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The Effects of the Diet Fermented with Yoghurt on Growth, Ileum Histomorphology and Fecal Microbiota of Quails

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ABSTRACT

This study aimed to evaluate the impact of fermented yogurt-supplemented diets on growth performance, fecal microbiota composition, and ileal histology in male quails. A total of 120 male quails, each 21 days old, were randomly assigned to three dietary treatment groups. Each group consisted of four replicates, with 10 chicks per replicate, and all chicks had equal body weights. The experimental groups were as follows: T_1 (control), T_2 (diet containing 5% fermented yogurt), and T_3 (diet containing 10% fermented yogurt). Chicks were housed in floor-covered cages measuring 50 × 100 × 50 cm. Feed consumption (FC) and live weight gain (LWG) were recorded on a weekly basis, and feed conversion ratio (FCR) were subsequently calculated. At the conclusion of the trial, no significant differences were observed among the groups in terms of growth performance, internal organ development, or fecal microbial microbiota. However, the T2 group exhibited significantly increased villus length, villus thickness, and lamina muscularis mucosae measurements compared to the control group. These findings suggest that dietary supplementation with fermented yogurt can enhance gut histomorphology and support intestinal function in male quails without compromising performance outcomes. It is thought that the reason for the lack of change in performance is due to the fact that the number of bacteria in yogurt is less than the amount in commercial probiotics. This study shows that there is a need to determine the effectiveness of yogurts obtained by different methods that will increase the number of bacteria in yogurt.

Yoğurtla Fermente Edilmiş Rasyonun Bıldırcınların Büyüme, İleum Histomorfolojisi ve Dışkı Mikrobiyotasına Etkileri

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ÖZET

Bu çalışmada, erkek bıldırcınlarda fermente yoğurt takviyeli diyetlerin büyüme performansı, dışkı mikrobiyota kompozisyonu ve ileum histoloji üzerine etkisinin değerlendirilmesi amaçlanmıştır. Her biri 21 günlük toplam 120 erkek bıldırcın, rastgele üç muamele grubuna ayrıldı. Her grup, her tekrar 10 civciv olacak şekilde dört tekerrürden oluşturuldu ve tüm civcivler başlangıç vücut ağırlıkları benzerdi. Deney grupları: T_1 (kontrol), T_2 (%5 fermente yoğurt içeren diyet) ve T_3 (%10 fermente yoğurt içeren diyet). Civcivler, 50 x 100 x 50 cm ölçülerindeki zeminle kaplı kafeslerde barındırıldı. Yem tüketimi (YT) ve canlı ağırlığı artışı (CAA) haftalık olarak tartıldı ve daha sonra yemden yararlanma oranı (YYO) hesaplandı. Denemenin sonunda, gruplar arasında büyüme performansı, iç organ gelişimi veya dışkı mikrobiyal popülasyonları açısından önemli bir fark gözlenmedi. Ancak T2 grubu, kontrol grubuyla karşılaştırıldığında önemli ölçüde artmış villus uzunluğu, villus kalınlığı ve lamina muskularis mukoza ölçümleri sergiledi. Bu bulgular, fermente yoğurtla yapılan diyet takviyesinin, erkek bıldırcınlarda bağırsak histomorfolojisini artırabileceğini ve performans sonuçlarını tehlikeye atmadan bağırsak fonksiyonunu destekleyebileceğini göstermektedir. Performansın değişmemesinin yoğurdun içerdiği bakteri miktarının ticari probiyotikleri içerdiği miktardan az

olmasından kaynaklı olduğu düşünülmektedir. Bu çalışma yoğurt içerisindeki bakteri miktarını arttıracak farklı yöntemlerle elde edilen yoğurtların etkinliklerinin belirlenmesine ihtiyaç duyulduğunu göstermektedir.

