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RESEARCH ARTICLE

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Growth, body characteristics and blood parameters of ostrich chickens receiving commercial probiotics

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Abstract

This study was undertaken to determine the effect of four commercial probiotics on growth, body characteristics and haematological parameters of ostrich chicks. A total of 25 ostrich chicks (937±68.1 g) were individually allocated and fed the experimental diet for six weeks (n=5 per treatment). Experimental diets consisted of a corn/soybean meal-based diet unsupplemented (T1: Control), and four diets supplemented with probiotics according to the recommendations of the manufacturer (T2: 0.04% Bioplus 2B; T3: 0.09% Primalac; T4: 0.1% Thepax; and T5: 0.03% Protexin). Feed intake (FI), body weight (BW) and seven body characteristics (*e.g.* height) were measured every week. Blood samples and other body characteristics were also taken in the last week. There was an interaction effect between diet and time on all the growth variables and body characteristics ($p<0.05$). Both the BW and the BW gain of the ostrich chicks were, in general, higher for those fed the diet T2 than those fed the control diet (0.42, 1.07, 0.99, 1.09, 2.51, and 1.66 kg BW gain *vs* 0.28, 0.41, 0.83, 0.94, 1.15, and 1.15 kg BW gain at 7, 14, 21, 28, 35, and 42 days respectively), while for those fed the other diets containing probiotics differences were only observed at 42 days ($p<0.05$). Consuming probiotics over an extended period influenced several of the haematological parameters differently compared to those fed the control diet ($p<0.05$). T2 and T3 increased the concentration of total cholesterol (157 and 210 mg/dL respectively), when compared to those fed the control diet (119 mg/dL), while total cholesterol was slightly reduced ($p>0.05$) for those fed the diet containing Thepax (T4, 79 mg/dL). In conclusion, the effects of commercial probiotics on growth performance, body characteristics and haematological parameters varied among probiotics.

Additional key words: blood parameters; growth performance; ostrich chicks; poultry nutrition

Abbreviations used: BW (body weight); FCR (feed conversion ratio); FI (feed intake); HDL (high density lipoproteins); LDL (low density lipoproteins); VLDL (very low density lipoproteins)

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Introduction

The use of probiotics in poultry nutrition has gained great importance during the last 20 years due to their useful effects on production, health and quality of carcasses when compared to antibiotics (Hajjaj *et al*., 2005; Kabir, 2009). Probiotics may stabilise microbiota populations throughout the gastrointestinal tract by producing specific metabolites (*e.g*. bactericins, hydrogen peroxide, short chain fatty acids) that help overcome the adverse effects of pathogens (Gabriel *et al*., 2006; Kabir, 2009; Khan & Naz, 2013). In addition, probiotics have been found to improve feed intake (FI) and digestion (Gabriel *et al*., 2006), reduce blood cholesterol (Mohan *et al*., 1996; Hajjaj *et al*., 2005) and triglycerides (Santoso *et al*., 1995), and improve bone strength (Khan & Naz, 2013).

The effects of probiotics on poultry have been mainly focused on broilers and little information has been reported in the literature regarding its effects on ostriches. Ostriches have a different gastrointestinal tract compared to broilers, which allows them to digest dietary fibre more efficiently (Cilliers *et al*., 1992, 1997; Brand *et al*., 2000; Sales, 2006). The length of the colon in an adult ostrich represents approximately 57% of the intestines compared to only 3% in an adult broiler (Angel, 1996). This may explain the higher apparent metabolizable energy of feed ingredients in adult ostriches compared to cockerels (*e.g*. barley 15 and 11 MJ/kg respectively) (Cilliers *et al*., 1997). This important difference in gastrointestinal tract physiology may also allow the ostrich to have different microbiota populations in terms of diversity and amount (Ahir *et al*., 2012; Oakley *et al*., 2014; Waite & Taylor, 2014). Thus, it may be expected that the effects of probiotics in ostriches may differ to those in broilers.

Hasan-Rezaie *et al*. (2013) reported that inclusion in the basal diet of the Primalac probiotics (included *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium thermophilum,* and *Enterococcus faecium*) improved body weight (BW), feed conversion ratio (FCR) and blood parameters (glucose, cholesterol, uric acid and urea), and those given 0.135% Primalac had the highest body weight, lowest FCR and lowest amounts of blood cholesterol, uric acid and urea (p<0.05) in ostriches. Xu *et al*. (2010) reported that the morbidity and mortality in ostriches fed probiotics (15 and 2.6%) was lower than ostriches fed a control diet (24.1 and 3.8%) respectively, while the daily gain was higher (26.0 *vs* 23.2 g/d, p≤0.01).

