INFLUENCE OF DIETARY ZINC SUPPLEMENTATION ON SERUM MINERALS (ZINC, CALCIUM, PHOSPHORUS) AND LIVER ENZYME LEVEL (ALP, ALT, GGT) IN LAYER CHICKEN

ABSTRACT

The Indian industry is presently focussed in production of eggs that have higher or enriched levels of certain nutrients. These eggs are capable of safeguarding the health of consumers. Designer eggs have high market demand because of the consumers' willingness to purchase designer eggs due to its' nutritional qualities additional to regular eggs. Considering these facts the current study was planned to produce zinc enriched eggs by supplementing laying hens' diet with required levels of zinc. The present study was conducted at the Department of Veterinary Physiology, College of Veterinary and Animal Sciences, Mannuthy, for 12 weeks. A total of 28 week old thirty two laying hens, belonging to crossbred (White Leghorn N strain and Desi) housed in the animal house attached to the department were used in the experiment. The treatments of this study included a control diet (according to BIS, 2007) fed group and zinc-supplemented group. Each of the experimental treatments had four replicates with four birds with all standard managemental conditions. The functional liver status of birds was not altered due to zinc supplementation, since the present study neither caused any toxicity nor imparted any negative effect on the production performance. Highly significant effect was observed on serum concentration of zinc and calcium. However, the phosphorus levels of treated birds were not significantly different from the control group.

Key words: Calcium, Layer chicken, Liver enzymes, Zinc

INTRODUCTION

Zinc is an essential trace mineral micronutrient which is an integral part of over 300 enzymes involved in carbohydrate, nucleic acid and protein metabolism. Zinc has a systemic role in fertility, antioxidation, skeletal and neurobehavioural functions. Zinc In addition, zinc plays a key role in the immune system, transport and the use of vitamin A. Many plant foods have high zinc content particularly cereal grains and legumes, but bioavailability is less due to presence of phytates. Though richest zinc sources are animal products such as beef, pork and shellfish, they are expensive. Therefore, zinc deficiency is widely felt in the population.

REVIEW OF LITERATURE

The zinc content of hen's eggs can be upgraded by relatively simple and economical 3 dietary approaches, by adding zinc compounds especially organic zinc complexes like zinc methionine (Stahl *et al.,* 1988) or inorganic zinc sources such as zinc sulphate, zinc carbonate and zinc oxide to the laying hen's diet. Due to its easy availability and low cost, inorganic zinc sources are commonly chosen for enrichment. If zinc enriched eggs are made available at a reasonable price, they will be the most effective zinc source for consumers.

Kaya *et al.* (2001) reported that"plasma zinc concentration was affected only by dietary zinc supplementation.The highest plasma zinc level was 4.39 µg/mL in the group fed 100 mg zinc/kg in the diet, and was very close to the plasma zinc level of the control group. However, plasma zinc level declined after feeding more than 100 mg zinc/kg".

Al-Daraji and Amen (2011) evaluated "the effect of zinc as feed additive on some the blood plasma traits of males and females of broiler breeder chickens. Outcomes of the study indicated that dietary zinc supplementation increased blood plasma calcium concentration in broiler breeder males and females during 58 and 66 weeks of age as compared with control group".

Bahakaim *et al.* (2014) noticed that "increasing zinc levels lead to differences in plasma phosphorus levels and the highest plasma P was recorded for control group which contain recommended level of inorganic zinc (50mg/kg diet) without supplementation"

"An atomic absorption spectrophotometric method for evaluating zinc in biological materials was developed" by Fuwa*et al.* (1964). Destruction or removal of organic material by ashing or precipitation was not necessary in this method. The sample was simply diluted with distilled water and aspirated directly into the hydrogen air flame of device. The limit of detection was 0.002 µg of Zn/mL. Zinc determination by atomic absorption was 100 times more efficient than the widely used dithizone method.

