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THE EFFECT OF INORGANIC OR ORGANIC ZINC ON THE MORPHOLOGY OF THE INTESTINE IN BROILER CHICKENS

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ABSTRACT

This study compared the effect of dietary supplementation with an inorganic or organic zinc source on the gut morphology in the jejunum of broilers. One-day-old chickens were fed a basal diet (Control group: BD — 32 mg Zn.kg^{-1} DM), or the same BD supplemented with 30 mg or 70 mg of Zn per kg of DM in the form of $ZnSO_4$. H_2O (Group 1: 30 mg $ZnSO_4$; Group 2: 70 mg $ZnSO_4$), and 30 mg or 70 mg of Zn per kg of DM in the form of zinc chelate of glycine hydrate (Group 3: 30 mg Zn-Gly; Group 4: 70 mg Zn-Gly) for 40 days. The villus height was increased in the groups which received 30 mg $ZnSO_4$ and 70 mg $ZnSO_4$ and or 70 mg $ZnSO_4$, as compared to the BD and 30 mg Zn-Gly. The villus surface was higher in all groups receiving the Zn supplements in comparison to the BD.

Key words: chickens; intestine; morphometry; zinc

INTRODUCTION

The nutritional importance of zinc has been known for a long time, but in the past decades its importance in immune modulation has gained increased recognition. Zn can come from organic or inorganic sources. The organic forms of zinc include: amino acid chelates, bioplexes, proteins, as well as lactates and acetates. Zinc from amino acid complexes has been reported to be more bioavailable than Zn from the inorganic sources [1, 15]. Zinc-glycin (Zn-Gly) complex has a slightly higher stability constant for Zn than methionine which could be important for better availability for absorption. Zn-Gly can directly or indirectly influence the function of intestinal mucosa and improve the utilization of dietary energy. The immune response of chickens may be modified by the level of zinc in the diet. Supplementation of Zn in diets also improves intestinal morphology by increasing the villus height and reducing the crypt depth in animals [4, 10].

The National Research Council [9] recommended 40 ppm for broiler chickens, which appeared to be based on the results that considered growth performance as the only

criterion [5, 12]. However, there are several reports that demonstrate that higher Zn levels [60—180 ppm] produce better immune growth performance and intestinal function of broiler chickens [13, 14].

These discrepancies prompted us to evaluate the morphology in the caudal part of jejunum after feed supplementation with different levels of organic and inorganic zinc.

MATERIALS AND METHODS

Animals

A total of 210 one-day-old ROSS 308 hybrid broilers (MACH Hydina Budmerice Ltd., Slovakia) of both sexes were randomly assigned into 5 treatment groups consisting of 6 replicate pens with 7 chickens in each pen. Dietary treatments included the unsupplemented basal diet (BD, Control) and the same BD supplemented with 30 or 70 mg.kg^{-1} added Zn from $ZnSO_4.H_2O$ (Sigma-Aldrich, USA) or zinc chelate of glycine hydrate (Glycinoplex-Zn 26, Phytobiotics, Germany). Commercial broiler starter (1—19 days) and grower (20—39 days) diets were formulated as a basal diet (BD) with no supplemental zinc for the control treatment (Table 1). The mean analysed values of the Zn content in the starter and grower basal diets were 31.6 and 31.9 mg.kg^{-1}, respectively. During the 40-day feeding trial, all birds were offered the BD supplemented with two levels of the inorganic Zn source ($ZnSO_4$ 30 mg.kg^{-1}, $ZnSO_4$ 70 mg.kg^{-1}) or organic Zn chelate (Gly-Zn 30 mg.kg^{-1}, Gly-Zn 70 mg.kg^{-1}).

The birds were housed in large pens on wood shavings. The environmental temperature was kept at about 35 °C during the first week and then was gradually reduced to reach a final temperature of about 24 °C. The broilers were exposed to 23 h constant light/1 h darkness light schedule and had free access to experimental diets and tap water throughout the experiment.

All procedures were in accordance with European Community guidelines (Directive 2010/63/EU) for animal experiments and the experimental protocol was approved by the Ethics Committee of the Institute of Animal Physiology of the Slovak Academy of Sciences and by the State Veterinary and Food Administration (Ro-4160/13-221).

