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Effects of Different Doses of Probiotic Supplementation to Diet on Performance, Organ Weight, and Some Blood Parameters in Broilers

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ABSTRACT
Objective: Probiotics are live microorganisms such as bacteria, fungi, or yeast that support the gastrointestinal flora and promote growth performance when administered in sufficient amounts. The current study was conducted to examine the impact of different doses of probiotic mix (*Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophiles, Lactobacillus acidophilus, Lactococcus lactis, Propionibacterium*) on performance, organ weights, and some blood parameters in broilers. **Materials and Methods:** For this aim, a total of 360 two-day-old Ross-308 mixed-sex broiler chickens were randomly divided into five groups, with six replicates containing 12 chicks per replicate. All of them were fed with five different dietary intakes for 40 days as follows: Control (Con; basal diet), Trial I (TI; 0.05% adding probiotics), TII (0.075% adding probiotics), TIII (0.10% adding probiotics), and TIV (0.125% adding probiotics). After the treatments, performance parameters and organ weights were evaluated. Also, blood specimens were collected for sero biochemical analysis. **Results:** Data showed that the highest body weight (BW), average daily weight gain (ADWG), and feed conversion rate (FCR) were determined in the TII group (p<0.05). There was no difference in the liver, heart, gizzard, spleen, and intestine weights among the groups. The lowest proventriculus weight was observed in the TII group (p<0.05). Probiotic supplementation did not affect the serobiochemical parameters, aspartate aminotransferase (AST), alanine aminotransferase (ALT), triglycerides (TG), glucose (GLU), and total protein (TP) (p>0.05). **Conclusion:** 0.075% mixed probiotics could be added to broiler diets to increase growth performance.
Keywords: Blood Parameters, Broiler, Organ Weight, Performance, Probiotic.

Broylerlerde Diyete Farklı Düzeylerde Probiyotik İlavesinin Performans, Organ Ağırlığı ve Bazı Kan Parametreleri Üzerine Etkileri

ÖZ
Amaç: Probiyotikler, yeterli miktarda kullanıldığında gastrointestinal florayı düzenleyen ve büyüme performansını arttıran bakteri, mantar veya maya gibi canlı mikroorganizmalardır. Bu çalışma, farklı düzeylerde probiyotik karışımının (*Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophiles, Lactobacillus acidophilus, Lactococcus lactis, Propionibacterium*) piliçlerde performans, organ ağırlıkları ve bazı kan parametreleri üzerindeki etkisini incelemek amacıyla yapılmıştır. **Gereç ve Yöntem**: Bu amaçla toplam 360 adet iki günlük Ross-308 karışık cinsiyetli etlik piliç, her tekrarda 12 civciv içeren 6 tekrarlı 5 gruba rastgele ayrıldı. Hepsi 40 gün boyunca 5 farklı diyet ile beslendi: Kontrol (Kon; bazal diyet), Deneme I (TI; %0,05 probiyotik katkısı TII (%0,075 probiyotik katkısı), TIII (%0,10 probiyotik katkısı) ve TIV (%0,125 probiyotik katkısı). Çalışma sonunda performans parametreleri ve organ ağırlıkları değerlendirildi. Ayrıca serobiyokimyasal analiz için kan örnekleri toplandı. **Bulgular:** Çalışmada, en yüksek canlı ağırlık (CA), ortalama günlük ağırlık artışı ve yemden yararlanma oranı (YYO) TII grubunda görüldü (p<0.05). Gruplar arasında karaciğer, kalp, taşlık, dalak ve bağırsak ağırlıkları açısından fark bulunmadı. En düşük proventrikulus ağırlığı ise TII grubunda gözlendi (p<0.05). Probiyotik takviyesi serobiyokimyasal parametreleri, aspartat aminotransferazı (AST), alanin aminotransferazı (ALT), trigliseritleri (TG), glikozu (GLU) ve toplam proteini (TP) etkilemedi (p>0.05). **Sonuç:** Büyüme performansını artırmak için piliç rasyonlarına %0,075 oranında karışık probiyotikler eklenebilir.
Anahtar Kelimeler: Kan Parametreleri, Etlik Piliç, Organ Ağırlığı, Performans, Probiyotik.

