

OPTIMIZATION OF BROILER TURKEY HEALTH AND PERFORMANCE USING FUNCTIONAL FEED ADDITIVES

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ABSTRACT

This study investigated the impact of functional feed additives (dietary supplementation with probiotics (*Lactobacillus acidophilus* and *Bacillus subtilis*) and prebiotics (mannose oligosaccharide and inulin) on the growth performance, carcass characteristics, immune response, gut morphology, and microbiota in broiler turkeys. A total of 135 broiler turkeys were randomly assigned to three groups: a control group (G1) with no supplementation, a probiotic-supplemented group (G2), and a prebiotic-supplemented group (G3). The birds were fed these diets for 70 days. Results showed that probiotics (G2) is significantly higher and improved body weight gain, feed intake, and white blood cell count ($p < 0.05$), while prebiotics enhanced gut morphology and nutrient absorption. Both treatments resulted in a nonsignificantly higher villus height with significant implication in *Lactobacillus spp.* counts, contributing to improved gut health. No significant differences were observed in feed conversion ratio, carcass yield, or *E. coli* counts. The findings suggest that dietary supplementation with probiotics and prebiotics positively affects turkey growth performance, immune function, and gut health, with probiotics showing the most pronounced impact on body weight gain and immune response. The inclusion of these functional additives enhances turkey production efficiency without compromising carcass quality. Thus, probiotics and prebiotics supplementation can be a valuable strategy to optimize broiler turkey performance and health.

Key words: broiler turkey; probiotics; prebiotics; gut health; immune response

INTRODUCTION

Although the turkey (*Meleagris gallopavo*) is a popular food in the West, it is still not well-developed commercially in many other regions of the world, particularly in poorer countries (Cauble *et al.*, 2020). Nonetheless, commercial turkey farming is becoming more popular in places like India and has potential in some African farming systems (Cloft *et al.*, 2018). The world's turkey industry works to provide high-quality meat at competitive prices because to the rising demand for poultry (Czech *et al.*, 2020). Since the healthcare industry

faces obstacles such as fierce global competition, worries about food safety, environmental issues, and regulatory changes, it is imperative that efficient techniques be used to prevent disease (Dittoe *et al.*, 2019). Furthermore, turkeys can only be treated with a restricted range of veterinary products that are permitted for use as food-producing animals (Gernat *et al.*, 2021). Turkey's significance in the poultry industry is demonstrated by the fact that its production rose from 2.45 million tons in 2000 to 2.71 million tons in 2016 in the United States (Feye *et al.*, 2016). Nonetheless, raising turkeys has drawbacks, such as a high rearing mortality rate that influences

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financial results (Feye *et al.*, 2016; Hafez, H. M. & Shehata, A. A., 2021). Turkey mortality is influenced by a number of factors, including breeder flock age, genetic strain, and hatchery conditions. There are now more worries in turkey production due to the ban on in-feed antibiotics (Erdoğan *et al.*, 2018). Probiotics have been the subject of much research on broilers and laying hens, but little to no study has been done on turkeys, and the results have been conflicting (Gulmez *et al.*, 2019).

Turkey performance depends on the gastrointestinal tract's (GIT) ability to adjust to environmental stress. High-protein diets are necessary for turkeys because undigested protein can enter the hindgut and cause hazardous bacterial activity as well as the production of poisonous chemicals including ammonia (NH₃) and hydrogen sulfide (H₂S) (Gulmez *et al.*, 2019; Khomayez & Adewole, 2022). Since the European Union banned antibiotic growth promoters in 2006, there has been a greater interest in substitute solutions (Lipiński *et al.*, 2021). Live microorganisms known as probiotics have gained popularity as an alternative because they support gut health (Markowiak & Śliżewska, 2017). They boost immunological responses, strengthen resistance to infections, and maintain the equilibrium of microorganisms (Markowiak & Śliżewska, 2017; Mohammadigheisar *et al.*, 2019). In a similar vein, non-digestible oligosaccharides called prebiotics encourage the development of good gut bacteria and advance gut health in general (Vahabi-Asil *et al.*, 2017). Short-chain fatty acids, which lower pH levels and enhance mineral absorption while creating an unfavorable environment for harmful bacteria, are produced in the colon during the breakdown of prebiotics and probiotics (Markowiak & Śliżewska, 2017).