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1. INTRODUCTION

Over the past two decades, the prohibition of antibiotic growth promoters in animal production, coupled with rising consumer demand for healthier animal-derived products, has driven the search for alternative feed additives. For this reason, functional feed additives that can be an alternative to antibiotics in animal production are intensively researched by researchers today. Poultry production represents an important place among farm animals. Therefore, the use of functional feed additives in poultry production is quite important. Alternative feed additives to antibiotics in poultry nutrition are mostly organic acids, plant extracts, probiotics, essential oils or their mixtures. In poultry production, these additives are intended to enhance animal health, lower production expenses, and support more cost-effective farming practices. Probiotics, in particular, have a positive effect on the digestive system and immune system, increasing the absorption of Mg, Zn, Ca minerals in the digestive system. On the other hand, they have a more important place due to their suppressive effects on pathogenic bacteria (Ergün et al., 2004, Yalçınkaya and Leblebiciler 2012). In recent years, the use and research of probiotics in poultry nutrition has increased, and it has been reported that functional products supported by fermented dairy products can also be used as a probiotic source. Yogurt is also a fermented dairy product, and it has been reported that it has a competitive exclusion culture in the intestines, regulates the immune system, and has antimutagenic and anti-carcinogenic effects (Ayivi and Ibrahim, 2022). Sultana et al. (2025) reported that yogurt increases performance in broiler chickens. Palamidi et al. (2024) reported that yogurt is effective on intestinal health by increasing the lactic acid bacteria population in the intestines without affecting performance. Research on the use of yogurt as a probiotic source in poultry remains limited, with even fewer studies focusing specifically on its impact on intestinal histomorphology. Therefore, this study aims to evaluate the effects of a yogurt-fermented diet a functional food and potential alternative feed additive on performance, internal organ development, fecal microbiota, and ileal histomorphology in quails.

2. MATERIAL VE METHOD

2.1. Animal Material

In this study, 21 days old, 120 male Japanese quails with standardized in weight were used as animal material. This study was approved by the Kırşehir Ahi Evran University Animal Experiments Local Ethics Committee with the ethics committee document dated 07/11/2018/21/5. In the study, 120 quails were randomly distributed into 3 treatment groups with 4 replications, and the experiment was continued for 21 days. In the study, 12 floor-type wire cages with wooden borders, 50 cm wide, 100 cm long and 50 cm high were used. Clean wood shavings, 5 cm thick, were used as cage base so as not to harm the quails and not to create dust.

2.2. Feed Material

The feed used in this experiment was obtained from a commercial supplier located in Kırşehir province. The nutritional composition of the feed is presented in Table 1.

Table 1. The nutritional composition of the feed

Feed ingredients	%	Vitamin premix*	0.25
Zea mays	44.00	Mineral premix#	0.25
Soy bean (44)	41.15	**Nutritional contents**	
Meat and bone meal	4.00	ME [kcal/kg]	3080
Soy oil	6.50	Crude protein	22.39
DCP	2.50	Crude Fiber	2.80
L-lysine HCl	0.70	Ether extracts	8.50
DL-methionine	0.35	Ca	2.00
Salt	0.30	Available P	3.80

2.3. Fermentation

Homemade yogurt was used as a fermentation source. In the analysis conducted in the Animal Husbandry Department feed and animal nutrition laboratory, 6.2×10^8 LAB were detected in the yogurt. A 50-liter capacity-covered plastic container was used for fermentation. First, 10 kg of commercial feed was poured into the plastic container and 10 liters of water was added. Then, 1 liter of homemade yogurt was added and mixed. The mixture was fermented at room temperature for 24 hours. After fermentation, the fermented feed was poured onto a sterilized bench, cleaned with alcohol on and around it, and dried for 3 days by mixing at certain intervals. After the drying process was completed, it was determined in the lactic acid bacteria analysis that the fermented feed contained 6×10^7 live LAB.

