Shell Zn (ppm)	126.54±1.50 ^e	136.30±1.8 ^d	156.80±3.00 ^b	154.79±2.00 ^b	145.50±2.50 ^c	161.14±3.50 ^a

^{abc}Means on the same row with the same or without superscript are not significantly different (p<0.05)

Table 5: Effects of zinc salts supplementation on lymphoid organs and white blood cell count

Parameters	Supplemental form of zinc					
	Control	ZnCl ₂	ZnSO ₄	ZnO	ZnCO	ZnP
Spleen (g/kg BWT)	1.11±0.07 ^c	1.89±0.09 ^b	1.99±0.12 ^b	1.92±0.10 ^b	1.96±0.10 ^b	2.14±0.14 ^a
WBC (10 ⁶ /mm ³)	1.82±0.06 ^c	2.11±0.09 ^a	2.27 ±0.11 ^a	2.19±0.10 ^b	2.12±0.09 ^b	2.41±0.12 ^a
H:L ratio	0.52±0.04 ^a	0.44±0.02 ^b	0.43±0.02 ^b	0.45±0.03 ^b	0.44±0.03 ^b	0.41±0.01 ^b

^{abc}Means on the same row with the same or without superscript are not significantly different (p<0.05) H:L = Heterophil-to-Lymphocyte ratio

suggesting that supplementation of Zn was useful in reducing stress. H:L ratio however was the same across Zn supplemented diets with ZnP group having lowest value. A normal H:L ratio for hens is about 0.4 and it rises to 0.8 in birds under severe stress (Gross and Siegel, 1983).

Conclusion: For better laying performance, higher Zn retention and alleviation of stress, 140 ppm of Zn in bioplex form (Zn proteniate) was recommended for laying chickens in the tropics.

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