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Probiotic level effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers

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ABSTRACT

Probiotic effects on growth performance, carcass traits, blood parameters, cecal microbiota, and immune response of broilers were studied. Two hundred one-day-old male chickens were allocated to one of five treatments (four replicates of 10 birds per treatment): control, and the same control diet supplemented with 0.005%, 0.01%, 0.015% and 0.02% probiotics. Probiotics in feed at 0.01% or higher levels of supplementation improved body weight gain (+12%) and feed conversion rate (-5%) compared with the control. There were no effects on carcass traits, but the relative weights of drumsticks and wings showed increasing and decreasing linear responses, respectively, to probiotic supplementation level. Blood plasma glucose and albumin contents linearly increased (from 167.1 to 200.5 mg dl^{-1}, and from 1.70 to 3.25 g dl^{-1}) with increasing probiotic supplementation. Triglycerides and cholesterol contents were lower in probiotic supplemented treatments (average contents 71.3 and 125.3 mg dl^{-1} vs. 92.6 and 149.9 mg dl^{-1} in the control). Probiotics decreased cecal *Escherichia coli* counts, but had no effects on immunity related organs or immune response. The linear trends, either positive or negative, observed in many of the parameters studied, suggest that more studies are needed to establish the optimal concentration of probiotics in broiler feed.

Key words: digestion, immunity, poultry, probiotics, production.

INTRODUCTION

Enteric diseases are an important burden to the poultry industry because of lost productivity, increased mortality, and the associated contamination of poultry products for human consumption (Patterson and Burkholder 2003). As a result, the banning of subtherapeutic antibiotic usage in several countries, due to consumers' concerns regarding food safety and antibiotic-resistant bacteria in humans, has brought about a challenge for the productive efficiency of the poultry industry. Therefore, several alternatives to growth-promoting antimicrobials have been investigated in recent years (Huyghebaert et al. 2011). Those strategies have focused on preventing the proliferation of pathogenic bacteria and modulating beneficial gut microflora so that the health, immune status and performance are improved (Adil and Magray 2012).

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Probiotics are single or mixed cultures of live microorganisms, which when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2001). Observed effects after probiotic supplementation are related to a more beneficial microbial population in the gut due to pathogen inhibition. Mechanisms of pathogen inhibition may include stimulation of the immune system, competition for available nutrients, and direct antimicrobial effects by secretion of inhibitory substances or competition for adhesion receptors to intestinal epithelium (Yang et al. 2009, Lee et al. 2010).

Several papers have shown that probiotics in broiler diets improve the growth performance compared with non-supplemented diets, being as effectives as antibiotic growth promoters (Kalavathy et al. 2003, Mountzouris et al. 2010, Shim et al. 2010). Some authors have investigated the effects of adding a single level of probiotics in broiler diet (Khosravi et al. 2010, Mountzouris et al. 2007, Zakeri and Kashefi 2011), while others have tested two (Anjum et al. 2005, Mehr et al. 2007, Nayebpor et al. 2007, Panda et al. 2006) or three or more levels of probiotic supplementation (Apata 2008, Li et al. 2008, Mountzouris et al. 2010, Wang and Gu 2010). However, the results obtained are contradictory and highlight the importance of evaluating probiotic administration level for maximizing efficacy. Hence, the aim of the present work was to investigate the effects of increasing levels of probiotic supplementation on growth performance, carcass traits, blood plasma constituents, cecal microbiota and immune response of broiler chickens.

MATERIALS AND METHODS

Animals, Housing, Diets and Treatments

Use and care of birds and procedures employed on this study were approved by the Islamic Azad University Ethics Committee. Before starting the trial, the research facility was thoroughly cleaned and disinfected. Two hundred one-day-old male chickens of the Ross 308 strain (Aviagen, Newbridge, UK), purchased from a commercial hatchery, were used. The broiler chicks were placed in 1.5 × 1.0 m cages, in which the floor was covered with shredded paper. Each cage was equipped with a pan feeder and a manual drinker. The research facility was an open sided poultry barn having thermostatically controlled curtains and equipped with thermostatically controlled gasoline rocket heaters, overhead sprinklers, wall-mounted fans on both ends of the barn, and fluorescent tubes in ceiling fixtures. Ambient temperature was set at 32 °C at placement and then decreased gradually until it reached 24 °C from week 3 onwards. Lighting was constant at day 1. From day 2 to the finish of the study, light regime was 21L:3D. Feed (mash form) and water were provided *ad libitum* throughout the whole trial.