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INTRODUCTION

The poultry industry has become an essential pillar of national income in many countries worldwide (Tarabees et al., 2019). The world's population is increasing quickly, and it is difficult for people to obtain healthy, cheap food due to drought, climate change, war, and migration (Akhalf et al., 2010). Poultry meat provides a viable option for many low-income families to compensate for the lack of other types of animal protein (Tarabees et al., 2019; Zengin et al., 2022). The poultry industry has recently become one of the most dynamic and ever-expanding sectors worldwide (Akhalf et al., 2010). It must fill the space between the necessity and availability of high-quality protein for human consumption. For people to consume animal foods such as meat, milk, eggs, and fish in sufficient quantities, it is necessary to increase their productivity and the number of animals. For this purpose, different feed additives must be tried to protect animal health, increase their efficiency, and increase the quality of animal foods (Castanon, 2007). Antibiotics have been used as a unique prevention way to fight infections for many years. Overusing antimicrobials as growth promoters in animal feed increased the number of multidrug-resistant pathogens. As a result of this overuse of antimicrobials, animals turned into potential reservoirs of antibiotic resistance genes (Gao et al., 2017; Tarabees et al., 2019). For this reason, many countries around the world have banned the addition of antibiotics to animal feeds. Therefore, the scientific community has started to seek economical and efficient alternatives with high safety margins to replace in feed antimicrobials (Tarabees et al., 2019). Probiotics are non-pathogenic, live microorganisms that, when provided in sufficient quantity, offer a range of health benefits to the host (Tarabees et al., 2019). Probiotics enhance immunity and a healthy equilibrium of bacteria in the gastrointestinal tract, stimulating gut integrity and maturation, enhancing feed intake and digestion by improving the activity of the digestive enzyme and reducing the efficiency of a bacterial enzyme, neutralizing enterotoxins, and enhancing immune function (Kheiri et al., 2018). Because of their useful effects, many probiotics have been used in the poultry industry, containing strains of *Lactobacillus, Streptococcus, Enterococcus, Bacillus, Bifidobacterium, Aspergillus, Candida*, and *Saccharomyces* (Lutful Kabir, 2003). Probiotic supplements have an important influence such as the secretion of antimicrobial agents, competitive adhesion to the mucosa and epithelium, and reinforcement of the intestinal epithelial barrier. For probiotics to be considered functional, bacteria must be an ingredient of the intestinal microflora, be durable to the acid environment, and smoothly adhere to the intestinal epithelium. *Bifidobacterium, Lactobacillus*, and yeast are among the most commonly used probiotics in poultry breeding. Besides, *Propionibacteria* strains could be used to improve the health and production of cattle, beef, and pigs (Cousin et al., 2012). The contribution of *Propiobacterium* to poultry health status with combined probiotics has not been fully characterized. The utility of probiotics can be strengthened by various methods, containing strategic strain selection, gene manipulation, and a mix of components that act synergistically (Jha et al., 2020). The most adopted application in modern poultry production is a combinational approach.The effects of probiotics on some blood parameters related to their growth efficiency and carcass weight in broilers have yet to be well known. The current research aimed to examine the effect of the addition of commercial probiotics containing *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophiles, Lactobacillus acidophilus, Lactococcus lactis, and Propionibacterium* in different doses to broiler feed on performance parameters, organ weights, and some blood parameters.

MATERIALS AND METHODS

Animal material and experimental design

A total of 360 two day-old-broiler chicks (Ross-308) were provided from a hatchery (Karahallılar, Balikesir). The study was conducted from November to December 2022 at Balikesir University Animal Husbandry Application and Research Center. The chickens were randomly allocated into five groups (72 chicks/group) and reared in pens of identical size (1.5 × 1 m) in a deep litter system with a wood shaving floor. Each group had six replicates (12 chicks/pen). Water and feed were provided ad libitum.

Experimental design

Chicks were divided into five dietary treatments as follows:

- Control (Con): Basal diet without probiotic
- Trial I (TI): 0.05 % probiotics to the basal diet
- Trial II (TII): 0.075 % probiotics to the basal diet
- Trial III (TIII): 0.10 % probiotics to the basal diet
- Trial IV (TIV): 0.125 % probiotics to the basal diet

The chicks were fed a basal diet (21.80 % CP, 2990 kcal ME/kg) from 1d to 10d, grower diets from 11 to 20d, and finisher diets from 21 to 40d. Dry matter (DM), crude ash, ether extract, crude protein, and crude cellulose were analyzed according to the guidelines of AOAC, 2000 (5).

The component and analyzed nutrients in the basal diet are represented in Table 1. The mix probiotic consisted of *Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophiles, Lactobacillus acidophilus, Lactococcus lactis, Propionibacterium* (not less than – 2.107 cfu/g) (Zoovit, Plovdiv, Bulgaria).

The experiment lasted for forty days. On the last day of the study, six broiler chickens were randomly chosen from each group, and one chicken from each replicate. After blood specimens were collected from the jugular vein for biochemical analysis, they were culled by cervical dislocation. Visceral organs (liver, heart, gizzard, proventriculus, spleen, and intestine) were exposed and weighed to determine organ weights.

Table 1. Composition of basal diets (as fed basis, %).

