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Effects of Using a Multi-Strain Probiotic on Performance, Immune Responses and Cecal Microflora Composition in Broiler Chickens Reared Under Cyclic Heat Stress Condition

Research Article

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ABSTRACT

This experiment was conducted to examine the effect of multi-strain probiotic (Primalac) on performance, immune responses and cecal microflora in broiler chickens under cyclic heat stress condition. A total of 96 one-d-old mixed sex broiler chicks (Ross 308) were weighed and randomly allocated to two treatment groups, each with 4 replicate pens of 12 chicks. The dietary treatments were basal diet (control) and control +0.9 g Primalac/kg diet. Body weights of broilers were determined at d 1, 21 and 42, feed intake was determined at the same periods, and feed conversion ratio was calculated accordingly. The populations of *Lactobacilli spp* and coliforms were enumerated in the cecum. Antibody titers against Newcastle, Bronchitis, and Gumboro were measured as immune responses at 28 d of age. As a result of this study, use of probiotic significantly ($P<0.05$) increased broiler performance by enhancing body weight, daily feed intake and decreasing the feed conversion ratio. The *Lactobacilli spp*. population in birds supplemented with probiotic significantly was higher and coliforms population was lower than control groups at 42 d of age ($P<0.05$). Also administration of the probiotic appeared to improve the antibody responses to Newcastle disease virus and Bronchitis, Gumboro disease vaccination. In conclusion, the results indicate that supplementing broiler reared under heat stress condition with 0.9 g Primalac/kg diet could induce favorable influences on performance, immune responses and cecal microflora.

KEY WORDS broiler, cecal microflora, cyclic heat stress, immunity, performance, probiotic.

INTRODUCTION

Intestinal microbiota is generally considered important for its nutritional, health, and immunomodulatory activities (Vispo and Karasov, 1997) and diet is one of the important factors that can influence the intestinal microbial ecology (Rehman *et al.* 2007). The composition of the intestinal microbiota remains stable in healthy birds. Apart from pathogens and antibiotics that primarily affect the intestinal microbiota, the environmental stressors such as transportation to the growing site, overcrowding, vaccination, chilling and high or low temperature can also disrupt the normal balance of the intestinal microbial ecology (Lan *et al.* 2004). It has been reported that broiler reared under high ambient temperature and humidity exhibit physiological changes like, decreased feed intake, poor body weight gain, suppressed immune responses and high mortality (Howlinder and Rose, 1989).

Various nutritional strategies have been suggested to overcome the negative impact of heat stress (HS) in broilers (Leeson, 1986; Teeter and Belay, 1996; Yahav *et al.* 1996). Probiotics have been defined as a live microbial feed supplement that can beneficially affect the performance (Haung *et al.* 2004; Panda *et al.* 2006; Ayasan *et al.* 2006),

nutrient digestibility (Apata 2008; Li *et al.* 2008), modulation of intestinal microflora (Mountzouris *et al.* 2007; Yu *et al.* 2008), pathogen inhibition (Higgins *et al.* 2008; Mountzouris *et al.* 2009), immunomodulation and gut mucosal immunity (Farnell *et al.* 2006; Teo and Tan, 2007) and reduced mortality in broiler chickens (Panda *et al.* 2006). Furthermore, Zulkifli *et al.* (2000) and Rahimi and Khaksefidi (2006) observed the beneficial influence of probiotic supplementation on intestinal microbial composition in birds exposed to HS. Also Lan *et al.* (2004) reported that supplementation of broiler with probiotic *Lactobacillus agilis* and *Lactobacillus salivarius* increased the prevalence of these species in broilers reared under HS.

The present study was designed to evaluate the efficacy of multi-strain probiotic on performance, immune responses and cecal microflora in broiler chickens under cyclic heat stress condition.

MATERIALS AND METHODS

Animals and dietary treatments

Ninety six, 1-d-old broiler chickens of mixed sexes (Ross-308) were weighed and randomly assigned to each of the 2 treatment groups, each with 4 replicate pens of 12 chicks. The probiotic Primalac (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Enterococcus faecium* and *Bifidobacterium bifidium*) was supplemented to basal diet with no additives. The dietary treatments were basal diet (control) and control +0.9 g Primalac/kg diet. Considering the previous research, the amount of probiotic supplementation was chosen (Talebi *et al.* 2008; Falaki *et al.* 2011; Nayebpor *et al.* 2007; Chichlowski *et al.* 2007).