Table 1. Composition of the basal diets (BD)

Ingredient [%]	Starter diet (Days 1 to 19)	Grower diet (Days 20 to 39)
Wheat, ground	30.96	26.56
Maize, ground	35.00	45.00
Soybean meal, extracted	28.20	25.00
Fish meal	2.50	–
Monocalcium phosphate	0.90	0.95
Limestone	1.70	1.70
Feed salt	0.35	0.36
Coccidiostat	0.05	0.05
Lysine	0.10	0.10
Methionine	0.16	0.20
Vitamin premix a	0.04	0.04
Mineral premix b	0.04	0.04
Nutrient composition		
Dry matter [g.kg^{-1}]	883.02	881.55
Crude protein [g.kg^{-1}]	210.75	182.19
Crude fat [g.kg^{-1}]	28.27	27.44
Crude fibre [g.kg^{-1}]	30.85	29.97
Lysine [g.kg^{-1}]	12.10	9.90
Methionine [g.kg^{-1}]	5.09	4.89
Zinc [mg.kg^{-1}]	31.64	31.86
Manganese [mg.kg^{-1}]	90.99	88.48
Copper [mg.kg^{-1}]	14.23	13.58
ME [MJ.kg^{-1}]	12.08	12.24

[a]The vitamin premix provided per kg of diet: vitamin A 12.000 IU; vitamin D3 4000.0 IU; vitamin K 3.0 mg; vitamin E 45.5 mg; vitamin B1 2.0 mg; vitamin B2 6.0 mg; vitamin B6 4.0 mg; vitamin B12 0.02 mg; niacin 40.0 mg; pantothenic acid 12.0 mg; biotin 0.2 mg; folic acid 1.5 mg.
[b]The mineral premix provided per kg of diet: I 0.64 mg; Mn 64.0 mg; Cu 6.4 mg; Se 0.1 mg; Fe 48.0 mg.

Sample collection

After the 40-day feeding period, two birds from each replicate (12 birds/group) were slaughtered for sample collection. Tissue samples from the terminal section of the jejunum were collected for the determination of villus height and surface area.

Intestinal histomorphology

Jejunum samples were fixed in 10 % neutral buffered formalin and prepared using paraffin embedding techniques. Three consecutive sections (5 µm) from each jejunum were stained using haematoxylin and eosin and observed for histomorphology. The villus height and its area (from the tip of the villus to the crypt opening) were measured from 70 to 100 randomly selected villi with one section per chicken at 100× magnification (Fig. 1). The morphometry was evaluated using the NIS-Elements Advanced Research 3.0 Programme (commercial purchased programme).

Statistical analysis

Statistical analysis of the data was done by one-way analysis of variance (ANOVA) with the post hoc Tukey multiple comparison test using GraphPad Software (USA). The differences between the mean values for the different treatment groups were considered statistically significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$. The values are expressed as means ± standard deviation (SD).

RESULTS

Intestinal morphometry

The dietary Zn supplementation increased the villus height of the jejunum in both groups fed the diets enriched with inorganic Zn source ($ZnSO_4$ 30 mg, $ZnSO_4$ 70 mg.kg^{-1}) and also in Gly-Zn 70 mg.kg^{-1} group (ab $P < 0.001$) compared to the BD and Gly-Zn 30 mg.kg^{-1} groups (Table 2).

