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ORIGINAL ARTICLE

Effect of zinc oxide nanoparticles and *Bacillus coagulans* as probiotic on growth, histomorphology of intestine, and immune parameters in broiler chickens

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Abstract Two hundred eighty-eight 1-day-old-male broilers were assigned to six dietary treatments to assess the influences of nanoparticles Zinc oxide (Zn-nan) and probiotic (*Bacillus coagulans*) supplements and their interaction on growing, histomorphology of intestine and immune parameters in chicks. Three amounts of dietary supplemental Zn source (100 mg/kg diet Zinc oxide, 25, and 50 mg/kg of diet Zn-nan) and two amounts of probiotic (0, 10^{10} CFU/kg of diet) were mixed as a randomized basic design with a 3 × 2 factorial experiment. The interaction effect of Zn source and probiotic was not effectual to change growth performance items, whereas probiotic supplementation had significant positive influence on bodyweight gain (BWG) and feed conversion ratio (FCR) in the whole trial phase ($P < 0.001$). The relation between Zn Source and probiotic was observed for villus height, width, and crypt depth, which was due to probiotic having greater improvement of villus height and width in birds fed Zn-nan50 than those fed ZnO. In comparison with the ZnO and Zn-nan25, the chicks fed diets with supplemental Zn-nan50 had greater villi length, villi length to crypt depth ratio, and antibody titer against sheep red blood cells (SRBC) at 28 and 42 days ($P < 0.05$). The chicks consumed probiotic had greater villus height, width, and villi length to crypt depth ratio and antibody titer against SRBC than those fed no probiotic. In conclusion, combination of probiotic (10^{10} CFU/kg) and Zn-nan (50 mg/kg) supplementation caused an improvement in performance parameters, immune responses, and intestinal morphology.

Keywords Broiler chickens · Zinc oxide nanoparticles · Probiotic · Intestinal morphology · Immune

Introduction

Zinc (Zn) is a crucial trace mineral and supplemented to diets of poultry because that plays important roles in several biological functions of animals, and also many feed are slightly deficient in Zn. For years, the dietary importance of Zn has been known, but its crucial roles in immune variation and functioning and development of the intestines has risened (Hu et al. 2013; Bonaventura et al. 2015). The forms of Zn in the diet of poultry could be as inorganic forms like Zn oxide (ZnO) and Zn Sulfate (ZnSO4) and also as organic shapes like Zn propionate and Zn acetate. However, the Zn bioavailability in organic forms is better than that of mineral Zn forms, but the use of organic Zn forms in poultry diets is restricted due to its price (Zhao et al. 2014). A new form of this inorganic salt is zinc oxide nanoparticles (Zn-nan) that has produced by nanotechnology (Song et al. 2010). Nanoparticles have larger surface area that allows higher interactions with other molecules, and also the larger surface area enhances the bioavailability and the intestinal absorption of these tiny structures (Nel et al. 2009; Tsai et al. 2016). In general, limited studies have indicated that the application of nano minerals in broilers production, immunity, and intestine morphology is promising. Mohammadi et al. 2015 suggested that application of nano organic form of Zn (zinc-nanomethionine) improved growth and immune responses. In another study, use of Zn-nan, caused an improvement in intestinal morphology (Ahmadi et al. 2013).

One of the best favorable spore creating lactic acid-creating types is *Bacillus coagulans*. *B. coagulans* is a worthy applicant for probiotic usage because it generates biological acids,

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have the ability to sporulate, and is simply cultivated in mass and also more resistant to heat (Hyronimus et al. 2000). It has been revealed that addition of *B. coagulans* in chicken feed promotes their growth performance and intestinal health (Hung et al. 2012). Many surveys have been showed the special effects of probiotic bacteria on the immune system. Kabir et al. (2004) showed in their study improved antibody creation in reaction to SRBC antigen by feeding probiotic *Protexin* in broiler chickens. Abd-El-Samee et al. (2013) reported that supplementing diets of growing Japanese quails with 1.0 g/kg prebiotic in combination with Bioplex zinc/kg improved immunity. Also in the recent years, researchers have used probiotics in combination with some nano trace minerals, such as nano-selenium, to improve the growth parameters in chicks (Saleh 2014). In addition, in the other study demonstrated that probiotics decreased intestine pH (Scholz-Ahrens et al. 2007); therefore, it increased creation of short-chain fatty acids because of the alteration of the gut microflora with a sequential increased mineral solubility and absorbability (Gilman and Cashman 2006).