Table 1 lists the basal diet formulated to meet or exceed the nutrient requirements of broilers (NRC, 1994). The birds were fed a starter diet from 0 to 21 d, and grower diet from 22 to 42 d. A commercial multi-strain probiotic (Primalac) was added to the basal diets at the expense of corn. Chicks were raised on floor pens (120×120×80 cm) for 6 wk and had free access to feed and water throughout the entire experimental period. The lighting program consisted of a period of 23 h light and 1 h of darkness. Temperature and relative humidity (RH) on 1 d were maintained at 32 ˚C and 65%, respectively. Temperature was decreased 2.8 ˚C per week until it reached 26.7 ˚C on 21 d, with RH 65%, which was designated as the thermoneutral zone (TN). Birds were kept at TN or subjected to cyclic heat stress by exposing them to 35 ˚C and 75% RH for 8 h/d from d 22 to the conclusion of the study (d 42).

Performance

Body weights of broilers were determined at 1, 21, and 42 d of age. Daily weight gain and daily feed intake (DFI) were recorded in different periods and feed conversion ratio (feed intake/weight gain) was calculated. Mortality was recorded as it occurred.

Table 1 The ingredient and calculated composition of basal starter, and grower diets

Item	Starter	Grower
Ingredient, g/kg		
Corn	584.2	584.9
Soybean meal	315.8	300.7
Soybean oil	25.2	55.0
Fish meal	45	30
Dicalcium phosphate	9.3	8.0
$CaCO_3$	10.6	11.7
NaCl	2.0	2.9
Oyster shell	1	1
Trace mineral premix[1]	2.5	2.5
Vitamin premix[2]	2.5	2.5
DL-Methionine	1.5	0.8
L-Lysine	0.4	0
Analysis results		
Metabolizable energy (kcal/kg)	3000	3200
Crude protein (g/kg)	215.6	200.0
Calcium (g/kg)	9.7	9.0
Available phosphorus (g/kg)	4.2	3.5
Methionine + cysteine (g/kg)	8.4	7.2
Lysine (g/kg)	12.7	11.2

1. Mineral premix per kg of diet: Fe ($FeSO_4.7H_2O$, 20.09% Fe): 50 mg; Mn ($MnSO_4.H_2O$, 32.49% Mn): 100 mg; Zn (ZnO, 80.35% Zn): 100 mg; Cu ($CuSO_4.5H_2O$): 10 mg; I (KI, 58% I): 1 mg; Se ($NaSeO_3$, 45.56% Se): 0.2 mg.
2. Vitamin premix per kg of diet: vitamin A (retinol): 2.7 mg; vitamin D3 (Cholecalciferol): 0.05 mg; vitamin E (tocopheryl acetate): 18 mg; vitamin k3: 2 mg; Thiamine: 1.8 mg; Riboflavin: 6.6 mg; Panthothenic acid: 10 mg; Pyridoxine: 3 mg; Cyanocobalamin: 0.015 mg; Niacin: 30 mg; Biotin; 0.1 mg; Folic acid: 1 mg; Choline chloride: 250 mg and antioxidant 100 mg.

Cecal microflora composition

At 42 d of age, 2 birds per replicate were randomly chosen, based on the average weight of the group and slaughtered through cutting carotid arteries and partial slicing of the neck by a manual neck cutter. The carcasses were subsequently opened and the entire gastrointestinal (GI) tract was removed aseptically, and ceca separated from the small intestine. For the bacterial enumeration in cecal digesta per bird, appropriately stored ceca, frozen at -80 ˚C, were thawed and removed from storage bags.

Cecal digesta contents were then aseptically emptied in a new sterile bag and were immediately diluted 10-fold (i.e., 10% wt/vol) with sterile ice-cold anoxic PBS (0.1 *M*; pH 7.0) and subsequently homogenized for 3 min in a stomacher (Bagmixer 100 Minimix, Interscience, Arpents and France).