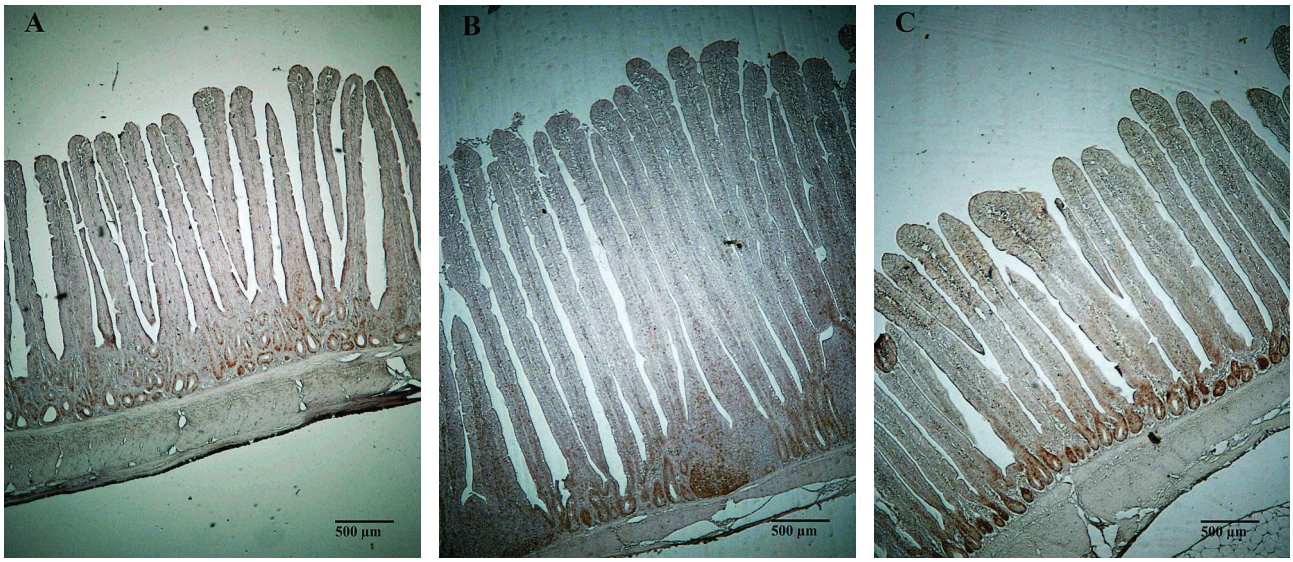

Fig. 1. Histological sections of the jejunum of broilers supplemented with different amount and Zn source (A) — BD; (B) — $ZnSO_4$ 30 mg.kg^{-1}; (C) — Gly-Zn 70 mg.kg^{-1}

Table 2. Effect of Zn supplementation on the histomorphology of the jejunum in broilers

Group	BD	$ZnSO_4$.30 mg^{-1}	$ZnSO_4$.70 mg^{-1}	Gly-Zn.30mg^{-1}	Gly-Zn.70 mg^{-1}
Villus height [µm]	1284 ± 180^{bd}	1462 ± 360^{a}	1492 ± 330^{a}	1204 ± 240^{b}	1376 ± 310^{c}
Villus surface [µm^2]	$152^3 \pm 680^{f}$	$192^3 \pm 1370^{ae}$	$184^3 \pm 910^{a}$	$160^3 \pm 580^{bd}$	$184^3 \pm 640^{c}$

All values are expressed as means ± SD. Means with different superscripts within a row differ significantly (^{ab} — $P < 0.001$; ^{cd} — $P < 0.01$; ^{ef} — $P < 0.05$)

The villus height was higher in the group Gly-Zn 70 mg.kg^{-1} (cd $P < 0.01$) compared to the jejunal villi of broilers fed the BD only.

Low-dose supplementation with inorganic Zn source ($ZnSO_4$ 30 mg.kg^{-1}) led to the increase of the villus surface area in compare to the BD (ef $P < 0.05$) and Gly-Zn 70 mg.kg^{-1} groups (ab $P < 0.001$). Similarly, the higher villus surface area was measured in the $ZnSO_4$ 70 mg.kg^{-1} group (ab $P < 0.001$) compared to the Gly-Zn 30 mg.kg^{-1} as well as in the group Gly-Zn 70 mg.kg^{-1} (cd $P < 0.01$) compared to Gly-Zn 30 mg.kg^{-1}.

DISCUSSION AND CONCLUSIONS

Zinc is known to influence the intestinal morphology and improve absorptive capacity, and enhance growth performance [5, 6]. Moreover, zinc is essential for cell proliferation and differentiation, especially for the regulation of DNA synthesis and mitosis [2). Southon et al. [11] demonstrated that Zn deficiency in rats is accompanied with a reduction of the jejunal villus height, while a short period of zinc supplementation returned the morphology into its normal condition in experimental animals. On the other hand, 42-day-old chickens fed the diet supplemented with 90 mg.kg^{-1} Zn-Gly increased the villus height and decreased the crypt depth of the jejunum [5]. In our experiments, the intake of both diets supplemented with the inorganic Zn source and also with the higher-dose organic chelate of zinc (Gly-Zn 70 mg.kg^{-1}) increased the height of the jejunal villi. Similarly, the surface villus area followed the same pattern as the height of the villi. It should be stressed that the villus height and surface villus area of broilers fed the diet with the addition of 70 mg.kg^{-1} of Zn chelate reached the similar value of both morphometric parameters as the broilers supplemented with the low-dose inorganic source of zinc.

The height of villi and their area can influence the source of supplemented zinc in diets [8]. It is known, that organic zinc chelate used in our trial improves zinc absorption comparing to the inorganic form of zinc. The absorption difference of zinc between the organic and inorganic forms can influence the growth of the intestinal villi. Our results suggest that organic zinc chelate after part absorption, supported better growth of the villi after supplementation of the diet with higher doses of organic zinc chelate. On the other hand, recently published immunological parameters from that experiment [3, 7] as quantification of the expression of MUC-2, IgA gene, and evaluation of secretory IgA in the lumen of the intestine, resulted in better affects found in birds fed diet supplemented with low doses of organic source of zinc.

In conclusion, our results demonstrated that an inorganic zinc source increased the height of villi and surface area of villi already after supplementation of feed with a low dose of zinc. On the other hand, the positive effect on the growth of villi was seen only after administration of a high dose of organic zinc in the feed. The villus surface was higher in all groups receiving the Zn supplements in comparison to just the basal diet.

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