According to the above evidences, it appears that combination of Zn-nan and probiotic caused synergies effects between the two, and their impact on performance compared with using each alone is more improved. Therefore, in the current study, for the initial period, the synergistic properties of Zn-nan in combination, a probiotic on the growth, histomorphology of intestine, and immune reactions of broilers were studied. Thus, the ideas of current study were to assess the special effects of *B. coagulans* and different levels of Zn-nan on the growth, histomorphology of intestine, and immune reactions.

Material and methods

Birds, management, and experimental diets

Two hundred eighty-eight 1-day-old-male broiler chickens (Ross 308) were randomly allocated to six treatments with four replicate cages and 12 birds per replicate. The broilers were raised in pens of equal size (100 × 100 cm floor area and 80 cm height) for the 42-day experimental period. All treatments were applied to similar management practices like lighting, feeding, and watering during the experiment except for the diets presented. Broiler chickens were provided 24 h/day lighting (20-lx light intensity). Feed and water were provided ad libitum. The preliminary temperature was 32 °C and then slowly reduced until reaching 22 °C at the end of the experiment.

The starter diet was fed up to day 21 and a finisher diet until day 42. The compositions of the trial basal diets are shown in Table 1. All diets within a period had the same composition. Diets were formulated to meet or top the Ross 308 management manual requirements for broiler chickens especially for mineral requirements (Aviagen 2014). The experiment was directed according to the animal wellbeing procedures at the Veterinary Control and Research Institute of Kerman, Iran. Three levels of dietary supplemental Zn source (100 mg/kg of DM diet Zinc oxide, 25, and 50 mg/kg of DM diet Zn-nan) and two levels of probiotic (0, 10^{10} CFU/kg of DM diet) were pooled as an entirely randomized design with 3 × 2 factorial arrangement. Basal diet was prepared to meet nutrient supplies according to the Ross 308 manual, aside from Zn (included premix without Zn) (Table 1). The treatment diets were prepared daily in mash form.

A Trade company provided the ZnO product (Merk, Germany; CAS No: 1314–13-2) with 99.9% purity. The Zn-nan was a white fine particles with a purity ≥ 99% (ZnO ≥ 99%, Cu ≤ 3 ppm, Mn ≤ 5 ppm, Pb ≤ 9 ppm, and Cd ≤ 9 ppm) is provided by the US Research Nonmaterial's, Inc. (Stock #: US3590, USA). The sizes of the Zn-nan are 10 to 30 nm with an average of 20 nm. The spores of *Bacillus coagulans* strains

Table 1 Ingredients and composition of the basal diet

Item	Diets	
	Starter diet (1 to 21 days)	Grower diet (22 to 42 days)
Ingredients (g/kg, as-fed basis)		
Corn	578.50	640.70
Soybean meal	338.70	284.30
Soybean oil	38.00	33.40
Dicalcium phosphate	23.50	19.00
Calcium carbonate	10.50	12.50
DL-methionine	1.80	1.10
Mineral premix[a]	2.50	2.50
Vitamin premix[b]	2.50	2.50
Salt	4.00	4.00
Calculated chemical composition (g/kg, dry-matter basis)		
Metabolizable energy (Mj/kg)	12.60	13.10
Crude protein	225.00	180.00
Calcium	9.50	8.20
Available phosphorous	4.50	3.60
Methionine + cysteine	9.20	8.00
Lysine	12.70	9.80

The basal diet (included premix without Zn) was added Zn as ZnO (100 mg Zn) in an amount of 100 mg/kg (control diet) and Zn-nan in an amount of 25 and 50 mg/kg

