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Effect of a probiotic blend in broiler chicken diets and its effect on growth performance, carcass traits, and haematological profile

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Abstract

The study aimed to assess the effect of different dietary levels of a probiotic blend (Probio Enzyme®) during the first 14 days of age (DOA) or up to 42 DOA, on growth performance, carcass and digestive tract traits, and haematological profiles of broiler chicks. A total of 540, one-day-old broiler chicks were randomized assigned to nine treatments: four dietary probiotic blend levels (250, 500, 750, and 1,000 g/ton) within two feeding periods (0–14 and 0–42 d, respectively), whereas the ninth treatment was a control diet without any dietary probiotic supplementation. The feed intake (FI) was found to be higher in broilers fed 1,000 g/ton of probiotic blend when fed during the first 14 d; however, BW gain and feed efficiency were not influenced by the treatments. Carcasses (deplumed and full) from broilers fed 250 g/ton of probiotic blend (0–14 DOA) were heavier than the other groups; the same was observed for leg portions. Broiler duodenum, jejunum and ileum weights, and ileum percentage were greater when fed diets without any supplementation. The haematological profile of broilers was not affected by the dietary treatments.

Keywords: broiler, diet, feed additive, growth, probiotics
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Introduction

Increases in the incidence of human infections from antibiotic-resistant bacteria have been hypothesized to be directly related to the overuse of antibiotics required for human medical prophylaxis and to therapeutics in food animal production (Hume, 2011; Chand *et al.*, 2022). Considering the severe restriction or total ban on using antibiotics as growth promoters in poultry production, probiotics and enzymes have been suggested as an alternative (Cimrin *et al.*, 2020; Khan *et al.*, 2021). Khaksefidi and Ghoorchi (2006) reported some improved immunocompetence in broiler chicks against Newcastle disease virus when *Bacillus subtillis* was used as a probiotic additive in diet. Ashyerizadeh *et al.* (2009) concluded that a probiotic blend containing *Lactobacillus acidophilus, L. casei, Enterococcus faecium, and Bifidobacterium bifidium* inoculations could be used as a substitute for flavomycin growth promoters. In another study, probiotics displayed a growth-promoting effect comparable to the antibiotic, avilamycin (Mountzouris *et al.*, 2007). However, some researchers have reported that probiotics are not capable of preventing pathogenic bacteria in intestinal microbial flora (Lin *et al.*, 2009). Timmerman *et al.* (2006) point out that, according to the examination of 13 published studies, the high productivity rates of broiler chicks reduce the effect of probiotics. Exogenous enzymes may be added to broiler chick diets containing these by-products as an aid for

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fibre digestion or to solubilize phytic phosphorus (phytase), thereby reducing their negative effects on broiler chick production parameters (Choct, 2006). Some of these indigestible fibre compounds, especially the soluble fraction, are capable of improving the proliferation of undesirable microorganisms in the intestinal wall and lumen. Supplementation with enzymes markedly decrease the viscosity and increase the dry matter of digesta in the intestinal lumen (Malayoğlu *et al.*, 2010). These implications underline the idea that the addition of enzymes can help to reduce the multiplication of pathogenic microorganisms on diets without any antibiotics (Bhogoju & Nahashon, 2022).

Probiotic supplements for the development and stabilization of intestinal flora are used particularly before stressful changes such as new housing and environments, while enzymes are used to help with the breakdown of a wide variety of feed components that would normally be left unused and would maybe act as a substrate for the growth of bacterial pathogens (e.g., *E. coli* and *Salmonella* spp.). The combination of both additives could bring a significant number of advantages to animal production and management, such as a more economic feed efficiency, a better availability of key nutrients to animals, less manure with a lower level of phosphorus and nitrogen, as well as cleaner animals.

Therefore, the aim of this study was to determine the effect of different dietary levels of a probiotic blend as a natural growth promoter, supplemented during different rearing periods, on growth performance, carcass traits, and haematological profiles of broiler chickens.