In an effort to provide potential answers to the problems plaguing the turkey farming business, this study attempts to assess the impact of probiotics and prebiotics on turkey performance.

MATERIAL AND METHODS

Experimental location and materials

The Department of Agriculture's research farm, in partnership with the Department of Life Sciences at Mewar University in India, served as the site of the study. The Local Ethics Commission for Animal Experiments provided ethical clearance for the study. The farm is situated at Mewar University in Gangrar, between lati-

tudes 23°32'N and 25°13'N and longitudes 74°12'E and 75°49'E. Probiotics, prebiotics, broiler turkey poults, feed ingredients, weighing scales, haemocytometers, dissection kits, sterile needles, syringes, and sterile tubes were among the supplies used in the study.

Experimental design and treatments

A total of 135 broiler turkeys (mixed-sex) were randomly divided into three treatment groups (G1, G2, G3), with each group comprising 45 birds, further subdivided into three replicates of 15 birds each. The experiment lasted for 10 weeks. The groups were fed the following diets:

G1 (Control): Basal diet with no supplementation.

G2: Basal diet supplemented with a mixture of probiotics (*Lactobacillus acidophilus* and *Bacillus subtilis*).

G3: Basal diet supplemented with prebiotics (Mannose oligosaccharide and Inulin).

Source and management of the experimental animals

A total of 135 broiler turkeys (mixed-sex) poults, aged one month, were obtained from Tamil Nadu Agricultural University (TNAU), located in Saidapet, Chennai, Tamil Nadu, through the Agricultural College and Research Institute. The poults were weighed individually (average initial body weight: 0.9 kg ± 0.2) and distributed at random among 9 250 × 250 cm wooden shaving-lined enclosures. Three pens (45 birds per diet) were distributed randomly among the three experimental diets. The samples were subjected to 16 hours of light and 8 hours of darkness (16 L:8D). Probiotics and prebiotics were not added to the normal meal that the control group (G1) was fed. A probiotic mixture containing 1.0 × 10⁴ CFU/g *Lactobacillus acidophilus* and *Bacillus subtilis* at 0.5 % was given to group two (G2). Group three (G3) was fed a diet containing 5 % prebiotics. Feed was provided ad libitum according to the NRC (National Research Council, 1994). Nutrient Requirements of Poultry (9th Revised Edition). National Academies Press., n.d.) recommendations.

Experimental procedures and samples collection

Probiotics, prebiotics and diets

Turkey feed was made as part of the diet formulation process and utilized components from the Tamil Nadu Research Institute and small mixing equipment. To guarantee even mixing, the sunflower meal was

first processed into smaller particles, and the maize was processed into a fine powder. The probiotics (combination of *Lactobacillus acidophilus* *Bacillus subtilis* strains) and prebiotics (combination of inulin and Mannan oligosaccharides) were maintained under cool, dry conditions to preserve their potency. Each group was fed a maize-based diet with sunflower meal as the protein source. The diets were formulated to meet the nutrient requirements of turkeys according to standard recommendations. The diet formulation is reported in Table 1.

DATA COLLECTION

Growth performance

Initial body weight (IBW), final body weight (FBW), body weight gain (BWG), feed intake (FI), and

the feed conversion ratio (FCR) were among the growth performance metrics that were measured. While FBW was assessed on day 70 of the trial, IBW was recorded at the start of the investigation (4 weeks of age). To calculate body weight gain (BWG), the difference between FBW and IBW was used. The amount of feed left over after the amount offered was deducted was used to calculate feed intake (FI). As a gauge of feed efficiency, the feed conversion ratio (FCR) was computed as the ratio of FI to BWG.