[a] Composition of the premix per kg of starter and grower diet: Mn100 mg, Cu16 mg, Se 0.18 mg, I 1 mg, Fe 40 mg

[b] Provided per kilogram of diet: 15,000 IU of vitamin A (retinol), 3750 IU of vitamin D3 (Cholecalciferol), 37.5 mg of vitamin E (tocopheryl acetate), 2.55 mg of vitamin K3, 3 mg of thiamin, 7.5 mg of riboflavin, 4.5 mg of vitamin B6 (pyridoxine), 24 μg of vitamin B12 (cyanocobalamin), 51 mg of niacin, 1.5 mg of folic acid, 0.2 mg of biotin, 13.5 mg of pantothenic acid, 250 mg of choline chloride, and 100 mg of antioxidant

(10^{11} CFU/g) were used as the probiotic bacterium (Pardis Roshd Mehregan Co., BioExir®, Iran).

Performance parameters

Birds and feeds were weighed after 12-h feed removal at days 1, 21, and 42 on a replicates basis. The body weight (BW), feed intake (FI), and feed conversion ratio (FCR) were determined. Mortality was checked twice a day and they were weighed during the study.

Intestinal morphology

The tissue samples from lower ileum (from Meckel's diverticulum to the ileocecal colonic junction) including 10 cm proximal to the ileocecal junction were processed for histopathological evaluation. In brief, 1-cm segments of the lower ileum ($n = 4$/treatment) were fixed in 10% buffered formalin, then embedded in paraffin waxes, and finally 5-μm sections were stained with hematoxylin and eosin (Sakamoto et al. 2000). Samples were stained with hematoxylin and eosin following a standard procedure to make histological slides by the paraffin infusion method. The slides were examined using an optical microscope (Micromaster, Fisher Scientific, Cat. No. 12-562-27, Fisher Scientific, Waltham, MA) fitted with a digital camera, and the intestinal criteria including villus height, villus width, and crypt depth ($n = 5$/bird) were examined and measured using the Image Pro Plus v 4.5 software package (Media Cybernetics, Silver Spring, MD, USA). For this purpose, villus height was defined from the top of the villus to the top of the lamina propria, and the width was measured at the bottom of the villi. In addition, crypt depth was considered from the base upwards to the region of transition, between the crypt and villus (Afsharmanesh et al. 2013).

Humoral immune response

For assessing the humoral immune in this experiment, we used the blood cells, a nonpathogenic antigen, in broiler chickens. Two birds from each replicate (8 birds/treatment) were inoculated with 1 mL of 0.5% SRBC suspension into brachial vein on the days 21 and 35, and blood samples were collected on day 7 of post inoculation. Before analysis, complement was heat inactivated (56 °C, 30 min). Each well of a 48-well plate received 0.05 mL of a diluent buffer containing PBS with 0.05% BSA. The first well received 0.05 mL of plasma, which was then serially doubly diluted by transferring 0.05 mL to the next well. Then, 0.05 mL of 2% SRBC in PBS was added to each well. The plates were shaken for 1 min, incubated for 1 h (SRBC), shaken again for 1 min, incubated for 24 h at room temperature, and then scored. The antibody titer was expressed as the log2 of the highest titer with 50% agglutination (Afsharmanesh and Sadaghi 2014).

Statistical analysis

Data were analyzed on a two-factorial ANOVA by using the SAS Institute's GLM method (SAS Institute, Cary, NC). Differences were considered significant at $P < 0.05$, and Duncan's test was used to determine the effect of treatments. Means were presented with their standard error of means (SEM).

Results

Broiler performance

Mortality through the trial was little (0.5%), and the mortalities were not related with any particular treatment. The effects of the dietary different form of Zn and probiotic supplementation on broiler performance are presented in Table 2. In the whole of experimental period, there was no interaction between Zn Source and probiotic on growth performance, whereas main effects of probiotic on broiler performance were significant *($P < 0.050$)* (except for feed intake). Birds fed probiotic showed improvement in BWG and FCR ($P < 0.001$) compared to those fed no probiotic. Dietary of Zn Source had no influence on growth items of broilers.