Therefore, the aim of this study was to determine the effects of four different commercial probiotics on growth, body characteristics and haematological parameters in ostrich chicks. The commercial probiotics were selected based on their difference in microbiota composition and in previous studies done in both broilers (Kabir *et al*., 2004; Gunal *et al*., 2006; Mutus *et al*., 2006; Nayebpor *et al*., 2007; Paryad & Mahmoudi, 2008; Boostani *et al*., 2013) and ostriches (Hasan-Rezaie *et al*., 2013).

Material and methods

Probiotics and dietary treatments

Probiotics containing different microbiota compositions were obtained from the producer companies: Bioplus 2B (Chr. Hansen A/S, Horsholm, Denmark) (*Bacillus subtilis* and *Bacillus licheniformis*); Primalac (Star Labs, Clarksdale, MO, USA) (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Streptococcus facium*, *Bifidobacterium thermophilum*); Thepax (Doxal Co, Italy) (*Saccharomyces cerevisiae*); and Protexin (Probiotics Int. UK, Ltd) (*Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Bifidobacterium bifidum*, *Streptococcus thermophilus*, *Enterococcus faecium*, *Aspergillus oryzae* and *Candida pintolopesii*).

A basal diet was formulated based on the nutritional recommendations reported in previous studies of ostrich nutrition (Angel, 1996; Cilliers *et al*., 1998). The diet (Table 1), used as the basal diet throughout the experimental period, was either unsupplemented (*i.e.* control diet) or supplemented with one of the four probiotics described above. The probiotic was added to the basal diet according to the manufacturer's recommendations: 0.04% Bioplus B2, 0.09% Primalac, 0.1% Thepax, and 0.03% Protexin.

Animals and housing

Approval for the animal trials was obtained from the Animal Ethics Committee, Rasht Branch, Islamic Azad University, Rasht, Iran. A total of 25 two-week-old ostrich chicks, from the blue and black Neck African breed, and with an initial live weight of 937 ± 68.1 g, were used. The chicks were housed individually, in cages of 2.0×1.7 m, with an open area of 1×1 m. Each cage had a single compartment dry feeder and a drinking bowl.

Experimental design

Each treatment group was made up of five chicks, two males and three females. Experimental diets were randomly allocated to 3 repetitions together of each treatment goup, so that the five diets were represented in each group, with gender being equalised across the groups, in a randomized complete block design. All the ostrich chickens were fed *ad libitum* during 42 experimental days. Body weight (BW), feed intake (FI) and body characteristics [total body height (from head to floor); neck length; circumferences at the shoulder joint (thoracic), abdominal (at the lap), bottom of the neck, hip and tail] were recorded weekly. On day 42, blood samples (10 mL) were collected from the neck vein of three male ostrich chicks from each treatment. In addition on day 42, other body characteristics were also measured (top and middle neck circumference, tibiotarsus, tarsometatarsus, tail and head circumference, and leg, wing, neck and beak length), to build a three-dimensional (3-D) ostrich figure representing the mean of each treatment using the Design Modeler ANSYS™ 14.5 software (Canonsburg, PA, USA).

Blood sample collection and analysis

Blood samples using a syringe containing heparin were taken without anesthesia. Blood plasma was isolated by centrifugation at 3000 rpm for 20 min at 4ºC and aliquots were stored at –20ºC for analysis. Aliquots were analysed for glucose (Barham & Trinder, 1972), alkaline phosphatase (Bessey *et al*., 1946), uric acid, blood urea nitrogen, creatinine, total cholesterol, triglycerides, high density lipoproteins (HDL), low density lipoproteins (LDL) and very low density lipoproteins

Table 1. Ingredients and calculated chemical composition of the experimental diets.

	Experimental diets	
	Control	**Probiotic**
Ingredients (g/kg/dry matter)		
Corn	433.8	433.8
Soybean meal	333.0	333.0
Alfalfa meal	75.2	75.2
Barley	100.0	100.0
Dicalcium phosphate	31.3	31.3
Limestone	13.0	13.0
Vitamins and mineral premix[1]	10.0	10.0
DL-Methionine 98%	0.7	0.7
Salt	3.0	3.0
Probiotic	–	0.3-1.0
Calculated chemical composition (g/kg/dry matter)		
Crude protein	210.0	
Lipids	24.0	
Acid linolenic	12.8	
Crude fiber	46.7	
Calcium	15.0	
Total phosphorus	9.2	
Available phosphorus	1.5	
Methionine	3.9	
Sulphur amino acids	7.0	
Threonine	8.0	
Tryptophan	3.2	
Metabolizable energy (kcal/kg)	2665	

[1]Supplied per kg of diet: 12000 IU vitamin A, 10 mg vitamin E, 2200 IU vitamin D, 35 mg niacin, 12 mg D-pantothenicacid, 3.63 mg riboflavin, 3.5 mg pyridoxine, 2.4 mg thiamine, 1.4 mg folic acid, 0.15 mg biotin, 0.03 mg vitamin B, 60 mg manganese, 40 mg zinc, 1280 mg iron, 8 mg copper, 0.3 mg iodine, and 0.2 mg selenium.