$$\text{Body weight gain (g)} = \text{Final body weight (g)} - \text{Initial weight gain (g)}$$

$$\text{Total feed intake (g)} = \text{Daily feed intake multiplied by 84 days}$$

$$\text{FCR} = \frac{\text{Total feed intake}}{\text{Total body weight gain}}$$

Table 1. Ingredients composition of experimental diets

Ingredients	Treatments (Diets)		
	G1 (Control)	G2 (Probiotics)	G3 (Prebiotics)
Diet for turkeys (4 – 14 Weeks)			
Maize (%)	60	60	60
Sunflower meal (%)	30	30	30
Wheat bran (%)	5.95	3.95	4.45
Probiotics	None	$1.0 \times 10^8 + 1.0 \times 10^8$ CFU/g	None
Prebiotics	None	None	1% + 0.5 %
Limestone (%)	1.20	1.20	1.20
Dicalcium phosphate (%)	1.80	1.80	1.80
Vitamin premix	0.20	0.20	0.20
Mineral premix	0.10	0.10	0.10
Salt (%)	0.30	0.30	0.30
Methionine (%)	0.30	0.30	0.30
Lysine (%)	0.15	0.15	0.15
Total	100	100	100
Calculated nutrient composition			
CP (%)	15.65	15.33	15.41
ME (kcal/kg)	2714.10	2687.56	2687.88
Ca (%)	0.99	0.99	0.99
P (%)	0.83	0.83	0.83
Fat (%)	3.15	3.15	3.15
CF (%)	8.79	8.79	8.79

CP: crude protein; ME: metabolizable energy; Ca: calcium; P: phosphorus; CF: crude fibre; CFU: Colony Forming Unit. G1: control group (no probiotics and prebiotics); G2: probiotics containing; G3: prebiotics containing diet. Probiotics (combination of *Lactobacillus acidophilus* *Bacillus subtilis* strains) and prebiotics (combination of inulin and Mannan oligosaccharides).

Health and immune response parameters

Blood haematology and biochemistry analyses

Three (3) birds per replicate were chosen at random when they were 84 days old, and needles were used to draw blood samples for biochemical analysis. Blood samples were subsequently placed in blood collection tubes along with 10 milligrams of ethylene diamine tetra acetic acid (EDTA), an anticoagulant. The samples were subsequently centrifuged for 20 minutes at 3000 rpm. After being pipetted, the resulting plasma was stored at -20 °C in 2 ml Eppendorf tubes until analysis as presented in Figure 1. Total cholesterol, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels were measured via a clinical chemistry analyser. A hematocytometer was used to assess hematological components, such as red blood cells, white blood cells, and platelets.

Immune response

Three turkeys, one from each replication, were killed at the end of the study. The spleen, bursa, and thymus from each dead bird were weighed via an electronic sensitive weighing balance to determine the immune status of each organ. The weights of each organ were recorded for comparison between groups, and the results are displayed in the Results section.

Mortality rate

During the research trial, the mortality rate was recorded by dividing the number of deaths by the total number of turkeys in the group and multiplying by 100.

$$MR = \frac{\text{Numer of death}}{\text{Total number}} \times 100$$

Gut health and microbiota parameters

Gut morphology (villus height and crypt depth)

Nine birds from each group (3 birds per replicate) were chosen on the 70th day of the study, weighed separately, and transported to a nearby commercial slaughterhouse to undergo the standard killing procedure. After receiving approval from the Ethical Commission, electrically stunned birds were killed. The duodenum, caecum, and jejunum were analysed to prepare them for histological evaluation. The tissue samples that were collected were preserved in 10 % buffered formalin. Haematoxylin and eosin (H&E) were applied to the prepared tissue sections, and the villus height and crypt depth were measured under a microscope. An external camera was used to take the images, which were then placed onto the eyepiece port of the microscope and measured via ImageJ analysis software (Schneider *et al.*, 2012).

Gut microbiota composition

For microbial counting, the digesta was removed from each bird's ileum, placed in a sterile container, and chilled to 4 °C. Colony-forming units were counted via culture techniques (CFUs). The day before the collected samples were placed in Petri dishes, the culture media were ready. *Escherichia coli* (Eosin methyl blue agar, incubated aerobically for 24 hours) and *Lactobacillus* (Man Rogosa Sharpe agar, incubated anaerobically for 48 hours) were used to count the bacteria. In separate tubes, 9 ml of preproduced salt medium was filled with one milliliter of the digest. The suspension was made via a 10⁻¹ dilution, and successive dilutions were performed. The media was then cultured via serial dilutions of 10⁻³ and 10⁻⁵. A 0.1 ml sample from the dilution was plated onto suitable medium to count



Figure 1. Gut microbiota count with a colony counter

the bacteria. Using a colony counter, discrete colonies on plates were counted, and the quantity of bacteria per 1 g sample (log₁₀ CFU/g) was estimated as shown in Figure 1.

