Short Paper

Comparison of the effect of probiotic, prebiotic, salinomycin and vaccine in control of coccidiosis in broiler chickens

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Summary

Background: Coccidiosis is the most common parasitic disease in poultry, ionophore antibiotics are preferred drugs for controlling this disease. However, prolonged use of ionophores will result in *Eimeria* deformation and resistance to these drugs. **Aims:** The aim of this study was to compare the effects of probiotic and prebiotic, that is used to boost digestive system health, with salinomycin and vaccine in controlling coccidiosis. **Methods:** A completely randomized design, including 6 treatments, 4 replications and 20 birds in each experimental unit was applied. All experimental groups except negative control were challenged with suspension containing a mixture of three common species in Iran by oral inoculation in the crop at 28 days of age. **Results:** The results showed that the reduction of oocyst excretion was significant in coccidiostat (salinomycin), vaccine (Livacox® T), and probiotic (Primalac®) compared to the positive control group ($P<0.05$). Also, in the intestinal tract injuries, there was a significant reduction between the vaccine and salinomycin compared to the positive control group only in the cecum section ($P<0.05$). The effect of treatments on performance index (PI) was investigated and it was found that the best performance between infected groups was for salinomycin and vaccine groups ($P<0.05$). **Conclusion:** Finally, it can be concluded that probiotic and prebiotic are not effective in controlling coccidiosis and its complications like vaccine and salinomycin.

Key words: Prebiotic, Probiotic, Salinomycin, Vaccine, Coccidiosis

Introduction

Coccidiosis is created by a single-cell parasite belonging to different species of *Eimeria* and has a cycle of evolution of life between the external and internal environment of the host (Lillehoj and Lillehoj, 2000). Since 1971 preferred drugs for control of coccidiosis have been ionophore antibiotics. However, the long-term use of coccidiostat inevitably leads to an unexpected *Eimeria* deformation that will increase resistance to all anti-coccidiosis ionophore drugs (Allen and Fetterer, 2002). Extending drug-resistance in avian coccidiosis, the drug residue in poultry products, the consumer's pressures to avoid using chemical drugs, and the European's (EU's) announcement that bans the use of anti-coccidiosis have led to attention being paid to vaccination of poultry against coccidiosis (Williams, 2002). Livacox® is an attenuated vaccine that has low proliferation ability, so it does not risk the entry of unwanted species into the environment (Kitandu and Juranova, 2006). The innate immune responses such as interferon gamma (IFN-γ) production are critical for confronting the coccidiosis (Lillehoj *et al*., 2004). According to Dalloul *et al*. (2003) adding *Lactobacillus* (Primalac®) probiotics to broiler chicken feed during coccidial infection is effective in initial IFY-γ production. It has been reported that feeding Mannan-oligosaccharide (MOS) has been associated with reduction in the number of asexual stage schizonts in the lamina propria of the caecum of broiler chicken and this is because of increased immunity of broiler chicken (Elmusharaf *et al*., 2006). The aim of this study was to compare the protective effect of probiotic and prebiotic with vaccine and salinomycin to control coccidiosis in broiler chickens under experimental condition.

Materials and Methods

The study was performed in the Poultry Research Center, Faculty of Agriculture, Tarbiat Modares University. Through a completely randomized design, a total of 480 one-day old male broiler chicks after weighting were divided into 6 treatments and 4 replication groups with 20 birds in each replication.

Except for first three days (24 h light: 0 h dark), a 23 h light: 1 h dark lighting program was applied during the experiment. Treatments included negative control (non-contaminated), positive control (contaminated), probiotic (Primalac®: containing a minimum of 1.0×10^8 CFU of *Lactobacillus* sp. organisms per g [StarLabs/Forage

Research, Inc., Clarksdale, MO, USA]), prebiotic (Fermacto®: the commercially accessible fermentation product of *Aspergillus orizae* [PetAg, Inc., Hampshire, IL, USA]), coccidiostat (salinomycin), and vaccine (Livacox® T). Salinomycin were obtained from Kimiafaam Company (Iran) for veterinary products under a trade name (Kimiasalino 12®), and Livacox® T from Hezarteb Tehran Company (Tehran, Iran).

