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A comparison between the effects of a probiotic (Bioplus 2B) and an antibiotic (virginiamycin) on the performance of broiler chickens under heat stress condition

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Summary

An experiment was conducted on 400 broilers to evaluate the effects of dietary supplementation of antibiotic and probiotic on performance of birds under heat stress condition. The first group of chickens, the control group, received a diet without any antibiotic and probiotic. The second group received diet with 0.02% virginiamycin antibiotic; the two other groups were fed diets with 0.05% and 0.1% Bioplus 2B probiotic, respectively. The addition of antibiotic to the diet significantly improved body weight gain during 0–3 and 4–6 weeks (heat stress) periods as compared to the control and 0.05% probiotic-supplemented groups (P<0.05). Feed intake of different groups did not differ significantly (P>0.05). Addition of antibiotic or various levels of probiotic to the diet significantly improved feed conversion ratio during 4–6 weeks (heat stress) period as compared to the control group (P<0.05). Supplementation of diet with antibiotic had no significant effect on antibody production against sheep red blood cells (SRBC), Newcastle disease virus (NDV) vaccine, white blood cell (WBC) count, heterophil to lymphocyte ratio (H/L), serum cholesterol and haemoglobin content (P>0.05). Supplementation of diet with various levels of probiotic had no significant effect on antibody titer against SRBC, NDV vaccine and haemoglobin content (P>0.05). However, supplementation of diet with 0.1% probiotic significantly increased WBC count and decreased H/L as compared to the control group (P<0.05) which is important in reduction of stress effects on birds.

Key words: Antibiotic, Probiotic, Performance, Broiler chickens

Introduction

To achieve high levels of economic efficiency, poultry are raised under intensive production systems in densely populated colonies of flocks. During the process of intensive production, chickens are stressed by several factors such as transportation to the growing site, overcrowding, vaccination, chilling and/or overheating (Panda *et al.*, 1999). These stressors can be alleviated by early establishment and maintenance of a favourable microbial population in the digestive tract which in turn improve the assimilation of the food particle and performance (Panda *et al.*, 2000). Evidence is presented showing that treatment with probiotics moderates growth retardation in chickens and the probiotic protection occurs whether the disease is induced by an environmental, microbial or nutritional stressor (Dunham *et al.*, 1998). However, results under field conditions have generally been inconsistent (Stavric *et al.*, 1992). Results from trials conducted with broiler fed various probiotics were inconsistent. Some researchers reported positive responses of weight gain and feed conversion ratio in chickens due to consumption of probiotics (Chiang and Hsieh, 1994; Mohan *et al.*, 1996; Cavazzoni *et al.*, 1998; Jin *et al.*, 1998; Joy and Samuel, 1997; Kumprecht and Zobac, 1998; Fritts *et al.*, 2000), while others reported no beneficial effects (Maiolino *et al.*, 1992; Choudhury *et al.*, 1998; Panda *et al.*, 1999; Kahraman *et al.*, 2000). Scarce information are available relating to probiotic feeding on the performance and immune response of broiler chicks under heat stress condition. Keeping this in mind, the present study was conducted to compare the effects of “Bioplus 2B” probiotic and “virginiamycin” antibiotic on the performance and on immune system of broiler chicks under heat stress condition.

Materials and Methods

Four hundred one-day-old male broiler chicks (Ross) were wing banded, weighed and distributed randomly into four groups of 100 chicks with four replicate in each group. The replicate groups were housed separately in pens under the same environmental conditions on wood shaving litter. Each group was subjected to a feeding treatment: a) basal diet without any additive (control group); b) basal diet supplemented with 0.02% virginiamycin; c) basal diet supplemented with 0.05% Bioplus 2B; and d) basal diet supplemented with 0.1% Bioplus 2B. The probiotic used in the experiment, Bioplus 2B, is a commercial probiotic preparation containing two strains of organisms namely *Bacillus subtilis* CH201 and *Bacillus licheniformis* CH200 containing 3×10^9 colony forming units per each gram of the product. The antibiotic and probiotic supplements were added to 1/10 of the diet mixed with it and finally incorporated into the whole diet by a cochlear mixer. Chicks were allowed to have free access to a starter diet during the first three weeks and then to a finisher diet during the second three weeks (Table 1). They also had free access to water. Weight gain, feed intake and feed conversion (gain:food) of the birds were determined weekly. The chicks were exposed to heat stress by increasing the poultry house temperature (34°C) three hrs in a day at grower period (4–6 weeks of age). On the 22nd and 35th day of age, 20 birds from each group were injected intravenously (brachial vein) with 0.1 ml of 0.5% sheep red blood cells (SRBC). Antibody production against SRBC was measured after 7 days of inoculation. Titers were expressed as the log 2 of the reciprocal of the highest dilution in which there was agglutination (Wegmann and Smithes, 1996). The SRBCs used for inoculation and antibody titration were obtained from the same donor sheep.