Butrimovitz and Purdy (1977) used atomic absorption spectrometry for routine plasma zinc analysis because of its low operating costs, sensitivity and simplicity. Dilution techniques reduce plasma viscosity and permit direct analysis. It was recommended to dilute plasma directly (1:4) with deionized water. Working zinc standards were prepared in 5% glycerol to estimate the viscosity and aspiration levels in par with the diluted plasma

specimens. The method's accuracy was 99% and the recovery of inorganic zinc added to the 22 pooled plasma was 99.8% on an average. The system worked without clogging of nebulizers and interference with matrixes

MATERIALS AND METHODS

The study was conducted using thirty two numbers of 28 weeks old crossbred (White Leghorn N strain X Desi) layer birds procured from All India Coordinated Research Projects (AICRP) on Poultry for Eggs, Mannuthy, KVASU. The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Mannuthy. The birds were randomly distributed in a completely randomized experimental design and they were placed into two treatment groups, each with four replicates having four birds in each replicate. Control with standard layer diet (BIS 2007) was fed for birds in group I and Zn enriched diet for group II birds. "All birds were fed with a standard layer diet and raised under standard managemental conditions up to 28 weeks of age at AICRP on Poultry for Eggs Mannuthy, KVASU. The birds were immunized against diseases as per standard protocol followed in the farm. On completion of 28 weeks of age, the birds (average body weight of 1.28±0.06 Kg) were brought and housed in well ventilated cages of animal house attached to Department of Veterinary Physiology. They were kept at 24.5 ± 0.5˚C ambient temperature and at relative humidity ranging from 60-80 per cent. A photoperiod of 16 h per day was ensured throughout the period" (Megha and Ramnath, 2021)

EXPERIMENTAL DIET

Table 1: Percent ingredient composition of experimental diet

Sl. No.	Feed ingredients	Percentage
1	Yellow maize	56.50
2	De-oiled rice bran	6.50
3	Soya bean meal	27.50
4	[1]Calcite powder	7.50
5	Dicalcium phosphate	1.50

6	Salt	0.50
Total		100.00
Feed Supplements (g/100 kg feed)		
1	L-Lysine	100
2	DL-Methionine	100
3	Vitamin premix	50
4	Toxin binder	100
5	Choline chloride	100
6	Trace mineral mixture	100
7	Liver tonic powder	25
Total		575

Zinc level in control diet was formulated according to BIS 2007 standards. Treatment group II was provided with a diet incorporating inorganic zinc sulphate at the level of 75 mg/kg mash diet (Megha *et al*., 2021). The total ration was provided as a single lot in the morning. Water was provided ad libitum throughout the experimental period (84 days).

BLOOD SAMPLE COLLECTION AND ANALYSIS

"Blood samples were collected once in every month from the wing vein under aseptic condition using 26 gauge hypodermic needle and syringe. After holding the collected blood samples in vials for 30 min at room temperature, they were subjected for serum separation by centrifuging at 3000 rpm for 15 minute. Serum samples were stored in eppendorf vial at -20˚C till further estimations". [17]

"Analysis of serum for zinc (ppm) and calcium (mg/dL) were done using Atomic Absorption Spectrometry (AAS). The AAS enabled a simple automated measurement of Zn using hollow cathode lamp of wavelength 213.86 nm, energy level 40% and lamp current of 15 mA. Calibration curve was plotted with standards ranging from 0 to 6 ppm with a

calibration equation non linear through zero. Slit width of 0.7 nm ensured accurate analysis. The flame atomizer with a temperature of 2400˚C was ideal for the measurement. Fuel and oxidant gases were acetylene (2.5L/min) and air (10 L/min) respectively".[17]

Table 2: Absorbance and calculated concentrations of zinc standards

Concentration of standard (mg/L)	Mean signal (Abs)	Calculated concentration (mg/L)
Blank	0.0000	0.0000
2.0	0.0769	1.837
4.0	0.1975	4.483
6.0	0.2515	5.583