The experiment lasted 42 days. The feeding programme was a commercial one and consisted of a starter diet until the chicks were 14 days old, followed by a grower diet up to 28 days of age, and then a finisher diet until the end of the experiment. All feeds were based on maize and soybean meal and did not contain any antibiotic feed additives (Table I). Chicks were assigned into one of the following treatments: control (basal diet without added probiotics), and the same basal diet supplemented with 0.005%, 0.01%, 0.015% and 0.02% of Protexin probiotics (P1, P2, P3 and P4 treatments, respectively). Protexin Compounder (Probiotics International Ltd, Somerset, UK) was obtained from a local provider. It is a multi-strain commercial preparation in powder form (2×10^9 CFU/g) that consists of *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopesii.* The manufacturer's recommended levels of Protexin

supplementation are 0.01% (0.10 g/kg feed) until four weeks of age and 0.005% (0.05 g/kg feed) thereafter. Each treatment had four replicates, thus there was a total of 20 groups of 10 birds.

Growth Performance and Carcass Measurements

Body weight (BW) of the chicks and feed consumption were weekly recorded by replicate. Following, body weight gain (BWG, g/period), feed intake (FI, g/period), and feed conversion ratio (FCR, feed to gain g/g) were determined within each treatment. At the age of 42 days, after 4 h of fasting for complete evacuation of the gut, four chickens per treatment (one from each replicate) that had weights closest to the mean weight for the cage were selected and euthanized by cervical dislocation to determine carcass traits. Birds were fully plucked by dry plucking method and the feet, head, and wingtips were removed. Broilers were eviscerated before determining the carcass weight. Weights of the breast, drumsticks, wings, abdominal fat, and organs were recorded.

TABLE I
Experimental diets fed to broiler chickens.

	Starter 1-14 d	Grower 15-28 d	Finisher 29-42 d
Ingredients, %			
Maize	55.60	61.56	64.31
Soybean meal 44	37.00	30.00	27.00
Soybean oil	1.20	2.30	3.60
Dicalcium phosphate	1.70	1.70	1.50
Calcium carbonate	1.50	1.40	1.20
Vitamin and mineral mixture[1]	2.00	2.00	2.00
DL-methionine	0.20	0.26	0.17
Salt	0.23	0.33	0.20
Sodium bicarbonate	0.17	0.17	0.15
L-lysine HCL	0.15	0.15	0.05
Choline chloride	0.10	0.10	0.10
L-treonine	0.03	0.03	0.04
Enzymes[2]	0.05	0.05	0.03
Phytase[3]	0.01	0.01	0.05
Calculated analysis[4]			
Metabolizable energy, MJ kg^{-1}	11.8	12.3	12.9
Crude protein, %	21.3	18.7	17.5
Lysine, %	1.26	1.09	0.93
Methionine + Cysteine, %	0.93	0.80	0.75
Treonine, %	0.83	0.72	0.69
Calcium, %	1.06	1.01	0.90
Phosphorus, %	0.71	0.68	0.63

[1] Supplied per kilogram of feed - Vitamin A: 12500 IU; vitamin D_3: 1250 IU; vitamin E: 18 IU; vitamin K_3: 3.7 mg; thiamine: 1.8 mg; riboflavin: 6.6 mg; calcium pantothenate: 10 mg; niacin: 37.5 mg; pyridoxine: 32.5 mg; vitamin B12: 2.5 mg; Mn: 50 mg; Zn: 37.5 mg; Fe: 25 mg; Cu: 7.5 mg.
[2] Yiduozyme 9680. GuangDong, VTR Bio-Tech Co. Ltd., China.
[3] Phyzyme XP 10000 TPT. Danisco Animal Nutrition, Marlborough, UK.
[4] According to National Research Council (1994).