Ingredients (%)	Rations		
	Starter	Grower	Finisher
Corn	60.00	65.00	65.00
Maize DDGS	1.00	1.00	1.00
Soybean meal, 44%	24.30	18.30	17.30
Corn gluten, 62%	7.70	7.70	7.70
Vegetable oil	-	1.00	2.25
Wheat	1.85	1.85	1.60
Barley	1.00	1.00	1.00
Rice bran	0.50	0.50	0.50
Dicalcium phosphate	1.40	1.40	1.40
DL-methionine	0.35	0.35	0.35
L-lysine	0.30	0.30	0.30
Sodium bicarbonate	0.20	0.20	0.20
Calcium carbonate	1.00	1.00	1.00
Salt	0.20	0.20	0.20
Vitamin-mineral *	0.20	0.20	0.20
Total	100.00	100.00	100.00
Nutritional Composition(%)			
Dry matter	89.90	89.60	89.70
Crude protein	21.80	19.50	19.10
Ether extract	2.20	3.16	4.25
Crude ash	5.22	5.20	5.18
Crude cellulose	2.70	2.99	2.98
ME kcal/kg	2990	3117	3202

* Vitamin-Mineral premix supplied per kg; vitamin D3 4,000 IU; vitamin E 70 IU; vitamin A 10,000 IU; Mn 80mg; Fe 30mg; Zn 80 mg; Cu 5 mg; Co 0.5mg; I 1.5mg; Se 0.30 mg. ME:Metabolizable energy.

Biochemical analyses

After taking blood specimens from the jugular vein, they were centrifuged at 3000 g for 10 min, and serum specimens were transferred into clean plastic microtubes and stored at -20°C until analysis. Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein (TP), glucose (GLU), and triglyceride (TG) were measured by using a biochemical autoanalyzer (Mindray, BS300, Shenzhen, China).

Performance parameters

Chicks and feed were weighed on the 2nd days, 20th, and 40th days of the study. Finally, arranged average daily weight gain (ADWG), average daily feed intake (FI), and feed conversion ratio (FCR) were calculated for each period (2 to 20 days, 21 to 40 days, and 2 to 40 days).

Statistical analysis

The experiment was performed according to the randomized plots experiment plan, with five groups and six replications in each group. Normality tests of the variables were done with the Shapiro-Wilk test. Data were subjected to analysis of variance (ANOVA) with the SPSS 20.0 (SPSS Inc., Chicago, IL) program. Duncan's multiple comparison ($P<0.05$) test was used to specify the differences between the means of the experimental groups (11). $Y_{ij} = \mu + T_i + e_{ij}$, Y_{ij} = observed value of trait i in experimental animals; μ = constant for all groups; T_i = i probiotic effect (i = 1: CON; 2: TI; 3: TII; 4: TIII; 5: TIV) and e_{ij} = random error related with the Y_{ij} observation.

Ethical considerations

Before the research, ethical approval was required from the Ethical Committee of Balikesir University (Date: 29.09.2022, Approval no: 2022/7–2).

RESULTS

The effects of probiotic mix supplementation on growth performance parameters, such as body weight (BW), average daily weight gain (ADWG), and feed conversion rate (FCR), are presented in Table 2. The body weight of the TII group at 20 d of age was significantly ($p<0.05$) higher than that of the TI, TIII, and TIV groups. On the 40th day age, the highest body weight was seen in TII ($p<0.05$).

The highest average daily weight gain at 20 d of age was observed in the TII group ($p<0.05$). There were no differences in FI between the groups at 2-20, 21-40, and 2-40 days of age. FCR was significantly lower in the TII group according to TIV ($p<0.05$) at 2-40 d of age. There were no differences in FCR on 2-20 d and 21-40 d. The effects of mixed probiotics on carcass weight are presented in Figure 1. The differences between the groups for liver, heart, gizzard, spleen, and intestine weights were not important. ($p>0.05$). However, proventriculus weights in the TIII and TIV groups were higher ($p<0.05$) in the TII group. The blood serum profiles in terms of composition (AST, ALT, TG, GLU, TP) are presented in Figure 2. The experimental trials had no important effects on blood biochemical parameters ($p>0.05$).

Table 2. The growth performance of the groups.

	Treatments Groups					SEM	p
	Con	TI	TII	TIII	TIV		
BW, g							
02. d	67.79	67.79	67.80	67.96	67.96	0.09	NS
20. d	663.44^{ab}	647.93^{b}	695.32^{a}	641.25^{b}	651.67^{b}	5.62	*
40. d	2320.28^{ab}	2286.42^{b}	2414.54^{a}	2282.06^{b}	2356.57^{ab}	17.56	*

Table 2 (Continued). The growth performance of the groups.

	Treatments Groups						
	Con	TI	TII	TIII	TIV	**SEM**	**P**
ADWG, g							
02-20. d	55.20[ab]	53.24[b]	57.93[a]	52.84[b]	53.71[ab]	0.66	*
21-40. d	87.20	86.80	90.98	87.69	90.83	0.92	NS
02-40. d	71.20[ab]	70.02[b]	74.46[a]	70.26[b]	72.27[ab]	0.61	*
FI, g							
02-20. d	54.50	52.33	56.33	52.00	55.16	0.96	NS
21-40. d	142.21	144.52	142.71	151.37	154.66	2.16	NS
02-40. d	98.36	98.43	99.52	101.69	104.91	1.19	NS
FCR							
02-20. d	0.986	0.983	0.971	0.985	1.026	0.01	NS
21-40. d	1.635	1.671	1.563	1.723	1.710	0.02	NS
02-40. d	1.383[ab]	1.406[ab]	1.336[b]	1.445[ab]	1.453[a]	0.01	*

NS: No significant, *: P<0.05. Con: Basal diet without probiotics, TI: Adding 0.05 % probiotics to the basal diet, TII: Adding 0.075 % probiotics to the basal diet, TIII: Adding 0.10 % probiotics to the basal diet, TIV: Adding 0.125 % probiotics to the basal diet. BW: Body weight, ADWG: Average daily weight gain, FI: Feed intake, FCR: Feed conversion ratio.