Intestinal morphology

The influences of different form of Zn and probiotic supplementation on intestinal morphology of broiler are shown in Table 3 and Fig. 1. The interaction among Zn Source and probiotic was observed for villus height, width, and crypt depth ($P < 0.05$), which was due to probiotic having greater improvement of villus height and width in birds fed Zn-nan50 than those fed ZnO. In comparison with the ZnO and Zn-nan25, the birds fed diets with supplemental Zn-nan50 had greater villi length, crypt depth, and villi length to crypt depth ratio ($P < 0.05$). The main effect of probiotic for birds fed with probiotic were significant such as those birds had greater villus height, width, and villi length to crypt depth ratio ($P < 0.05$) compared with those fed unsupplemented probiotic.

Humoral immune response

The influences of different form of Zn and probiotic supplementation on antibody titers against SRBC of broiler chickens are shown in Table 4. The antibody titer in contrast to SRBC at 28 and 42 days, were affected by main effect of Zn source as in broilers fed with Zn-nan50 were significantly better than the birds fed diets with ZnO and Zn-nan25 ($P < 0.05$). The birds fed probiotic had higher antibody titer against SRBC at 28 and 42 days ($P < 0.05$) than those fed no probiotic. There was no

Table 2 Effects of experimental diets on the growth performance of broilers

Item	Probiotic	BWG, g/bird			Feed intake, g/bird			Feed conversion ratio g/g		
		day 0–21	day 22–42	day 0–42	day 0–21	day 22–42	day 0–42	day 0–21	day 22–42	day 0–42
Zn source										
ZnO	–	32.43[ab]	74.10[bc]	53.08[ab]	42.10	145.99	93.75	1.28[a]	1.97[a]	1.76[a]
	+	33.19[a]	76.75[ab]	54.31[ab]	41.52	144.45	91.44	1.25[ab]	1.88[b]	1.68[bc]
Zn-nan25	–	30.53[b]	73.59[c]	51.67[b]	39.24	145.78	91.76	[a] 1.28	1.98[a]	1.77[a]
	+	33.88[a]	77.85[a]	55.87[a]	42.07	146.86	94.34	1.24[ab]	1.88[b]	1.69[bc]
Zn-nan50	–	32.36[ab]	74.43[bc]	53.19[ab]	41.24	147.31	93.24	1.27[a]	1.98[a]	1.75[ab]
	+	33.66[a]	78.52[a]	55.68[a]	40.50	146.06	91.72	1.20[b]	1.86[b]	1.64[c]
SEM		0.730	0.868	0.852	1.372	2.507	2.395	0.018	0.028	0.024
Main effect										
Zn source										
ZnO		32.81	75.42	53.67	41.81	145.22	92.59	1.274	1.926	1.724
Zn-nan25		32.23	75.72	53.77	40.65	146.32	93.05	1.261	1.934	1.731
Zn-nan50		33.01	76.47	54.43	40.87	146.68	92.48	1.238	1.919	1.699
SEM		0.516	0.614	0.602	0.970	1.773	1.693	0.013	0.019	0.017
probiotic										
–		31.79[b]	74.04[b]	52.65[b]	40.86	146.36	92.92	1.284[a]	1.977[a]	1.764[a]
+		33.57[a]	77.70[a]	55.29[a]	41.36	145.79	92.50	1.231[b]	1.876[b]	1.672[b]
SEM		0.421	0.501	0.492	0.792	1.447	1.382	0.010	0.016	0.014
Probability										
Zn source		0.549	0.472	0.642	0.676	0.832	0.969	0.182	0.865	0.396
Probiotic		0.007	< 0.001	< 0.001	0.659	0.782	0.834	0.003	0.0003	< 0.001
Zn source × probiotic		0.212	0.602	0.242	0.362	0.851	0.560	0.717	0.847	0.874

Means in columns with different letters (a, b, c) were significantly different ($P < 0.05$)

BWG body weight gain, *SEM* standard error of the means

[a] Data are means of four replicate cages of 12 birds each ($n = 48$)

[b] ZnO ,basal diet supplemented with 100 mg Zinc oxide/kg DM diet; Zn-nan25, basal diet supplemented with 25 mg Zinc oxide nanoparticles/kg DM diet; Zn-nan50, basal diet supplemented with 50 mg Zinc oxide nanoparticles/kg DM diet; Probiotic, without (−) or with (+) (10^{10} CFU/kg DM diet)

interaction ($P > 0.05$) between Zn source and probiotic on humoral immune response.

