Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org

Kafkas Univ Vet Fak Derg
28 (1): 131-138, 2022
DOI: 10.9775/kvfd.2021.26633

RESEARCH ARTICLE

Effect of Probiotic Mixture Supplementation to Drinking Water on the Growth Performance, Carcass Parameters and Serum Biochemical Parameters in Native Turkish Geese

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Article ID: KVFD-2021-26633 Received: 11.10.2021 Accepted: 10.01.2022 Published Online: 10.01.2022

Abstract

This study aims to evaluate the effects of a probiotic mixture (PM) supplement on the growth performance and the serum biochemical parameters of native Turkish geese. A total of 60 one-day-old goslings were randomly divided into three groups, and each group was divided into four subgroups with five animals each. While no supplement was added to the drinking water of the control group, 0.25 mL and 0.50 mL of PM were supplemented in the drinking water of the PM-1 and PM-2 groups, respectively. The trial was completed in 10 weeks. The results revealed that supplementation of 0.50 mL/L PM in drinking water improved the live weight, live weight gain, feed consumption, feed/gain ratio. The supplementation of PM improved dressing and the liver weight, but it did not have an impact on the heart and gizzard weight of geese. It was also observed that 0.50 mL of PM increased the serum biochemical parameters such as glucose, total protein, albumin, calcium, phosphorus levels. At the same time, it decreased the triglycerides, total cholesterol, LDL-Cholesterol, Aspartate Aminotransferase levels. The present study showed that PM added at 0.50 mL/L in drinking water could be used as a supplement by local breeders for the growth performance of geese.

Keywords: *Biochemical parameters, Carcass, Goose, Growth performance, Probiotic*

Yerli Türk Kazlarında İçme Suyuna Probiyotik Karışımı İlavesinin Büyüme Performansı, Karkas Parametreleri ve Serum Biyokimyasal Parametreleri Üzerine Etkisi

Öz

Bu çalışmada, probiyotik karışımı (PM) ilavesinin yerli Türk kazlarının büyüme performansı ve biyokimyasal parametreleri üzerine etkilerinin değerlendirilmesi amaçlanmıştır. Toplam 60 adet 1 günlük kaz civcivi rastgele üç gruba ayrıldı ve her grup her biri beş hayvan olmak üzere dört alt gruba ayrıldı. Kontrol grubu içme suyuna katkı maddesi eklenmezken, PM-1 ve PM-2 gruplarının içme suyuna sırasıyla 0.25 mL ve 0.50 mL PM ilave edildi. Deneme 10 haftada tamamlandı. Sonuçlar içme suyuna 0.50 mL/L PM ilavesinin canlı ağırlık, canlı ağırlık artışı, yem tüketimi, yem/ağırlık artışı oranını iyileştirdiği ortaya konmuştur. PM ilavesi, karkas ve karaciğer ağırlığını iyileşmiş, ancak kazların kalp ve taşlık ağırlığı üzerine bir etkisi olmamıştır. Ayrıca %0.50 PM seviyesinin glikoz, total protein, albümin, kalsiyum ve fosfor gibi serum biyokimyasal parametrelerini artırdığı, trigliserit seviyesini düşürdüğü görülmüştür. Bu çalışma, içme suyuna 0.50 mL/L düzeyinde ilave edilen PM'nin yerel yetiştirici koşullarında beslenen kazların büyüme performansına kullanılabileceğini göstermiştir.

Anahtar sözcükler: *Biyokimyasal parametreler, Karkas, Kaz, Büyüme performansı, Probiyotik*

How to cite this article?

Ölmez M, Şahin T, Özlem Ö, Öğün M, Yörük MA, Dalğa S: Effect of probiotic mixture supplementation to drinking water on the growth performance, carcass parameters and serum biochemical parameters in native Turkish geese. *Kafkas Univ Vet Fak Derg,* 28 (1): 131-138, 2022.
DOI: 10.9775/kvfd.2021.26633

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Introduction

The use of antibiotic supplements has for many years created risks of developing resistant bacteria, leaving residue in animal products, and resulting in adverse effects on human health, which, in turn, has led to concerns among the industry stakeholders [1]. Therefore, the use of antibiotics in animal production is prohibited. There is interest in seeing the effect of how probiotic, prebiotic, and herbal-originated natural alternative supplements have improved the efficiency as well as how they have helped to preserve the health of the animals [2,3].

