Effect of dietary zinc level of zinc on performance and cecal lesion score in broilers infested with *Eimeria tenella*

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SUMMARY

Coccidiosis has great economic impact on poultry production. One-day - old Ross 308 broiler chicks (n=144) were fed one of three levels of dietary Zn (40 as control treatment, 120 and 200 mg/kg). At 14 d of age, birds were inoculated orally with oocysts of *Eimeria tenella*. Body weight gain (BWG), feed intake, feed conversion ratio and mortality rate were obtained by pen on d 21 and 42. Also at 42 d heterophil/lymphocyte (H/L) ratio, the weights of carcass, spleen and bursa of Fabricius and scoring of lesions from the intestinal *Eimeria* challenge were measured. Treatments did not affect performance traits at ages 21 and 42. Adding Zn to diet decreased H/L ratio and cecal lesion score ($P< 0.05$). Adding levels of 120 and 200 mg Zn/kg diet significantly increased the weight of bursa and spleen respectively ($P< 0.05$). The overall results of this study show that levels of 120 and 200 mg Zn/kg diet could be effective to enhance immunity in broilers infested with *E. tenella*.

Keywords: broiler; coccidiosis; heterophil/lymphocyte ratio; lesion score; organs weight; performance; Zn

INTRODUCTION

Coccidiosis is recognized as the parasitic disease that has great economic impact on poultry production. Williams (1995) reported that the annual worldwide cost is estimated to be about 800 million $ and that for American broiler industry about $ 45 million. Depending on the localization, disease in poultry has two forms: coccidiosis of the caecum that is caused by *Eimeria tenella* and intestinal coccidiosis that is caused by a number of parasites: *E. necatrix*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. mitis*, *E. mivati*, *E. praecox* and *E. hagani* (Lilic *et al*., 2009). Also it is true that very high zinc concentrations can be microbicidal. For example, the relatively low frequency of urinary tract infections in men may be due, in part, to the very high microbicidal zinc

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concentration in semen (Fair and Heston, 1977). Substantial evidence has been reported that adding zinc above the requirement enhances disease resistance (Shankar and Prasad, 1998). However, excessive zinc also depressed immunocompetence. Zinc has both specific and nonspecific role in the immune defense mechanism. As nonspecific function, it protects integrity of skin and mucous membranes (mucosal barrier) and is indispensable to the natural killer activity. The objective of this study was to evaluate the effect of different levels of zinc on performance traits and lesion score of cecum in broiler infected by *E. tenella*.

Table 1: Ingredients and calculated composition of the starter and finisher diets*

Ingredients	Starter (%)	Finisher (%)
Corn	53.55	59.57
Soybean meal 44%CP	38.93	33.34
Monodibasic Phosphate	1.43	1.21
Limestone	1.35	1.38
Vegetable oil	3.84	3.51
Salt	0.41	0.43
DL-methionine	0.207	0.214
L-Lysine HCl	0.129	0.197
Choline HCl 60%	0.06	0.05
Mineral-vitamin premix[1]	0.1	0.1
Total	100	100
Calculated Nutrients		
Crude protein %	22	20
ME, kcal/kg	3,050	3,100
Calcium, %	0.9	0.85
Available phosphorus, %	0.4	0.35
Digestible Lys, %	1.15	1.07
Digestible Met,, %	0.49	0.48
Digestible Met+Cys %	0.81	0.77
Choline, mg/kg	1,420	1,300

*Basal diets Zn measured by atomic absorption spectrometer and zinc contents were 74 and 72 mg/kg in starter and finisher basal diets

[1]Composition (per kg): manganese, 75,000 mg; iron, 50,000 mg; copper, 8,000 mg; iodine, 750 mg; vitamin A, 8,000 kIU; vitamin D3, 2,000 kIU; vitamin K3, 1,800 mg; vitamin B1; 1,800 mg; vitamin B2, 6,000 mg; vitamin B6, 2,800 mg; vitamin B12, 12,000 µg; pantothenic acid, 10,000 mg; niacin, 40,000 mg; folic acid, 1,000 mg; biotin. 60,000 µg; selenium, 0.3 mg/kg.

MATERIAL AND METHODS

Birds and treatments

144 one-day- old Ross 308 broiler chicks (male and female) were used in the experiment. The study was carried out according to a completely randomized design, with three dietary zinc ($Zn\text{-}So_4$) levels and four replicates of 12 birds. The experimental diets were manufactured from a basal diet

(Table 1), which was formulated to meet the nutrient requirements of broiler chickens (NRC, 1994). Three zinc levels (40 as control treatment, 120 and 200 mg/kg) were added to the basal diet to establish the treatments. Zinc contents in starter, finisher basal diets and potable water were 72, 70 and 5 mg/kg respectively, as measured by atomic absorption spectrophotometer. Birds were kept in floor pens, and diets and fresh water were provided *ad libitum* from day one. The lighting program used was 24 hours of artificial light during the entire experimental period, which lasted 42 days.