(VLDL) cholesterol, aspartate amino transferase (EC 2.6.1.1), alanine amino transferase (EC 2.6.1.2), calcium, phosphorus, iron, total protein, albumin, and globulin (Schmid & Forstner, 1986; Thomas, 1998). All the reagents and kits used to analyse the blood sample were provided by Teif Azmoon Pars, Co. (Tehran, Iran).

Statistical analysis

The statistical analyses were performed using the Mixed Model procedure of SAS (SAS/STAT v. 9.3, SAS Inst. Inc., Cary, NC, USA). To examine the effect of diet, time and the interaction between diet and time on BW, FI, BW gain, feed conversion ratio (FCR) and body characteristics, a repeated measure analysis using a randomised complete block design was performed, using each ostrich chicken as an experimental unit and the gender as a block. The most appropriate covariance structure for each parameter was selected based on the smallest Akaike's and Bayesian's information criteria value when the covariance structures were compared (Littell *et al*., 1998). In addition, to examine the effect of the commercial probiotics on the haematological parameters and the body characteristics at day 42, a randomised complete block design analysis was performed, with gender as a block.

The model diagnostics (*e.g.* homogeneity of variance) of each parameter were tested combining the Proc Univariate and the ODS Graphics options of SAS. When the model assumptions were not fulfilled for an individual parameter, a transformation of its raw data was conducted to achieve those assumptions. In addition, when only the assumption of homogeneity variances was not fulfilled, an analysis with separated variances was conducted. When the F-value of the analysis of variance was significant for a specific response variable ($p<0.05$), the means of the diets containing the probiotics were individually compared with the control diet using the adjusted Dunnet's tests.

Results

Growth performance

The statistical analysis of the overall study showed a significant effect of the diet on FI, BW gain and FCR

(p<0.05) (Fig. 1 and Table 2). FI and BW gain were higher for the ostrich chicks fed T2 and T5 than for those fed the control diet (p<0.01). However, FCR was better for those fed the diets containing Primalac, Protexin and Thepax when compared to the control diet (p<0.05).

There was a highly significant effect of the interaction between diet and time on BW, FI, BW gain and FCR (p<0.001) (Fig. 1 and Table 2). When compared to the chicks fed the control diet, those fed the diet containing Bioplus 2B had a higher FI across all the time points, while those fed the diet containing Protexin had higher intake at day 7, 28, 35 and 42 (p<0.05). For the chicks fed with the other probiotic diets, FI was higher at 42 days only (p<0.05). Both, BW and BW gain were higher for those fed the diet containing Bioplus 2B when compared to those fed the control diet, while for those fed the other diets containing probiotics differences were observed at 42 days only (p<0.05). The FCR of the diets containing probiotics was better than the control diet at 14 (Bioplus 2B), 28 (Primalac), 35 (Bioplus 2B and Primalac) and 42 (Protexin) days (p<0.05).