Materials and Methods

Experimental protocols were approved by the Animal Care Committee of the Islamic Azad University (process #17-33-5-9013; 93-12-7) and were performed in accordance with recommendations of the Iranian Council for Control of Animal Experimentation.

A total of 540 one-day-old Ross 308 (Aviagen, Newbridge, Scotland, UK) male broiler chickens were purchased from a commercial hatchery. Birds were placed in cages with dimensions of 1.0×1.0×0.6 m, providing a floor area of 0.1 m^2 per bird, in a thermostatically-controlled, curtain-sidewall poultry barn. The cage floor was covered with paper roll litter. The feeding trial lasted up to 42 days of age. Each cage of 10 chicks (initial BW of 41.37 ± 2.1 g) was assigned to a specific dietary treatment. The ambient temperature inside the poultry barn was maintained with supplementary heat generated by thermostatically-controlled gasoline rocket heaters, and humidity was added to the barn atmosphere via a water spray to maintain a relative humidity of 55–65%. The ambient temperature was controlled at 33 °C at the time of placement and was decreased periodically to reach 23 °C when the chicks were three weeks old. This temperature was maintained until the end of the trial. Constant light was provided on day 1, but on day 2, the light was 21 h per day until the end of the study. A two-phase feeding program was used on this study and consisted of a starter diet from 1–21 d and a grower diet from 22–42 d (Table 1).

Diets met or exceeded Ross 308 catalogue recommendations according to the producer instructions. The treatments were randomized into nine dietary groups as follows: four dietary probiotic blend levels (250, 500, 750, and 1,000 g/ton) within two feeding periods (0–14 and 0–42 d, respectively), whereas the ninth treatment was a control diet without any dietary supplementation. The probiotic blend (Probio Enzyme®, Xvet, Germany) was a commercial probiotic supplement containing enzymes and included: *B. licheniformis, B. subtilis, Enterococcus faecium, L. acidophilus,* β-glucanase 3.2.1.4, β-glucanase 3.2.1.6, β-xylanase 3.2.1.8, α-amylase 3.2.1.1.9 (under the EU regulation No 234/2011), protease, and cellulase.

To determine the broilers' growth performance, the feed intake (FI) and weight gain (WG) were recorded at the end of the two periods. Feed efficiency (FE = WG/ FI), energy intake (EI = kcal consumed/day), energy efficiency (EE = kcal/g of WG), protein intake (PI = g of protein consumed/day), and protein efficiency (PE = g of protein consumed/g of WG) were assessed. Mortality rate was recorded to allow the correction of performance data. The birds and feed were weighed at 1, 21, and 42 d in order to calculate the WG, FI, FCR, viability, and production efficiency index (PEI) of 42-d-old birds, according to the following equations:

$$\text{Viability} = 100 - \text{MO} \quad (1)$$

$$\text{PEI} = [(\text{ABW} \times \text{viability})/\text{MA} \times \text{FCR}] \times 100, \quad (2)$$

where: MO = mortality, ABW = average body weight at slaughter, MA = market age, and FCR = feed conversion ratio (FCR = 1/FE).

Table 1 Ingredients and nutrient composition of basal diets fed to broilers

Ingredient, %	Starter period (1–21 d)	Grower period (22–42 d)
Corn	54.32	58.69
Soybean meal, 44% CP	39.43	31.87
Soybean oil	2.16	5.83
Dicalcium phosphate	2.05	1.68
Mineral oysters	0.90	0.79
Sodium chloride	0.37	0.37
Vitamin premix*	0.25	0.25
Mineral premix**	0.25	0.25
DL-Methionine	0.20	0.22
Lysine-Hydrochloride	0.07	0.05
Nutrient composition		
Metabolizable energy, kcal/kg	2,900	3,200
Crude protein, %	22.16	19.02
Calcium, %	1.00	0.85
Available phosphorus, %	0.50	0.42
Lysine, %	1.15	0.96
Methionine, %	0.50	0.48
Methionine + Cysteine, %	0.83	0.78
Threonine, %	0.79	0.71