E. tenella challenge

At 14 d of age, all birds were inoculated orally with 5×10^3 sporulated oocysts of *E. tenella* by potable water. Oocyst production and shedding were assessed as described by Lillehoj and Ruff (1987).

Performance traits

Birds were weighed on d 1, 21 and 42 as a group. Average body weight gain (BWG), average daily feed intake (ADFI), feed conversion ratio (FCR) and mortality rate were obtained by pen on d 21 and 42.

Heterophil/lymphocyte (H/L) ratio

The blood was collected (via wing vein) in tubes containing EDTA as anticoagulant. H/L ratio, an indicator of stress in birds, were counted to a total of 100 cells. For each bird two slides were counted and the mean was calculated.

Immune organs weight

At 42 day of age, eight birds from each treatment were chosen at random, weighed and then slaughtered. The weights of spleen and bursa of Fabricius were recorded. Organs weights were expressed on a relative live weight basis.

Lesion scoring

On d 42, 2 birds per pen (n = 8/treatment) were randomly selected and killed for scoring of lesions from the intestinal *Eimeria* challenge. Lesions were scored by personnel blinded to the treatment in the ceca according to the method of Johnson and Reid (1970) based on score range from 0 (no gross lesion) to 4 (most severe lesion).

Statistical analysis

Statistical analyses were conducted using the ANOVA general linear models procedure of SAS software (SAS, 1997). When ANOVA revealed

significant effects, means were separated by Duncan's multiple range tests. The values were considered significant at P< 0.05.

RESULTS

The influence of dietary treatments on performance traits is shown in Table 2. No dietary treatment significantly altered ADFI, BWG, FCR and mortality rate at days 21 and 42.

Table 2: Average daily feed intake (ADFI), body weight gain (BWG) and feed conversion ratio (FCR) of broilers fed with different levels of dietary zinc.

Treatment	0-21				0-42			
	ADFI (g/bird)	BWG (g/bird)	FCR (g/g)	Mortality %	ADFI (g/bird)	BWG (g/bird)	FCR (g/g)	Mortality %
40 mg	30.33	18.18	1.67	12.5	67.15	36.13	1.86	16.67
120 mg	29.78	18.56	1.6	12.5	63.95	35.9	1.87	12.5
200 mg	31.35	19.48	1.68	16.67	70.4	36.3	1.94	18.75
MSE	1.33	1.097	0.074	4.3	5.22	2.58	0.01	4.5

Figure 1 shows the effect of different treatments on H/L ratio. Adding Zn to diet had a significant effect on H/L ratio (P< 0.05). Although there was no significant difference between the levels of 120 and 200 mg Zn/kg diet, but both these levels decreased H/L ratio of broilers in compare with the control.

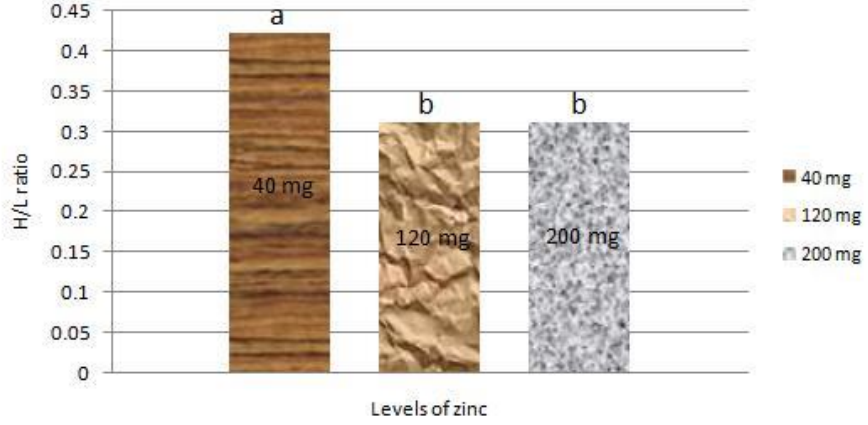

Figure 1: H/L ratio of broilers fed with different levels of dietary zinc
[a,b] Columns that do not share the same letters differ significantly (P< 0.05)

Immune organs (spleen and bursa of Fabrecius) weight were measured to evaluate the effect of different levels of zinc on broilers' immunity (Figure 2).

Adding levels of 120 and 200 mg Zn/kg diet significantly increased the weight of bursa and spleen respectively (P< 0.05) in compare with control.