Probiotics are defined as microorganisms that help to create a beneficial population in the gastrointestinal tract of poultry, exhibit an antagonistic effect on pathogens, and create beneficial conditions for the use of nutrients [4]. These microorganisms are composed of bacteria and yeast [2]. Various studies in the literature report the positive effect of probiotics on yield in animal feeding. However, the most important factors contributing to its effectiveness have been the probiotic strain used and the level of its use [5]. In this context, investigating new strains with good probiotic properties and effective optimization of their concentrations is quite important for the efficiency of the applications of probiotics. Strains such as *Lactobacillus, Bacillus, Bifidobacterium, Streptococcus, Enterococcus, Aspergillus, Candida,* and *Saccharomyces* have been used as probiotics for the nutrition of poultry, and they are still used today [6]. Among these strains, the species of *Lactobacillus* spp. and *Saccharomyces cerevisiae* have been the most commonly used ones [7]. According to numerous studies, *Lactobacillus* spp. and *S. cerevisiae* were found to prevent the development of harmful bacteria in the digestive system of poultry and to preserve the health of the flock [4,8]. Furthermore, *Lactobacillus* was found to be resistant to the high acidity of the stomach, shows resistance against salinity and bile, offers a high adaptation to ambient temperature [9]. Besides, It was determined that by increasing the concentrations of *Lactobacillus* species present in the digestive tract of geese, they showed higher antimicrobial activity against pathogens [10].

Geese are raised under traditional conditions in Turkey, both as an alternative to the consumption of animal protein and as important contributions to family economies. Geese are regularly fed on the pasture once they are 2-3 weeks of age until they have reached the appropriate weight for slaughter. Geese are released to pasture during the daytime and kept in houses at night. Concentrated feed is added as well as pasture in goose ration [11]. In the literature, a limited number of studies have been conducted on the improvement of the growth performance of native Turkish geese [11,12].

Probiotic mixture (PM) has been hypothesized to have beneficial impacts on growth performance. Probiotic supplementation also improves the metabolic response due to regulating serum biochemical profiles of poultry. This research was designed to evaluate the growth performance of native Turkish geese and various serum biochemical parameters by using a PM.

Material and Methods

Ethical Statement

Ethical Approval: This study was approved by the Kafkas University Animal Experiments Local Ethics Committee (KAÜ - HADYEK/2019-117).

Animal, Feed and Experimental Design

The study was carried out at the Research Farm of Kafkas University. A total of 60 male one-day-old native Turkish goslings were used in the study. The animals were subjected to 70 days (14 days of adaptation and 56 days of feeding period) feeding program. After the goslings were fed the uniform basal diet (20% CP and 2900 kcal/kg ME) for an adaptation period through 1-14 days of age, they were grazed *ad libitum* on the pasture for the trial period lasting from day 15 to day 70. On day 15, based on the homogeneous average weight of geese per subgroup, all geese were weighed and assigned to three groups, with four subgroups per group and five birds per subgroup. An enclosed space of about 0.4 to 0.5 m^2 was provided per animal. The PM was added at 0 (control group), 0.25 mL and 0.50 mL to drinking water. The geese were given 450 mL/day of drinking water in individual drinkers and it was confirmed that the geese consumed the water. The PM liquid solution was administered via drinking water from day 15 to day 70 at a dose rate of 0.25 and 0.50 mL per liter of drinking water by way of a dosage pump. The drinking water was non purified well water with a pH of 7.4 and no residual chlorine at 0.5 mg/L. The geese were fed in the pasture between 08.00 and 16.00. After grazing, the geese were supplemented with as much barley meal as they could consume. The feed was provided *ad libitum*. The pasture samples were collected from five different parts of the pasture at the beginning, middle and end of the trial. The grass was mixed, and its dry matter and crude protein contents were analyzed *(Table 1)*. The nutrient content of the feed used in the study was determined following the methods reported in [13]. In the calculation of metabolized energy, the formula recommended by TSE was used [14].

The results of the nutrient analysis of feed and barley meals are presented in *Table 2* and *Table 3*. The PM, which was used in the experiment, was obtained from a commercial manufacturer (EM Premium® - EM Agriton Ltd. Sti. Izmir/Türkiye). PM (1×10^7 cfu/g; pH: 3.00-3.85) contains five bacteria and one yeast strain: *Lactobacillus fermentum, L. plantarum, L. rhamnous, L. casei, L. delbrueckii,* and *S. cerevisiae*.