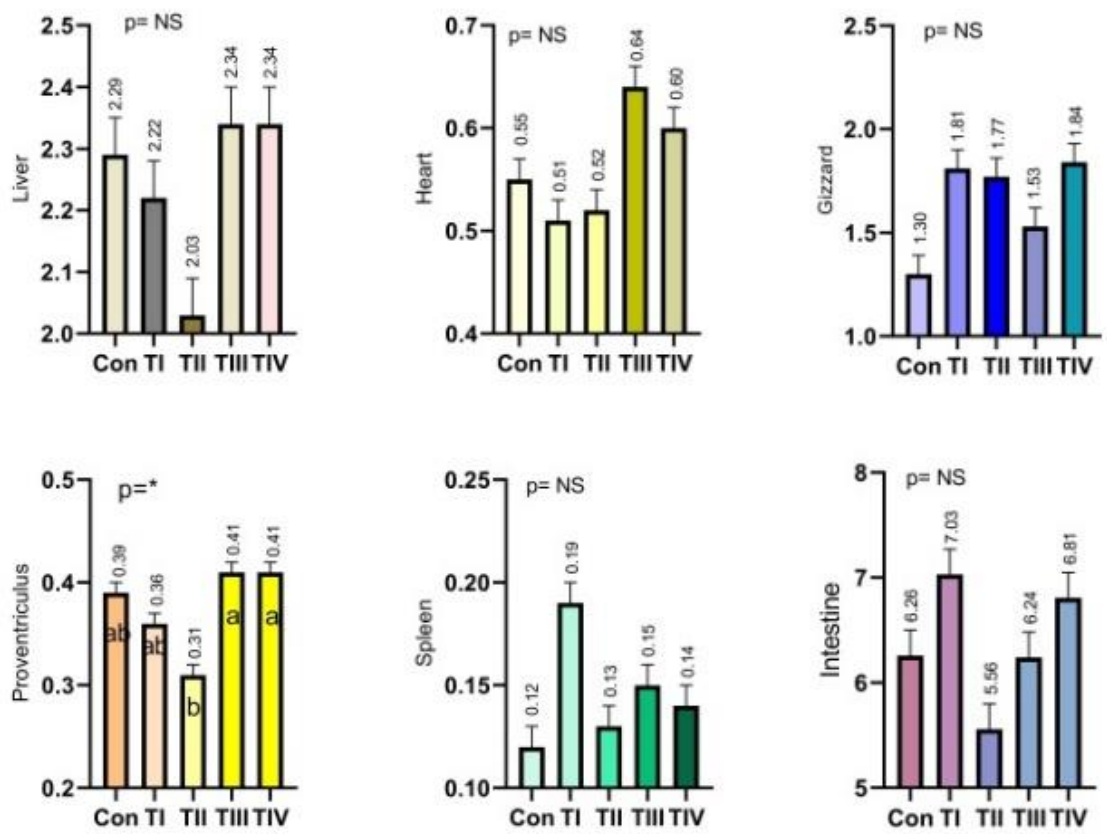

Figure 1. Internal organ weights of the experimental groups (g/100 g body weight).

NS: No significant, *: p<0.05. Con: Basal diet without probiotics, TI: Adding 0.05 % probiotics to the basal diet, TII: Adding 0.075 % probiotics to the basal diet, TIII: Adding 0.10 % probiotics to the basal diet, TIV: Adding 0.125 % probiotics to the basal diet