All the groups had the same corn-soybean meal basal diet (Table 1). Salinomycin was supplemented at the rate of 50 ppm in feed. The experimental diet for probiotic and prebiotic groups was prepared by adding Primalac® and Fermacto® at a level of ~1 g/kg diet. All three of these treatments were given to the birds of each group from day 1 to the end of the experimental period. Livacox® T [Whitish suspension made up of live attenuated oocysts of the main three lines of coccidiosis (*E. tenella*, *E. acervulina* and *E. maxima*)] was given at the age of 5 days with drinking water to the vaccine treatment chickens.

Table 1: Nutrient content of diets of broilers (as-fed basis): starter (day 1-14), grower (day 15-28), and finisher (day 29-42)

Item	Starter	Grower	Finisher
Ingredients (%)			
Corn	42	50	50
Soybean meal (44%)	34	28	25.5
Wheat	18	16	17.5
Soybean oil	2.0	2.0	2.0
Dicalcium phosphate [1]	1.6	1.8	1.9
$CaCO_3$ (38%)	1.4	1.1	0.9
Sodium chloride	0.34	0.34	0.34
L-Lysine HCl	0.05	0.18	0.18
DL-methionine	0.10	0.14	0.14
Vitamin premix [2]	0.25	0.25	0.25
Vitamin premix [3]	0.25	0.25	0.25
Contents by calculation			
ME (kcal/kg)	2950	3000	3050
CP (%)	21	19	18
Met (%)	0.48	0.45	0.42
Met + Cys (%)	0.91	0.87	0.81
Lys (%)	1.21	1.14	1
Available phosphorus (%)	0.72	0.71	0.69
Calcium (%)	1.05	0.95	0.9

[1] Contained 20% P and 23% Ca, [2, 3] Supplied the following per kg of diet: 9,000 IU of retinyl acetate, 2,000 IU of cholecalciferol, 12.5 IU of dl-α-tocopheryl acetate, 1.76 mg of menadione sodium bisulfite, 0.12 mg of biotin, 1.2 mg of thiamine, 3.2 mg of riboflavin, 6.4 mg of calcium d-pantothenate, 1.97 mg of pyridoxine, 28 mg of nicotinic acid, 0.01 mg of cyanocobalamine, 320 mg of choline chloride, 0.38 mg of folic acid, 60 mg of $MnSO_4.H_2O$, 80 mg of $FeSO_4.7H_2O$, 51.74 mg of ZnO, 8 mg of $CuSO_4.5H_2O$, 0.8 mg of iodized NaCl, 0.2 mg of Na_2SeO_3

All experimental groups except negative control were challenged with 3 × 100 μL of suspension containing a mixture of three common species of *Eimeria* in Iran (containing 24×10^4 sporulated oocysts of *E. acervulina*, 6×10^4 sporulated oocysts of *E. maxima* and 4×10^4 sporulated oocysts of *E. tenella*), by gavage in the crop at 28 days of age.

Seven days after challenge, by placing a white card in each pen, faecal samples were collected on a daily basis for 5 days (Pourali *et al*., 2014). Total faecal samples were homogenized, and diluted in saline solution for oocyst counting (McMaster counting chamber technique was used).

In order to compare the performance, the following formula was used:

Performance index = (total weight gain/total feed conversion) × 100

Two weeks after challenge, 3 birds per replication (12 birds per treatment) were selected and the intestinal mucosal lesions were measured according to Johnson and Reid (1970) method in severity grades of 0-4.

The date obtained through the experiment was analyzed using the general linear models (GLM) procedure in SAS 9.1 software and means of experimental groups were compared using Duncan's multiple-range test at 5% level of significance.

Results

The effect of treatments on quantitative performance of broiler chickens including body weight (BW), gain (g), FI (g), feed conversion ratio (FCR), and performance index (PI) have been reported in Table 2. The results of the pre-challenge period indicated that salinomycin group had a better weight gain than other groups ($P<0.05$). In the post-challenge period (29 to 42 days) the highest weight gain was observed in the negative control group and the lowest weight gain was seen in the positive control group ($P<0.05$). In the whole experiment period the highest FI was related to salinomycin and negative control groups ($P<0.05$). In the post-challenge period and the whole period of the experiment the worst FCR was related to positive control and prebiotic groups ($P<0.05$). Performance index of all treatments showed a significant difference with positive control expect Fermacto® treatment ($P<0.05$).