Microbial Enumeration

At 42 days of age, four chickens per treatment (one from each replicate) were selected as above, and euthanized. From each euthanized bird, the caeca were quickly dissected and their contents were collected in sterilized sampling tubes. From those contents, 10-fold serial dilutions of 1 g of sample were serially made in phosphate buffer solution (10^{-1} - 10^{-6}). Subsequently, 100 µl were removed from 10^{-4}, 10^{-5}, and 10^{-6} dilutions and poured onto Petri dishes containing the culture media. *Lactobacilli* were cultured in De Man, Rogosa and Sharpe agar and incubated at 37 °C in anaerobic conditions for 72 h. Total aerobes, *Escherichia coli* and *Enterococci* were cultured in nutrient agar, eosin methylene blue agar and Slanetz-Bartley agar, respectively, and incubated at 37 °C under aerobic conditions for 48 h. Bacterial colony forming units (CFU) in the Petri dishes were counted using a colony counter. The counts were reported as $\log_{10}$ CFU per one g of sample.

Blood Sampling and Analysis and Immune Response Study

For measuring blood plasma metabolites, enzymes and minerals at 42 days of age, four chickens per treatment (one from each replicate) were selected as above to collect 5 ml of blood from their wing veins into EDTA tubes. After centrifuging blood samples (3000 x g, for 20 min at room temperature), plasma was harvested and stored in Eppendorf tubes at -20º C until assayed. Biochemical analysis was according to standard protocols using commercial laboratory kits (Pars Azmoon Co., Tehran, Iran). Parameters measured were glucose, total protein, albumin, uric acid, triglycerides, cholesterol (total, HDL, LDL and VLDL), alkaline phosphatase, calcium and phosphorus.

Production of antibodies in response to different antigens was assessed during the experiment. The birds were vaccinated against infectious bronchitis disease (1st and 9th day of age), Newcastle disease (1st, 14th and 20th day of age), influenza disease (1st day of age) and Gumboro disease (14th and 23rd day of age). All vaccines were provided by Razi Co. (Tehran, Iran). Additionally, one bird per replicate was injected under the breast skin with 0.5 ml of a 10% suspension in phosphate buffered saline of sheep red blood cells (SRBC) at the 15th and 29th day of age. To determine the systemic antibody response, blood samples were collected from one chick per replicate via the wing vein at the 21st and 27th day of age (Newcastle disease and influenza disease), and at the 22nd and 36th day of age (SRBC). Blood samples were processed and analyzed as described by Pourhossein et al. (2014). To determine the antibody response to Newcastle disease and influenza disease a hemagglutination inhibition assay was used. Total immunoglobulin (Ig) and immunoglobulin G (IgG) titers to SRBC were also determined by hemagglutination assay; then, immunoglobulin M (IgM) titers to SRBC were calculated as total Ig minus IgG titers. The hemagglutination inhibition titer was expressed as the $\log_2$ of the last dilution of serum that completely inhibited haemagglutination activity.

Statistical Analyses

The GLM procedure of SAS 9.1 (SAS Institute Inc., Cary, NC) was used in the statistical analyses. The statistical design was: $Yij = \mu + Ti + eij$; where Yij is the observation, µ is the overall mean, Ti is the fixed effect of the treatment, (i = 5), and eij is the residual error. Tukey's test was used to compare least squares means. The responses to probiotic supplementation were investigated through preplanned contrasts, both orthogonal (control vs. probiotic supplemented diets) and polynomial (linear and quadratic effects of supplementation levels). Statistical significance was declared at $P<0.05$.

