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RESEARCH ARTICLE

Effects of Probiotics (*Bacillus subtilis*), Prebiotics (MOS + β-Glucans) and Their Combination on Growth Performance, Duodenal Histomorphology and Meat Quality in Broiler Chickens

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Abstract

The aim of this study was to evaluate the effects of dietary supplementation with probiotics, prebiotics, and synbiotics on growth performance, carcass traits, meat quality, and histomorphological characteristics in broiler chickens. A total of 320 one day old broiler chicks were allocated to four dietary treatment groups, each comprising 8 replicates of 10 chicks in completely randomized design. The groups included: a control group (no supplement), a prebiotic group (1 g/kg β-glucan + mannan-oligosaccharide; BM), a probiotic group (1 g/kg *Bacillus subtilis*; BS), and a synbiotic group (0.5 g/kg β-glucan + mannan-oligosaccharide + 0.5 g/kg *Bacillus subtilis*; BM + BS). The results revealed that body weight (BW), body weight gain (BWG), and average daily feed intake (ADFI) significantly increased in the BM + BS group, while feed conversion ratio (FCR) improved compared to the control group ($P<0.001$). Furthermore, slaughter weight, hot and cold carcass weights, as well as heart and gizzard weights, were significantly higher in the BM + BS and BS groups ($P<0.05$). Histomorphological analysis showed that villus height to crypt depth ratio (V/C) was significantly greater in the BM + BS and BS groups, but lower in the BM group ($P<0.001$). Regarding meat quality, the BM + BS and BS groups showed increased brightness (L*), redness (a*), and yellowness (b*) values ($P<0.05$). In conclusion, the dietary synbiotics supplementation in broiler diets was shown to enhance growth performance, improve intestinal morphology.

Keywords: Growth performance, broilers, prebiotics, probiotics, synbiotics

INTRODUCTION

The extensive use of antibiotics in animal husbandry has contributed to resistant strains of bacteria and antibiotic residues in meat, causing serious health risks for human beings and the environment [1]. This has prompted a move from antibiotic growth promoters to developing alternative measures. It has been made possible for modern poultry farmers to rear chickens to the slaughter weight in a short time because of advancements made in chicken genetics and feeding techniques [2]. Nevertheless, the rapid growth rates of these broiler strains are accompanied by greater vulnerability to stressors, which can impair growth efficiency and ultimately compromise production outcomes [3]. Moreover, stress leads to pronounced biochemical and physiological alterations in the animals, resulting in antioxidant depletion, hence the degradation of meat quality [4].

As a result, meeting higher consumer expectations and improving meat quality has created a need for new approaches to animal nutrition [5]. In this regard, researchers aiming at replacing antibiotics with natural additives like probiotics, prebiotics, and synbiotics have gotten traction [6-9]. Probiotics, in particular, have attracted substantial scientific interest since the pioneering studies on replacing antibiotics with live microorganisms in poultry [10]. They are defined as a selective mixture of microorganisms primarily *Lactobacilli, Streptococci*, and *Bacillus* species that support intestinal health by modulating the gut flora through antagonism against pathogenic bacteria [11-13].

Prebiotics are substrates selectively metabolized by gut microorganisms to confer health benefits to the host [14]. Probiotics and prebiotics improve intestinal health, control

foodborne pathogens, and strengthen the immune system [15,16]. These practices modify the composition of the gut microbiota by augmenting the populations of useful bacteria *(Bifidobacteria* and *Lactobacilli)* and decreasing harmful bacteria *(E. coli* and *Campylobacter)* [17]. Moreover, the results suggest improvement in gut structure, serum immunological responses, and production of short chain fatty acids [18].

The combined use of probiotics and prebiotics is referred to as a synbiotic approach [19]. Prebiotics bind to the fimbriae of the harmful bacteria, facilitating their removal via the fecal bolus while simultaneously promoting the growth and metabolism of beneficial microorganisms. Additionally, probiotics enhance enterocyte nutrition, stimulating the digestive system and promoting intestinal balance and health in birds [20].