As seen in Table 3, the OPG shedding of faecal in terms of logarithm (Log (x+1)) in different post-challenge sampling days, in the vaccine and salinomycin groups showed a significant decrease in the contaminated groups compared to the positive control group ($P<0.05$). There was a significant difference between probiotic treatment compared to the positive control group on 6-9th day after challenging, although this difference was significantly lower than the vaccine and salinomycin treatments. Reducing the amount of oocyst excretion in Fermacto® was less than positive control group but it was not significant.

In Table 4, the lesion scores after 2 weeks of challenge were reported. Among the infected groups there was no great significant difference between the lesions of different segments of the small intestine, but the vaccine and salinomycin have shown a beneficial effects in ceca ($P<0.05$).

Table 2: Effect of treatments on BW (g), FI (g), FCR, and PI of broiler chickens at different periods of experiment

Item	Period	Treatment						P-value	SEM
		Negative control	Positive control	Primalac®	Fermacto®	Livacox® T	Salinomycin		
BW (g)	1-28 day	1031.82^{b}	1012.87^{b}	1047.73^{b}	1042.43^{b}	1005.63^{b}	1151.03^{a}	*	13.58
	28-42 day	1216.67^{a}	510.00^{d}	625.00^{c}	525.00^{d}	837.43^{b}	817.67^{d}	*	59.58
	1-42 day	2291.67^{a}	1566.67^{e}	1716.67^{d}	1611.67^{e}	1887.43^{c}	2012.67^{b}	*	61.24
FI (g)	1-28 day	123.667^{b}	122.167^{b}	123.547^{b}	123.570^{b}	122.620^{b}	141.383^{a}	*	1.69
	28-42 day	174.553^{a}	149.763^{c}	154.050^{c}	150.240^{c}	160.477^{b}	164.763^{b}	*	2.19
	1-42 day	4175.07^{a}	3806.95^{b}	3886.33^{b}	3833.33^{b}	3963.33^{ab}	4286.00^{a}	*	4.43
FCR	1-28 day	1.590	1.610	1.580	1.583	1.643	1.636	NS	0.01
	28-42 day	2.010^{d}	4.170^{a}	3.456^{b}	4.023^{a}	2.686^{c}	2.823^{c}	*	0.19
	1-42 day	1.730^{d}	2.466^{a}	2.206^{b}	2.396^{a}	1.990^{c}	2.030^{c}	*	0.06
PI	1-42 day	132.427^{a}	63.773^{d}	77.873^{c}	67.427^{d}	94.867^{b}	99.087^{b}	*	3.16

BW: Body weight, FI: Feed intake, FCR: Feed conversion ratio, and PI: Performance index. [a, b, c, d, e] Means within a row with no common superscript differ significantly (* P<0.05). SEM: Standard error of the means. NS: Non-significant

Table 3: Effect of treatments on faecal oocyst counts [log (x+1)] on 6-10th days post challenge

Experimental days	Treatment						P-value	SEM
	Negative control	Positive control	Primalac	Fermacto	Livacox T	Salinomycin		
6	0^{e}	5.1003^{a}	4.6213^{b}	4.8633^{ab}	4.0140^{c}	3.5813^{d}	*	0.42
7	0^{d}	5.1517^{a}	4.7963^{b}	5.0640^{ab}	3.9897^{c}	3.8757^{d}	*	0.43
8	0^{d}	5.1907^{a}	4.8887^{b}	5.0350^{ab}	3.9850^{c}	3.8423^{c}	*	0.43
9	0^{d}	4.6127^{a}	4.2287^{b}	4.4933^{ab}	3.4997^{c}	3.6177^{c}	*	0.38
10	0^{c}	4.1307^{a}	3.7930^{ab}	3.9770^{ab}	3.7730^{b}	3.6973^{b}	*	0.35

[a, b, c, d, e] Means within a row with no common superscript differ significantly (* P<0.05). SEM: Standard error of the means

Table 4: Intestinal lesion scores at 2 weeks after challenge

Segment	Treatment						P-value	SEM
	Negative control	Positive control	PrimaLac	Fermacto	Livacox T	Salinomycin		
Duodenum	0^{b}	1.66^{a}	1.16^{ab}	1.66^{a}	1.33^{a}	1^{ab}	*	0.20
Jejunum	0^{b}	1^{ab}	0.83^{ab}	1.33^{a}	0.66^{ab}	0.66^{ab}	*	0.15
Ileum	0^{b}	0.33^{a}	0.33^{a}	0.33^{a}	0.15^{ab}	0.16^{ab}	*	0.11
Cecum	0^{c}	4^{a}	3.83^{a}	4^{a}	2^{b}	1.83^{b}	*	0.39