2.4. Conducting Experiment

The experiment was established in the Poultry Unit of the Animal Husbandry Department of Faculty of Agriculture of Kırşehir Ahi Evran University. Only male quails were included in the experiment. Male quails sourced from the hatchery were individually weighed, and their mean live body weight was determined. Quails equal to or closest to the average live weight were divided into 12 groups of 10 chicks in each. Treatment groups were T_1= Control, T_2= 5% diet mixed with yoghurt fermented diet, T_3= 10% diet mixed with yoghurt fermented diet supplementation. The cage floor was covered with wood shavings supplied by a commercial enterprise for the natural and healthy ground needs of the animals. 120 male quails were placed in each cage, 10 in 4 replications and 3 treatment groups, individually weighed on an electronic scale with ± 0.01 gr sensitivity. The animals were provided with food and water ad libitum. The drinking water needs of the quails were provided by 1 hanging water in the middle of the cages. Their feed needs were provided by hanging feeders on the edges of the cages. The environment where the study was conducted was cleaned and disinfected 2 days in advance. The environment started to be heated 1 day before the start of the experiment. The ambient temperature was set to 27°C, which is considered appropriate for 21-day-old chicks. At the beginning of the trial, the first feed given was weighed and given in 1 kg. When the amount of feed in the cages decreased, all feeders were collected, the remaining feed was measured, 1 kg of feed was added again and placed in the cages. The waterers were cleaned daily without considering the size and placed back in the cages. The ventilation of the environment was provided by 2 automatic ventilation fans. The ambient temperature was controlled by a thermometer and the temperature was provided with 2 electric heaters. Adequate lighting was maintained to ensure the animals had continuous access to feed throughout the day. Quails and feeders were constantly checked at certain times during the 21-day trial period. At the end of study, feeders were collected from the cages of 42-day-old quails to empty their digestive systems and the slaughtering process was started 8 hours later.

2.5. Slaughtering and Sample Collection

Prior to slaughter, all animals were reweighed by group, and the average live weight for each group was calculated. After the weighing process, 2 quails were randomly selected from each group. The slaughtering process was carried out in sterile conditions in the slaughterhouse room. After the randomly selected quails were individually weighed again, they were cut from the throat with a scalpel in accordance with ethical rules. Then, the rib cage was opened with scissors and a scalpel in a way that would not damage the internal organs. All internal organs were removed without damage. The weights of the heart, liver, gizzard, and proventriculus were weighed. The weight and length of the digestive system starting from the pancreas were measured. For histological analyses, 1 cm thick samples were cut from the ileum regions of the digestive system and placed in closed containers containing 10% formaldehyde liquid.

2.6. Fecal Microbiota

First of all, all quails in the treatment groups were separated individually for fecal microbiota analyses. They were waited to defecate by placing sterile white paper under them. Immediately after the quails defecated, fecal samples on the paper were placed in sterile petri dishes. The groups from which the fecal samples were taken were written on the petri dishes and brought to the laboratory for analyses. 1 gr of the fecal samples were taken and mixed with peptone water using a Vortex until a homogeneous distribution was obtained. Certain dilutions were prepared from the obtained homogeneous mixture and inoculated onto agar media and 3M counting plates. *E. coli*, Lactic Acid Bacteria (LAB), Coliform,

Total Live Bacteria (TLB) and Enterobacter were taken into analysis to investigate the amount. Inoculations were made onto MRS Agar media for Lactic Acid Bacteria analysis. The incubation was carried out at 37°C for a duration of 3 days. For bacterial enumeration, 3M E. coli count plates were used to assess E. coli levels, 3M Enterobacter count plates for Enterobacter quantification, and 3M TLB count plates for determining the total viable bacterial count.

2.7. Ileum Histology

Ileum sections taken from slaughtered quails were placed in containers containing 10% formaldehyde liquid for histology analysis and brought to the laboratory. The brought samples were cut into 1 cm thick and placed vertically in previously prepared paraffin blocks with the cut surfaces facing upwards. Heat was applied in the incubator for the paraffin to penetrate the tissues. After the paraffin was applied to the tissues, they were cooled in that way and the paraffin tissues that cooled and became solid were cut into 5-micron thicknesses with the help of a micro cutter. The cut ileum sections were allowed to stick to the slide. The slide and the tissue section on it were washed with xylene to remove paraffin residues from the tissue. Then, the slide and tissues were passed through alcohol, and xylene was removed from the tissues. Afterwards, the tissues were stained with Hematoxylin & Eosin dye and photographs were taken with an AM Scope MU853B digital camera integrated microscope (ZEISS Primo Star, Germany) with 8.5 MP resolution for appropriate imaging. Statistical analyses were performed by measuring the villi length and villi lengths of the photographs obtained for each treatment group and each sample with the AM Scope 3.7 image analysis program.