Carcass and meat quality parameters

Carcass characteristics

Nine birds from each group (3 birds per replicate) were chosen on the 84th day of the study, weighed separately, and transported to a nearby commercial slaughterhouse to undergo the standard killing procedure. After receiving approval from the Ethical Commission, electrically stunned birds were killed. To calculate the carcass yield, the carcasses were weighed as a percentage of the live weight.

$$\text{Carcass Yield (\%)} = \left(\frac{\text{Dressed Carcass Weight}}{\text{Live Weight}} \right) \times 100$$

Meat drip loss

Drip loss was measured by storing muscle samples under controlled conditions (refrigerator set at 40°C), and the percentage of weight lost due to water leakage was calculated after 24 hours.

$$\text{Drip Loss (\%)} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Meat pH

All the fat and skin were removed, exposing the muscles. pH 4.0 and pH 7.0 standard buffer solutions were used to calibrate the pH meter. The pH of each sample was measured 30 min after the turkeys were killed (for the initial pH) and 15 h later (for the final pH). Make sure that. The electrode was placed straight into the thickest area of the breast tissue, ensuring that it was deep enough. After the pH meter was stable, the pH was recorded. Each time the electrode was used, it was properly cleaned to prevent contamination.

Feeding behaviour and digestibility of the feed

Feeding behaviour

Feeding behaviour, such as feeding frequency and duration, was carefully observed and recorded.

Digestibility

At the conclusion of the feeding trial, samples of the feed given and the faeces produced by each group were gathered throughout a three-day period. After the litter samples were homogenized in plastic bags, the moisture content was determined by placing the samples in an oven at 80 °C for 48 hours. Samples of the diet and excreta that had been air-dried were finely ground. The levels of DM, N, crude fat, crude fibre, nitrogen retention, and phosphorus retention were measured in each sample. Standard operating procedures were followed to determine the DM (AOAC International. (n.d.). Official Methods of Analysis. AOAC International., n.d.).

STATISTICAL ANALYSIS

Data gathered on growth performance, health indices, gut morphology, and carcass features were subjected to analysis of variance (ANOVA) via Minitab 21 software (Minitab, LLC. (2023). Minitab Statistical Software (Version 21.1) [Computer Software]., n.d.). To evaluate the primary effects of probiotics, prebiotics, and their interactions, the factorial arrangement of treatments was analysed via a general linear model. The treatment means were compared via Tukey's honestly significant difference (HSD) test, which was used to establish statistical significance at $P < 0.05$.

RESULTS

In comparison to the control group (G1), the addition of probiotics (G2) and prebiotics (G3) increased feed intake and body weight gain (Table 2). Probiotics were found to have the greatest effect on feed intake ($p = 0.05$) and body weight increase ($p = 0.05$) in turkeys, suggesting that they can improve the animals' metabolism and absorption of nutrients (Lambo *et al.*, 2021). Nevertheless, there was no discernible variation in the feed conversion percentages between the groups.

Between the treatment groups, there was no discernible variation in either yield or carcass weight as shown in Table 3. Nonetheless, the probiotic group's breast muscle pH was considerably higher ($p = 0.05$), which may be related to higher-quality meat and superior muscle glycogen stores. Since there was no discernible difference in drip loss, the groups' muscle water retention

was probably comparable. Prebiotics, albeit to a slightly lesser extent, similarly positively impacted immunological response, while probiotics considerably boosted WBC count ($p = 0.05$), indicating higher immune function as presented in Table 4. The treated groups exhibited

a substantial improvement in total cholesterol and spleen weight ($p < 0.05$), indicating an improved overall health state of the turkeys.

Gut morphology was improved by probiotics and prebiotics (Table 5); villus height showed a tendency

Table 2. Means for growth performance of turkeys

Parameters	Treatment Groups			F – Value	P – Value
	G1	G2	G3		
IBW (kg/bird)	4.17	4.31	4.32	0.51	0.684
BWG (kg/bird)	5.68 ^b	6.39 ^a	6.02 ^{ab}	3.95	0.053
FI (kg/bird)	10.32 ^b	11.12 ^a	11.62 ^a	14.94	0.001
FCR	1.75	1.71	1.82	0.75	0.552

Keys: ^{a, b} means with the same superscript along the row are not significantly different ($P > 0.05$). IBW: initial body weight; BWG: body weight gain; FI: feed intake; FCR: feed conversion ratio; G1: control group (no probiotics and prebiotics); G2: probiotics containing; G3: prebiotics containing diet.