RESULTS AND DISCUSSION

The effects of diet supplementation with increasing levels of probiotics on growth performance are

presented in Table II. All performance traits were improved by probiotic supplementation. However, compared with the control, the worst results in BWG and FCR were observed at the lowest level of supplementation. Both BWG and FCR showed a linear response ($P<0.001$), either positive (BWG) or negative (FCR), to increasing probiotic supplementation, while the response of FI was mostly quadratic ($P<0.01$). The BWG response to probiotic supplementation level was also quadratic ($P<0.05$), the highest value being observed in the P2 treatment. Khosravi et al. (2010) did not find improvements in BWG and FCR of broilers fed Protexin at the recommended level, compared with those fed the control diet. However, in agreement with our results, Anjum et al. (2005) and Mehr et al. (2007) found that diet supplementation with Protexin at two levels (recommended or 10 and 20% over recommendations, respectively) improved BWG and FCR in broilers compared with the control treatment, but both authors observed that the improvements were clearer at the highest level of supplementation. The fact that Protexin supplementation above recommendation levels seems to improve growth performance might be due to a limited effect on nutrient and energy cost for both the growth and proliferation of live microbes in the gut, and the development of an immune response by the host, or to a more profound effect of the supplied microbes on gut health and function (Mountzouris et al. 2010). Moreover, despite the significant linear trends observed in BWG and FCR in the present work, no significant differences ($P>0.05$) were found between P2, P3 and P4 treatments, which suggests that the average manufacturer's recommended level was appropriate to improve the productive results.

Several papers have reported contradictory effects on growth performance when comparing different levels of probiotic supplementation (Apata 2008, Li et al. 2008, Nayebpor et al. 2007, Panda et al. 2006, Wang and Gu 2010). Mountzouris et al. (2010) pointed out that no consistent conclusions can be drawn regarding the effect of increasing probiotic administration level on growth performance due to the contradictory results found in the literature and suggested the occurrence of an optimal strain-dependent concentration of each of the probiotics tested. On the other hand, it has been suggested that efficacy for most probiotics in animals could be achieved with a daily intake of 1 x 10^7 to 1 x 10^9 microorganisms (Mountzouris et al. 2010, Shim et al. 2010). In the present work, according to the manufacturer´s specifications, the calculated average daily intake of microorganisms was 1 x 10^6 and 2 to 4 x 10^7 in the P1 treatment and the P2, P3 and P4 treatments, respectively, which could explain why the P1 treatment did not improve the performance traits compared with the control treatment. On the other hand, most of the above-mentioned works and the present one were carried out with chickens raised in cages or do not specify the rearing system. The rearing system (floor vs. cage) may affect the observed productive results (Santos et al. 2008). Furthermore, the effects of broiler feed supplementation with alternatives to growth-promoting antimicrobials, such as probiotics, may depend on the rearing system due to differences in the hygienic conditions (Pirgozliev et al. 2014). Thus, rearing conditions should be taken into account for a more complete interpretation of the experimental data from research on probiotic supplementation effects.

Table II shows final BW, carcass traits and organ weights. BW was higher ($P<0.05$) in the P4 treatment and showed a positive linear response ($P<0.05$) to the increasing levels of probiotics. Except for wings and abdominal fat, no differences ($P>0.05$) were found in carcass traits among treatments. Nevertheless, carcass weight showed a positive linear trend ($P=0.06$) with increasing probiotic supplementation. Anjum et al. (2005) and Awad et al. (2009) did not find differences in BW and

TABLE II

Body weight gain (BWG), feed intake (FI), feed conversion rate (FCR), carcass traits and organ weights of 6-week old broilers fed diets containing either no probiotics (control) or 0.005%, 0.01%, 0.015% and 0.02% of probiotics (P1, P2, P3 and P4, respectively). Carcass and organ measures were obtained from four birds per treatment.