The study aimed to determine the effects of dietary supplementation with probiotics, prebiotics, and synbiotics on growth performance, intestinal health, and meat quality in broilers. Although several studies have been conducted to evaluate the effects of probiotics, and prebiotics on broiler performance, gut health and serum biochemical parameters. However, results have been inconsistent and information on the the the synbiotic effects of Bacillus subtilis with β-glucan and mannan-oligosaccharide on both intestinal histomorphology and meat quality is limited. Therefore, this study hypothesized that probiotic and prebiotic individually and synbiotic supplementation would synergistically improve growth performance, duodenal histomorphology, and meat quality characteristics in broiler chickens.

Material and Methods

Ethics Statement

All methods employed in this study were conducted following the guidelines approved by the Kafkas University Ethics Committee (KAÜ-HADYEK/2024-128).

Experimental Birds, Husbandry, and Diets

The experiment was carried out at the Broiler Unit, Faculty of Veterinary Medicine, Kafkas University. Two sources of supplementation included beta-glucan + mannan-oligosaccharide (Vimar Company, Türkiye) and *Bacillus subtilis* (Kartal Kimya, Türkiye). A total of 320 one day-old mixed-sex Ross 308 broiler chicks were obtained from a local commercial producer, weighed, and assigned to four different dietary treatment groups in a completely randomized design, with eight replicates of 10 chicks each (initial weight: 44.66±0.08 g). The chicks were housed in floor pens with dimensions of 130 cm in width, 108 cm in length, and 54 cm in height. The temperature control started with a reduction from an initial temperature of 35°C on day one, decreasing gradually at a rate of 0.5°C per day until a temperature of 26°C, from which point it remained steady at that temperature until day 42. During the experiment, the average relative humidity ranged from 60% to 75%, and a lighting regimen of 23 hours of light and 1 hour of darkness was applied until day 42. The broilers were fed according to a two-phase feeding program, consisting of a starter diet (0-21 days) and a finisher diet (21-42 days). The control group received no dietary supplementation, whereas the experimental groups were fed as follows: basal diet + 1 g/kg prebiotic (β-glucan + mannan-oligosaccharide [BM]); basal diet + 1 g/kg probiotic *(Bacillus subtilis* [BS]); basal diet + 0.5 g/kg prebiotic + 0.5 g/kg probiotic (BM + BS) synbiotic group. Prebiotic (ß-glucans and mannanoligosaccharides) and probiotic BS supplements were sourced from a commercial supplier. The base diets for each phase were formulated according to the nutritional requirements of Ross 308 broiler chickens, as defined by the NRC *(Table 1)* [21]. Prebiotic and probiotic premixes were manually incorporated into the mash feed. All experimental groups were provided with powdered feed, and drinking water was available *ad libitum* throughout the study.

Growth Performance and Organ Index

Birds were individually weighed using a digital weighing scale on days 1, 21, and 42. Body weight gain (BWG) was calculated using a differential method. Feed intake (FI) was determined on intervals by measuring the difference between unconsumed and offered feed. Feed conversion ratio (FCR) was calculated by dividing FI by BWG. Adjustments were made to the BWG, FI, and FCR calculations to account for mortality and to ensure accurate data representation [22]. On day 42 of the study, one broiler chicken per cage was randomly selected from each of the four experimental groups (eight birds per group) and sacrificed by cervical dislocation. Defeathering was performed with the hard scaling process as described by Shung et al. [23]. Slaughter weight and hot carcass weight were recorded (g). The weight of internal organs (heart, liver, gizzard and spleen) was recorded. Cold carcass weight was measured after being stored at 4°C for 24 h. Hot and cold carcass yields were determined based on recorded carcass weights.