Discussion

Results of the present research demonstrate that there is no interaction between Zn Source and probiotic on performance. In other study, feeding *Aspergillus* probiotic alone or its combination with selenium nanoparticles improve BW and FCR of broilers (Saleh 2014). Also, Abd-El-Samee et al. (2013) indicated that the supplementation diets of growing Japanese quails with zinc alone or in mixture with prebiotic had no significant effect on growth performance. In the current study, the main effect of probiotic on growth performance was significant such as the BWG and FCR were improved due to probiotic supplementation. In other study, broilers fed probiotic diets had greater BWG and lower FCR with no change in FI than those fed control diet (Zhang and Kim 2014). Jawad et al. (2016) showed that supplementation with probiotic significantly improved BW and FCR. The possible mechanism for this improvement is that probiotic applies beneficial influence via holding of valuable microbial population in the digestive tract, improving digestion and absorption of feed. In addition, probiotics may possibly improve growth performance through enhanced VFA production in the intestine and control of insulin signaling in different tissues (Ajuwon 2015). Also, probiotics can enhance nutrient availability and absorption by directly protecting epithelial barriers and stimulate digestive enzyme activities (Awad et al. 2010 and Wang and Gu 2010), which also may result in enhanced growth.

Our data showed that supply of zinc in the composition of Zn-nan at lower concentrations (25 and 50% ZnO) did not adversely affect growth performance. In another study, dietary nano-Zn supplementation improved weight gain compared to control group, but no change was observed in feed conversion ratio (Mohammadi et al. 2015). Also, results of a study showed that the BWG of broilers fed 40 mg/kg nano-ZnO

Table 3 Effect of experimental diets on intestinal morphology in broiler chickens

Item	Probiotic	Villus height	Villus width	Crypt depth	Villus height/crypt depth
Zinc source					
ZnO	–	1311[f]	169.10[b]	110.75[b]	11.84[c]
	+	1407[d]	192.50[a]	106.66[b]	13.19[ab]
Zn-nan25	–	1366[e]	174.16[b]	110.41[b]	12.39[bc]
	+	1707[b]	192.91[a]	124.16[a]	13.75[a]
Zn-nan50	–	1649[c]	188.75[a]	130.41[a]	12.68[b]
	+	1737[a]	187.91[a]	125.08[a]	13.90[a]
SEM		8.502	3.380	2.412	0.266
Main effect					
Zinc source					
ZnO		1359.16[c]	181.25	108.79[c]	12.52[b]
Zn-nan25		1536.29[b]	183.54	117.29[b]	13.07[ab]
Zn-nan50		1693.33[a]	188.33	127.75[a]	13.29[a]
SEM		6.011	2.390	1.705	0.188
Probiotic					
–		1442.11[b]	177.64[b]	117.19	12.30[b]
+		1617.08[a]	191.11[a]	118.64	13.62[a]
SEM		4.908	1.951	1.392	0.154
Probability					
Zinc source		< 0.0001	0.1304	< 0.0001	0.0265
Probiotic		< 0.0001	0.0001	0.4728	< 0.0001
Zinc source × probiotic		< 0.0001	0.0061	0.0013	0.956

Means in columns with different letters (a, b, c) were significantly different ($P < 0.05$)

SEM standard error of the means

[a] Data are means of four replicate cages of two birds each ($n = 8$)

[b] ZnO, basal diet supplemented with 100 mg Zinc oxide/kg DM diet; Zn-nan25, basal diet supplemented with 25 mg Zinc oxide nanoparticles/kg DM diet; Zn-nan50, basal diet supplemented with 50 mg Zinc oxide nanoparticles/kg DM diet; Probiotic, without (−) or with (+) (10^{10} CFU/kg DM diet)

was significantly greater, and FCR was significantly lesser than that of the control (Fathi 2016).