Table 3. Carcass and meat quality of turkeys

Parameters	Treatment groups			F – Value	P – Value
	G1	G2	G3		
CW (kg)	7.79	8.03	7.91	0.53	0.673
CY (%)	74.99	75.02	75.09	0.71	0.573
BM Ph	5.70 ^{ab}	5.13 ^b	5.87 ^a	5.23	0.027
Drip Loss (%)	1.90	2.06	1.73	2.14	0.173

Keys: ^{a, b} means with the same superscript along the row are not significantly different ($P > 0.05$). CW: carcass weight; CY: carcass yield; BM pH: breast muscle pH; G1: control group (no probiotics and prebiotics); G2: probiotics containing; G3: prebiotics containing diet.

Table 4. Immune response and blood parameters of the turkeys

Parameters	Treatment groups			F – Value	P – Value
	G1	G2	G3		
WBC ($\times 10^3/\mu\text{L}$)	19.00 ^b	22.67 ^a	25.33 ^a	16.24	0.001
RBC ($\times 10^6/\mu\text{L}$)	2.43	2.67	2.53	1.97	0.197
Platelets ($\times 10^3/\mu\text{L}$)	23.33	24.67	23.67	0.32	0.808
LDL (mg/dL)	108.33	115.33	114.00	3.48	0.070
HDL (mg/dL)	55.33 ^b	59.33 ^{ab}	60.67 ^a	3.67	0.063
TC (mg/dL)	190.00 ^b	198.33 ^a	196.00 ^a	11.63	0.003
Spleen (g)	11.67 ^b	14.67 ^a	15.00 ^a	15.52	0.001
BF (g)	1.03 ^b	1.40 ^a	1.33 ^{ab}	7.67	0.010
Mortality (%)	6.66	2.22	2.22	0.41	0.753

Keys: ^{a, b} means with the same superscript along the row are not significantly different ($P > 0.05$). WBC: White Blood Cells; RBC: red blood cells; LDL: low-density lipoprotein; HDL: high-density lipoprotein; TC: total cholesterol; BF: bursa of Fabricius; G1: control group (no probiotics and prebiotics); G2: Probiotics containing; G3: prebiotics containing diet.

toward significance ($p = 0.05$), which may indicate improved nutrient absorption (Lambo *et al.*, 2021). While *E. coli* levels stayed the same, *Lactobacillus* spp. counts were considerably higher ($p = 0.05$) in the therapy groups, suggesting a favourable shift in gut microbiota. Tables 6 and 7 present the apparent digestibility and behavioural observation results. There were significant differences in the digestibility of crude protein (CP), nitrogen retention (NR), and activity level (AL) but not

in the other parameters, even though there were some numerical differences between the groups.

DISCUSSION

Growth performance

Regarding growth performance, there were significant differences ($P < 0.05$) between the treatment

Table 5. Gut morphology and microbiota composition of turkeys

Parameters	Treatment groups			F – Value	P – Value
	G1	G2	G3		
Villus height (μm)	973.33	1050.00	1096.67	3.61	0.065
Crypt depth (μm)	180.00	190.00	206.67	2.75	0.112
<i>L. spp.</i> (CFU/g)	$6.83 \times (10^6)^b$	$7.53 \times (10^6)^a$	$7.0 \times (10^6)^{ab}$	15.19	0.001
<i>E. coli</i> (CFU/g)	1.27×10^4	1.23×10^4	1.23×10^4	0.04	0.990

Keys: ^{a, b} indicate that means with the same superscript along the row are not significantly different ($P > 0.05$). CFU: colony-forming unit; *E. coli*: *Escherichia coli*; *L. spp.*: *Lactobacillus* spp.; G1: control group (no probiotics and prebiotics); G2: probiotics containing; G3: prebiotics containing diet.