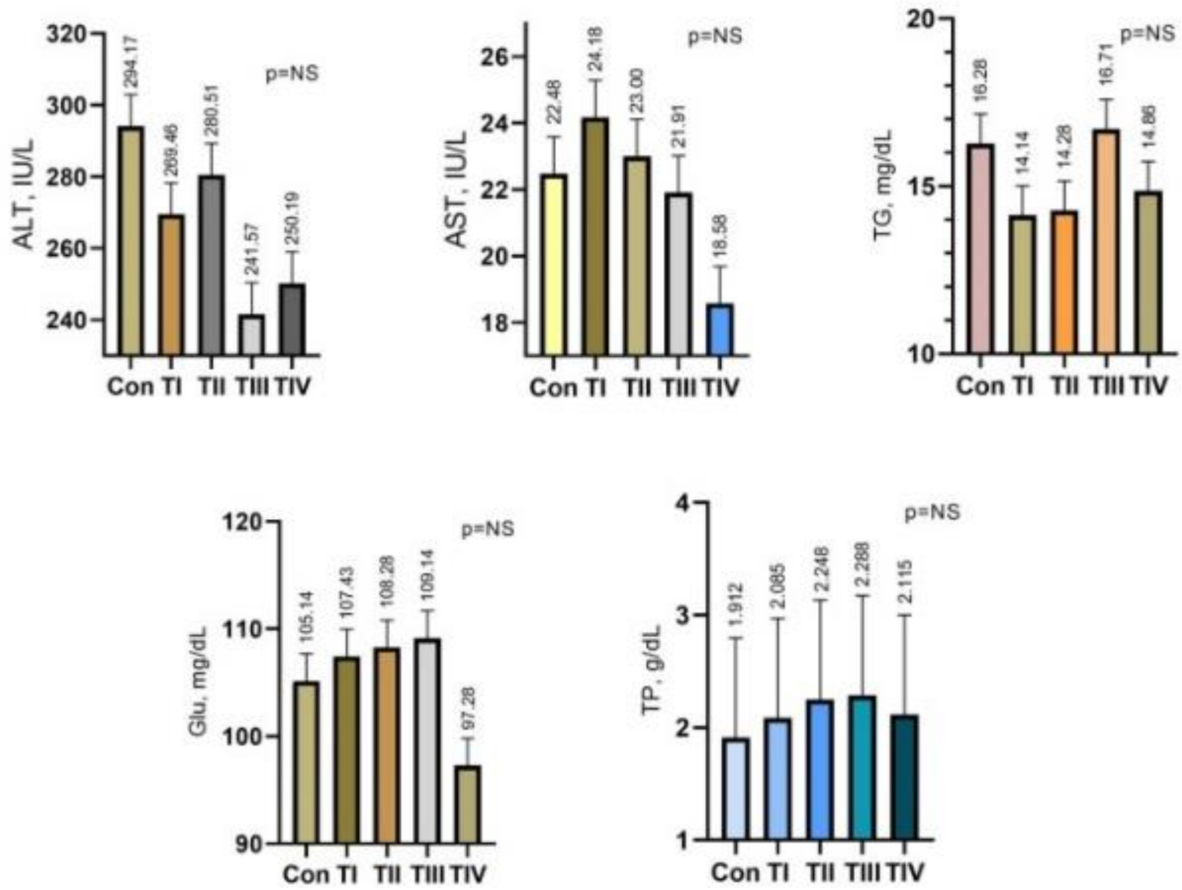

Figure 2. Effects of probiotic on some blood parameters.

NS: No significant, *:p<0.05. Con: Basal diet without probiotics, TI: Adding 0.05 % probiotics to the basal diet, TII: Adding 0.075 % probiotics to the basal diet, TIII: Adding 0.10 % probiotics to the basal diet, TIV: Adding 0.125 % probiotics to the basal diet. ALT: Alanin aminotransferaz; AST: Aspartat aminotransferaz; TG: Triglycerides; GLU: Glucose; TP: Total protein.

DISCUSSION

Recently, researchers have stated that the additive of probiotic species (*Lactobacillus*, *Streptococcus*, *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Aspergillus*, *Candida*, and *Saccharomyces*) in broiler diet has a useful impact on growth performance (GP), intestinal health, immune functions and meat quality (Yazhini et al., 2018). In addition, studies show that improving growth performance and feed efficiency after probiotic addition and probiotics could be an alternative to feed additive antibiotics by protecting against diseases (Ahmat et al., 2021). Advances in GP and FCR of broiler chickens fed probiotics are linked to the total effects of probiotic action containing the maintenance of useful microbial population (Samli et al., 2007). Lactic acid bacteria compete with pathogenic bacteria and inhibit their activity, reducing the breakdown of proteins to nitrogen and reducing dietary protein's effectiveness. Due to this, the use of amino acids and proteins is enhanced (Yazhini et al., 2018). In some studies (Siadati et al., 2017; Yazhini et al., 2018), it was reported that probiotic addition enhanced the plasma protein and increased the GP in quail, but the result of the current research did not concur with these studies. Moreover, Abdel Hafes et al., (2017) showed that the serum total protein concentration of chickens supplemented with probiotics was significantly lower than that of control birds, which concurs with the current study.

The probiotic used from the first day positively affects intestinal microbial balance by ensuring the normalization of the intestinal microflora and protection against pathogenic microbes. Bacteria, part of probiotics, ensure better digestion and absorption of feed (Khabirov et al., 2020). Ahmat et al., (2021) showed that including *Bacillus amyloliquefaciens* LFB112 in broiler diets significantly enhanced the growth performance of broilers. Similarly, Anjum et al., (2005) indicated that the including mixed probiotics in broiler diets positively affects BW and

FCR. Khabirov et al., (2020) recomment the possible advantage of probiotic addition in increasing the growth and quality of broilers.