	Treatments						Probability		
	Control	P1	P2	P3	P4	SEM	Control vs probiotics†	Linear‡	Quadratic‡
BWG, g period^{-1}	64.2^{b}	65.7^{b}	72.5^{a}	71.4^{a}	71.9^{a}	0.90	<0.001	<0.001	<0.05
FI, g period^{-1}	118.2^{b}	120.8^{ab}	128.1^{a}	125.3^{ab}	121.6^{ab}	1.15	<0.05	0.10	<0.01
FCR, g g^{-1}	1.84^{a}	1.84^{a}	1.77^{ab}	1.76^{ab}	1.69^{b}	0.016	<0.05	<0.001	0.47
Body weight (BW), g	2794^{bc}	2692^{c}	3020^{ab}	2968^{ab}	3040^{a}	45.0	0.15	<0.01	0.91
Carcass weight (CW), g	1699	1577	1761	1824	1836	39.4	0.59	0.06	0.65
Breast, % CW	43.9	42.0	44.1	39.7	41.4	0.55	0.09	0.05	0.85
Drumsticks, % CW	37.1	35.3	36.5	41.2	40.5	0.77	0.42	<0.05	0.30
Wings, % CW	8.69^{ab}	10.4^{a}	9.19^{ab}	7.57^{b}	7.87^{b}	0.324	0.91	<0.05	0.15
Abdominal fat, % CW	2.22^{a}	1.25^{ab}	1.31^{ab}	1.01^{b}	1.75^{ab}	0.136	0.24	0.77	0.87
Organ weights, % BW									
Left caecum	0.192^{b}	0.290^{a}	0.259^{ab}	0.252^{ab}	0.262^{ab}	0.010	<0.01	0.08	<0.05
Liver and bile	2.59	2.64	2.32	2.69	2.73	0.068	0.97	0.48	0.25
Spleen	0.113	0.114	0.100	0.125	0.097	0.033	0.69	0.50	0.63
Thymus	0.237^{b}	0.509^{a}	0.334^{ab}	0.276^{ab}	0.261^{ab}	0.004	0.13	0.34	0.06
Bursa of Fabricious	0.096	0.065	0.110	0.086	0.085	0.007	0.57	0.97	0.86

SEM: standard error of the mean.

^{abc} In a row, least squares means with a different superscript differ significantly (P<0.05) by Tukey's test.

† Probability of the probiotic supplementation effect.

‡ Probability of the linear and quadratic responses to the increasing levels of probiotics in the diet.

carcass percentage between a control and a probiotic supplemented treatment. However, Mehr et al. (2007) observed higher body and carcass weights and breast percentage with higher level of probiotic supplementation compared with a lower level and the control treatment. Abdominal fat expressed as percentage of carcass weight was higher (P<0.05) in the control treatment and did not show linear or quadratic trends (P>0.05). Some authors have observed that probiotic supplemented diets reduce abdominal fat weight in broilers compared with the controls (Anjum et al. 2005, Mehr et al. 2007), and others have reported a simultaneous decrease of blood triglyceride content (Kalavathy et al. 2003, Mansoub 2010, Santoso et al. 1995). Santoso et al. (1995) found that abdominal fat could be related to a decrease in the activity of acetyl-CoA carboxylase, the rate limiting enzyme in fatty acid synthesis, after *Bacillus subtilis* culture supplementation, which in turn could explain the decreased blood triglyceride content that was observed in their work. In agreement with that, in the present work lower (P<0.05) blood plasma triglyceride contents were observed in the supplemented treatments (Table III). However, no significant correlation could be found between abdominal fat and blood plasma triglyceride contents. Regarding organ weights, left caecum and thymus weights were higher (P<0.05) in the P1 treatment, and were higher or tended to be higher in the supplemented treatments (P<0.05 and P=0.13) compared with the control, showing quadratic trends (P<0.05 and P=0.06). Awad et al. (2009) did not find significant differences in the weights of caecum, liver, spleen, thymus and bursa of Fabricious, as a proportion of BW, between broilers fed a control or a probiotic supplemented diet. Other authors have also reported no effects of probiotic supplementation on lymphoid organs

(Ahmadi 2011, Naseem et al. 2012). The enlarged caecum observed in the supplemented treatments of the present work could be explained by an increase of the length and density of the microvilli of the cecal tonsils due to the probiotics (Yurong et al. 2005).

TABLE III

Blood plasma constituents of 6-week old broilers fed diets containing either no probiotics (control) or 0.005%, 0.01%, 0.015% and 0.02% of probiotics (P1, P2, P3 and P4, respectively). All data were obtained from four birds per treatment.