Each cecal digesta homogenate was serially diluted from 10-1 to 10-7. Dilutions were subsequently plated on duplicate selective agar media for enumeration of target bacterial groups.

Lactobacillus spp and coliforms were enumerated using Rogosa agar and MacConkey agar. Plates were then incubated at 39 ˚C for 24 to 72 h aerobically (Mac Conkey

agars) or 48 to 120 h anaerobically (Rogosa agar) and colonies were counted. Anaerobic incubation was achieved using appropriate catalysts (Anaerocult A, Merck and Darmstadt Germany) in sealed anaerobic jars (Oxoid, Basingstoke, UK). Results were expressed as base-10 logarithm colony-forming units per gram of caeca.

Immunity

The broilers were vaccinated against infectious bronchitis by coarse *spray* and infectious bursal disease virus D78 (Nobilis†Gumboro D78) via the drinking water at 1 and 14 d of age, respectively. Birds were vaccinated against Newcastle disease virus (NDV) on d 9 (intraocular; live attenuated) and d 21 (Lasota).

Antibody titers against NDV, Bronchitis, and Gumboro were measured as immune responses. At 28 d of age, 2 male broilers from each pen were randomly selected, and blood samples were taken by puncture of the brachial vein for analysis of antibody titers against NDV, Bronchitis, and Gumboro.

Serum antibody titers against NDV were measured by the hemagglutination inhibition test (HI), according to Allan and Gough (Allan and Gough, 1974) and serum antibody titers against infectious Bronchitis and infectious bursal viruses measured by the enzyme-linked immunosorbent assays (IDEXX Laboratories, B.V., The Netherlands) test.

Statistical analysis

The data were subjected to analysis of variance procedures appropriate for a completely randomized design using the General Linear Model procedures of SAS (SAS, 1996). Means were compared using Tukey test. Statements of statistical significance are based on P<0.05.

RESULTS AND DISCUSSION

Performance

The effect of probiotic supplementation on performance indices are summarized in Table 2. Broilers receiving probiotic had higher body weights compared to control group at 21 and 42 d of age (P<0.05). The inclusion of probiotic to the diet significantly increased DFI at different periods of the trial compared to control group (P<0.05).

Broilers receiving basal diet supplemented with probiotic had lower feed conversion ratio (FCR) compared to broilers receiving basal diet during starter and grower period and entire experimental period (P<0.05).

The daily weight gain obtained in broilers fed diet containing 0.9 g Primalac/kg diet was significantly higher than control group at different growth periods (P<0.05). No differences because of treatment effects were observed on mortality.

Cecal microflora composition

Data on cecal bacteria populations of broiler chicks at d 42 of age are summarized in Table 3. The *Lactobacilli spp*. population in birds supplemented with the probiotic was significantly higher than control group at 42 d of age (P<0.05).

The coliforms population in broilers fed the diet containing probiotic was significantly lower than control group (P<0.05).

Table 2 Effect of experimental diets on performance indices of broilers at different ages (Mean±SE)

Variables	Dietary treatments	
	Control	Probiotic
Body weight (g)		
21 d	478.00^{b}±9.53	516.43^{a}±8.65
42 d	1552.68^{b}±14.68	1619.16^{a}±13.52
Daily feed intake (g/d)		
0-21 d	36.53^{b}±0.33	37.40^{a}±0.36
21-42 d	137.11^{b}±1.33	140.55^{a}±1.39
0-42 d	86.82^{b}±1.42	89.00^{a}±1.38
Feed:gain (g:g)		
0-21 d	1.57^{a}±0.03	1.47^{b}±0.03
21-42 d	2.75^{a}±0.02	2.69^{b}±0.02
0-42 d	2.15^{a}±0.03	2.08^{b}±0.04
Daily weight gain (g/d)		
0-21 d	21^{b}±0.37	22.83^{a}±0.35
21-42 d	50.18^{b}±0.41	52.56^{a}±0.43
0-42 d	35.6^{b}±0.27	37.7^{a}±0.28

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Table 3 Effects of dietary treatments on cecal bacteria populations of broiler chicks at d 42 of age (Mean±SE)

Variables	Dietary treatments	
	Control	Probiotic
Lactobacilli spp.	8.08^{b}±0.53	9.96^{a}±0.64
Coliforms	11.59^{a}±0.48	10.18^{b}±0.52

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

Immune responses

The effect of experimental diets on humoral immune responses is presented in Table 4.