*Supplied per kg of diet: 12,000 IU Vitamin A, 10 mg Vitamin E, 2200 IU Vitamin D, 35 mg niacin, 12 mg D-pantothenic acid, 3.63 mg riboflavin, 3.5 mg pyridoxine, 2.4 mg thiamine, 1.4 mg folic acid, 0.15 mg biotin, and 0.03 mg Vitamin B

**Supplied per kg of diet: 60 mg manganese, 40 mg zinc, 1280 mg iron, 8 mg copper, 0.3 mg iodine, and 0.2 mg selenium

At the age of 42 d, before the blood collection, the feed was removed from all the birds for a period of four hours in an attempt to facilitate the stabilization of plasma constituents; all blood sampling was done in the morning to further reduce the variability of the plasma constituents. Then, a 5 ml sample of venous blood was collected from the ulnaris vein of the wing, sampled from each replicate (Hasan *et al.*, 2022). The whole blood sample was transferred from the syringe into a tube coated with 10 mg of the anticoagulant, EDTA; blood samples were centrifuged at 3000 *rpm* × 20 min. Plasma was collected and stored at -20 °C until analyses following standard protocols. Serum TG (triglycerides), CHOL (total cholesterol), VLDL (very-low density lipoproteins), LDL (low-density lipoproteins), HDL (high-density lipoproteins), plasma glucose, uric acid, total protein, albumin, and globulin were analysed using commercial enzymatic kits (Wako Pure Chemicals Industries, Ltd., Richmond, VA).

After blood collections, three birds from each replicate were selected and weighed; the averages from these birds for each parameter were calculated and used as one experimental unit to assess carcass traits. Birds were fully defeathered via the dry method. Feet were separated from the carcass at the tibio-tarsal joint. Neck, wing tips, digestive tract, and liver were removed, and the carcass was weighed (cold carcass weight, after chilling). Economically relevant cuts of carcass and offal were separated. Breast muscle, including the skin and sternum, were dissected free from the carcass. Legs (thighs and drumsticks) were dissected by the ex-articulation at the hip joint and by dissecting tissue from the iliac bone. All abdominal fat, including that around the rectum, gizzard and proventriculus, was collected. Collected cuts including breast, wings, thighs, and drumsticks (legs), heart, neck, gizzard, digestive tract, and abdominal fat were weighed. The total weight of dissected parts was related to the totally eviscerated carcass. Relative portion percentages were calculated according to the following equation:

Portion percentage = [(weight of component(s)/eviscerated carcass weight)×100]. (3)

Data were subjected to analysis of variance (ANOVA) using a two-way procedure based on the following model:

$$Y_{ijk} = \mu + A_i + e_{ijkl} \quad (4)$$

Where: μ = general average, A_i = treatment effect, and e_{ijkl} = incidental residual effect of observation. After statistical difference confirmation, the General Linear Model (PROC GLM) was applied, and the differences among means (P <0.05) were assessed using Duncan's multiple range

test (SAS, 2012). Relative percentage was used as a descriptive statistic to determine the ratio between carcass components and eviscerated carcass total weight.

Results

The performance traits of broilers fed diets including different levels of probiotic blend during the first 14 days of age or over the entire rearing period (1–42 DOA) are reported in Table 2. Feeding the probiotic at 1000 g/ton during the starter period led to higher FI (P = 0.027), energy intake (P = 0.026), and protein intake (P = 0.033) compared to the other treatments. Furthermore, broilers on the control diet resulted in higher final BW (P = 0.009) than on the experimental diets. Feeding birds the control diet resulted in the best deplumed and full carcass weights (P = 0.004), as well as leg cut weight (P = 0.042) (Table 3). The intestinal measurements of broilers under the different dietary treatments are provided in Table 4. Most of the examined traits differed markedly among groups; the weight of the duodenum, jejunum, and ileum resulted in heavier in birds on the control diet (P <0.05). Moreover, broilers fed the control diet had a higher jejunum and ileum yield (P <0.0001) and ileum width (P <0.0001). The colon tract was not influenced by treatments. The haematological profile of broilers fed diets containing different levels of probiotic blend over different supplementation periods was not influenced by treatments.