[a, b, c] Means within a row with no common superscript differ significantly (* P<0.05). SEM: Standard error of the means

Discussion

The results of the pre-challenge period indicated that salinomycin group had a greater weight gain than other groups. Demirulus *et al*. (2006) reported that increasing the level of salinomycin in diet resulted in a decrease in BW, FI, weight gain, and feed efficiency. However, in their experiment the group that received 1 ppm salinomycin had higher BW and a better FCR than the control group, which could indicate the effect of growth promoter on low salinomycin levels. The lack of distinction in the production indexes between probiotic and prebiotic treatments and control groups in our experiment can be due to short time of usage of the feed additives (4 weeks).

After infection, weight gain was significantly improved in the probiotic receiving group, compared to the positive control group but the weight gain of the chickens in the final period (625 g) was much less than the negative control group (1216 g). As shown in Table 3, probiotic has reduced OPG excretion in faeces, which is similar to the results of Najafi *et al*. (2009). Although it was predicted that there was a direct and meaningful correlation between performance parameters and reducing the secretion of the OPG, but according to Dalloul *et al*. (2005) use of probiotic did not always lead to lowering the OPG excretion and enhancing the weight gain of chickens. Other causes of weight loss in all contaminated treatments especially probiotic, prebiotic, and positive control, as shown in Table 4, can be attributed to severe intestinal injuries in the cecal region which caused a severe hemorrhage. *Eimeria* species must attack the host cell in order to replicate and adhere to the epithelial surface. Intestinal-compatible probiotic bacteria may compete with *Eimeria* cells to grip the intestinal mucosa and absorb receptors in the epithelial cells, which delayed the penetration and secretion of *Eimeria* sporozoites into the intestinal mucosa and as a result the proliferation and shedding of oocysts decreased (Dalloul *et al*., 2003). It seems that Primalac® (probiotic with *lactobacillus* source) affect the small intestine especially at the beginning of it, and this is probably one of the reasons why intestinal injuries are more evident in the ceca. In the present study, the contamination was a mixture of three species of *Eimeria* and dose rate was much higher than the amount that chickens confront in farm and even the vaccine trial challenge levels (Dalloul *et al*., 2005), that is why the OPG excretion and the lesion severity was so high.

In a study that compared the protective effect of salinomycin and Inovocox® vaccine, it has been pointed out that the use of the vaccine led to higher weight gain, more antibody production, and augmented pro-inflammatory immune status (Lee *et al*., 2013); which is somewhat inconsistent with our results. It should be noted that the doses and composition of *Eimeria* species during the challenge, as well as the type of vaccine and its compounds contribute to the diversity of the results.

In a comparative study between Primalac®, Fermacto®, butyric acid glycerides, the mixtures of them, and salinomycin, it was reported that all treatments had a positive effect on decreasing the duodenal and cecal lesions. There was no significant difference in their protective role in the cecum and the highest protective role in the duodenum was for salinomycin. Also, the level of faecal oocyst excretion for salinomycin and probiotic treatments suggested that they have a better protective role than prebiotic (Taherpour *et al*., 2012), which is somewhat consistent with our findings. In a study performed in 2007 on infected with a combination of several species of *Eimeria*, it was shown that by adding 10 g MOS/kg diet only lesions caused by *E. acervulina* were significantly reduced in the intestines of infected chicks and prebiotic had no protective effect on the injuries of *E. tenella*, *E. maxima* types and enhancement of functional traits (Elmusharaf *et al*., 2007). It has also been stated that the protective effect of MOS may be due to the increase of the villus length and improvement of the integrity of the digestive tract (Loddi *et al*., 2002).

As explained, the type and source of feed additives, the duration and amount of their use in the diet, levels of oocyst inocula, and *Eimeria* types affect the indicators measured in different experiments. Our results showed that the amount of the Fermacto® prebiotic that was used in this experiment had almost no protective effects on the challenged chickens. Primalc® probiotic was partially effective, but its protective role was not so high that it could be used as a substitute for coccidiostat drugs.

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