Table 6. Apparent nutrient digestibility of turkeys

Parameters	Treatment groups			F – Value	P – Value
	G1	G2	G3		
ICP (%)	73.00 ^b	78.00 ^{ab}	79.67 ^a	6.38	0.016
CF (%)	59.00	59.33	61.00	0.50	0.693
C Fat (%)	72.67	76.67	75.33	1.76	0.231
NR (%)	69.33 ^b	71.27 ^a	71.00 ^{ab}	5.63	0.023
PR (%)	56.00	58.00	58.00	0.61	0.626

Keys: ^{a, b} means with the same superscript along the row are not significantly different ($P > 0.05$). CP: crude protein; CF: crude fibre; C Fat: crude fat; NR: nitrogen retention; PR: phosphorus retention; G1: control group (no probiotics and prebiotics); G2: probiotics containing; G3: prebiotics containing diet.

Table 7. Behavioural observations of turkeys

Parameters	Treatment groups			F – Value	P – Value
	G1	G2	G3		
FF (times/day)	10.33	11.67	12.00	1.40	0.311
FD (min/feeding session)	5.17	5.33	5.00	0.09	0.964
AL (active minutes/day)	270.00 ^b	297.00 ^{ab}	300.00 ^a	6.56	0.015

Keys: ^{a, b} means with the same superscript along the row are not significantly different ($P > 0.05$). FF: feeding frequency; FD: feeding duration; AL: activity level; G1: control group (no probiotics and prebiotics); G2: probiotics containing; G3: prebiotics containing diet.

groups in terms of body weight gain (BWG) and feed intake (FI). The results of (Mookiah *et al.*, 2014) and (Lambo *et al.*, 2021), who proposed that probiotics and prebiotics boost body weight gain (BWG), the feed conversion ratio (FCR), and chicken productivity overall, are corroborated by this investigation (Abdelqader *et al.*, 2020). The turkeys in group four (G3) fared better in terms of BWG and FCR than the turkeys in the control group (G1) after receiving both probiotics and prebiotics.

Turkeys in treatment groups receiving probiotics (G2) and prebiotics (G3) had increased body weight gain (BWG) in comparison to the control group (G1), according to the examination of growth performance. Group G2 exhibited the highest BWG, and G3 outperformed G1 by a significant margin. The advantages of probiotics and prebiotics on nutritional digestion and gut health can be linked to this growth improvement (Li *et al.*, 2016). Probiotics are known to improve the balance of gut bacteria, facilitating improved nutrient absorption and utilization. *Lactobacillus* and *Bacillus* in particular are known to do this (Feye *et al.*, 2016; Halder *et al.*, 2024). The substantial variation in body weight gain between the groups is consistent with the literature (Halder *et al.*, 2024), which reports that probiotic administration increases the effectiveness of food absorption, improving growth performance (Blajman *et al.*, 2015). In addition, feed intake (FI) was considerably higher in the probiotic and prebiotic-treated groups. Better gut health and feed palatability, which encourage better digestion and nutrition utilization, may account for these groups' higher FI (Halder *et al.*, 2024). The turkeys were able to maintain growth efficiency in all treatment groups, even while FI rose, as seen by the feed conversion ratio (FCR), which stayed statistically similar across all groups.

Carcass and meat quality of turkeys

Both carcass weight (CW) and carcass yield (CY) did not substantially differ significantly across treatment groups ($P > 0.05$), suggesting that although probiotics and prebiotics enhanced growth performance overall, their effects on final carcass weight or yield % were not statistically ($P > 0.05$) significant. However, there were notable variations in the pH of breast muscle (BM pH), with the probiotic group (G2) having the lowest pH values. Because lower muscle pH may result in better water-holding capacity and softness, lower muscle pH has been linked to higher-quality meat (Elshagabee

et al., 2017). This finding implies that probiotic supplementation may improve meat quality, which is something that customers find appealing (Halder *et al.*, 2024). Although muscle pH varied, the general quality of meat in terms of water retention was unchanged, as evidenced by the fact that drip loss, a measurement of meat water retention, was unaffected by the treatments. BM was lowest in G1, indicating that the level of post-mortem glycolysis, which influences the ability of meat to hold water, softness, and colour, may be related to the lack of probiotics and prebiotics (Reuben *et al.*, 2021). These results support these of a previous study by Reuben *et al.* (Reuben *et al.*, 2021) reported similar improvements in carcass traits when probiotics and prebiotics were included in poultry diets.