Discussion

Broiler chicks fed during the probiotic blend over the initial 14 d presented higher total feed and nutrient intakes. However, these results did not produce an improvement in final weight at 42 DOA. High feed and nutrient intakes without any performance gain are uneconomical and undesirable by the poultry industry.

Timmerman *et al.* (2007) gathered information based on 13 published studies and suggested that with the higher productivity rates of the broiler chicks, the effect of probiotics becomes smaller. Araújo *et al.* (2014) reported that the inclusion of an enzyme blend did not affect the feed intake of broiler chicks. Additionally, Abdelrahman & Saleh (2007) did not find any influence of the inclusion of glucanase on diets. Sarica *et al.* (2005) observed no significant differences in BWG, FI, and FCR of the broilers that were fed with enzyme blend treatment. Other authors gathered different information about probiotic and enzyme nutritional and immunological modulatory effects in broiler chickens.

Mountzouris *et al.* (2007), when studying the efficacy of a multi-species probiotic in broiler chick nutrition and comparing it to avilamycin antibiotics, verified a modulated composition and the activities of the cecal microflora resulted in a significant probiotic effect, but they did not report any performance results. In contradiction to the results presented in the current study, Mountzouris *et al.* (2010) showed that the use of probiotics on the diets was similar to the use of an antibiotic growth promoter (avilamycin) and superior to the use of control diets without any antibiotics. Regarding enzyme supplementation, Cao *et al.* (2010) reported that supplementing xylanase and phytase increased the weight gain of broiler chicks from 1–21 d. These authors inferred that this higher performance was explained by the improvement of the apparent metabolisability of energy and nitrogen. However, none of these studies worked with the association of enzymes and probiotics in a unique Probio Enzyme® dietary additive.

The efficiency of probiotics and enzymes depends on several factors such as the period of use (as shown in this study's results), the bird's age, the environment, the bacterial challenger strains, the nutrient contents in feed, the solubility of dietary fibre, and other factors (Choct, 2006; Timmerman *et al.*, 2006). Due to these facts, the results among the experiments can be very different in relation to performance results. Other undesirable results could be witnessed with the use of Probio Enzyme® additives in broiler chicks' feeds. No statistical difference was observed in carcass traits, resulting in equal or inferior parameters to those on the control diet without any additives. Yang *et al.* (2009), in an extensive review, focused on gathering information about alternatives to in-feed antibiotics that are capable of dietary modulation of the digestive tract microflora in broiler chickens. In most sources of the review, probiotics did not appear to be effective as a substitute for these antibiotic growth promoters without impacting broiler performance or carcass parameters. Ahmad (2006), when also aiming to review the impact of probiotics on broiler chick performance, gathered some contradictory observations among the trials. One of the reasons for the incongruity in the data identified by the author is the probiotic dosage. Another factor is the viability of these microorganisms in the digestive tract wall and lumen and their capacity for colonization and adhesion.