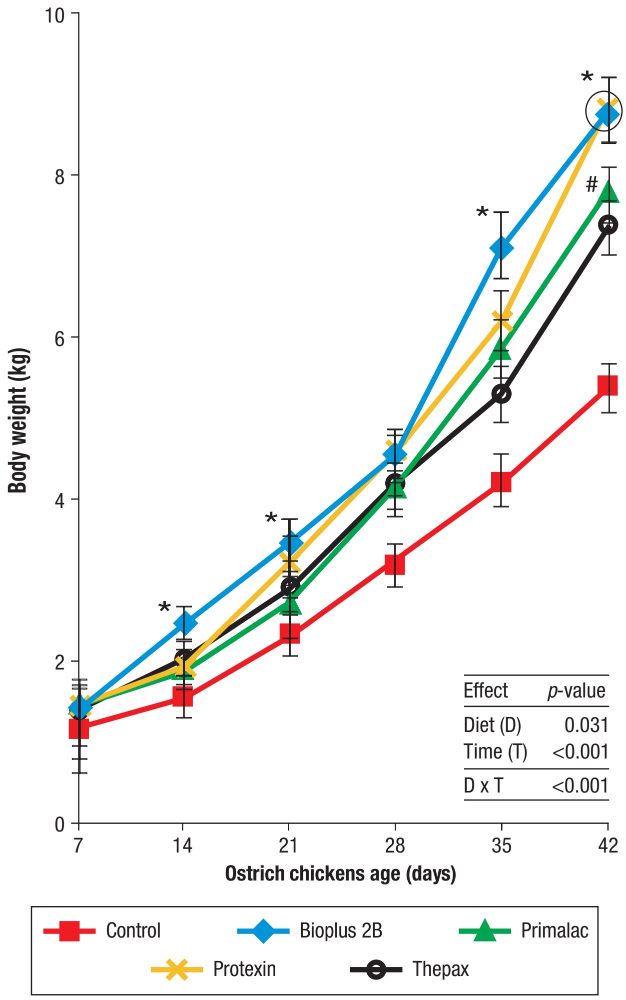

Figure 1. Body weight changes over time in ostrich chickens fed diets containing different commercial probiotics and a control diet. Values are least square mean ± standard error, n=5. Values with the symbol *,# for a given time point, differ significantly (p<0.05) or non-significantly from the control diet, respectively.

Body characteristics

All the body characteristics measured in the ostrich chicks throughout the study were influenced by the interaction between diet and time (p<0.05) (Table 3). In general, at 35 and 42 days, chicks fed the diets containing Bioplus 2B, Primalac and Protexin were higher with a longer hip circumference than those fed the control diet (p<0.05). In addition, those fed Bioplus 2B also had greater thoracic (at 35 and 42 days) and abdominal (at 35 days) circumferences and neck length (at 35 days) (p<0.05).

Several of the body characteristics (top neck, bottom neck, hip, tibiotarsus, tarsometatarsus and head circumferences, neck, wing and beak lengths) of the chicks at day 42 were influenced by the diet (p<0.05), as shown in the 3-D figure build to represent the 'average' ostrich chicken for each diet (Fig. 2 and Table 5). In general, these body characteristics were greater for the chicks fed the probiotic diets than those fed the control diet (p<0.05). However, other body characteristic variables (thoracic, abdominal, middle neck circumferences, tail and neck lengths and height) were not influenced by the diet (p>0.05).

The estimated volume of the body trunk, obtained from the built 3-D figure, was greater for the chicks fed the diets containing probiotics (11548-13751 cm^3) than those fed the control diet (9636 cm^3). Similarly, the estimated total body area of chicks fed the diets containing probiotics was greater (4310-4841 cm^2) than those fed the control diet (3784 cm^2).

Haematological parameters

The haematological profile of the samples collected at day 42 was influenced by the probiotic supplementation (creatinine, total and LDL cholesterol, and HDL/LDL ratio and albumin) (p<0.05) (Table 4). The creatinine content and the HDL/LDL ratio were lower for the those fed the diets containing Bioplus 2B and Primalac when compared to control (p<0.05). In contrast, their total and LDL cholesterol contents were higher (p<0.05). The albumin content of the chicks fed the control diet was lower than for those fed the diets containing Thepax (p<0.05).

Table 2. Growth performance and body characteristic variables in ostrich chickens fed diets containing different commercial probiotics from 7 to 56 days of age.