Table 1. *Nutrient composition of pasture used in the experiment*

Items *	Periods		
	Begining	Middle	Finishing
Dry matter (%)	18.90	28.50	32.30
Crude protein (%)	12.20	13.15	13.65
** Analyzed values*			

Table 2. *Nutrient and chemical values of starter diet*

Ingredients	%
Maize, yellow	55.30
Soybean meal, 44% CP	29.26
Barley meal	2.85
Wheat bran	4.40
Sunflower meal, 36% CP	3.50
Vegetable oil	2.20
Limestone	0.85
Dicalcium phosphate	0.90
DL-Methionine	0.10
L-Lysine HCl	0.03
Salt	0.35
Vit-Min Mix[1]	0.25
Chemical Analysis	
Dry matter (%)	89.50
Crude protein (%)	20.00
Ca (%)	0.65
Available P (%)	0.31
Metabolic Energy (kcal/kg)	2900

[1] In each kg of diet: 7.000.000 I.U. Vit. A; 60.000 I.U. Vit. D_3; 20.000 I.U. Vit E; 2.000 mg Vit. K_3; 1.500 mg, Vit. B_6; 7 mg Vit. B_{12}; 5.000 mg, Nicotinamide; 40 mg Folic acid; 0.40 mg, Zinc sulphate; 0.50 mg, Iron sulphate; 0.04 mg, Mn sulphate; 0.15 mg, Copper sulphate

Table 3. *Chemical analysis of barley meal*

Items	Values
Dry matter (%)	87.10
Crude protein (%)	11.50
Ash (%)	2.50
Crude fiber (%)	5.00
Neutral detergent fiber (%)	20.90
Acid detergent fiber (%)	6.50
Starch (%)	58.70
Metabolic Energy (kcal/kg)	2700

Growth Performance

The LW and live weight gain (LWG) values of the geese were determined by weighing them individually each week. Also, feed consumption (FC) was determined weekly. Thus, the feed/gain ratio (F/G) was calculated by dividing FC by LWG (FC/LWG). The concentrate was considered while determining feed consumption and feed/gain ratio.

Carcass

Six animals from each group were randomly selected at the end of the experiment. After the slaughtering, the processes of plucking, separating the legs, and removing internal organs were carried out consecutively. Then, their carcass and visceral organ weights were determined. The dressing was found by calculating the ratio of cold carcass weight to the LW.

Collection and Analysis of Serum Samples

At the end of the trial, blood samples (6 geese/per group) were taken from *Vena brachialis*. Then, serum samples were obtained by centrifuging the blood samples at 4000 rpm for 10 min. Then the samples were stored at -20°C until analysis. After the serum was thawed at room temperature, the values of glucose (GL), total protein (TP), albumin (Alb), bilirubin (Bil), calcium (Ca), phosphorus (P), triglyceride (TG), Total cholesterol (TC), HDL-Cholesterol (HDL), Aspartate Aminotransferase (AST), Alanine aminotransferase (ALT) and Gama Glutamyl Transferase (GGT) were measured spectrophotometrically by using a Colorimetric Assay kit (Elabscience, UK).

Statistical Analysis

The data were evaluated using the SPSS 20 (IBM Inc., Chicago - IL) software package. The results were analyzed using the ANOVA test. The Tukey's range test was conducted to determine the mean separation among the groups. The level of statistical significance was assumed to be $P<0.05$.

Results

At the end of the experiment, significant differences were found between the groups in LW and LWG *(Table 4)*. According to the results of the experiment, it was found that the supplementation of 0.5 mL/L (PM-2) probiotic mixture in the drinking water of the geese significantly increased the LW and LWG compared to the control group ($P<0.05$). Also, the PM-2 group was found to have the highest FC value and the best F/G ($P<0.05$).

Significant differences were found between the groups in terms of dressing ($P<0.05$). Internal organ weights were affected by the supplementation of PM *(Table 5)*.

It was found that the addition of PM supplied a significant increase in biochemical parameters of GL, TP, Alb, Ca, and P levels. It caused a significant decrease in the TG level ($P<0.05$). Bil level was not influenced by PM supplementation ($P>0.05$) *(Fig. 1)*.