Table 2 Performance traits of broilers fed diets containing different levels of probiotic blend at different supplementation periods

Item		Feed intake (g/day)	BW gain (g/day)	Feed efficiency (g/g)	Energy intake (kcal/day)	Energy efficiency (kcal/g)	Protein intake (g/day)	Protein efficiency (g/g)
14 d	250*	102.02^{ab}	51.41	1.962	302.9^{ab}	5.80	19.4^{bc}	0.381
	500	99.52^{abc}	49.19	2.034	295.5^{bc}	6.02	19.0^{abc}	0.396
	750	99.44^{bc}	47.05	2.062	295.2^{bc}	6.10	18.9^{abc}	0.401
	1000	102.84^{a}	47.90	2.061	305.4^{a}	6.10	19.6^{a}	0.400
1–42 d	250	99.02^{bc}	47.55	1.993	294.0^{bc}	5.90	18.8^{bc}	0.387
	500	98.03^{c}	46.69	2.063	291.0^{c}	6.10	18.7^{c}	0.401
	750	101.85^{ab}	49.14	1.997	302.4^{ab}	5.91	19.4^{bc}	0.388
	1000	98.02^{c}	48.53	1.957	291.0^{c}	5.79	18.7^{c}	0.380
Control		99.65^{abc}	49.69	1.999	295.7^{bc}	5.91	19.1^{abc}	0.390
P-value		0.027	0.149	0.467	0.026	0.471	0.033	0.459
RSD		0.010	0.023	0.021	0.010	0.021	0.010	0.020

Item		BW at 42 d (g)	Production index
14 d	250	2,393^{ab}	256.9
	500	2,067^{c}	235.3
	750	2,153^{b}	233.6
	1000	2,050^{c}	238.1
1–42 d	250	2,191^{b}	243.5
	500	2,028^{c}	228.5
	750	2,081^{c}	250.4
	1000	2,242^{abc}	249.9
Control		2,486^{a}	255.6
P-value		0.009	-
RSD		0.037	-

* Probio Enzyme® g/ton. $^{a-c}$ Values within a row with different superscripts differ significantly at $P < 0.05$

Different strains of probiotic bacteria may exert different effects based on specific capabilities and enzymatic activities, even within one species (Ahmad, 2006). The proposed mechanisms of pathogen inhibition by the intestinal microbiota include nutrient competition, production of toxic conditions and compounds (volatile fatty acids, low pH, and bacteriocins), and the contest of binding sites on the intestinal epithelium (Yang *et al.*, 2009). The probiotic blend mechanism inoculated five different strains of probiotic bacteria (*B. licheniformis, B. subtilis, E. faecium,* and *L. acidophilus),* which may be due to the exclusion of competitive mechanisms as one or more of these bacteria strains have antagonistic functions on the development of others, thereby resulting in a low effectiveness of probiotic functions. Lin *et al.* (2009) clarified this phenomenon: they reported that the multiple probiotic supplements had no significant effect on preventing bacterial infections. The researchers might have attributed it to the antagonism among the different strains of probiotics in the multi-strain supplement.

Table 3 Carcass cut weights and yield of broilers fed diets containing different levels of a probiotic blend for different supplementation periods

Item		Deplumed carcass (g)	Full carcass (g)	Empty carcass (g)	Carcass yield (%)	Breast (g)	Breast (%)	Legs (g)	Legs (%)	Wings (g)
14 d	250*	2,103^{ab}	1,941^{ab}	1,499	77.2	708	33.7	615^{a}	29.3	85.5
	500	1,784^{c}	1,634^{c}	1,255	76.8	564	31.6	537^{ab}	30.2	74.8
	750	1,891^{bc}	1,740^{bc}	2,469	84.4	610	32.3	595^{a}	31.4	80.3
	1000	1,767^{c}	1,630^{c}	1,235	75.7	546	30.8	534^{ab}	30.2	73.5
1–42 d	250	1,879^{bc}	1,738^{bc}	1,299	74.7	606	32.2	537^{ab}	28.5	76.6
	500	1,770^{c}	1,627^{c}	1,282	78.7	624	35.1	501^{b}	28.3	74.2
	750	1,804^{c}	1,659^{c}	1,256	75.7	574	31.8	532^{ab}	29.6	76.6
	1000	1,949^{bc}	1,800^{bc}	1,343	74.4	595	30.5	598^{a}	30.6	72.7
Control		2,211^{a}	2,053^{a}	1,459	71.1	666	30.2	601^{ab}	27.9	93.1
P-value		0.004	0.004	0.400	0.416	0.157	0.124	0.042	0.051	0.142
RSD		0.038	0.039	0.073	0.048	0.062	0.036	0.047	0.025	0.063