Table 4 Effect of experimental diets on antibody titers against Newcastle, Bronchitis and Gumboro viruses at d 28 (Mean±SE)

Variables	Dietary treatments	
	Control	Probiotic
New castle	2.4^{b}±0.4	4.62^{a}±0.3
Bronchitis	1580.0^{b}±286	2261.5^{a}±311
Gumboro	1672.3^{b}±233	2298.5^{a}±235

The means within the same row with at least one common letter, do not have significant difference (P>0.05).

The NDV antibody titer observed in birds fed the diet containing probiotic was significantly greater than those fed the control diet (P<0.05). The antibody titres against Bronchitis and Gumboro in probiotic treated groups were significantly greater than control group (P<0.05).

The results of this trial indicated that dietary supplementation of probiotic significantly improved performance indices of broiler chicks reared under heat stress condition. Zulkifli *et al.* (2000) reported an enhancement in body weight gain and DFI of broilers offered diet containing probiotic based on *Lactobacillus* strain and raised under heat stress condition than those treated with supplemental oxytetracycline or the control group. In another trial, Rahimi and Khaksefidi (2006) reported that, addition of probiotic (Bioplus 2B) to the diet of broiler chicks reared under heat stress condition significantly improved FCR, and treatments had not any significant effect on DFI. Consistently, in other trials BW and FCR obtained in birds fed diet supplemented with Primalac did not significantly improve compared with the control group (Ashayerizadeh *et al.* 2011).

Probiotics can improve broiler performance by increasing the villous height in the small intestine (Gunal *et al.* 2006; Panda *et al.* 2006). Probiotic can also change the bacterial population in the poultry gastrointestinal tract (Netherwood *et al.* 1999) and modifies mucin biosynthesis or degradation, which in turn influences gut function resulting in improved nutrient uptake (Smirnov *et al.* 2005). Mountzouris *et al.* (2010) reported positive effect of probiotic on cecal microflora composition resulted on higher broiler growth responses and ileal nutrient digestibility. In this trial, the potential of the probiotic to fortify beneficial members of the intestinal microflora was evidenced because the Primalac treatments resulted in a significant higher *Lactobacillus* concentration and lower coliforms concentration in the cecal digesta of 42-d-old broilers.

Results of this experiment suggest that the probiotics may have had an immunomodulatory role. The observations are in agreement with that of others (Talebi *et al.* 2008). In another trial in mice oral administration of probiotic *Lactobacillus* strains had positive effects on the IgG production (Maassen *et al.* 2000), and administration of lactic acid bacteria produced higher levels of anti-pneumococcal serum IgG and bronchoalveolar lavage IgA in mice (Racedo *et al.* 2006). Serum globulins, being used as an indicator of immune response, are source of antibodies (Abdel Fattah *et al.* 2008) and immunoglobulin production.

The research is required to find the mechanism of immunomodulation by probiotics but they may stimulate different subsets of immune system cells (Maassen *et al.* 2000; Christensen *et al.* 2002; Bal *et al.* 2004). The contribution of gut microflora to the development and physiological status of the humoral and cellular mucosal immune system is well understood (Christensen *et al.* 2002; Cebra 1999). Also, probiotics can significantly affect the systemic immune responses (Dallout *et al.* 2003). It is now well recognized that bioactive peptides released during fermentation by lactic acid bacteria could contribute to the known immunomodulatory effects of probiotic bacteria (Leblanc *et al.* 2004). Also, probiotics can increase the systemic antibody production to some antigens in broilers (Haghighi *et al.* 2005).

CONCLUSION

In conclusion, the supplementation of a multi-strain probiotic (Primalac) at the level of 0.9 g/kg in the diet could induce favorable influences on performance, immune responses and microflora composition of broiler chickens reared under heat stress.

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