Table 4. *Effect of PM on performance parameters of geese*

Parameters	Groups			P
	Control	PM-1	PM-2	
Initial LW (g/bird)	551.69±2.30	546.96±2.21	548.63±2.97	0.408
Final LW (g/bird)	3612.90±21.87^{b}	3573.25±16.88^{b}	3701.05±18.67^{a}	0.001
ADG (g/bird/day)	54.66±0.41^{b}	54.04±0.29^{b}	56.29±0.34^{a}	0.001
ADFC (g/bird/day)	198.96±1.11^{b}	197.79±1.08^{b}	201.52±1.05^{a}	0.047
F/G	3.64±0.03^{a}	3.66±0.02^{a}	3.58±0.02^{b}	0.025

LW: *Live weight,* ***ADG:*** *Average daily gain,* ***ADFC:*** *Average daily feed consumption;* ***F/G:*** *Feed/Gain;* ***PM-1:*** *0.25 mL/L Probiotic mixture;* ***PM-2:*** *0.50 mL/L Probiotic mixture;* $^{a-c}$ *Values in the same row with different superscripts were significantly different (P<0.05)*

Table 5. *Effect of PM on carcass parameters and visceral organ weights of geese*

Parameters	Groups			P
	Control	PM-1	PM-2	
Dressing (%)	67.04±0.24^{b}	66.67±0.26^{b}	68.12±0.15^{a}	0.010
Heart (g)	22.80±0.97	21.80±1.66	23.13±0.80	0.317
Liver (g)	133.80±6.76^{b}	118.80±2.73^{c}	145.40±4.77^{a}	0.002
Gizzard (g)	152.40±7.87^{ab}	145.20±8.00^{b}	161.73±6.88^{a}	0.013

PM-1: *0.25 mL/L Probiotic mixture;* ***PM-2:*** *0.50 mL/L Probiotic mixture;* $^{a-c}$ *Values in the same row with different superscripts were significantly different (P<0.05)*

