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Effect of Zn-amino Acid Complex (ZAA) Supplementation on Broiler Performance, Physiology and Gut Histomorphology

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ABSTRACT

Study objective was to evaluate effects of dietary different levels of zinc-amino acid complex (ZAA) on growth performance, physiological parameters and gut histomorphology on broiler (ROSS308). A total 180 birds were randomly assigned to three treatments (60 birds per treatment) and three replicates per treatment with 20 birds in each replicate). Diets were prepared using a basal diet, 120 and 140 mg/kg Zn amino acid complex. Treatments showed significance in growth performance with reduced feed intake in all zinc-amino acid complex supplemented treatments in comparison to the control. Glucose, triglycerides, ALT and AST showed non-significant effects among different treatments, otherwise, cholesterol was significant in treatments 120 and 140 mg/kg compared to the basal control. In conclusion, the results showed the zinc-amino acid complex supplementation in the basal diet significantly improved and enhanced for increased growth performance and gut histomorphology. It also showed impact on cholesterol serum level.

Key words: Broiler, cholesterol, growth performance, zinc-amino acid

INTRODUCTION

Zinc plays an important role in many biological activities that occur in the body and is considered an important nutrient for poultry (Mohammed *et al.,* 2023). Zn is commonly added as a supplement to all formulated poultry diets at a rate of 0.012-0.018% (Naz *et al.,* 2016). The biological activity of Zn is required for growth and skeletal development (Tomaszewska *et al.*, 2017) and also improves antioxidant activity as it involves the production of glutathione peroxidase (Luis-Chincoya *et al.,* 2021). De Grande *et al.* (2020) noted that Zn aided in the synthesis, catalytic activity and stability of numerous protein forms. However, Štenclová *et al.* (2016) reported that supplementation of 20 or 40 mg Zn oxide (ZnO) had no significant effect on the growth performance of broiler chickens.

An additional, zinc complex with amino acids can lead to better bioavailability as compared to inorganic forms. On the other hand, Mohammed *et al.* (2023) found that zinc oxide nanoparticles in drinking water increased body weight in broiler. Therefore, the important activity of the Zn-amino acid complex in broiler feed and its effect on performance, biochemical parameters and gut morphology was estimated.

MATERIALS AND METHODS

One hundred and eighty broilers were kept as used chicks for two weeks before the start of the experiment and randomly assigned to three groups of twenty birds each. The birds were divided into three groups of three replicates of 20 birds each. The diets were prepared in accordance with the National Research Council (Table 1).

Where, experimental diets were basal diet; Control=without any addition; Control+ZAA = zinc-amino acid complex added to provide 120 mg/kg zinc above control; Control+ZAA = zinc-amino acid complex added to provide 140 mg/kg zinc above control. The Institute of Animal Production, College of Agriculture, Tikrit University, approved all procedures applied in this study.

The body weight and feed intake of the broiler were measured weekly. The body weight gain and feed conversion ratio were calculated from these data. On day 42, blood samples were collected from six randomly selected birds per

Table 1. Ingredients and chemical composition of the basal diet used during experiment

Ingredients	Start	Finisher
Yellow maize	46.18	53.88
Wheat	9.92	9.0
Soybean 44%	36.9	29.5
Vegetable oil	2.8	4.2
Primix	2.5	2.5
Di-calcium phosphate	1.0	0.2
Salt	0.3	0.5
Methionine	0.1	0.1
Lysine	0.3	0.12
Chemical composition of the start and growth diets		
ME (Kcal/kg)	3027.74	3202.42
Crude protein (%)	23.02	20.02
Lysine	1.48	1.12
Methionine	0.57	0.54
Methionine+cysteine	0.92	0.85
Calcium	0.97	0.83
Phosphor	0.61	0.45