Intestinal Relative Index and Histomorphological Analysis

On day 42 of the study, mid-segment duodenal samples (approximately 2 cm in length) were collected from eight birds per treatment group (the same birds used for carcass evaluation, one per cage) and preserved in 10% buffered formalin for fixation. Following fixation, the samples were washed, dehydrated, cleared, and paraffin embedded. Sections of 5 µm thickness were cut out of the

Table 1. Ingredients and nutrient composition of the basal diet for broilers chicken (g/kg of diet on an as-fed basis, at 90% DM)

Ingredients (%)	Starter	Finisher
Corn, yellow	30.00	46.10
Barley	10.00	7.20
Wheat	5.70	8.00
Bran	2.80	2.50
Wheat middlings	3.50	2.40
Wheat offal	10.00	2.00
Vegetable oil	3.10	3.80
Rice bran	2.00	2.00
Sunflower meal, 45% CP	4.55	5.50
Corn gluten meal, 62% CP	11.00	10.25
Soybean meal, 48% CP	13.00	6.50
Dicalcium phosphate	1.80	1.40
DL-methionine	0.25	0.20
L-Lysine	0.50	0.50
Threonine	0.25	0.20
Marble dust	1.15	1.00
Salt	0.20	0.25
Vitamin-mineral premix[1]	0.20	0.20
Nutrients levels2 (%)		
Metabolizable energy, kcal/kg	3001	3201
Dry matter	89.90	90,00
Crude protein	22.50	19.50
Phosphorus	0.48	0.38
Calcium	0.95	0.78
Methionine +Cystine	1.04	0.92
Lysine	1.16	0.99
Ether extract	5.44	6.20
Crude fiber	4.26	3.73
Ash	6.02	5.11

1 Supplied per kilogram of diet: 1.537.200 mg vit. A, 6.28 mg vit. E, 0.64 mg vit. K3, 37.36 mg Mn, 25 mg Zn 89, mg Fe, 0.03 mg Co, 8.76 mg Cu, 0.05 mg Mg, 0.91 mg Se
2 Calculated compositions.

paraffin blocks and stained with Hematoxylin and Eosin for histomorphometric analysis of villi length and crypt depth *(Fig. 1)*. Measurements were manually conducted on an area of 30.000 µm (the size of 20 fields of view) using the Cameram SLR 6.1 software for digital analysis (Mikro Sistem Ltd., Türkiye), and the corresponding arithmetic means were computed. The villus height to crypt ratio (V/C) was calculated [20].

Meat pH Value

At 15 min and 24 h after slaughter, pH values of the breast muscles were measured at a depth of 2.5 cm

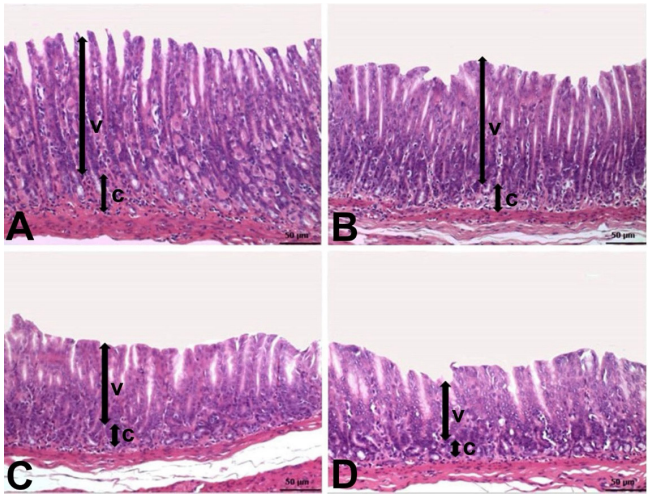

Fig 1. Broiler duodenum, Control: basal diet; BM: 1 g/kg prebiotic powder (β-glucan + mannanoligosaccharide), BS:1 g/kg probiotic powder (Bacillus subtilus), BM+BS: 0.5 g/kg probiotic (Bacillus subtilus) + 0.5 g/kg prebiotic (β-glucan + mannanoligosaccharide), V = villus height, C = Crypt depth. H&E staining

below the surface from three different points a combined glass penetrating electrode (Hanna instruments, Inc. Woonsocket, USA) [24].

Meat Color

Color measurements were taken on the carcass surface of the breast muscles, as well as the freshly exposed cut surface of the muscle. The L* (lightness), a* (redness), and b* (yellowness) values were determined using a chromameter (Hangzhou CHNSpec Technology Co., Ltd., China) [24].