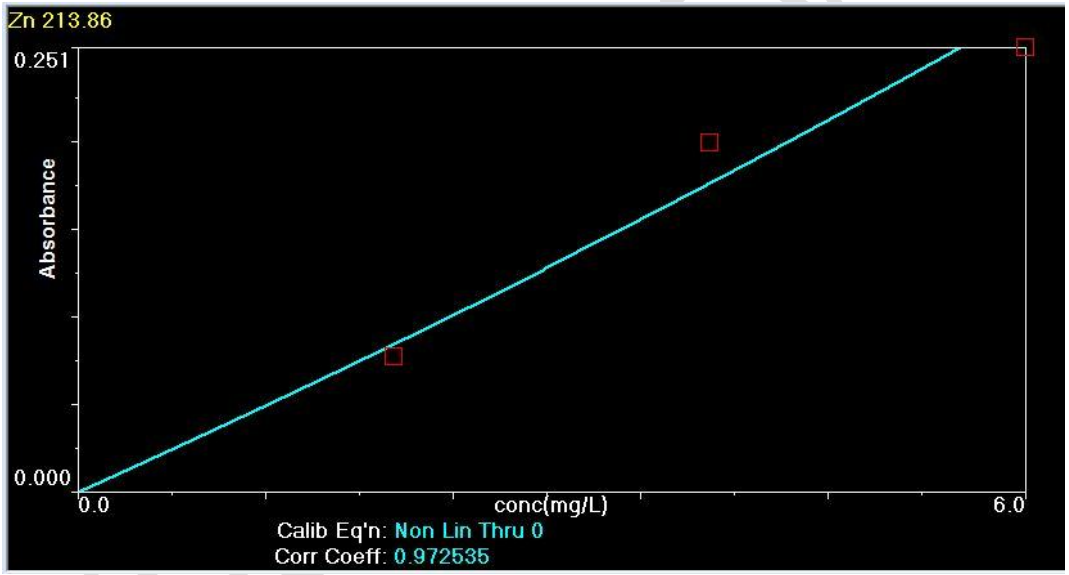

Fig. 1: Calibration curve of zinc with different standards

"The serum samples were analysed for calcium by Atomic Absorption Spectrometry standard method" (AOAC., 2016). "The PinAAcle 900H Series AAS enabled a simple automated measurement of Ca using hollow cathode lamp of wavelength 422.67 nm, energy level 66% and 35 lamp current of 10 mA. Calibration curve was plotted with standards ranging from 0 to 100 ppm with a calibration equation nonlinear through zero". [17]

Table 3: Absorbance and calculated concentrations of calcium standards

Concentration of standard (mg/L)	Mean signal (Abs)	Calculated concentration (mg/L)
Blank	0.00	0.00
3.0	0.2046	2.937
6.0	0.4073	6.181
10.0	0.6114	9.891
15.0	0.8570	15.189
50.0	1.6422	47.385
100.0	2.0530	105.918

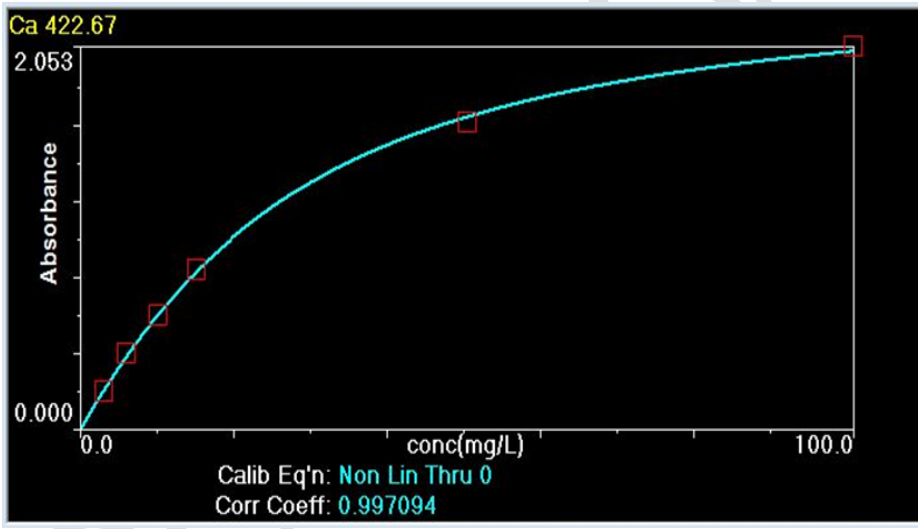

Fig. 2: Calibration curve of calcium with different standards

Serum phosphorous level (mg/dL) and activity of serum enzymes like Alanine aminotransferase (ALT: IU/L), Alkaline phosphatase (ALP: IU/L) and Gamma glutamyl transferase (GGT: IU/L) were quantified by semi-automated biochemical analyser (Megha and Ramnath, 2023) using commercial kits supplied by M/s Agappe diagnostics Ltd, Maharashtra.