Immune response and blood parameters of turkeys

Turkeys given probiotics and prebiotics showed notable changes in their immunological markers. The probiotic and prebiotic groups (G2 and G3) had considerably greater counts of white blood cells (WBC) than the control group (G1), suggesting an enhanced immunological response. This is consistent with earlier research that suggests probiotics can improve immunity through modifying gut microbiota and affecting the proliferation of immune cells (Asgari *et al.*, 2016).

Furthermore, the supplemented groups showed no significant difference ($P > 0.05$) in low-density lipoprotein (LDL) levels, but significant differences were observed in total cholesterol (TC) and high-density lipoprotein (HDL) levels (Table 4), especially in G3, which suggests improved lipid metabolism (Zheng *et al.*, 2016). This shows that prebiotic administration, which encourages the development of *Lactobacillus* and other good gut bacteria, can favourably affect lipid profiles and improve the health of turkeys' cardiovascular systems (Asgari *et al.*, 2016; Hume, 2011).

One study revealed that protein levels, not probiotics or prebiotics, had an effect on HDL and TC blood parameters, in contrast to earlier findings (Vahabi-Asil *et al.*, 2017). The probiotic and prebiotic groups had considerably bigger spleen weights, a measure of immune organ development, which lends more credence to the idea that these additions improve immune function. Furthermore, the bursa of Fabricius (BF) and spleen results indicated significant differences ($P < 0.05$) among the treatment groups; turkeys in the G2 diet group seemed to have the best statistical values for both BF and spleen, followed

by turkeys in the G2 diet group. By increasing the synthesis of mucins, defensins, and antimicrobial peptides (AMPs), probiotics enhance the epithelial barrier and promote immunological function by inducing mucosal immunity (Bermudez-Brito *et al.*, 2012). Through their interactions with macrophages, lymphocytes, epithelial cells, and dendritic cells, they alter the immune system and cause an increase in immunological responses (Ashraf & Shah, 2014). The fact that bursa of Fabricius weight (BF), a measure of lymphoid organ growth, increased significantly also highlights the fact that probiotics and prebiotics strengthen the immune system (Khan *et al.*, 2013).

Gut morphology and microbiota composition of turkeys

Nutrient absorption and general gut health are significantly influenced by gut shape. The probiotic and prebiotic groups showed higher villus height (VH) in the results despite no statistical ($P > 0.05$) variation, with G3 (prebiotics) showing the maximum villus height. Because of the increased surface area of the intestinal lining, an increase in villus height is linked to a better capacity for nutritional absorption (Latha *et al.*, 2016). According to research, prebiotics encourage the growth of good gut bacteria, which in turn improves gut health and nutrient absorption. This conclusion is in line with those findings (Lindgren & Dobrogosz, 1990; Mookiah *et al.*, 2014).

There were no appreciable variations in the levels of Crypt Depth (CD) among the treatment groups. On the other hand, both the probiotic and prebiotic groups showed improvements in the ratio of villus height to crypt depth (VH), a crucial measure of intestinal health and functionality, suggesting that these additions support a more favourable gut environment (Dittoe *et al.*, 2018; Wang *et al.*, 2015).

Lactobacillus spp. numbers significantly increased in the probiotic and prebiotic groups, especially in G3, according to the gut microbiota research. It is well known that *Lactobacillus* species improve gut health by inhibiting the growth of dangerous bacteria like *Escherichia coli* (Callaway *et al.*, 2008; Wang *et al.*, 2015). All treatment groups showed lower levels of *E. coli* than the control group, suggesting that probiotics and prebiotics work to decrease dangerous bacteria and improve gut health and nutrient utilization (Gernat *et al.*, 2021).