Data Analysis

All the experimental data were processed using SPSS (PASW Statistics 22) software. One way ANOVA followed by Tukey's multiple comparisons were used to assess the effects of dietary treatments on the measured values. Differences were considered significant at $P<0.05$.

Results

Growth Performance

Dietary supplementation with prebiotics, probiotics, and synbiotics significantly ($P<0.005$) improved body weight and body weight gain compared to the control group *(Table 2)*. The most notable improvement was observed in the BM + BS group, followed by the BS and BM group. Birds receiving the BM + BS had higher average daily feed intake (ADFI), while the control, BM, and BS groups exhibited similar ADFI values (21-42 d and 0-42 d). The BM + BS group observed the best FCR during days 0-21, 21-42, and across the entire study period (0-42 days) ($P<0.001$). Additionally, the BS group showed improved FCR compared to the control group, whereas the BM was similar to the control group but exhibited a tendency toward improvement.

Carcass parameters

As shown in Table 3, dietary inclusion of BS and BM + BS resulted in a significant improvement in slaughter, hot carcass, and cold carcass weights relative to the control group (P<0.001). Improvements were observed in the BM+BS group than in the BS group (P<0.001). Both BS and BM + BS feeding also resulted in significant increases in heart weight and gizzard weight. No significant variations were seen in liver weight, spleen weight, or in the percentages of hot and cold carcass yield among groups (P>0.05).

Intestinal Histomorphology

As presented in *Fig. 2*, the V/C ratio was significantly increased by BS and BM + BS supplementation, whereas BM addition resulted in a significant decrease (P<0.001). Interestingly, despite the increase in the V/C ratio, the

Table 2. The effect of dietary supplementation with prebiotics, probiotics, and synbiotics on the performance parameters of broilers

Item	Dietary Treatments1				P-value
	Control	BM	BS	BM+BS	
d 1-21					
BW (g/bird)	350.89±2.41^{c}	365.62±2.86^{b}	373.68±2.29^{ab}	385.62±4.62^{a}	0.001
BWG (g/bird/ d)	28.12±0.22^{b}	28.96±0.43^{ab}	29.7±0.24^{ab}	30.61±0.75^{a}	0.005
ADFI (g/bird/ d)	38.94±0.31	39.41±0.58	39.65±0.19	40.04±1.05	0.659
FCR	1.36±0.01^{c}	1.34±0.01^{bc}	1.32±0.01^{ab}	1.3±0.01^{a}	0.001
d 22-42					
BW (g/bird)	1714.5±5.95^{d}	1809.92±12.23^{c}	1863.9±15.93^{b}	1990.58±13.07^{a}	0.001
BWG (g/ d)	81.35±0.33^{c}	84.87±1.01^{bc}	87.32±1.50^{b}	93.38±1.33^{s}	0.001
ADFI (g/bird/ d)	125.5±1.03^{b}	127.62±1.3^{b}	129.61±2.35^{ab}	136.38±0.68^{a}	0.001
FCR	1.53±0.01^{b}	1.5±0.01^{ab}	1.48±0.02^{ab}	1.46±0.0^{2a}	0.032
d 1-42					
BW (g/bird)	1032.69±2.48^{d}	1087.77±5.78^{c}	1118.79±7.41^{b}	1188.10±7.59^{a}	0.001
BWG (g/ d)	54.73±0.12^{c}	56.92±0.33^{b}	58.51±0.70^{b}	61.99±0.45^{a}	0.001
ADFI (g/bird/ d)	82.22±0.48^{b}	83.52±0.56^{b}	84.63±1.17^{b}	88.21±0.46^{a}	0.001
FCR	1.45±0.01^{c}	1.42±0.01^{bc}	1.40±0.01^{ab}	1.38±0.01^{a}	0.001