On the 15th and 28th day of age, the chicks were vaccinated against Newcastle disease virus (NDV) by eye drop. Serum samples of 20 birds from each treatment group were collected on the 10th day of post-immunization (on the 25th and 38th day of age) and serum antibody titers were determined by the haemagglutination inhibition (HI) test. The HI titer was expressed as the log 2 reciprocal of the highest serum dilution producing 100% inhibition of HA activity. At the end of the 6th week, the serum cholesterol concentration was determined for 20 birds taken from each group. Serum cholesterol was measured by method of Zlatkis *et al*., (1993). On the day 42, blood samples were taken from 20 broilers taken from each group for determination of white blood cell (WBC) count, heterophil to lymphocyte ratio and haemoglobin concentration. Blood samples were collected in EDTA tubes. Blood smears were prepared using May-Grünwald-Gimsa stain and 100 leukocytes, including granular (heterophils, eosinophils and basophils) and non-granular (lymphocytes and monocytes), were counted and the heterophil to lymphocyte ratio was calculated. Haemoglobin was assayed by the cyanmethaemoglobin method of Dacie and Lewis (1998) using a haemoglobin diagnostic kit.

Table 1: Composition of basal diet (%)

Ingredients and composition	Starter	Grower
Corn	54.5	60.5
Soybean meal	38	32
Fish meal	2.5	2.5
Vegetable oil	1.4	1.6
Dicalcium phosphate	1.5	1.5
Calcium carbonate	1.3	1.2
Vitamin-mineral premix*	0.5	0.5
DL-methionine	0.2	0.15
Lysine	0.05	0.00
Salt	0.05	0.05
Calculated composition		
Crude protein	21.5	19.5
ME, kcal/kg	2900	3050

*The broiler premix provided the following per kg of diet: vitamin A, 10000 IU; cholecalciferol, 82.5 µg; vitamin E, 25 IU; riboflavin, 8 mg; niacin, 50 mg; d-pantothenic acid, 15 mg; folic acid, 1 mg; vitamin B_{12}, 15 µg; choline chloride, 1000 mg; thiamine, 2.5 mg; biotin, 0.1 mg; ethoxyquin, 100 mg; menadione sodium bisulfite, 3.3 mg; pridoxine, 1 mg; manganese, 15 mg; zinc, 50 mg; iodine, 1.5 mg; iron, 30 mg; copper, 6 mg; selenium, 0.2 mg

Data were analysed following the general linear model procedure of SAS

(6.12) and comparison of means was made using Duncan's multiple range test. Significance was considered at p<0.05.

Results

Body weight gain, feed intake and feed conversion ratio

The effects of probiotic and antibiotic supplementation on body weight gain, total feed intake and feed conversion ratio (FCR) for 0–3 and 4–6 (heat stress) weeks periods are presented in Table 2. The addition of antibiotic to the diet significantly improved body weight gain during the periods mentioned as compared to the control and 0.05% probiotic-supplemented group (P< 0.05). However, supplemented diet with antibiotic had no significant effects on body weight gain during 4–6 weeks (heat stress) period as compared to 0.1% probiotic-supplemented group (P>0.05).

Feed intake of different groups do not differ significantly (P>0.05). The addition of antibiotic or various levels of probiotic to the diet significantly improved FCR during 4–6 weeks (heat stress) period as compared to the control group (P<0.05). No significant differences were found between the groups supplemented with 0.05 and 0.1% probiotic.

Antibody production against SRBC and in response to NDV vaccination

The effects of probiotic and antibiotic on antibody production against SRBC and antibody against NDV vaccination are

Table 2: Effects of probiotic and antibiotic supplementation on the performance of chicks