STATISTICAL ANALYSIS

Results were expressed as mean (±S.E.). The statistical significance of difference or relation between the two treatments were analysed by CRD using the software Statistical

Product and Services (SPSS) version 24.0 and the differences were considered statistically significant at 95% level ($p<0.01$).

RESULTS AND DISCUSSIONS

Zinc level in the serum of birds

Samples were analyzed for zinc by AAS, using hollow cathode lamp of wavelength 213.86 nm. The findings of zinc concentration in serum, when hens were supplemented with inorganic zinc sulphate at a level of 75 mg/kg mash diet are presented in the table4

Zinc concentration in the serum was significantly higher ($p<0.01$) for birds supplemented with zinc in the diet when compared to the control group

Table 4: Mean ±S.E. value of serum zinc concentration [mg/L (ppm)] in different treatment groups

Treatment groups	**No of samples**	**Mean value of amount(ppm)**	**p-value**
		Mean±S.E.	
Control group	12	2.54^{a}±0.06	0.000**
Supplemented group	12	3.09^{b}±0.11	

Means bearing different superscripts within a column differ significantly ($p<0.01$)

* Significant ($p<0.05$)

** Highly significant ($p<0.01$)

Zinc concentration in the serum was significantly higher ($p<0.01$) for birds supplemented with 75 mg/kg zinc in the diet compared to control group (2.54±0.06 ppm). Similar observations were reported by Aghai *et al.* (2017)who found that **s**erum zinc concentration was increased when quails fed at 120 mg Zn/kg feed level.These results are in agreement with reports of Kaya *et al.* (2001) who reported that only dietary zinc supplementation affected the plasma zinc concentration and the highest plasma zinc level recorded was 4.39 µg/mL in 100 mg zinc/kg fed group. On contrary, plasma zinc declined significantly after feeding of more than 100 mg Zn/kg, which might be due to the homeostatic mechanism with respect to the absorption, distribution, metabolism and excretion of zinc in tissues.

On contrary to current findings, Mitchell and Carlisle (1991) put forward a positive correlation between plasma zinc level and egg production since laying birds exhibited much

higher levels of zinc than male or immature female birds. The zinc level was consistent with the appearance of vitellogenin in the plasma, and the circulating vitellogenin was considered as an indicator for egg yield in layers.

Bahkaim *et al.* (2014)found that when layer hens which were supplemented zinc as zinc methionine at 150 mg/kg diet yielded the highest values for egg and plasma zinc content. However in the present study, a similar situation was noticed in birds fed with inorganic zinc supplemented diets which showed higher zinc concentration in eggs and serum compared to the other groups which received control diet.

Calcium level in the serum of birds

Results of calcium levels in serum of birds are given in table 5. The zinc supplementation brought about significant ($p<0.01$) changes in serum calcium level of birds. The calcium level was significantly higher for Zn fed group compared to the control.

Table 5: Mean (±S.E.) value of serum calcium (mg/dL) concentration in different treatment groups

Treatment group	**No of samples**	**Mean value of amount(mg/dL)**	**p-value**
		Mean±S.E.	
Control group	12	$8.65^{a} \pm 0.15$	0.000**
Supplemented group	12	$9.25^{b} \pm 0.16$	

Means bearing different superscripts within a column differ significantly ($p < 0.01$)

* Significant ($p<0.05$)

** Highly significant ($p<0.01$)

In present study serum calcium levels of birds were significantly ($p<0.01$) influenced by zinc supplementation. The serum calcium level was significantly ($p<0.01$) higher for the Zn supplemented group compared to the control groupwhich was in agreement with the observations of Idowu *et al.* (2011) who reported that the addition of either inorganic or chelated zinc increased the utilization of calcium. Similarly findings of Bahakaim *et al.* (2014) revealed that plasma calcium level was significantly increased by increasing Zn levels in diets. Hens which received 100 mg/kg zinc methionine diets, recorded the highest plasma calcium level.In broiler breeder male and female during the age of 58 to 66 weeks Al-Daraji and Amen (2011) found that zinc supplementation resulted in significant increase of blood plasma calcium.