Gut health is vital for the poultry wellbeing and efficiency. Larger and shorter villi result in a larger and shorter surface area that enables intesine of bird for greater and poor absorption of nutrients, respectively (Hung et al. 2012; Hu et al. 2012). The large crypt indicates tissue turnover is higher and a high demand for new tissue (Hu et al. 2012).

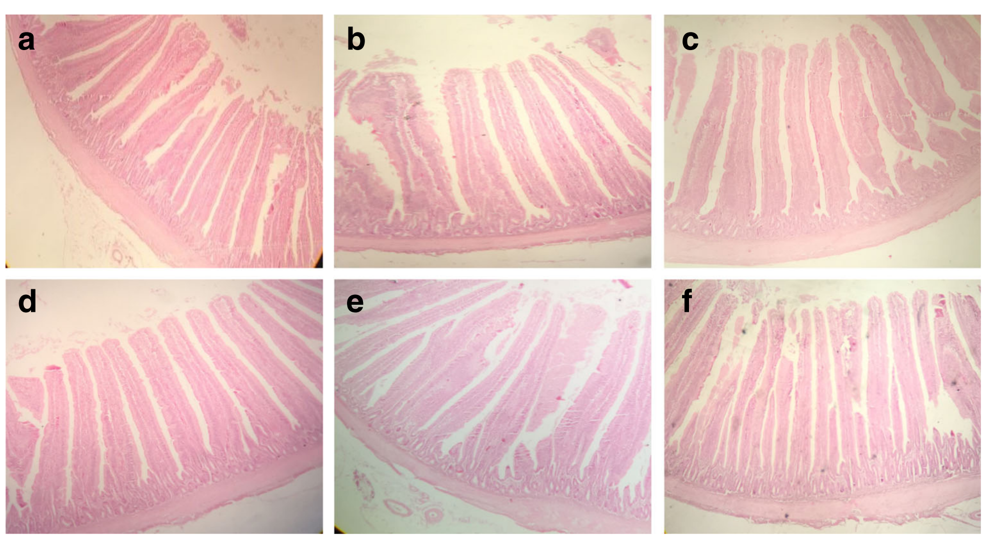

Fig. 1 **a** ZnO without probiotic. **b** Zn-nan25 without probiotic, **c** Zn-nan50 without probiotic, **d** ZnO with probiotic, **e** Zn-nan25 with probiotic, **f** Zn-nan50 with probiotic. H&E. Bar = 250 µm

Table 4 Effect of experimental diets on antibody titers against sheep red blood cells in broiler

Item[b]	Probiotic	Primary (28 days)	Secondary (42 days)
Zinc source			
ZnO	–	4.12c	4.87b
	+	4.62c	6.12ab
Zn-nan25	–	4.25c	5.00b
	+	5.12bc	6.12ab
Zn-nan50	–	5.87ab	6.75a
	+	6.25a	7.37a
SEM		0.338	3.390
Main effect			
Zinc source			
ZnO		4.37b	5.50b
Zn-nan25		4.68b	5.56b
Zn-nan50		6.06a	7.06a
SEM		0.239	0.276
Probiotic			
–		4.75b	5.54b
+		5.33a	6.54a
SEM		0.195	0.225
Probability			
Zinc source		0.0002	0.0011
Probiotic		0.0491	0.0057
Zinc Source × probiotic		0.7477	0.7040

Means in columns with different letters (a, b, c) were significantly different ($P < 0.05$)

SEM standard error of the means

[a] Data are means of four replicate cages of two birds each ($n = 8$)

[b] ZnO, basal diet supplemented with 100 mg Zinc oxide/kg DM diet; Znnan25, basal diet supplemented with 25 mg Zinc oxide nanoparticles/kg DM diet; Znnan50, basal diet supplemented with 50 mg Zinc oxide nanoparticles/kg DM diet; Probiotic, without (−) or with (+) (10^{10} CFU/kg DM diet)