Time (days)	Dietary treatment	Growth performance[1]		
		FI (kg)	BW gain (kg)	FCR (kg/kg)[2]
7	Control	0.78c	0.28b	2.79a
	Bioplus 2B	1.08a	0.42ab	2.56a
	Primalac	1.00ab	0.39ab	2.61a
	Protexin	1.06ab	0.45ab	2.36ab
	Thepax	0.90bc	0.56a	1.64b
14	Control	0.99b	0.41b	2.61a
	Bioplus 2B	1.34a	1.07a	1.25b
	Primalac	1.20ab	0.45b	2.13a
	Protexin	1.26a	0.46b	2.77a
	Thepax	1.20ab	0.65b	1.87a
21	Control	1.49bc	0.83b	1.55a
	Bioplus 2B	1.90a	0.99ab	1.92a
	Primalac	1.29c	0.66b	1.98a
	Protexin	1.57b	1.35a	1.16b
	Thepax	1.40bc	0.86b	1.67a
28	Control	1.52b	0.94b	1.69a
	Bioplus 2B	2.06a	1.09ab	1.93a
	Primalac	1.62b	1.52a	1.05c
	Protexin	1.90a	1.22ab	1.56ab
	Thepax	1.67b	1.34ab	1.26bc
35	Control	1.79c	1.15c	1.50a
	Bioplus 2B	2.40a	2.51a	0.95c
	Primalac	1.81c	1.79b	1.02bc
	Protexin	2.06b	1.71bc	1.21ab
	Thepax	1.88c	1.21bc	1.51a
42	Control	2.01d	1.15c	1.76a
	Bioplus 2B	2.98a	1.66bc	1.54ab
	Primalac	2.43c	1.93b	1.26b
	Protexin	2.62b	2.63a	1.10c
	Thepax	2.37c	2.01b	1.18bc
SEM[3]		0.088	0.150	0.278
p-value				
Dietary treatment (D)		<0.001	0.002	0.129
Time (T)		<0.001	<0.001	<0.001
D × T		<0.001	<0.001	<0.001
Overall[4]	Control	8.3c	4.8b	1.78a
	Bioplus 2B	11.3a	7.8a	1.46ab
	Primalac	9.3bc	6.7a	1.39b
	Protexin	10.5ab	7.8a	1.34b
	Thepax	9.3bc	6.6a	1.41b
SEM[3]		0.41	0.59	0.087
p-value		0.001	0.009	0.049

Labeled least square means within each column for each time point or the overall analyses, differ significantly to the control. Values are least square mean of five replicates. [1]FI: feed intake; BW gain: body weight gain; FCR: feed conversion ratio. All data was recorded and presented weekly. [2]A reciprocal transformation was required of the raw data to achieve the model diagnostic assumptions. The values presented in this table were obtained after back transformation. [3]SEM: standard error of the mean. [4]The FCR values, for the overall statistical analysis were obtained after dividing the value of cumulative FI (from day 1 to day 42) / cumulative BW gain (from day 1 to day 42).

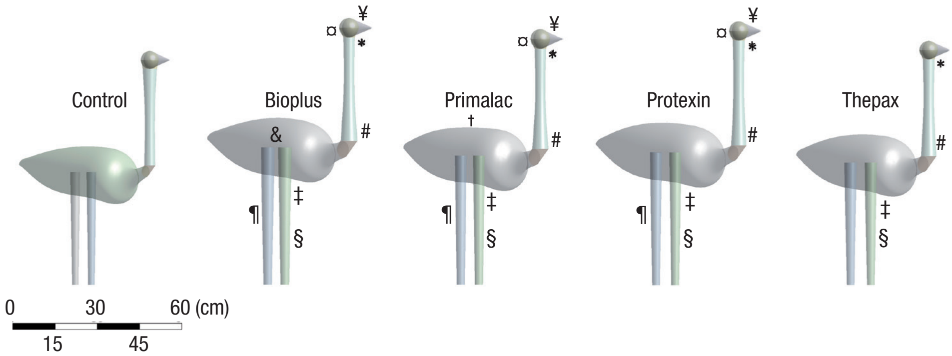

Figure 2. A 3D-figure of the ostrich chickens fed diets containing different commercial probiotics and a control diet at 42 days. Values to create the 3D-figures are least square mean, n=5. Body characteristics with symbols differ significantly (p<0.05 to p<0.01) from the control diet for: ¤ head circumference; * top neck circumference; #bottom neck circumference; † hip circumference; ‡tibiotarsus circumference; §tarsometatarsus circumference; ¶ leg length; & wing length; ¥ beak length.

Discussion

Growth performance and body characteristics

In contrast with a previous study conducted on ostrich chicks over 37 days with the Lactosym probiotic (Dube *et al*., 2009), this study shows a beneficial effect of supplementing the diet with commercial probiotics on several growth performance parameters, over an extended period of 42 days as suggested by Hasan-Rezaie *et al*. (2013). The chicks were in general able to increase their voluntary FI which was reflected in greater BW gain when compared to the control diet. Interestingly, those fed the probiotic diets increased, in different ways, the size of several body components of commercial interest (*e.g*. meat, skin, feathers). Bioplus 2B was the only probiotic able to increase the length of the wing and Bioplus 2B and Protexin were able to increase the length of the legs.