Item		Wings (%)	Abdominal fat (g)	Abdominal fat (%)	Gizzard (g)	Gizzard (%)	Heart (g)	Heart (%)	Neck (g)	Neck (%)
14 d	250	4.07	41.69	1.97	50.87	2.42	8.41	0.401	52.25	2.48
	500	4.20	29.84	1.69	46.34	2.61	10.32	0.576	46.28	2.68
	750	4.25	30.84	1.63	51.45	2.72	9.49	0.501	49.44	2.61
	1000	4.15	41.59	2.37	52.32	2.97	9.16	0.521	44.22	2.51
1–42 d	250	4.07	36.14	1.92	57.99	3.11	9.10	0.487	43.88	2.34
	500	4.19	28.51	1.62	45.98	2.61	8.38	0.475	45.21	2.56
	750	4.24	36.23	2.01	53.31	2.96	9.99	0.554	45.18	2.50
	1000	3.72	56.00	2.91	50.32	2.59	9.38	0.483	50.49	2.59
Control		4.20	45.34	2.05	64.34	2.91	8.39	0.379	48.88	2.21
P-value		0.535	0.067	0.219	0.115	0.570	0.534	0.058	0.165	0.080
RSD		0.042	0.148	0.163	0.077	0.09	0.080	0.084	0.048	0.030

* Probio Enzyme® g/ton. $^{a-c}$ Values within a row with different superscripts differ significantly at $P < 0.05$

Regarding the enzyme component of the probiotic blend that was used, other authors did not demonstrate effects when promoting the increased broiler carcass traits. Araújo *et al.* (2014) did not verify an influence on carcass parameters of broiler chicks when they were fed with dietary enzymes during the entire husbandry period. The use of a combination of endo-1,4-β xylanase (equivalent to 1,400 xylanase units g^{-1}) and endo-1,3-β glucanase (200 glucanase units g^{-1}) in feed did not affect the breast but it decreased the thigh and drumstick weights in broiler chickens. Choct (2006) gathered information in a review paper concerning the enzymes commonly used in poultry industry. Results were shown in studies around the world reporting the effectiveness of these feed additives, or a lack thereof. The most significant reason for the non-effectiveness of enzymes is because they are substrate-dependent. In diets where the substrate is low, the effectiveness of these enzymes is impaired.

Some desirable results were observed in the carcass trait in the present study. Similar to the results that were reported in the current study, Mutuş *et al.* (2006) did not observe an impact on the live performance of the birds throughout the 6-week feeding trial when they were fed with diets inoculated with *B. licheniformis* and *B. subtilis* (containing 2.3×10^8 CFU/g of spores for each strain). However, they reported that the thickness of the medial and lateral wall of the tibia, tibio-tarsal index, ash, and P content were substantially improved by the probiotic. These facts can explain the high thigh and drumstick percentages without affecting the performance parameters that were witnessed.

Table 4 Intestinal measurements of broilers fed diets containing different inclusions of a probiotic blend at different supplementation periods