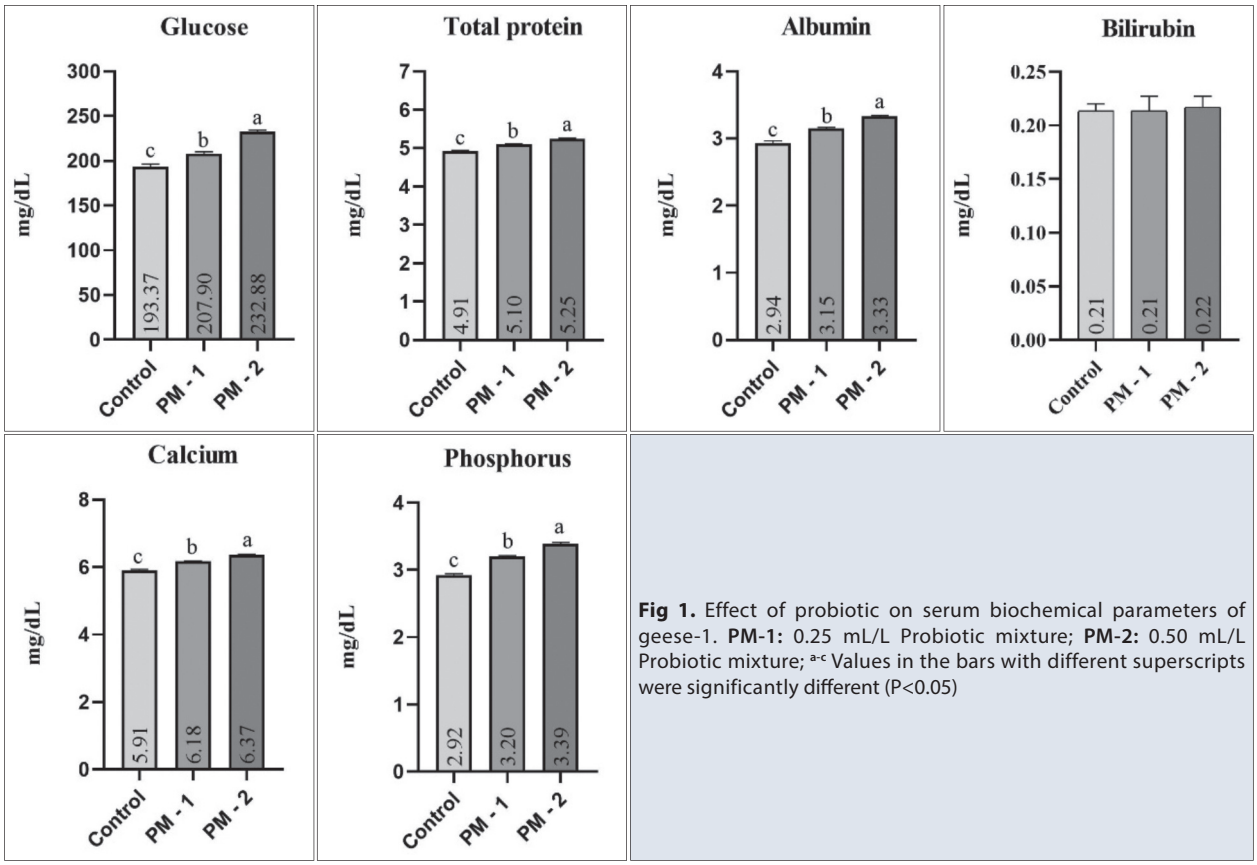

Fig 1. Effect of probiotic on serum biochemical parameters of geese-1. **PM-1:** 0.25 mL/L Probiotic mixture; **PM-2:** 0.50 mL/L Probiotic mixture; $^{a-c}$ Values in the bars with different superscripts were significantly different (P<0.05)

As presented in *Fig. 2*, levels of serum TG, TC, and LDL in PM groups decreased compared to the control group (P<0.05). HDL level was not impacted by PM supplementation (P>0.05). While dietary PM decreased significantly on activities of AST (P<0.05), it did not have any significant effect on activities of ALT and GGT (P>0.05).

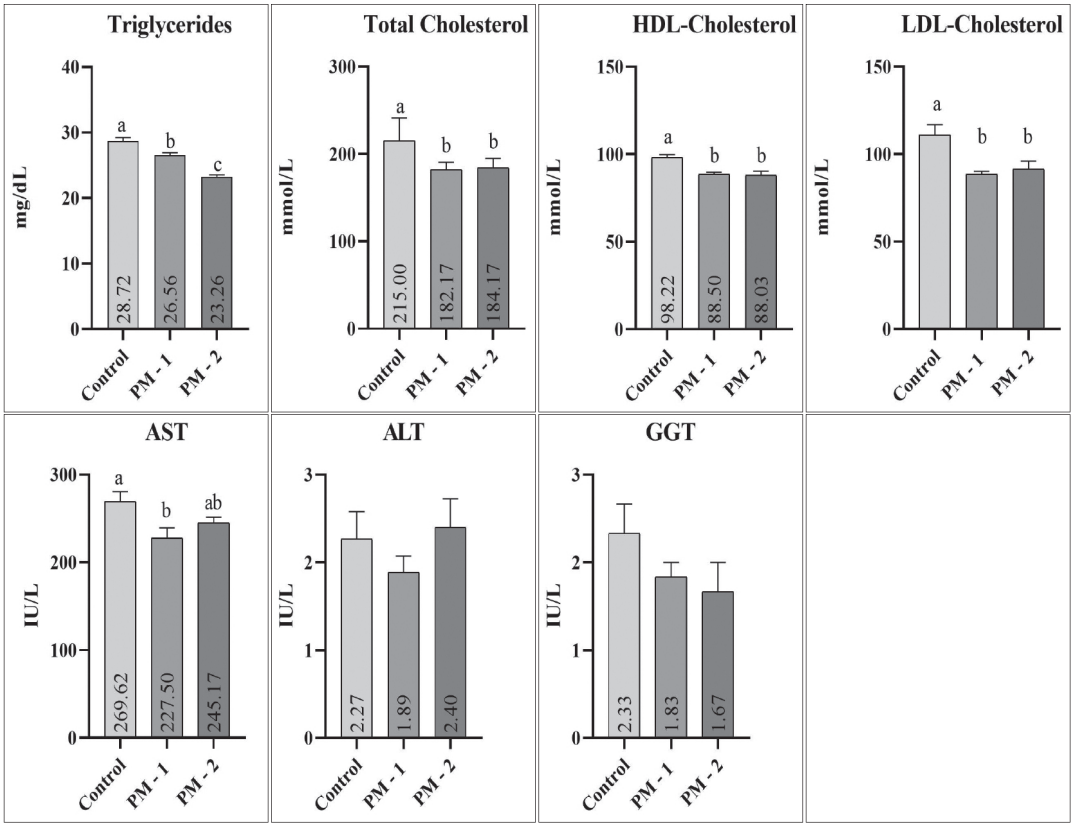

Fig 2. Effect of probiotic on serum biochemical parameters of geese-2
PM-1: 0.25 mL/L Probiotic mixture; **PM-2:** 0.50 mL/L Probiotic mixture; [a-c] Values in the bars with different superscripts were significantly different (P<0.05)