The higher trunk volume may indicate higher meat deposition and the higher total body area may indicate more skin from chicks fed the diets containing probiotics compared to the chicks not fed probiotics. The latter may suggest that the probiotic composition (*i.e.* the microbiota profile) was able to modulate specific body characteristics. Previous studies in broiler chickens showed that adding into diets some of the commercial probiotics studied here (*e.g*. Bioplus 2B and Protexin), improved carcass yield and several body characteristics (*e.g*. thickness of the medial and lateral wall of the tibia, weight of the legs) (Kabir *et al*., 2004; Mutus *et al*., 2006; Ashayerizadeh *et al*., 2011). However, few studies with ostrich chicks have shown that commercial probiotics to the diet improved carcass yield and body characteristics (Greenhill, 2007; Juste-Poinapen, 2007; Ebrahimzadeh *et al*., 2009; Greenhill, 2010). Further studies considering the effect of probiotics on the quality of the carcass, feathers and skin on ostriches are warranted.

Improving the FCR in the ostrich chicks should result in a higher profit margin for the producer. Nutrition represents up to 80% of total production costs (Delgado *et al*., 1999). In this regard, the diets supplemented with the probiotics Primalac, Protexin and Thepax appear to be a potential option to improve the profit margin for producers. However, it is necessary to mention that the production costs and the economic benefits of supplementing the diet with probiotics were not considered in this study. Therefore, an economic evaluation of supplementing the diet with probiotics is needed for the producers.

Previous studies have shown that the inclusion of commercial probiotics (*e.g*. Thepax and Protexin) in diets for broiler chickens increased several growth performance variables (*e.g*. BW, FCR) (Mohan *et al*., 1996; Yeo & Kim, 1997; Kabir *et al*., 2004; Gunal *et al*., 2006; Nayebpor *et al*., 2007; Paryad & Mahmoudi, 2008). In this study, the higher growth performance observed in the ostrich chicks consuming the diets containing probiotics may be due to a change in their gastrointestinal tract microbiota populations, as reported for broiler chickens (Schrezenmier & Vrese, 2001; Gunal *et al*., 2006; Alloui *et al*., 2013). This change in the microbiota population may be beneficial in several ways, including reducing pathogenic bacteria, stimulating the immune system and improving bone strength (Gunal *et al*., 2006; Boostani *et al*., 2013; Khan & Naz, 2013). A further benefit could also be the effect of the microbiota present in the probiotics on fermenting nutrients, mainly fibre, compared to a probiotic-free diet (Gabriel *et al*., 2006). Improved fermentation may increase the production and absorption of short-chain fatty acids throughout the gastroin-

Table 3. Growth performance and body characteristic variables in ostrich chickens fed diets containing different commercial probiotics from 7 to 56 days of age.

Time (days)	Dietary treatment	Body characteristic[1]					
		Height (cm)	NC[2] (cm)	TC (cm)	AC[3] (cm)	HC (cm)	NL (cm)
7	Control	41.8b	8.5	28.2b	34.6	20.2	15.6
	Bioplus 2B	46.8a	8.9	30.6ab	35.6	20.8	16.0
	Primalac	45.4a	9.6	32.0a	34.0	21.2	16.0
	Protexin	43.4ab	9.3	29.4ab	33.2	21.4	16.3
	Thepax	46.0a	9.0	28.8b	33.1	21.2	16.4
14	Control	51.4	10.8	32.8	38.5ab	21.7b	18.4ab
	Bioplus 2B	54.4	10.9	35.4	40.8a	26.4a	19.8a
	Primalac	53.4	10.5	35.2	41.3a	24.3ab	19.7a
	Protexin	53.4	10.5	33.6	36.8b	23.2ab	19.2ab
	Thepax	52.0	10.5	32.6	37.5ab	25.5a	16.6b
21	Control	56.6b	11.5	38.0	44.7	23.2bc	21.4ab
	Bioplus 2B	62.8a	12.5	40.3	44.1	27.3a	23.5a
	Primalac	62.8a	13.4	37.6	43.9	21.4c	20.4b
	Protexin	61.2ab	12.2	40.8	48.2	28.4a	23.4a
	Thepax	61.2ab	11.8	39.4	44.9	25.8b	23.2a
28	Control	67.0b	12.9	43.6	49.0b	28.0	25.4bc
	Bioplus 2B	75.3a	13.2	47.6	55.0a	31.3	28.8a
	Primalac	64.4b	13.9	45.4	53.2ab	31.2	25.9bc
	Protexin	73.6a	14.4	45.2	50.8ab	28.2	27.8ab
	Thepax	69.2ab	13.6	42.8	50.9ab	30.6	24.8c
35	Control	74.2b	14.3	46.4b	52.1b	30.0c	29.6b
	Bioplus 2B	86.6a	16.6	53.6a	65.3a	35.4ab	34.0a
	Primalac	79.6ab	15.3	49.6ab	57.5b	33.8ab	28.4b
	Protexin	85.0a	16.0	50.2ab	56.0b	36.8a	30.0b
	Thepax	85.2a	15.5	50.0ab	56.0b	32.2bc	29.9b
42	Control	83.8b	15.2	51.0	58.3b	31.8b	33.8b
	Bioplus 2B	97.3a	18.3	59.9	65.5a	37.1a	36.3a
	Primalac	92.4ab	17.8	56.8	63.8ab	38.7a	35.0ab
	Protexin	95.0a	16.6	54.8	65.4a	39.2a	36.6ab
	Thepax	91.2ab	17.0	54.0	63.4ab	33.6b	33.8b
SEM[4]		2.18	0.21	1.64	0.46	1.16	0.93
p-value							
Dietary treatment (D)		0.035	0.213	0.169	0.204	0.010	0.063
Time (T)		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
D × T		<0.001	0.058	0.043	<0.001	<0.001	<0.001
Overall[5]	Control	42.0b	6.8	22.8b	24.0	11.6b	18.2
	Bioplus 2B	51.0a	9.8	29.7a	30.5	16.8a	20.5
	Primalac	47.0b	8.2	24.8ab	30.0	17.5a	19.0
	Protexin	51.6a	7.4	25.5ab	32.2	17.8a	20.3
	Thepax	45.2b	8.0	25.2ab	30.4	14.4ab	17.4
SEM[4]		2.49	3.87	2.16	2.95	1.47	1.45
p-value		0.111	0.101	0.377	0.344	0.032	0.514