2.8. Statistical Analyses

Data obtained from the study was analyzed using one-way ANOVA based on a randomized parcel experimental design. Mean comparisons were conducted using Duncan's multiple range test. All statistical analyses were carried out with SPSS version 15.0 (Windows).

3. RESULTS AND DISCUSSION

At the of the study performance parameter are given in Table 2. No significant differences were observed among the groups regarding performance parameters. While live weight gain did not differ statistically, the T_2 group receiving 5% yogurt-fermented feed supplementation showed numerically higher gains compared to the Control T_0 and T_3 groups. Similarly, although feed conversion ratio (FCR) values were not statistically different, the T_2 group exhibited numerically improved FCR compared to the other groups

Table 2. Effects of yogurt fermented diet supplementation on performance parameters in quails

Groups	T_1	T_2	T_3	SEM	*p* values
FI (g)	490.58	476.88	478.44	6.43	0.67
BWG (g)	148.50	156.89	147.72	2.67	0.32
FCR	3.32	3.04	3.24	0.08	0.12

[a-b], Means with different letters in the same row are statistically different ($p < 0.05$). FI:Feed intake. BWG: Body weight gain. FCR: Feed convertion ration. SEM: Standart error of means.

Table 3. Effects of yogurt fermented diet supplementation on internal organ development in broiler quails (g-cm/100 gr live weight)

Groups	T_1	T_2	T_3	SEM	*p* value
DSL	22.70	22.47	21.86	0.55	0.83
DSW	2.32	2.58	2.35	0.09	0.60
Heart	1.33	1.26	1.30	0.06	0.91
Liver	1.72	1.88	1.81	0.05	0.51
Gizzar	2.14	2.24	2.14	0.06	0.80
Proventriculus	0.37	0.37	0.37	0.01	0.98

SEM: Standart error of means. DSL: Digestive system length, DSW: Digestive system weight

The impact of yogurt and fermented feed supplementation on internal organ development is summarized in Table 3. No significant differences were found among the groups in terms of internal organ development following the dietary treatments.

Table 4 presents the effects of yogurt-fermented feed supplementation on the fecal microbiota of broiler quails. By the end of the study, no significant differences were observed among the groups regarding Enterobacter, *E. coli*, Coliform bacteria, total viable bacteria (TCB), and lactic acid bacteria (LAB) counts.

Table 4. Effects of yogurt fermented diet supplementation on fecal microbiota in broiler quails

Groups	T_1	T_2	T_3	SEM	*p* value
Enterobacter	7.01	7.05	6.91	0.16	0.94
E. coli	7.13	6.98	7.07	0.14	0.93
Coliform	7.03	6.78	6.87	0.10	0.64
TLB	7.58	6.91	7.49	0.16	0.20
LAB	7.28	7.40	6.80	0.13	0.08

SEM: Standart error of means, TLB: Total live bacteria, LAB: Lactic acid bacteria.

Table 5 summarizes the impact of yogurt-fermented feed supplementation on ileum histological parameters in broiler quails. At the study's conclusion, the T_2 group receiving 5% yogurt-fermented feed showed significantly greater villus length and lamina muscularis mucosa thickness compared to the Control and T_3 groups. Additionally, villus thickness in the T_2 group was significantly higher than that of the Control group. No significant differences were found between groups regarding crypt depth.

Table 5. Effects of yogurt fermented diet supplementation on ileum histomorphological parameters in quails

Groups	T_1	T_2	T_3	SEM	*p* value
Villi length (µm)	247.85^{b}	308.62^{a}	237.78^{b}	4.14	0.00
Villi width(µm)	34.86^{b}	42.28^{a}	39.00^{ab}	0.92	0.00
Crypt depth(µm)	14.96	15.36	14.06	0.32	0.28
LMM (µm)	17.50^{b}	21.12^{a}	18.96^{b}	0.54	0.01

$^{a-b}$, Means with different letters in the same row are statistically different ($p < 0.05$). SEM: Standart error of means, LMM: Lamina muscularis mucosae thickness.