	Control	Antibiotic	Probiotic 0.05%	Probiotic 0.1%
1–3 week				
Body weight (g)	$568.75^{a} \pm 19.86$	$621.50^{b} \pm 4.55$	$585.00^{a} \pm 19.52$	$575.25^{a} \pm 28.09$
Feed intake (g)	1120.00 ± 36.98	1166.25 ± 12.55	1141.00 ± 42.43	1089.50 ± 68.76
FCR (feed/gain)	1.97 ± 0.06	1.88 ± 0.04	1.95 ± 0.02	1.89 ± 0.03
4–6 week				
Body weight (g)	$987.50^{a} \pm 35.03$	$1112.00^{b} \pm 52.04$	$1015.00^{b} \pm 59.91$	$1050.75^{ab} \pm 41.67$
Feed intake (g)	2276.00 ± 96.06	2399.50 ± 127.71	2206.75 ± 122.12	2276.50 ± 118.44
FCR (feed/gain)	$2.30^{a} \pm 0.07$	$2.16^{b} \pm 0.04$	$2.17^{b} \pm 0.05$	$2.17^{b} \pm 0.04$

$^{a,\,b}$Means in the same row with different superscripts differ significantly (P<0.05)

Table 3: Effects of probiotic and antibiotic supplementation on antibody production against SRBC antigen and antibody against NDV vaccination at different ages

	Control	Antibiotic	Probiotic 0.05%	Probiotic 0.1%
Antibody against SRBC (first time)	5.42 ± 0.95	5.80 ± 0.73	5.62 ± 0.70	6.00 ± 0.50
Antibody against SRBC (second time)	5.47 ± 0.47	6.07 ± 0.37	6.12 ± 0.64	6.20 ± 0.54
Antibody against NDV vaccination (25 days of age)	4.05 ± 0.48	4.4 ± 0.60	4.37 ± 0.42	4.7 ± 0.36
Antibody against NDV vaccination (38 days of age)	3.00 ± 0.35	3.25 ± 0.25	3.37 ± 0.54	3.40 ± 0.41

Table 4: Effects of probiotic and antibiotic supplementation on WBC count and heterophil to lymphocyte ratio

	Control	Antibiotic	Probiotic 0.05%	Probiotic 0.1%
WBC count	$22275^{a} \pm 4000$	$24875^{ab} \pm 2250$	$24500^{ab} \pm 2692$	$27500^{b} \pm 1280$
Heterophil to Lymphocyte ratio	$0.80^{a} \pm 0.02$	$0.73^{ab} \pm 0.04$	$0.74^{ab} \pm 0.04$	$0.70^{b} \pm 0.05$

$^{a,\,b}$Means in the same row with different superscripts differ significantly (P<0.05)

Table 5: Effects of probiotic and antibiotic supplementation on serum cholesterol and blood haemoglobin concentration

	Control	Antibiotic	Probiotic 0.05%	Probiotic 0.1%
Serum cholesterol (mg/dl)	$124.25^{a} \pm 14.69$	$130.25^{a} \pm 12.28$	$115.00^{ab} \pm 7.07$	$111.75^{b} \pm 11.00$
Haemoglobin (mg/dl)	9.95 ± 0.9	10.05 ± 0.50	9.70 ± 0.60	9.60 ± 0.7

$^{a,\,b}$Means in the same row with different superscripts differ significantly (P<0.05) presented in Table 3. The supplemented diet with antibiotic or various levels of probiotic had no significant effects on antibody production against SRBC or in response to NDV vaccination during heat stress.

WBC count and heterophil to lymphocyte ratio

The effects of probiotic and antibiotic on WBC count and heterophil to lymphocyte ratio are presented in Table 4. The supplemented diet with 0.1% probiotic significantly increased WBC count in comparison to the control group. However, no significant differences were found among 0.1 and 0.05% probiotic- and antibiotic-supplemented groups.

Serum cholesterol and blood haemoglobin concentration

The effects of probiotic and antibiotic supplementation on serum cholesterol and blood haemoglobin concentration are presented in Table 5. The serum cholesterol concentration in 0.1% probiotic-supplemented group was significantly decreased in comparison with both of the control and antibiotic-supplemented groups; however, no differences were observed between 0.05 and 0.1% probiotic-supplemented groups.

Haemoglobin levels were not significantly different among treatment groups. Therefore, it was concluded that inclusion of these antibiotic and probiotic at the recommended doses has no adverse effect on the chicks.

Discussion

Feed intake, weight gain, feed conversion ratio

The results of this experiment indicate that supplementation of diet with probiotic and antibiotic significantly improves FCR during heat stress condition. Moreover, the data suggest that there is a large phase of 21 days before the effects of this probiotic preparation (on FCR) becomes apparent. Concomitant to the finding of the experiment, Takahashi *et al*., (1997) reported that the feeding probiotic (*Bacillus cereus* CIP 5832) improves feed efficiency in chicks raised in the dirty environment, but not the body weight gain and feed intake. Choudhury *et al*., (1998) found no growth promoting effects of probiotics. However, chicks reared on the rations containing antibiotic, had a higher growth rate than other groups.