Labeled least square means within each column for each time point or the overall analyses, differ significantly to the control. Values are least square mean of five replicates. [1]NC, TC, AC and HC: neck, thoracic, abdominal and hip circumference, respectively; NL: neck length. [2]A reciprocal transformation was required of the raw data to achieve the model diagnostic assumptions. The values presented in this table were obtained after back transformation. [3]A natural logarithm transformation of the raw data was required to achieve the model diagnostic assumptions. The values were obtained after back transformation. [4]SEM: standard error of the mean. [5]The phenotypic characteristics values, for the overall statistical analysis, were obtained after subtracting the values of the day 0 of the study to those obtained at day 42 for each ostrich chicken.

Table 4. Haematological parameters in ostrich chickens fed diets containing different commercial probiotics from 14 to 56 days of age.

	Dietary treatment					SEM[1]	*p*-value
	Control	Bioplus2B	Primalac	Protexin	Thepax		
Glucose (mg/dL)	195	174	197	202	220	12.5	0.219
Creatinine (mg/dL)	0.26ab	0.21c	0.21c	0.24bc	0.27a	0.010	0.002
Uric acid (mg/dL)	6.6	9.9	10.5	8.1	4.7	1.95	0.103
Total cholesterol (mg/dL)	119c	157b	210a	155b	79d	10.7	<0.001
Triglycerides (mg/dL)	144	96	116	128	127	32.2	0.388
HDL cholesterol (mg/dL)	51ab	46ab	32b	71a	33b	6.9	0.050
LDL cholesterol (mg/dL)	39cd	91b	155a	58c	21d	6.7	<0.001
HDL/LDL	1.36a	0.53bc	0.20c	1.23abc	1.69a	0.218	0.024
VLDL cholesterol (mg/dL)	28.7	19.3	23.3	25.7	25.3	6.44	0.875
AST (U/L)[2]	499	556	497	522	553	32.3	0.567
ALT (U/L)[2]	13.0	9.0	8.3	12.7	11.7	1.21	0.066
Alkaline phosphatase (U/L)	1704	1481	1691	1391	1213	213.7	0.478
Calcium (mg/dL)	10.0	9.5	9.3	9.8	10.2	0.47	0.715
Phosphorus (mg/dL)	6.3	6.8	8.2	6.3	5.5	0.77	0.244
Serum iron (µg/dL)	64	111	80	67	90	25.4	0.694
Total protein (g/dL)	2.8	3.1	2.9	3.0	3.0	0.16	0.636
Albumin (g/dL)	1.4b	1.7b	1.5b	1.6b	2.0a	0.09	0.005
Globulin (g/dL)	1.4	1.5	1.4	1.4	1.3	0.13	0.865
Globulin /Albumin	0.94	0.87	0.91	0.87	0.62	6.44	0.875

Labeled least square means within each row differ significantly to the control. Values are least square mean of three replicates. [1]SEM: standard error of the mean. [2]AST and ALT: aspartate and alanine amino transferase, respectively.

Table 5. Body characteristic variables in ostrich chickens fed diets containing different commercial probiotics at 42 days of age.