The current research data indicated that different levels of applying *L. bulgaricus*, *L. acidophilus*, *Streptococus thermophilus*, *L. lactis*, and *Propioni bacterium* did not significantly impact the parameters of GP. However, the GP data indicated that the highest body weight values on the 20th and 40th days were obtained from the TII group, which fed 0.075% supplemented probiotics to the diets. The difference between the groups in terms of FI during the experiment was not important. The lowest FCR was seen in the TII group. Rehman et al., (2020) indicated that no interaction was seen for weight gain at the starter, finisher, and overall phases in their study using probiotics in broilers (p>0.05). In general, studies report that the inclusion of probiotics in broiler diets improves GP and resistance to pathogen microorganisms (Hooge et al., 2004; Samli et al., 2007; Jadhav et al., 2018).

The probiotic levels did not affect the relative weights of intestinal tracts of broilers after 40 d of feeding. Huang et al., (2004) reported that the probiotic addition of *Lactobacillus* spp. did not affect organ weights. In the present study, proventriculus weight was the lowest in the TII group and the highest in the TIII and TIV groups (p<0.05). Molnar et al., (2011) in their study of adding *B. subtilis* to broiler diets, stated that the liver weight of the group that added probiotics to mixed feeds was lower than the control group, and the groups were similar in terms of spleen weights. Reporting similar results to the data of this study, Hidayat et al., (2016) showed that the inclusion of probiotics in diets did not affect the weight of the heart, liver, and spleen.

Due to various metabolic processes in the body that can be controlled by the liver, AST and ALT are values that can be used to determine hepatic cell damage and healing, as well as the effects of toxic substances on birds. AST is an enzyme involved in protein metabolism. It has many functions, including participation in constructing the cell membrane and synthesizing amino acids. A constant level of AST indicates that the cells are not impaired and are functioning normally. Excessive ALT concentration in serum is known to indicate the development of organ dysfunction and disease progression, and increased ALT levels are associated with liver pathology (Khabirov et al., 2020). In one study, Khabirov et al., (2020) examined the effect of a probiotic Normosil (containing a mixture of living cultures of *Lactobacillus* and *Enterococcus* strains at a concentration of 1×106 and 1×107 CFU/mL) on serum AST and ALT levels. The result showed that AST and ALT levels remained within the physiological limits. According to these data, both enzymes in the serum suggested that "Normosil" had low toxicity in broiler chickens during the growing period. In this study, the experimental treatments had no important effects on AST and ALT values (p>0.05), similar to Khabirov et al., (2020).

The cell's important energy source is glucose, which acts as a metabolic substrate. The chicks that were administered probiotic-addition diets indicated a significant increase ($P \leq 0.05$) in their glucose levels (Hussein et al., 2020). Similarly, Khabirov et al., (2020) showed that the addition of probiotic feed additive "Normosil" in the diet of broiler chickens at a concentration of 1×10^6 CFU/mL increased the metabolism of carbohydrates in the body, which was depicted by the increase in the concentration of blood glucose. In the current study, the probiotic mix did not affect the glucose levels (P>0.05). Also, treatments containing probiotics have led to a numerical decrease in blood serum cholesterol. Lactobacilli, which have high hydrolytic activity of bile salts, respond to the conjugation of bile salts, and in general, the microorganisms of the digestive system can inhibit the production of cholesterol (Hernández-Gómez et al., 2021). Different data about blood biochemical parameters are reported in the literature, probably due to the different species involved, different probiotic resources, or different levels of probiotics in diets. The fat ingredient of bovine colostrum is high, therefore its utilization in quail diets has enhanced the LDL and TG serum concentrations, as seen in broiler by Arjomand et al. Differently, other authors noticed contradictory data with a decline of LDL serum levels in quails that assumed colostrum with diet (Gorbannejhad Parapary et al., 2021). These could be linked to the probiotic source as well as to the age of the used animals. By making bile salts unpaired, microorganisms reduce their ability to be absorbed in low intestinal ph. As a result, a large part of bile salts is excreted from the body as feces. This process requires the conversion of cholesterol into bile acids in the liver. It decreases the blood serum cholesterol concentration (Sachdev et al., 2021).

CONCLUSION

Inclusion of a probiotic mix (*Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophiles, Lactobacillus acidophilus, Lactococcus lactis, Propionibacterium*) in a broiler's diet could be a useful way to improve the performance. According to our study data, adding 0.075% of mixed probiotics to broiler diets improves chickens' BW and increases their growth performance by improving feed efficiency.

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Conflict of Interest

The author declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: ED, MAA, GK; **Material, methods and data collection:** MZ, ED; **Data analysis and comments:** MZ, HS, GK, ED; **Writing and corrections:** MZ, HE, AS.

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Ethical Approval

Institution: Balikesir University Animal Experiments Local Ethics Committee

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REFERENCES

Abdel-Hafeez, H.M., Saleh, E.S.E., Tawfeek, S.S., Youssef, I.M.I., & Abdel-Daim, A.S.A. (2017). Effects of probiotic, prebiotic, and synbiotic with and without feed restriction on performance, hematological indices and carcass characteristics of broiler chickens. *Asian Australasian Journal of Animal Science*, *30*(5),672-682. https://doi: 10.5713/ajas.16.0535.