In contrast, Cavazzoni *et al*., (1998) believed that addition of probiotic to the chicken diet significantly improves chicken performance as compared with chickens that had no additive or had antibiotic as a growth-promoting prophylactic additive. Zulkifli *et al*., (2000) vaccinated broiler for Newcastle disease and exposed the treatment group to cyclic heat stress and found that birds on the lactobacillus regimen had greater body weights and higher feed intake than those treated with supplemental oxytetracycline or the control group while feed conversion was significantly improved with lactobacillus prior to heat stress. The reverse was true after exposure to heat stress; the oxytetracycline did not improve body weight, feed intake or feed conversion during heat stress. However, prior to heat stress it significantly improved weight gain and feed efficiency.

Yeo and Kim (1997) reported a significant improvement in the mean daily gain but not in feed conversion efficiency in broiler chicks in the probiotic-supplemented group during the starter phase as compared to the control and antibiotic-supplemented groups. Nonetheless, no significant improvement in daily gain or feed conversion efficiency was observed in the finisher phase. The reasons for these discrepancies are still not known.

Differences in management and sanitary conditions, hybrids of broilers, microbial strains, product stability, type and dosage of probiotics or feed processing (e.g., pelleting), may affect the results of such experiments. For instance, Kahraman *et al*., (2000) showed that probiotic and antibiotic had no positive effects on the performance of broiler chicks. These researchers implied that addition of probiotic and antibiotic to the diet did not influence broiler performance kept under good hygienic condition.

Antibody against SRBC and in response to NDV vaccination

Concomitant to the findings of the

present investigation, Panda *et al*., (1999) reported that supplementation of probiotic did not have any effects on antibody production against SRBC. In contrast, Panda *et al*., (2000) supplemented the broilers diet with various levels of probiotic (100, 150, 200 mg/kg diet) and reported the significant difference in antibody production in 100 mg probiotic-supplemented group on the 5th and 10th day post-inoculation (PI) with SRBC antigen when injected on the 14th and 21st day of age, respectively, as compared to the control group. However, after 28 days of age, no significant difference was observed in the antibody production on days 5, 10 and 15 PI. Zulkifli *et al*., (2000) exposed two lines of commercial broilers (Hubbard and Shaver) to a heat stress regimen and superimposed a lactobacillus and oxytetracycline treatment birds were given vaccination against Newcastle on the 7th and 21st day when (21st day) the heat stress treatments were imposed. Hubbard birds fed on lactobacillus, had a higher antibody production rate than Shaver birds suggesting that there may have been some strain differences.

WBC count and heterophil to lymphocyte ratio

Results of this research indicate that the group fed 0.1% probiotic showed higher resistance to heat stress than the control group. Shoeib *et al*., (1997) reported the effect of dietary supplements of pronifer (probiotic) on immune response in 80-day-old chicks. Haematologic profiles showed an increase in total erythrocyte and leukocyte counts and marked increase in the percentage of lymphocytes and monocytes. These researchers implied that use of pronifer, as a feed additive, enhances immune response in chickens.

Serum cholesterol and blood haemoglobin concentration

A similar cholesterol depressing effect in chicks had been observed by Mohan *et al*., (1996); Jin *et al*., (1998) and Panda *et al*., (2001). Endo *et al*., (1999) reported that the cholesterol level of the liver and serum was significantly decreased in the cocks fed on the cholesterol-enriched probiotic-supplemented diet. Mohan *et al*., (1996) also reported that inclusion of probiotic (probiolac) in layer diets reduced serum/yolk cholesterol.

This reduction in serum cholesterol could be attributed to reduced absorption and/or synthesis of cholesterol in the gastrointestinal tract by probiotic supplementation (Mohan *et al*., 1996; Panda *et al*., 2001).

Concomitant to the findings of the experiment, Baidya *et al*., (1994) reported that inclusion of probiotic and antibiotic in broiler diet did not cause significant effect on haemoglobin concentration. In contrast, Mohan *et al*., (1996) reported a significant reduction in haemoglobin concentration by addition of probiotic (probiolac). These researchers implied that this reduction may be due to competition of probiotic with the host for folic acid or other nutrients.

In conclusion, the results showed that diet supplementation with virginiamycin has a significant effect on body weight gain and FCR during the heat stress. The supplemented diet with 0.1% probiotic (Bioplus 2B) has a favourable effect on FCR, WBC count, heterophil to lymphocyte ratio and serum cholesterol during the heat stress.

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