group. About 3-5 ml blood samples were taken from the wing vein by a 5 ml syringe of a 22 gauge needle and transferred into non-heparinized tubes. The clotted blood samples were centrifuged at 3000 rpm for duration of 15 min then clear serum got separated and stored in a -20°C freezer for the upcoming biochemical analysis. Serum glucose, cholesterol and triglycerides were determined using a kit. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined by using spectrophotometric methods (Biolabo/French). The intestinal samples collected on day 42, two birds from each pen (n=6 per treatment), were selected, and approximately 5 cm lengths of duodenum was preserved in formalin. For a maximum of 24 h, they were fixed in 10% formal saline, treated and infiltrated with paraffin wax. Section 5 µm thick was made, stained with haematoxylin and eosin (H and E), examined with the light microscope and analyzed for villus height (VH), crypt depth (CD) and villus height/crypt depth (VH/CD).

Data analysis was conducted by a fully randomized arrangement in which pen was the main experimental unit using SAS one way ANOVA procedure of SAS software in CRD. The multiple range test of Duncan was applied to partition means ($P<0.05$).

RESULTS AND DISSCUSION

There were non-significant group differences at day 14 of age (Table 2). During days 21, 28, 35, 42 and overall of 21-42 day growth

Table 2. Effect of zinc-amino acid complex on broiler growth

Items	Groups			*p* value
	Control (0.0)	Control+Zn AA (120 mg/kg)	Control+Zn AA (140 mg/kg)	
Body weight (kg)				
Day 14	231.33	236.66	235.33	0.1564
Day 21	488.33^{b}	521.67^{b}	565.00^{a}	0.0118
Day 28	980.00^{c}	1142.33^{b}	1220.00^{a}	0.0001
Day 35	1440.00^{b}	1715.67^{a}	1777.67^{a}	0.0003
Day 42	2035.00^{b}	2338.33^{a}	2400.00^{a}	0.0002
Body weight gain (g)				
Day 21	257.00^{b}	285.00^{b}	329.67^{a}	0.0198
Day 28	491.67^{b}	620.67^{a}	655.00^{a}	0.0011
Day 35	460.00^{b}	573.33^{a}	557.67^{a}	0.0168
Day 42	595.00	622.67	622.33	0.6072
Day 21-42	1803.67^{b}	2101.67^{a}	2164.67^{a}	0.0002
Feed intake (g/bird)				
Day 21	563.33^{a}	460.00^{b}	520.00^{a}	0.0051
Day 28	960.00	901.67	941.67	0.1597
Day 35	1171.67	1053.33	1036.67	0.0550
Day 42	1381.67^{a}	1350.00^{ab}	1313.33^{b}	0.1068
Day 21-42	4076.67^{a}	3765.00^{b}	3811.67^{b}	0.0014
Feed conversion (g:g)				
Day 21	2.20^{a}	1.61^{b}	1.59^{b}	0.0219
Day 28	1.96^{a}	1.45^{b}	1.44^{b}	0.0042
Day 35	2.57^{a}	1.84^{b}	1.85^{b}	0.0094
Day 42	2.32	2.17	2.11	0.1008
Day 21-42	2.26^{a}	1.77^{b}	1.76^{b}	<.0001

Different superscripts differ significantly ($P<0.05$).

performance increased in all ZAA groups (120 and 140 mg/kg+ basal diet) in comparison to the basal diet control. ZAA addition to the broiler diet in both groups at 120 and 140 mg/kg was significantly increased in the body weight for each day (Table 2). The same result applied to body weight gain for each day and for the whole period, except on day 42 when there was no significant difference in comparison to the control group. Feed intake was significantly lower on day 21 due to adding ZAA (120 mg/kg), while, it was lower on day 35 and throughout the period in both 120 and 140 mg/kg ZAA groups. Also, feed conversion improved in ZAA groups compared to the control group.