	Treatments						Probability		
	Control	P1	P2	P3	P4	SEM	Control vs probiotics†	Linear‡	Quadratic‡
Glucose, mg dl^{-1}	167.1	180.8	199.5	197.4	200.5	4.96	<0.05	<0.05	0.28
Total protein, g dl^{-1}	4.02	3.56	3.27	4.40	4.23	0.165	0.69	0.25	0.14
Albumin, g dl^{-1}	1.70^{b}	1.92^{ab}	2.54^{ab}	2.98^{ab}	3.25^{a}	0.184	0.07	<0.01	0.62
Uric acid, mg dl^{-1}	2.37	2.41	2.47	2.03	2.03	0.121	0.69	0.26	0.58
Triglycerides, mg dl^{-1}	92.6^{a}	74.2^{ab}	73.8^{ab}	69.6^{b}	67.4^{b}	2.91	<0.01	<0.01	0.16
Cholesterol, mg dl^{-1}									
Total	149.9^{a}	129.6^{abc}	136.2^{ab}	111.2^{c}	124.0^{bc}	3.66	<0.01	<0.01	0.11
HDL	75.0^{b}	92.8^{a}	91.5^{a}	73.8^{b}	84.5^{ab}	2.26	<0.05	1	0.03
LDL	56.4^{a}	22.0^{b}	30.0^{b}	23.6^{b}	26.0^{b}	3.18	<0.001	<0.01	<0.01
VLDL	18.5^{a}	14.9^{ab}	14.8^{ab}	13.9^{b}	13.5^{b}	0.58	<0.01	<0.01	0.16
Alkaline phosphatase, U L^{-1}	139^{c}	330^{a}	355^{a}	235^{b}	258^{b}	21.8	<0.001	<0.001	<0.01
Calcium, mg dl^{-1}	10.18	9.42	7.81	9.04	9.32	0.338	0.13	0.37	0.09
Phosphorus, mg dl^{-1}	5.01^{b}	7.41^{a}	7.47^{a}	6.10^{ab}	6.04^{ab}	0.266	<0.01	0.56	<0.001

SEM: standard error of the mean.

[abc] In a row, least squares means with a different superscript differ significantly (P<0.05) by Tukey's test.

† Probability of the probiotic supplementation effect.

‡ Probability of the linear and quadratic responses to the increasing levels of probiotics in the diet.

Blood parameters are shown in Table III. Blood glucose was higher (P<0.05) and albumin tended to be higher (P=0.07) in the supplemented treatments, both showing a positive linear response (P<0.05) to probiotic supplementation. These effects could be explained by a higher absorptive capacity of the intestinal mucosa due to histomorphological changes (Awad et al. 2009, Aliakbarpour et al. 2012) and/or a more effective digestion of the diet due to a higher intestinal enzyme activity (Jin et al. 2000, Mountzouris et al. 2007, Wang and Gu 2010), thus increasing the nutrients available to the animals. As previously discussed, blood triglyceride contents were lower (P<0.05) in the supplemented treatments. Blood total cholesterol was also lower (P<0.05) in the supplemented treatments and there was a change in the contents of the different cholesterol fractions: HDL was increased (P<0.05) and LDL and VLDL were decreased (P<0.05) by probiotic supplementation. Blood total cholesterol, LDL and VLDL showed a negative linear response (P<0.05) to probiotic supplementation. The negative effect of probiotic supplemented diets on broiler blood cholesterol content is well-known (El-Baky 2013, Kalavathy et al. 2003, Mansoub 2010, Panda et al. 2006, Santoso et al. 1995). Although the mechanisms involved are not fully understood, it is hypothesized that some bacterial probiotic strains are able to incorporate cholesterol into the bacterial cells, hydrolyze bile salts or inhibit hydroxymethylglutaryl-CoA, the rate limiting enzyme of cholesterogenesis, thus reducing cholesterol in the body pool (Kalavathy et al. 2003). A decrease in blood LDL and VLDL cholesterol contents due to probiot-

ic supplementation was also reported by Kalavathy et al. (2003) and Panda et al. (2006). Blood alkaline phosphatase activity and phosphorus content increased (P<0.05) due to probiotic supplementation, while no effects (P>0.05) were observed in calcium contents. Blood alkaline phosphatase activity showed a linear positive response (P<0.05) to the increasing levels of probiotics; however, the response of phosphorus was quadratic (P<0.001). On the contrary, El-Baky (2013) and Panda et al. (2006) observed no effects on blood alkaline phosphatase activities and phosphorus contents and higher calcium contents in probiotic supplemented treatments compared with the controls.