The results of this study showed that adding different amounts of yogurt-fermented grower feed to quail diets had no negative effects on growth, internal organ development and intestinal microflora, while adding 5% (T_2) increased villi development in the ileum, primarily providing intestinal health and better digestion. Although no statistically significant differences were observed in performance parameters among the groups, the animals receiving 5% fermented feed (T_2) showed numerically higher live weight gains and improved feed conversion ratios compared to both the control and the 10% supplemented (T_3) groups. This enhancement in weight gain and feed efficiency in the T_2 group could be attributed to the increased development of intestinal villi, as a larger absorptive surface area likely contributed to better nutrient utilization. The present study focused on fermenting grower feed with yogurt, a probiotic source, followed by drying the feed to maintain bacterial viability and enhance its functional properties. Although the performance values did not increase statistically, the numerical increase in live weight and numerical decrease in feed conversion rate in the T_2 group, in addition to the increase in villi length, villi thickness and lamina muscularis mucosa in the ileum, indicate that the feed gained functional properties by fermenting with yogurt. Poultry fattening performance is influenced by genetic, environmental, and stress-related factors (Öztürk and Yıldırım, 2004). The reason why the performance increase was not greater in this study may be due to the three factors mentioned. This research study was conducted in the summer period in Kırşehir province. There was no stress factor that would affect the performance values of the animals during the experiment. Therefore, the results of this study show that meat quails reached the size allowed by their genetic capacity without receiving any functional food. This research study is one of the first studies in which yogurt was used as a fermentation source and fermented and used as a functional food in animals. The number of studies conducted on yogurt as a probiotic source in poultry is limited. In previous studies, yogurt was given to animals in liquid or dried form. Many

previous studies have demonstrated that using yogurt as a probiotic source in animals can enhance performance outcomes. Asad et al. (2006) reported that giving yogurt probiotics to broiler chicks had a significant effect on live weight and feed utilization rate. Coşkun et al. (2018) found that supplementing the drinking water of broiler quails with yogurt led to an increase in the intestinal population of lactic acid bacteria (LAB). Boostani et al. (2013) reported that including yogurt in the diet enhanced growth performance, improved FCR and decreased fecal *E. coli* counts in broiler chick. In addition to studies reporting the positive effects of yogurt, there are also studies reporting that it has no effect. Mahmmod et al. (2014) found that supplementation with yogurt powder had no significant effect on broiler performance or on the levels of total aerobic bacteria and coliforms in feces. Yogurt contains lactic acid bacteria and these are used as probiotics in animals. In addition to the studies reporting that lactic bacteria have positive effects on animals (Dilworth and Day, 1978; Crawford, 1979; Gülşen et al., 2002; Naseem et al., 2005; Şamlı et al. 2007; Awad et al., 2009; Angelakis and Raoult, 2010; Parlat and Göçmen, 2010;), there are also studies reporting that they do not have positive effects (Miles et al., 1981; Panda et al., 1999; Loddi et al., 2000; Özcan et al., 2003). Therefore, although it is known that probiotics increase the performance of animals, the lack of positive results in some studies may be due to the lack of stress factors. In our study, the absence of environmental stress factors may explain why the live weight gain and feed conversion rates in the T_2 group did not reach the expected levels. Nevertheless, the addition of yogurt-fermented feed increased the development of villi in the ileum and numerically increased the performance values.

4. CONCLUSIONS

As a result, it was determined that the addition of 5% yogurt-fermented grower feed improved the performance values of meat quails and provided better digestion by increasing the development of villi in the ileum. The results obtained from this study revealed the necessity of conducting studies using different stress factors in different animal species in addition to the addition of yogurt-fermented feed.

Explanation

This article is a research article produced from Abdurrahman KORKMAZ's Master's Thesis.

Conflict of Interest Declaration

There is no conflict of interest.

Researchers' Contribution Rate Declaration Summary

The research was produced with 20% contribution from İsa COŞKUN consultancy and 80% contribution from the Graduate Student.

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