Phosphorus level in the serum of birds

It was noticed that zinc supplementation did not significantlyinfluence the serum phosphorus levels as showed in Table 6.

Table 6: Mean (±S.E.) value of serum phosphorus (mg/dL) levels in different treatment groups

Treatment group	No of samples	Mean value of amount(mg/dL)	p-value
		Mean±S.E.	
Control group	12	$3.77^{a} \pm 0.20$	p >0.05
Supplemented group	12	$3.69^{a} \pm 0.20$	

Means bearing same superscript within a column do not differ significantly (p>0.01)

Kaya *et al.* (2001) reported that plasma phosphorus level was altered by zinc supplementation. But in the present study phosphorus value of treatment were statistically comparable. However increasing zinc levels in the diet led to decrease in plasma phosphorus concentration insignificantly. Highest plasma P was recorded in control group which was having recommended level of inorganic zinc (50mg/kg diet) without extra supplementation (Bahakaim *et al.*, 2014).

Liver enzyme activity of birds

The effect of Zn feeding triggered insignificant change in liver enzyme status of birds during the experimental period.

Table 7: Mean (±S.E.) value of liver enzymes in serum in different treatment groups

Treatment group	No of samples	Mean value of amount (IU/L) Mean ± S.E.		
		ALP	ALT	GGT
Control group	12	432.35±11.59	1.43±0.10	10.31±0.33
Supplemented group	12	422.00±9.95	1.33±0.11	10.86±0.42
p-value		0.898	0.591	0.780

Status of liver enzymes activity in serum was estimated to rule out the side effects of UVB exposure and zinc supplementation in birds. In the present study non significant changes in the activity of various liver enzymes were recorded. The activity of ALP (ranged

from 422.00±9.95 to 432.35±11.59 IU/L), ALT (ranged from 1.33±0.11 to1.43±0.10 IU/L), GGT (ranged from 10.31±0.33 to10.86±0.42) were summarised in table 7.

Various reports revealed that incremental Zn supplementation in diets caused its deposition in liver (117 to 143 ppm) of broiler chickens at 4 weeks of age (Sunder *et al.,* 2008). Similarly, Sandoval *et al*. (1997) observed rise in liver Zn deposition ($P < 0.01$) as dietary Zn increased. Diaz *et al.* (1999) pointed out that measurement of plasma enzyme activities particularly ALT, LDH, and GDH, was a valuable tool for diagnosis of liver damage in birds. In their study, White leghorn laying hens (UCD-003 strain) consistently had significantly higher value for plasma liver enzymes which was indicative of extensive hepatic lesions, while plasma activity of ALT averaged 1.0±1.0 IU/L in normal birds. The present study results closely matched with this reported value.

Similar to the present findings, Idowu *et al.* (2011) who evaluated the influence of various sources of zinc in the diets of laying birds in a 10 weeks trial, found thatserumALT activity did not vary due to Zn sources. On contrary, study of Al-Daraji and Amen (2011) indicated that dietary zinc supplementation led to a significant ($p<05$) increase in the activity of plasma alkaline phosphatase (ALP) in broiler breeders.Fernandez *et al.* (1994) whoconducted a study to find out the variations of clinical biochemical parameters of laying hens and broiler chickens fed with aflatoxin containing feed and reported that the activity of serum gamma glutamyl transferase (GGT) increased significantly (75.2±41.00 IU/L) compared to the control group (8.9±5.5 IU/L). This significant increase in serum GGT activity was coincided with high degree of hyperplasia of bile ducts observed in the liver of laying hen. When the aflatoxin was withdrawn from the diet, hyperplasia of bile ducts and GGT levels decreased to normal value.

CONCLUSION

The present study findings revealed that zinc supplementation did not cause any hepatic damage since all the estimated serum biochemical parameters of exposed and supplemented groups were statistically comparable with that of control group.

Ethical approval:

The experiment was approved by the Institutional Animal Ethics Committee (IAEC) of College of Veterinary and Animal Sciences, Mannuthy.

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