BWG: body weight gain; BW: body weight; FI: feed intake; FCR: feed conversion ratio
a,b,c,d Each superscript indicates the difference between the means within the row (P<0.05)
1 Control: basal diet without supplementation; BM: Basal diet supplemented with 1 g/kg prebiotic powder (β-glucan + mannanoligosaccharide), BS: Basal diet supplemented with 1 g/kg probiotic powder (Bacillus subtilis), BM+BS: Basal diet supplemented with 0.5 g/kg probiotic (Bacillus subtilis) + 0.5 g/kg prebiotic (β-glucan + mannanoligosaccharide)

Table 3. The effect of dietary supplementation with prebiotics, probiotics, and synbiotics on some carcass parameters in broilers

Item (g)	Treatments[1]				P-value
	Control	BM	BS	BM+BS	
Slaughter weight	2380.99±41.76^{c}	2443.11±27.99^{bc}	2521.61±19.04^{b}	2636.92±21.06^{a}	0.001
Hot carcass weight	1758.00±31.40^{c}	1802.67±22.57^{bc}	1863.33±14.41^{b}	1950.17±16.03^{a}	0.001
Cold carcass weight	1743.00±31.40^{c}	1787.67±22.57^{bc}	1848.33±14.41^{b}	1935.17±16.03^{a}	0.001
Hot carcass yield %	73.83±0.14	73.77±0.16	73.89±0.02	73.95±0.02	0.679
Cold carcass yield %	73.20±0.14	73.16±0.17	73.30±0.02	73.38±0.02	0.474
Liver	58.19±1.06	54.48±2.69	56.29±1.31	52.56±1.43	0.137
Gizzard	24.62±0.54^{b}	24.75±1.29^{b}	26.33±0.64^{ab}	29.10±1.02^{a}	0.030
Heart	12.99±0.37^{b}	14.65±0.30^{ab}	15.48±0.49^{a}	15.02±0.69^{a}	0.004
Spleen	4.05±0.35	3.69±0.14	3.46±0.10	4.10±0.18	0.110

a,b Each superscript indicates the difference between the means within the row (P<0.05)
1 Control: basal diet without supplementation; BM: Basal diet supplemented with 1 g/kg prebiotic powder (β-glucan + mannanoligosaccharide), BS: Basal diet supplemented with 1 g/kg probiotic powder (Bacillus subtilis), BS+BM: Basal diet supplemented with 0.5 g/kg prebiotic (β-glucan + mannanoligosaccharide) + 0.5 g/kg probiotic (Bacillus subtilis)

supplementation of BM, BS and BM+BS in the diet significantly (P<0.001) reduced both villus height and crypt depth in broilers *(Fig. 2)*.

Meat Quality

The impacts of prebiotic, probiotic and synbiotic supplementation on muscle pH and meat color values are presented in *Table 4.* All meat quality traits, including breast color, differed significantly among experimental group (P<0.001). Birds treated with BM + BS and BS had lighter breast meat compared to the control group, while their breast color was similar to that of the BM group. The redness (a*) of the meat was significantly higher in the BM + BS group compared to the control group. The BS group had the highest redness, followed by the BM group (P<0.001). The BM + BS group showed higher yellowness (b*) compared to the control group and BM treated birds, while the BS treated birds had similar yellowness to all other group (control, BM and BM + BS). The addition of BM, BS and BM + BS significantly increased the pH of the pectoral muscle compared to the control group (P<0.001).