Item		Duodenum					Jejunum				
		weight (g)	yield (%)	length (mm)	width (mm)	diameter (mm)	weight (g)	yield (%)	length (mm)	width (mm)	diameter (mm)
14 d	250*	16.28^{b}	0.781	322	7.99	0.63	62.3^{bc}	2.95^{cd}	1,249^{bcd}	9.49^{ab}	0.640
	500	14.99^{b}	0.866	323	12.08	0.62	66.8^{bc}	3.79^{bc}	1,295^{bc}	8.59^{abc}	0.610
	750	13.08^{b}	0.691	276	34.74	0.66	42.8 ^{c}	2.27^{d}	1,054^{d}	7.63^{abc}	0.660
	1000	17.30^{b}	0.984	332	8.12	0.667	52.9^{c}	3.00^{cd}	1,210^{bcd}	6.96^{c}	0.663
1–42 d	250	17.50^{b}	0.935	312	8.40	0.66	84.6^{b}	4.489^{b}	1,396^{b}	9.44 ^{ab}	0.627
	500	16.14^{b}	0.908	325	7.29	0.65	49.1^{c}	2.77^{cd}	1,118^{cd}	7.81^{bc}	0.683
	750	16.13^{b}	0.893	310	7.72	0.64	62.4^{bc}	3.45^{bc}	1,252^{bcd}	8.22^{c}	0.650
	1000	17.00^{b}	0.875	332	8.41	0.63	57.1^{c}	2.93^{cd}	1,286^{bc}	8.190^{abc}	0.640
Control		22.63^{a}	1.018	376	9.25	0.620	120.6^{a}	5.45^{a}	1,701^{a}	9.603^{a}	0.617
P-value		0.032	0.132	0.055	0.515	0.729	<0.0001	<0.0001	<0.0001	0.025	0.416
RSD		0.088	0.081	0.050	0.787	0.036	0.103	0.098	0.047	0.062	0.036
Item		Ileum					Colon				
		weight (g)	yield (%)	length (mm)	width (mm)	diameter (mm)	weight (g)	yield (%)	length (mm)	width (mm)	diameter (mm)
14 d	250	10.72^{bc}	0.50^{bc}	247	7.80^{bc}	0.63	2.790	0.133	61	10.31	0.647
	500	8.28 ^{bc}	0.47^{c}	239	6.30^{de}	0.61	3.050	0.174	62	9.44	0.643
	750	6.65^{c}	0.35^{c}	220	6.13^{de}	0.66	1.987	0.105	50	7.99	0.697
	1000	7.83^{bc}	0.44^{c}	245	6.31^{de}	0.65	2.333	0.132	51	8.51	0.663
1–42 d	250	12.57^{b}	0.67^{b}	258	8.58^{ab}	0.61	2.353	0.125	58	8.01	0.673
	500	7.71^{c}	0.43^{c}	217	6.43^{de}	0.64	2.397	0.135	46	10.57	0.683
	750	9.42^{bc}	0.52^{bc}	233	5.86^{e}	0.62	5.637	0.302	51	9.18	0,678
	1000	9.14^{bc}	0.47^{c}	229	6.98^{cd}	0.66	2.663	0.137	62	7.11	0.620
Control		21.02^{a}	0.95^{a}	321	8.91^{a}	0.60	3.560	0.163	65	9.46	0.637
P-value		<0.0001	<0.0001	0.095	<0.0001	0.603	0.662	0.647	0.306	0.087	0.470
RSD		0.138	0.110	0.087	0.046	0.040	0.443	0.429	0.103	0.086	0.039

* Probio Enzyme® g/ton. [a-e] Values within a row with different superscripts differ significantly at *P* <0.05

Table 5 Haematological profile of broilers fed diets containing different inclusions of a probiotic blend for different supplementation periods

Item		Glucose (mg/dl)	Uric acid (mg/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	LDL/HDL	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)
14 d	250*	235	5.97	120	50	14	77	29	0.373	3.77	1.30	2.47
	500	240	4.60	131	94	19	84	29	0.332	3.00	1.27	1.73
	750	235	6.27	144	144	29	82	33	0.403	3.47	1.53	1.93
	1000	245	4.00	141	105	21	86	34	0.395	3.5	1.50	2.00
1–42 d	250	231	5.03	145	135	27	87	30	0.379	3.87	1.57	2.30
	500	252	4.40	115	64	13	68	34	0.482	3.33	1.43	1.90
	750	259	6.60	168	112	22	109	37	0.343	3.87	1.77	2.10
	1000	264	6.57	124	139	28	81	28	0.349	2.80	1.10	1.70
Control		220	3.93	104	46	9	72	22	0.314	3.67	1.13	2.53
P-value		0.120	0.223	0.051	0.090	0.112	0.059	0.890	0.910	0.120	0.059	0.067
RSD		0.042	0.168	0.091	0.262	0.247	0.088	0.220	0.217	0.077	0.085	0.095