Ahmat, M., Cheng, J., Abbas, Z.,Cheng, Q., Fan, Z., Ahmad, B., Hou, M., Osman, G., Guo, Wang, J., & Zhang, R. (2021). Effects of bacillus amyloliquefaciens lfb112 on growth performance, carcass traits, ımmune, and serum biochemical response in broiler chickens. *Antibiotics*, *10*(11), 1427. https://doi:10.3390/antibiotics10111427.

Alkhalf, A., Alhaj, M., & Al-Homidan, I. (2010). Influence of probiotic supplementation on blood parameters and growth performance in broiler chickens. *Saudi Journal of Biological Sciences, 17,* 219-225. https://doi:10.1016/j.sjbs.2010.04.005.

Anjum, M.I., Khan, A.G., Azim, A., & Afzal, M. (2005). Effect of dietary supplementation of multi-strain probiotic on broiler growth performance. *Pakistan Veterinary Journal*, *25*(1), 25-29.

AOAC, 2000. AOAC Official methods of analysis of aoac international (17th ed.), Maryland, DC, USA.

Arjomand, M., Nobakht, A., & Mehmannavaz, Y. (2022). Effects of different forms of cattle colostrum for broiler chickens. *Iranian Journal of Applied Animal Science*, *12*(2), 371-378. https://doi:10.5958/0974-8180.2017.00027.7

Castanon, J.I.R. (2007). History of the use of antibiotic as growth promoters in European poultry feeds. *Poultry Science*, *86*(11), 2466-2471. https://doi:10.3382/ps.2007-00249.

Cousin, F.J., Foligne, B., Deutsch, S.M., Massart, S., Parayre, S., Le Loir, Y., Boudry, G., & Jan, G. (2012). Assessment of the probioticpotential of a dairy product fermented by Propionibacterium freudenreichii in piglets. *Journal of Agricultural and Food Chemistry*, *60*(32), 7917-7927. https://doi:10.1021/jf302245m.

Duncan, D.B. (1955). Multiple range and multiple F tests. *Biometrics*, *11*(1), 1-42.

Gao, P., Ma, C., Sun, Z., Wang, L., Huang, S., Su, Xu, & Zhang, H. (2017). Feed additive probiotics accelerate yet antibiotics delay intestinal microbiota maturation in broiler chicken. *Microbiome*, *5*(91),1–14. https://doi:10.1186/s40168-017-0315-1.

Gorbannejhad Parapary, M.H., Nobakht, A., & Mehmannavaz, Y. (2021). The effect of colostrum powder supplementation on performance, ıntestinal morphology, blood biochemical parameters, ımmunity and antioxidant status of broilers. *Iranian Journal of Applied Animal Science, 11*(4), 809-816. https://doi:20.1001.1.2251628.2021.11.4.17.5.

Hernández-Gómez, J.G., López-Bonilla, A., Trejo-Tapia, G., Avila-Reyes, S.V., Jimenez-Aparicio, A.R., & Hernandez-Sanchez, H. (2021). In vitro bile salt hydrolase (BSH) Activity screening of different probiotic microorganisms. *Foods, 10,* 674. https://doi:10.1186/s40168-017-0315-1.

Hidayat, M.N., Malaka, R., Agustina, L., & Pakiding, W. (2016). Abdominal fat percentage and carcass quality of broiler given probiotics Bacillus spp. *Scientific Research Journal*, 4 (20), 33–37.

Hooge, D.M., Ishimaru, H., & Sims, M.D. (2004). Influence of dietary bacillus subtilis c-3102 spores on live performance of broiler chickens in four controlled pen trials. *Journal of Applied Poultry Research*, *13*(2), 222–228. https://doi:10.1093/japr/13.2.222.

Huang, M.K., Choi, Y.J., Houde, R., Lee, J.W., Lee, B., & Zhao, X. (2004). Effects of Lactobacilli and an acidophilic fungus on the production performance and immune responses in broiler chickens. *Poultry Science*, *83*(5), 788-795. https://doi:10.1093/ps/83.5.788

Hussein, E.O.S., Ahmed, S.H., Abudabos, A.M., Aljumaah, M.R., Alkhlulaifi, M.M., Nassan, M.A., Suliman, G.M., Naiel, M.A.E., & Swelum, A.A. (2020). Effect of Antibiotic, Phytobiotic and Probiotic Supplementation on Growth, Blood Indices and Intestine Health in Broiler Chicks Challenged with Clostridium perfringens. *Animals*, 10(3), 507. https://doi:10.3390/ani10030507.

Jadhav, K., Sharma, K.S., Katoch, S., Mane, B.G., Chawala, S., & Sharma, VK. (2018). Biological performance, carcass and meat quality characteristics of broiler chickens supplemented with lactobacillus casei as probiotics. *Livestock Research İnternational*, *06* (4), 86-90.