Discussion

The probiotic supplemented drinking water resulted in significant differences in native Turkish geese in terms of LW and LWG during the ten-week experiment. The present study showed similar results with some studies in the literature [15,16]. It was reported that a 0.5% and 1% probiotic supplement increased live weight [17]. Also, a 150 g/ton commercial probiotic supplement was reported to increase the LW, LWG and FC values and improve the F/G [18] in broilers. It is thought that the improvement in performance values in the cited study was the result of the probiotic supplement to the diet, which increased the digestion and absorption of nutrients in the digestive tract, reduced the toxic components, and demonstrated an antagonistic effect against pathogenic bacteria [19].

Contrary to the results of the present study, it has been reported that the probiotic supplement did not affect the performance values of the geese [20]. Yaman et al.[20] reported that a 0.20% and 0.50% probiotic supplement to the goose feeds did not affect LW, LWG, FC, and F/G. In a study using similar bacteria and yeast to the current study, it was determined that Kefir contains strains of *L. fermentum, L. plantarum, L. acidophilus, L. casei, L. delbrueckii* and *S. cerevisiae* was increased the performance parameters of geese [8]. Moreover, the results are not consistent with the results of studies reporting that the combiotic supplement (probiotic + prebiotic) or just probiotic added to quail and broiler rations did not affect the LWG, FC, and F/G [21,22]. These differences in the results may be due to viability of the probiotic mixture. The viability of the product is not continuously examined before it is applied. In addition, it is important to choose strains with maximum capacity for survival and growth rate in the digestive system. It is important to note that the efficacy of probiotics not only depends on the strains it contains but also on the animal's digestive system and diet. common probiotics like *Lactobacillus* and *S. cerevisiae* were used in the present study [15,20]. According to the results of present study, the positive results obtained from the probiotic supplemented groups in terms of performance parameters indicate that the supplement used is an alternative feed supplement that supports growth.

At the 0.5% level of PM supplementation, the dressing and the organ weight of the geese increased. A similar result was reported by Toghyani et al.[23] who found differences in the dressing and liver of broilers when probiotics were supplemented into the diet. Alam and Ferdaushi [24] stated that probiotic supplementation improved dressing and liver weight but had no effect on the weights of the heart and gizzard of broilers. On the contrary, studies have reported that the supplementation

of probiotics had no effect on dressing and weights of internal organs [25,26].

The differences in results of dressing and internal organ weights are thought to be due to the probiotic strain, administration of the probiotic and animal varieties. An increase in dressing and liver and gizzard weights could be due to their great body weight in the current study.

The serum biochemical parameters are usually used as the indicator of the physiological status of animals. The inclusion of probiotic mixture had a significant effect on serum glucose, total protein, albumin, calcium, phosphorus, and triglycerides. These effects could be explained by the increasing absorptive capability of the intestinal mucosa due to supplementation of the probiotic mixture. Therefore, animals could benefit further from the nutrients [27]. This situation may explain the change in the above-mentioned parameters with a probiotic mixture supplement.

In this study, the commercial probiotic supplement was found to increase the serum GL levels in geese. These results are similar to the studies reporting that natural and commercial probiotics significantly increased the serum GL levels in quail, broiler, and ducks [9,28]. However, several studies reported that the supplementation of probiotics did not affect the GL level [22,29]. The differences between the results of the studies are caused by the difference in the types of the probiotic supplement and the levels of use as well as the diet content.

The results of the present study are consistent with the studies reporting that the probiotic supplement increased the serum TP and Alb levels in broilers and quails [9,30]. This is because lactic acid bacteria increase the use of amino acids and proteins by preventing the breakdown of proteins into nitrogen and hindering pathogenic bacteria that reduce the efficiency of protein in the feed [31]. However, the results of the present study conflict with the results of the studies by and Abdel-Hafeez et al.[32], which reported that the probiotic supplement reduced TP levels in the broiler compared to the control group, and the study of Sahin et al.[22], which reported that the combiotic added to quail diets did not affect the serum the TP and Alb levels. While some studies showed that serum Bil level was affected by supplementation of probiotic, another research demonstrated that bil level was not affected [33,34]. Capcarova et al.[35] also reported that serum Bil level was not influenced by probiotic supplementation in the drinking water for broilers.