Apparent nutrient digestibility in turkeys

The results of the nutritional digestibility trials are displayed in Table 6. Among the treatment groups, there was a significant difference ($p < 0.05$) in the digestibility of crude protein (CP) and nitrogen retention (NR). Prebiotics and probiotics composed treatment groups G2 and G3, which presented the highest CP digestibility at 78.007 % and 79.67 %, respectively, in comparison to the control group G1 (73.00 %). These findings suggest that the addition of probiotics and prebiotics enhances the ability of turkeys to digest and absorb protein (Bermudez-Brito *et al.*, 2012). The observed improvement may be ascribed to the beneficial impact of probiotics on the balance of the gut microbiota and the reduction in deleterious metabolites that hinder the consumption of nutrients, hence improving protein metabolism (Manie *et al.*, 1998). Group G2, which included probiotics, had the greatest nitrogen retention at 71.27 %, which was considerably greater than the retention of the control group (69.33 %). This implies that probiotics enhance nitrogen retention, which may lead to a better ability to use dietary protein for maintenance and growth (Afsharmanesh & Sadaghi, 2014). Although they did not differ substantially from those in group G1, group G3 (prebiotics) also demonstrated increased nitrogen retention. Owing to improved gut health and nutritional digestibility, adding probiotics, prebiotics, or both to turkey diets has a positive effect on how well the birds use nitrogen (Ribeiro Jr *et al.*, 2014). The groups that received probiotics and prebiotics generally presented higher values than did the control group for crude fat and crude fibre digestibility, even though there were no significant differences between the treatment groups ($p > 0.05$). Compared with the control, the prebiotic group (G3) presented improved crude fibre digestibility, whereas the probiotic group (G2) presented somewhat greater crude fat digestibility (76.67 %). The treatments had no significant effect on phosphorus retention ($p > 0.05$), with group G2 and G3 exhibiting the greatest value (58 %). This might be because probiotic and prebiotics, which have been demonstrated to increase nutrient absorption by enhancing gut architecture and functionality, improve mineral absorption (Zhao *et al.*, 2017).

Behavioural observations of turkeys

Table 7 displays how turkeys feed and how active they are when given various nutritional regimens. For

probiotics and prebiotics, there was no significant difference in feeding frequency (FF) or duration (FD) ($p > 0.05$). Nonetheless, compared with the control group (G1), there was a discernible increase in feeding frequency among the groups receiving these supplements. The prebiotic-treated group, G3, was fed 12 times a day on average, more frequently than the probiotic-treated group, G2, which was fed 11.67 times a day. The control group was fed 10.33 times a day, which was the lowest frequency. Thus, although not to the point where there was a statistically significant difference, prebiotics may increase hunger and feeding behaviour (Zoghi *et al.*, 2014). Although these changes were not statistically significant, the eating time in G2 (5.33 minutes per session) was marginally longer than that in the control group (5.17 minutes per session), suggesting possible behavioural effects of probiotics and prebiotics on feeding patterns (Bai *et al.*, 2013). The activity level (AL) of the turkeys had the greatest impact on the food treatments, with significant variations ($p < 0.05$) noted. With an average of 300 active minutes per day, the turkeys in G3, which prebiotics, demonstrated the highest level of activity. This percentage was considerably greater than the average of 270 minutes per day in the G1 control group. This improved gut health and nutrient absorption from prebiotics may have increased the birds' general energy levels, which in turn may have contributed to their increased activity (Wells, 2011).

CONCLUSION

The growth, digestion, immune system, carcass quality, gut health, and microbial communities of turkeys were studied in relation to the addition of probiotics and prebiotics. Compared with the results of the control group, the addition of probiotics and prebiotics had favourable effects on a number of metrics. The result of probiotic turkey presented increased activity levels ($p < 0.05$), nitrogen retention ($p < 0.05$), and crude protein digestibility ($p < 0.05$). They also had greater growth performance, including greater body weight gain and feed intake ($p < 0.05$). The features of the carcass, such as drip loss and breast muscle pH, were also impacted; the probiotic group presented substantial improvements in breast muscle pH ($p < 0.05$). The immune response data revealed that the supple-

mented group had significantly greater levels of spleen weight, total cholesterol (TC), high-density lipoprotein (HDL), and white blood cell counts than did the control group ($p < 0.05$). Furthermore, the prebiotic group presented substantial improvements ($p < 0.05$) in gut health indices, such as villus height and *Lactobacillus* spp. – populations, indicating enhanced gut function.

These results suggest that adding probiotic and prebiotic supplements to turkey diets can enhance gut health, immunological response, growth performance, and nutritional digestibility. Future research might examine the cost-effectiveness and using symbiotic in commercial poultry production as well as the long-term effects of doing so.

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DATA AVAILABILITY STATEMENT

All the data generated or analyzed during this study are included in this published article and its supplementary information files.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this study.

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