	Dietary treatment					SEM[1]	*p*-value
	Control	Bioplus	Primalac	Protexin	Thepax		
Height (cm)	83.8	97.0	92.4	95.0	91.2	3.39	0.107
Thoracic circumference (cm)	51.0	54.0	56.8	54.8	54.0	2.99	0.729
Abdominal circumference (cm)	58.6	66.0	64.0	65.4	63.6	2.81	0.388
Bottom neck circumference (cm)	15.3a	12.4b	12.5b	12.8b	12.7b	0.42	<0.001
Middle neck circumference (cm)	11.0	11.8	11.1	11.6	11.6	0.39	0.580
Neck length (cm)	33.8	36.3	35.0	36.6	33.8	1.25	0.376
Hip circumference (cm)	31.8b	37.0a	38.8a	39.2a	35.6ab	1.62	0.025
Tibio circumference (cm)	10.8b	15.1a	13.2a	13.2a	13.6a	0.66	0.004
Leg length (cm)	39.4b	48.0a	45.0a	46.2a	42.6ab	1.76	0.026
Wing length (cm)	25.2b	33.5a	28.8ab	30.2b	28.6ab	1.69	0.047
Tarsom circumference (cm)	7.0b	9.3a	8.7a	8.6a	8.3a	0.35	0.003
Tail circumference (cm)	11.7	14.0	12.3	12.2	12.0	0.66	0.202
Beak length (cm)	6.6b	7.6a	7.6a	7.3a	6.8b	0.17	0.001
Head circumference (cm)	19.6c	21.4a	21.6a	21.0ab	20.2bc	0.39	0.007

Labeled least square means within each row, differ significantly to the control. Values are least square mean of five replicates. [1]SEM: standard error of the mean.

testinal tract (Wong *et al*., 2006). Short chain fatty acids are a source of energy to the host. They have been associated with intestinal tissue proliferation, enhanced absorption of minerals and water and prevention of diseases (Williams *et al*., 2001).

Haematological parameters

In contrast to previous studies of probiotics conducted in broiler chickens (Mohan *et al*., 1996; Paryad & Mahmoudi, 2008), the total and LDL cholesterol concentrations was higher for the ostrich chicks fed the diets containing Bioplus 2B and Primalac probiotics when compared to those fed the control diet. However, the ostrich chicks fed the diet containing the Thepax probiotic reduced the concentration of total cholesterol and increased the concentration of albumin as reported in broiler chickens fed diets containing the same probiotic (*S. cerevisiae*) (Onifide, 1997; Paryad & Mahmoudi, 2008). Fat deposition is commonly accepted to be correlated with total cholesterol, LDL and VLDL blood concentration, which mainly depends on the triglycerides removed from the plasma (Hermier, 1997; Musa *et al*., 2006). In this regard, the results from this study suggest that the ostrich chickens fed the diet containing Bioplus 2B and Primalac may have had higher adipose tissue deposition than those fed the control diet.

A lower concentration of creatinine was observed in the ostrich chickens fed the diets containing the Bioplus 2B and Primalac probiotics when compared to those fed the control diet. A reduction in creatinine concentration in blood is related to a lower muscle metabolism (Brosnan & Brosnan, 2010). Therefore, it is possible that at 42 days the muscle deposition may have been influenced, in an unknown way, for the ostrich chicks fed the diet containing the Bioplus 2B and Primalac probiotics.

While there are studies of biochemical parameters in ostrich chicks (Spinu *et al*., 1999; Fallah *et al*., 2014), there are very few reports of the effects of probiotics on blood parameters in this species. Other haematological factors (*e.g*. triglycerides) were not affected when the diet of the chicks was supplemented with the commercial probiotics, contrary to the results of previous studies in broiler chickens (Isshiki, 1979; Kos & Witner, 1982; Santoso *et al*., 1995; Zobac & Kumperchov, 2000; Paryad & Mahmoudi, 2008). The contrasting effects of probiotics on haematological parameters between this study and those on broiler chickens could be explained by the probiotic composition (*i.e.* the microbiota profile), the composition of the diets used in the studies, and anatomical differences (*e.g*. gastrointestinal tract) between both species.

Further studies investigating the relationship between microbiota populations and haematological parameters after probiotics supplementation on ostrich diets are required.

In conclusion, the probiotics improved several of the growth performance variables (*e.g.* BW gain, FCR) and influenced several of the body characteristics (*e.g.* neck length, thoracic circumference) and haematological parameters (*e.g.* creatinine, total cholesterol) in ostrich chickens when compared to those fed a probiotic-free diet.

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