In the present study, the Ca and P values increased significantly due to the probiotic supplementation. Scholz-Ahrens et al.[36] reported that a probiotic might increase calcium absorption in the intestines by reducing the gastrointestinal pH of short-chain fatty acids (SCFAs) produced by certain probiotic bacteria. Siadati et al.[9] and Hosseini et al.[37] found the highest P level in the probiotic-supplemented group. Eizaguirre et al.[38] reported that the probiotic decreased pH in the intestinal tract and increased the absorption of mineral substances by increasing their solubility.

While the results of the present study were similar to the results of the studies reporting that natural and commercial feed supplements (probiotic, prebiotic) increased the P level in broilers and quails, they were not consistent with the results stating that these supplements did not the affect calcium levels [9]. Also, the results of the present study contradicted the results of the study reporting that the serum calcium level increased with the probiotic supplement but the phosphorus level did not change [39]. Furthermore, two studies reported that the serum Ca and P levels were not affected by the probiotic supplement [39,40].

The results of the present study were similar to the results of the study reporting that various levels of probiotic supplements to broiler diets decreased TG, TC and LDL levels significantly compared to control and antibiotic groups [17,18]. Several studies report that TG, TC and LDL levels in poultry were not affected by the probiotic supplement [29,41]. Studies have also been reported that HDL level was not affected by the addition of probiotics [42,43]. These results were similar to the Kalavathy et al.[44] who demonstrated that the addition of PM reduced serum LDL level and but had no influence on serum HDL level in broilers.

Geese blood biochemical indices have been reported, but activities of key enzymes of fat metabolism are rarely described. Probiotics affect digestive enzymes, amino acids, B vitamins, unknown factors that impact the animal gastrointestinal system. Probiotics can support entero-hepatic circulation and arrange bile acid synthesis to reduce cholesterol. Probiotics can also develop fat metabolism by affecting the activity of enzymes. They reduce TG synthesis and decrease TG concentrations [45]. Lactic acid bacteria lower cholesterol by absorbing cholesterol in the intestinal system, producing bile salt hydrolase, an enzyme responsible for the deconjugation of bile salts and helps to secrete more bile acids in the feces [30]. Besides, it was reported that the probiotic supplement reduced the serum TC level indirectly by limiting the activity of acetyl-CoA carboxylase [46]. In the present study, reduction in serum TG, TC and LDL were observed during the growth phase of native Turkish geese in response to probiotic supplementation in drinking water. There seemed to be some interaction between growth parameters and probiotics by resulting in advanced usage of nutrients and boosting the speed of lipid metabolism in the present study.

ALT, AST and GGT exist widely in the liver. They are also major markers of liver function and get a strong link with animal growth performance [47]. ALT and GGT levels,

exception AST, were not affected by PM addition. Similar results were noticed in some studies [35,48]. It was reported that *L. plantarum* and *B. infantis* added to the diet caused a diminished ALT level in rats [49]. It was also determined that dietary *S. cerevisiae* increased serum ALT [50]. PM by decreasing effects of stress can cause a lower enzyme activity and be a protective agent for liver against damage factors in geese. Also, these liver enzyme parameters measured in the native Turkish geese can also be used as reference values for the literature.

In conclusion, the present study revealed that a 0.5 mL/L supplementation of probiotic might have an improving effect on performance and biochemical parameters in native Turkish geese. It was also concluded that probiotic supplements added to traditional feeding methods could increase the yield performance and the effectiveness of the probiotic on the native Turkish geese should be examined in detail.

Availability of Data and Materials

The authors declare that data supporting the study findings are also available to the corresponding author (M. Ölmez).

Funding Support

This work was not supported by any institution.

Conflict of Interest

The authors declared that there is no conflict of interest.

Authors' Contributions

MÖ and TŞ designed the study. MÖ, TŞ, and SD performed the feeding period. ÖK and SD collected the data. MÖ carried out biochemical analysis. MÖ and MAY carried out the statistical analysis. The manuscript was written by MÖ, MAY and TŞ. All authors approved the final version.

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