Jha, R., Das, R., Oak, S., & Mishra Paravin. (2020). Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: a systematic review. Animals, *10*(10), 1863. https://doi:10.3390/ani10101863.

Khabirov, A., Khaziakhmetov, F., Kuznetsov, V., Tagirov, H., Rebezov, M., Andreyeva, A., Basharov, A., Yessimbekov, Z., & Muhammad, A. (2020). Effect of Normosil Probiotic Supplementation on the Growth Performance and Blood Parameters of Broiler Chickens. *Indian Journal of Pharmacutical Education and Research*, *54*(4),1046-55. https://doi:10.5530/ijper.54.4.199.

Kheiri, F., Faghan, İ.M., &Landy, N . (2018). Evaluation of thyme and ajwain. as antibiotic growth promoter substitutions on growth performance, carcass characteristics and serum biochemistry in Japanese quails (Coturnix japonica). *Animal Nutrition,4*(1),79–83. https://doi:10.1016/j.aninu.2017.09.002.

Lutful Kabir, S.M. (2003). The dynamics of probiotics in enhancing poultry meat production and quality, Master of Science (m.s.) in veterinary public health and food hygiene.

Molnár, A.K., Podmaniczky, B., Kürti, P., Tenk, I., Glavits, R., Virag, G., & Szabo Z. (2011). Effect of different concentrations of Bacillus subtilis on growth performance, carcase quality, gut microflora and immune response of broiler chickens. *British Poultry Science*, *52*(6), 658-665.https://doi:10.1080/00071668.2011.636029.

Rehman, A., Arif, M., Sajjad, N., Al-Ghadi, M.Q., Alagawany, M., Abd El-Hack, M.E., A. R. Alhimaidi, A.R., Elnesr, S. S., Almutairi, B.O., Amran, R.A., Hussein, E.O.S., & Swelum, A.A. (2020). Dietary effect of probiotics and prebiotics on broiler performance, carcass, and immunity. *Poultry Science,* 99: 6946-6953. https://doi:10.1016/j.psj.2020.09.043.

Sachdev V, Duta-Mare M, Korbelius M., Sachdev, V., Duta-Mare, M., Korbelius, M., Vujic, N., Leopold, C., De Boer, J.F., Rainer, S., Fickert, P., Kolb, D., Kuipers, F., Radovic, B., Gorkiewicz, G., & Dagmar, K. (2021). Impaired bile acid metabolism and gut dysbiosis in mice lacking lysosomal acid lipase. *Cells*, *10*, 2619. https://doi:10.3390/cells10102619

Samli, H.E., Senkoylu, N., Koc, F., Kanter, M., & Agma, A.(2007). Effects of Enterococcus faecium and dried whey on broiler performance, gut histomorphology and intestinal microbiota. *Archives of Animal Nutrition*, *61*(1), 42-49. https://doi:10.1080/17450390601106655.

Siadati, S.A., Ebrahimnezhad, Y., Salehi Jouzani, G., & Shayegh, J. (2017). Evaluation of probiotic potential of some native Lactobacillus strains on the growth performance and serum biochemical parameters of Japanese quails (Coturnix Coturnix japonica) during rearing period. *Brazilian Journal of Poultry Science*, 19(3), 399-408. https://doi:10.1590/1806-9061-2016-0393.

Sur, A., Zengin, M., Bacaksız, O.K., Gökçe, Z., Yılmaz, O., Gürses, M., Esen, V.K., Azman, M.A., & Esen, S. (2023). Performance, blood biochemistry, carcass fattyacids, antioxidant status, and HSP70 geneexpressions in Japanese quails reared under high stocking density: the efects of grape seed powder and meal. *Tropical Animal Health Production,* *55* (1), 53. https://doi:10.1007/s11250-023-03481-y.

Tarabees, R., Gafar, K.M., El-Sayed, M.S., Shehata, A.A., Ahmed, M. (2019). Effects of dietary supplementation of probiotic mix and prebiotic on growth performance, cecal microbiota composition, and protection against *Escherichia coli* O78 in broiler chickens. *Probiotics and Antimicrob Proteins.* 11 (3),981–989. https://doi:10.1007/s12602-018-9459-y.

Yazhini, P., Visha, P., Selvaraj, P., Vasanthakumar, P., & Chandran, V.(2018). Dietary encapsulated probiotic effect on broiler serum biochemical parameters. *Vet World*, *11*(9), 1344-1348. https://doi: 10.14202/vetworld.2018.1344-1348.

Zengin, M., Sur, A., İlhan, Z., Azman, M. A., Tavşanlı, H., Esen, S., Bacaksız, O.K., & Demir, E. (2022). Effects of fermented distillers grains with solubles, partially replaced with soybean meal, on performance, blood parameters, meat quality, intestinal flora, and immune response in broiler. *Research in Veterinary Science*, *150*, 58-64. https://doi:10.1016/j.rvsc.2022.06.027.