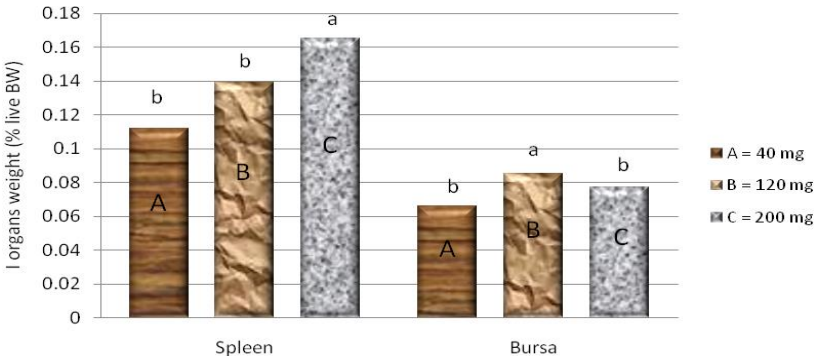

Figure 2: Immune organs weight of broilers fed with different levels of dietary zinc
[a,b] Columns that do not share the same letters differ significantly (P< 0.05)

Figure 3 shows the effect of different levels of zinc on lesion score of cecum created by *E. tenella*. Broilers supplemented with 120 and 200 mg Zn/kg diet showed a significant decrease in lesion score of cecum created by *E. tenella* in compare with the control.

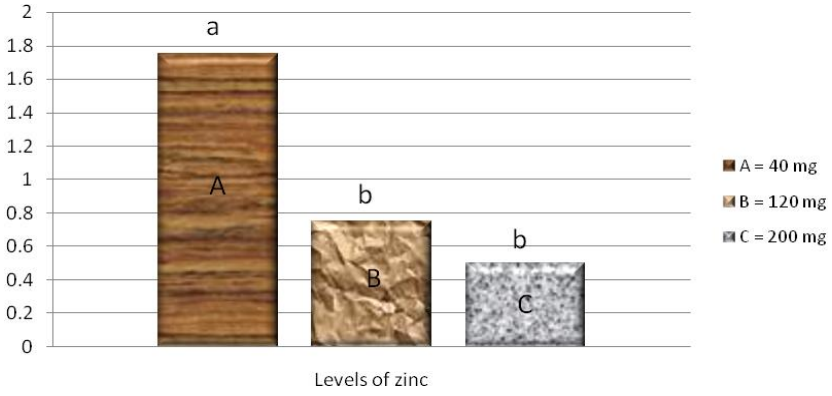

Figure 3: Lesion score of cecum in broilers fed with different levels of dietary zinc
[a,b] Columns that do not share the same letters differ significantly (P< 0.05)

DISCUSSION

Our findings about performance traits were in accordance with the results of Bartlett and Smith (2003) who found no difference in BWG, FI and FCR in broilers fed with 34, 68 and 181 mg Zn/kg. Also Kidd et al. (1994) observed no

differences in BW and FCR of broilers supplemented with 140 or 164 µg zinc/g diet. Additional levels of Zn in diet decreased H/L ratio in compared with the control group, indicating lower impact of stressors on organism. Sunder et al., (2008) suggested that supplementation of Zn was useful in reducing H/L ratio in young broilers. This suggests that supplementation of Zn is efficient in reducing stress in broilers. Zinc is required for the normal development of lymphocytes, peripheral T-cell numbers and T-cell helper functions (Kidd et al., 1996).

Levels of 120 and 200 mg Zn/kg diet were useful to improve the weight of bursa and spleen respectively. This finding was similar with result of Bartlett and Smith (2003) that showed a slight increase in lymphoid organ weights. Also Feng et al. (2011) reported that thymus, spleen, and bursa of Fabricius indexes were linearly increased with increasing dietary Zn. It could be due to role of zinc in lymphocyte proliferation (Tanaka et al., 1990). Also zinc is essential for thymulin, a thymic hormone that regulates T-lymphocyte maturation. Birds provided diets supplemented with a more available zinc source might have induced thymulin activity, and therefore promoted immune responses through increased maturation of T-lymphocytes and activation of B-lymphocytes by T-helper cells (Nassiri-Moghadam and Jahanian, 2009).

Both 120 and 200 mg Zn/kg diet were effective in reduction of lesion score in cecum created by *E. tenella.* It could be due to this matter that high levels of zinc are microbicidal. In other hand zinc has both specific and nonspecific role in the immune defense mechanism. As nonspecific function, it protects integrity of skin and mucous membranes (mucosal barrier) and is indispensable to the natural killer activity. In its specific role, zinc regulates the maturation and function of immune cells, among others by protecting developing lymphocytes from apoptosis (Fraker et al., 2000). Zinc also enhances the chemotaxic sensitivity and intensity of phagocytosis of neutrophils, monocytes and macrophages, moreover, supports complement activity (Fraker et al., 2000).

CONCLUSIONS

The overall results of this study show that levels of 120 and 200 mg Zn/kg diet could be effective in decreasing H/L ratio without altering performance traits. Also additional levels of zinc improved the weight of bursa and spleen and simultaneously declined the lesion score of cecum created by *E. tenella* in broilers. Then the enhancement of zinc in broiler's diet more than NRC (1994) recommendation could be considered as a natural promoter of safety in broiler chicks.

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