* Probio Enzyme® g/ton

However, other researchers have gathered contradictory results; for instance, Malayoğlu *et al.* (2010) did not verify the influence of enzyme supplementation on internal organ weights. Engberg *et al.* (2004) verified that the addition of xylanase increased chymotrypsin and lipase activities. Under these facts, the argument that physiology would be "lazy" and reduce the endogenous production of enzymes is fallacious. Another hypothesis is the influence of probiotic organisms on the size of the gastrointestinal tract. Probiotics induce beneficial effects on the host by improving the properties of the indigenous microflora and digestive tract size and weight (Ghadban, 2002). Thus, it is often implied that a more robust digestive tract will make a healthier animal, which, in turn, digests and uses nutrients more efficiently (Willis *et al.*, 2011), but this was not evidenced in this paper. Yan *et al.* (2007) reported that p75 and p40 were the first probiotic bacterial proteins that were demonstrated to promote intestinal epithelial homeostasis through specific signalling pathways, promoting antiapoptotic and proliferation responses. According to Smirnov *et al.* (2005), the dietary probiotic enlarged the goblet cell "cup" area throughout the small intestine, increased the presence of mucin glycoprotein in the jejunum, and the expression of mucin mRNA in the probiotic-fed chicks. Due to the lack of scientific publication of these results, there is still much controversy over the effects of Probio Enzyme® in the gastrointestinal tract of birds, and, consequently, there is an opening for further research on this issue in particular.

Classically, the first investigations in the area reported a high correlation between both total serum protein or albumin levels and the protein content in body composition (lean meat) (Thomas and Combs, 1967). Low serum total protein and albumin levels had a positive correlation with a marked decrease in weight gain and feed intake (Ologhobo, 1992). The results in the current study differ consistently from these authors, with a negative correlation between WG and carcass traits and serum albumin levels in Probio Enzyme®-supplemented birds. On the other hand, some more recent trials report that serum albumin levels are positively correlated with high stress levels and low performance. It was shown that preslaughter treatment (catching, crating, and transportation) during the summer increases blood albumin, which is a reliable indicator of stress in broilers (Yalçin*et al.*, 2004). Akşit *et al.* (2006) also reported that plasma albumin content was increased by high ambient temperature when heat-stressed broiler chickens were crated at 34 °C. Hernández *et al.* (2012), working with low-protein diets, found an increase of 3% in the feed conversion ratio when the plasma albumin levels were reduced. Thus, the positive correlation of serum albumin with high performance is very questionable. High serum albumin values in treatments where the birds showed low performance are therefore understandable.

Conclusions

The results of this research demonstrate that there are very few benefits in using this probiotic blend in the broiler diet. Some growth traits improved, but not sufficiently to justify the use of the product in terms of a cost-benefit analysis, particularly if the aim of using the probiotic blend is as an antibiotic growth promoter substitute. Thus, the search for an effective substitute for antibiotics in poultry diets must consider the factors that influence the efficacy of natural feed additives in order to increase digestibility, to balance the desirable gut microorganisms, and to promote poultry performance.

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Author contributions

All the authors approved the final version of the manuscript. MM, CL, AS, MN, WAGA, LFTA, VL, VT, and DDM: conceptualization, formal analysis, methodology, validation, writing-review & editing, writing-original draft.

Data availability

Data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interest Declaration

The authors declare no conflicts of interest.

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