TABLE IV

Cecal bacterial counts (log_{10} CFU g^{-1} digesta) of 6-week old broilers fed diets containing either no probiotics (control) or 0.005%, 0.01%, 0.015% and 0.02% of probiotics (P1, P2, P3 and P4, respectively). All data were obtained from four birds per treatment.

	Treatments						Probability		
	Control	P1	P2	P3	P4	SEM	Control vs probiotics†	Linear‡	Quadratic‡
Lactobacilli	7.53	7.85	7.81	7.70	7.54	0.082	0.36	0.83	0.19
Total aerobes	8.59[b]	8.74[ab]	8.71[ab]	8.88[ab]	9.08[a]	0.060	0.07	<0.01	0.22
Enterococci	6.53[b]	6.81[ab]	7.08[a]	7.00[ab]	6.98[ab]	0.065	<0.001	0.09	<0.05
Escherichia coli	7.99[a]	7.63[ab]	7.26[c]	7.76[ab]	7.51[bc]	0.063	<0.001	<0.001	<0.01

SEM: standard error of the mean.

[abc] In a row, least squares means with a different superscript differ significantly (P<0.05) by Tukey's test.

† Probability of the probiotic supplementation effect.

‡ Probability of the linear and quadratic responses to the increasing levels of probiotics in the diet.

No effects of supplemented treatments (P>0.05) could be observed in cecal *Lactobacilli* counts; however, total anaerobe counts tended to increase (P=0.07), *Enterococci* counts increased (P<0.05) and *Escherichia coli* counts decreased (P<0.05) due to probiotic supplementation (Table IV). These results are in partial agreement with those of Giannenas et al. (2012) who did not observe differences in *Lactobacilli*, *Enterococci* and total anaerobe counts, but did observe lower *Escherichia coli* counts in the caecum of broilers fed a probiotic supplemented diet compared with the control. On the contrary, Mountzouris et al. (2007) reported that including probiotics in the diet of broilers caused higher concentrations of *Lactobacilli* and gram-positive cocci (e.g., *Enterococci*, *Pediococci*) in the cecal microflora compared with the controls.

Probiotic supplementation had few significant effects on the immune response to the vaccines and SRBC administered to the animals (Table V). The P4 treatment showed the highest (P<0.05) antibody response to Newcastle disease at 27 days of age and IgM response to SRBC at 36 days of age. El-Baky (2013), Naseem et al. (2012) and Zakeri and Kashefi (2011) found higher antibody titers against influenza disease, infectious bursal disease and Newcastle disease virus, respectively, in broilers fed Protexin supplemented diets compared with the controls. Moreover, Rhee et al. (2004) and Haghighi et al. (2005) reported higher blood IgM against SRBC when probiotics were included in a broiler diet. However, Mountzouris et al. (2010) failed to show improvements in the overall broiler humoral immune status at systemic level in response to probiotic supplementation. Unclear immune response improvements in the supplemented treatments of the present work might be related to the lack of *Lactobacilli* count increases in the gastrointestinal tract (Table V), since those bacteria have been reported to have beneficial effects on the host's immune system (Xu et al. 2003).

TABLE V
Immune response after vaccination or injection of sheep red blood cells (SRBC) in broilers fed diets containing either no probiotics (control) or 0.005%, 0.01%, 0.015% and 0.02% of probiotics (P1, P2, P3 and P4, respectively). All data were obtained from four birds per treatment.