SEM standard error of the means

In the present study, interaction among Zn Source and probiotic was detected for the villus height, width, and crypt depth, so that dietary application of combined Zn-nan and probiotic improvement of villus height and width of the birds. We could speculate that combination of dietary Zn-nan and probiotics might cause synergistic effects on improvement of intestinal morphology. Hung et al. (2012) reported that birds fed *B. coagulans* had better villi in the jejunum compared with the control but no significant differences were found in crypt depth. They also found that dietary *B. coagulans* could sustain certain minerals related to intestinal health of broiler chickens. The possible mechanism for improvement in morphology of intestine due to the use of probiotic is that, the probiotics can increase the pH of intestine which decreases the growing of many pathogenic or non-pathogenic intestinal microbes, therefore, decrease intestinal settlement of harmful bacteria and decrease contagious processes, and finally reduce inflammatory processes at the intestinal mucosa, which rise villus height (Beski and Al-Sardary 2015).

Zn is an important mineral in the functioning and development of the intestines that has been proven in the current study and many other studies and research. In an experiment, ZnO supplementation enlarged the villus height and the villus height to crypt depth ratio at the intestine (Hu et al. 2013). Similarly, in another research, the birds fed with diet supplemented with Zn-nan, the height and width of villi, crypts depth, and the villus height to crypt depth ratio had significantly increased in jejunum (Ahmadi et al. 2013). In the current study, the villi length and villi length to crypt depth ratio in broilers fed with Zn-nan50 were significantly better than the birds fed diets with ZnO and Zn-nan25. The mechanism of supplementary Zn especially Zn-nan in increasing villus to crypt may be due to increasing in cell multiplying by protein synthesis in intestine (Neto et al. 2011). The improved villus health created in the current experiment due to the use of Zn-nan might be attributed to the increased absorption of Zn in broilers fed Zn-nan supplementation in comparison to ZnO (Tsai et al. 2016). A vigorous intestine not only affects nutrient use by the birds but also has a considerable effect on the immune position of the bird, which was seen in the immune data of the present experiment (Table 4).

In the present study, the antibody titer against SRBC in bird fed with Zn-nan50 were significantly greater than the birds fed diets with ZnO and Zn-nan25. Zn purposes as a modulator of the immune response is across its availability, which is strongly controlled by numerous carriers, regulators, and form of it. Zn deficiency affects cells that concerned in both intrinsic and adaptive immunity at the survival, mutiplying, and evolution levels (Bonaventura et al. 2015). Changes in Zn status of body influnced the T cell functions and the balance between the different T helper cell subsets (Bonaventura et al. 2015). In a study, the changes in Zn levels affected the antibody titers against SRBC inoculation, and also the measure of humoral immune response. Greatest antibody titers in broiler were detected at 80 ppm level compared with lower levels (Sunder et al. 2008). The higher antibody titers to SRBC was observed in broiler fed with 181 ppm of Zinc in comparison to 34 to 68 ppm levels of supplementation (Bartlett and Smith 2003). Abd-El-Samee et al. (2013) found that growing Japanese quails fed with zinc alone or mixture with prebiotic (mannan oligosaccharides) improved antibody titers against SRBC. In the present study, the birds fed probiotic had higher antibody titer against SRBC than those fed no probiotic. Consistent with our results, Kabir et al. (2004) verified in their research increased antibody production in answer to SRBC antigen by feeding probiotic *Protexin* in broiler.

Conclusions

In conclusion, the probiotic (*B. Coagulans*) (10^{10} CFU/kg of DM diet) and Zn-nan50 supplementation could be beneficial in promoting the performance, intestine morphology, and immunity of chicks. In addition, the current study showed that the supplementation of Zn-nan at a lesser dosage (50 and 25% of requirement) did not reduce the growth performance of broiler chickens, which suggests that these doses of Zn are adequate for optimum performance. Thus, Zn-nan could be a worthwhile substitute to ZnO in broiler diets where Zn-nan50 is superior than Zn-nan25 in immune and intestine morphology.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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