A study in which a zinc amino acid complex was added to broiler feed showed the high relational effect between growth performance results and gut histology in Tables 1 and 2. The results showed a highly significant difference at 120 and 140 mg/kg ZAA supplementation in broiler diet compared to the control diet. There were several reasons for increased body weight, body weight gain and FRC with reduced feed intake. First, Zn is a necessary element for normal growth and maintenance, and is included for bone development, enzyme components and function for all poultry species (De Grande *et al.*, 2020). Secondly, according to Esfahani *et al.* (2015) and Hafez *et al.* (2020) Zn in broiler feeding can improve immunity in the offspring of old and young broiler. De Grande *et al* (2020) showed that broiler performance was significantly improved by adding bioavailable zinc in the feed as an amino acid complex. Kumar *et al.* (2021) promoted Zn into the diet as ZnS, ZnM and HME Zn improved the growth performance. Saleh *et al.* (2018) showed that dietary zinc methionine and zinc nanoparticles improved body weight, weight gain and feed conversion efficiency in broilers, implying that bioavailability of other zinc sources was higher than organic sources (Mohammadi *et al.,* 2015).

The results of zinc-amino acid complex (ZAA) on biochemical parameters on broiler serum showed that there was no significant difference in glucose, triglycerides, ALT and AST between all groups, except cholesterol was higher in the group basal diet with 140 mg/kg ZAA compared with the control group (Table 3).

The zinc-amino acid complex (ZAA) increased villus height (VL), crypt depth (CD) and ratio (VL:CD) by supplementation at 120 and 140 mg/kg compared to the control (Table 4). ZAA supplementation at 140 mg/kg improved VH/CD in the 120 and 140 mg/kg groups compared to the control group. The results indicated that the addition of zinc amino acid complex (ZAA) had a significant impact on small bowel histology and morphology and increased villus length, and villus length to crypt depth ratio. De Grande *et al* (2020) showed that the crypts, the epithelial cells lining and the gut lumen were constantly renewed by migrating new cells from the crypts to the villi tip. Cells, during this migration, mature and become more attached to nutrient uptake. Zhang *et al.* (2015) showed that the villous epithelial cells directly contacted the lumen contents and were therefore vulnerable to damage, which often led to higher rates of villous epithelial cell loss in intestinal health problems. Improvements in gut histology and morphology led to an increase in digestion and absorption of nutrients, and increased in brush border enzymes and nutrient transport systems (Awad *et al.,* 2017; De Grande *et al.*, 2020). These findings may explain the higher growth performance in this study when the zinc-amino acid complex was added to the diet.

Table 3. Effect of zinc-amino acid complex on biochemical parameters on broiler serum on 42 day (n=6)

Items	Groups			*p* value
	Control (0.0)	Control+Zn AA (120 mg/kg)	Control+Zn AA (140 mg/kg)	
Glucose (mg/dL)	175.59	156.65	168.88	0.5901
Cholesterol (mg/dL)	85.55[a]	75.00[ab]	62.88[b]	0.0276
Triglycerides (mg/dL)	45.81	46.77	45.16	0.9783
ALT(I/U)	140.75	138.00	139.25	0.8233
AST(I/U)	64.25	69.50	70.25	0.7187

Different superscripts differ significantly (P<0.05).

Table 4. Effect of zinc-amino acid complex on duodenum on broiler on 42 day

Items	Groups			*p* value
	Control (0.0)	Control+Zn AA (120 mg/kg)	Control+Zn AA (140 mg/kg)	
Villus height (µm)	801.84[c]	1018.05[b]	1382.43[a]	<.0001
Crypt depth(µm)	237.27	285.11	294.29	0.2629
VH/CD	3.38[b]	3.57[b]	4.70[a]	0.0007

Different superscripts differ significantly (P<0.05).
N = 8 measurements/section/bird for villus height (?m) and crypt depth (?m).

CONCLUSION

The study proved that diet supplied with zinc-amino acid complex led to higher rates of bioavailability and to a significant improvement in broiler performance in addition to increasing villus length and villus length to crypt ratio which indicated improved gut morphology. The groups 120 and 140 mg/kg ZAA supplement showed the best results compared to the control basal diet.

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