	Treatments						Probability		
	Control	P1	P2	P3	P4	SEM	Control vs probiotics[†]	Linear[‡]	Quadratic[‡]
Newcastle disease, log_2									
21 d	3.50	3.50	3.25	2.25	3.50	0.186	0.39	0.31	0.23
27 d	3.75[ab]	2.50[b]	2.75[b]	2.25[b]	4.00[a]	0.223	0.07	0.84	<0.01
Influenza disease, log_2									
21 d	2.50	2.50	2.00	1.75	2.75	0.193	0.62	0.86	0.19
27 d	2.25	1.50	1.75	1.25	2.50	0.182	0.25	0.83	<0.05
SRBC, log_2									
Total Ig 22 d	3.50	3.50	4.75	4.25	4.50	0.270	0.29	0.18	0.59
Total Ig 36 d	4.75	6.25	6.00	4.00	6.50	0.394	0.32	0.64	0.94
IgG 22 d	1.75	1.25	2.50	2.75	1.75	0.192	0.46	0.21	0.16
IgG 36 d	2.75	2.75	2.75	2.75	2.75	0.250	1	1	1
IgM 22 d	1.75	2.25	2.25	2.00	2.75	0.236	0.39	0.34	0.91
IgM 36 d	2.00[bc]	3.50[ab]	3.25[ab]	1.25[c]	3.75[a]	0.270	0.05	0.33	0.87

SEM: standard error of the mean.
[abc] In a row, least squares means with a different superscript differ significantly (P<0.05) by Tukey's test.
[†] Probability of the probiotic supplementation effect.
[‡] Probability of the linear and quadratic responses to the increasing levels of probiotics in the diet.

CONCLUSIONS

Under the conditions of the present study, probiotic supplementation at manufacturer's recommended or higher levels in broiler feed was effective in improving BWG and FCR and had few effects on carcass traits. Increased blood glucose and albumin contents indicated a better digestion and absorption of nutrients in the supplemented treatments. Blood triglycerides and total, LDL and VLDL cholesterol were linearly decreased by probiotic supplementation. Probiotics increased *Enterococci* counts and decreased *Escherichia coli* counts in the cecal contents, and had no clear positive effects on immunity related organs or immune response. The linear trends, either positive or negative, observed in many of the parameters studied suggest that the optimal concentration of probiotics in broiler feed deserves further investigations.

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RESUMO

Os efeitos probióticos no desempenho produtivo, características de carcaça, parâmetros sanguíneos, microbiota cecal, e resposta imune de frangos de corte foram estudados. Duzentos animais, machos, de um dia de idade, foram alocados para um dos cinco tratamentos (quatro repetições de 10 aves por tratamento): controle, e mesma dieta controle suplementada com probióticos a 0,005%, 0,01%, 0,015% e 0,02%. A suplementação alimentar com probióticos a partir de 0,01% resultou no ganho de peso corporal (+ 12%) e taxa de conversão de ração (-5%) em comparação com o grupo controle. O

nível de suplementação de probióticos não apresentou qualquer efeito sobre as características de carcaça, apesar da alteração de pesos relativos de coxas e asas, com aumento e redução linear, respectivamente. Os níveis de glicose plasmática e conteúdo de albumina aumentaram linearmente no sangue (167,1-200,5 $mg.dl^{-1}$ e 1,70-3,25 $g.dl^{-1}$, respectivamente) conforme suplementação de probiótico. Níveis de triglicérides e de colesterol foram mais baixos nos tratamentos com probiótico (valores médios de 71,3 e 125,3 $mg.dl^{-1}$ vs. 92,6 e 149,9 $mg.dl^{-1}$ no grupo controle). A suplementação com probióticos resultou na diminuição da contagem de *Escherichia coli* cecais, mas não apresentou efeitos em órgãos relacionados com a imunidade ou ainda com resposta imune. As tendências lineares, positivas ou negativas, observadas em muitos dos parâmetros estudados, sugerem que mais estudos são necessários para estabelecer a concentração ideal de probióticos na alimentação de frangos de corte.

Palavras-chave: digestão, imunidade, aves, probióticos, produção.

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