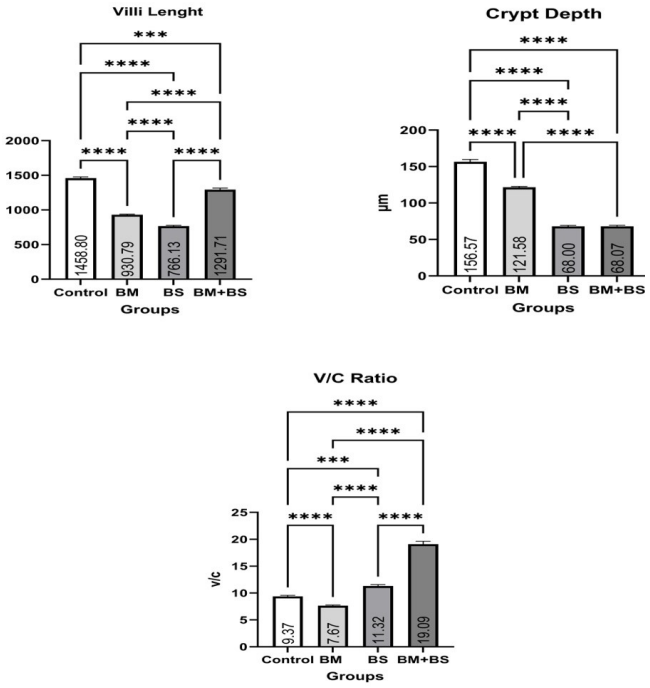

Fig 2. Impact of prebiotic, probiotic, and synbiotics supplementation on villus height, and villus height /crypt depth ratio. V/C: villus villus height/crypt depth. The X-axis displays villus length, crypt depth, and V/C ratio (µm), while the Y-axis shows their distribution in the control and experimental groups. Control: basal diet; BM: 1 g/kg prebiotic powder (β-glucan + mannanoligosaccharide), BS:1 g/kg probiotic powder (Bacillus subtilus), BM+BS: 0.5 g/kg probiotic (Bacillus subtilus) + 0.5 g/kg prebiotic (β-glucan + mannanoligosaccharide) Error bars indicate standard deviation. Control and other groups: ***P<0.05, ****P< 0.01

Discussion

Studies have demonstrated that prebiotics, probiotics, and synbiotics have a positive correlation with increased weight gain, FI, and FCR in broilers [25-27]. Our findings demonstrate that prebiotic and probiotic supplementation improved performance parameters, with synergistic increase in the synbiotic group. Our results corroborate previously established research, supporting the claim that supplementation with prebiotics, probiotics, and synbiotics has a positive correlation with broiler performance, particularly in the synbiotic supplemented group, which showed the highest response [28,29]. Conversely, Sahin et al.[19] observed that the administration of prebiotic and probiotic supplements did not yield any statistically significant impact on body weight gain and feed conversion ratio. Furthermore, feed intake increased in the synbiotic group, while there were no changes in the prebiotic and probiotic treatments. These findings are in accordance with Abdel-Fattah and Fararh [28] who did not find an effect of probiotic and prebiotic supplementations on feed intake. While the impacts of synbiotic supplementation on the growth performance of broilers are generally positive. The result depends on the type of synbiotic, the application method, and the chicken's genotype [30-32]. Our findings indicated that the FCR improved in both synbiotic and probiotic treatment groups, with the best improvement in the synbiotic group. These findings are in accordance with

Table 4. Effect of probiotic, prebiotic, and synbiotics supplementation on broiler meat quality

Item	Treatments[1]				P-value
	Control	BM	BS	BM+BS	
pH**15min**	5.96±0.03^{b}	6.32±0.06^{a}	6.25±0.04^{a}	6.27±0.06^{a}	0.001
pH**24h**	5.75±0.03^{b}	6.11±0.07^{a}	6.05±0.04^{a}	6.07±0.06^{a}	0.001
Lightness (L*)	44.28±0.67^{a}	42.97±0.81^{ab}	40.45±0.55^{b}	40.77±0.68^{b}	0.001
Redness (a*)	1.98±0.12^{b}	2.50±0.26^{ab}	2.70±0.31^{ab}	3.19±0.26^{a}	0.001
Yellowness (b*)	10.13±0.32^{b}	10.34±0.70^{b}	11.23±0.38^{ab}	12.21±0.52^{a}	0.018

a,b Each superscript indicates the difference between the means within the row (P<0.05)
1Control: basal diet without supplementation; BM: Basal diet supplemented with 1 g/kg prebiotic powder (β-glucan + mannanoligosaccharide), BS: Basal diet supplemented with 1 g/kg probiotic powder (Bacillus subtilis), BM+BS: Basal diet supplemented with 0.5 g/kg prebiotic (β-glucan + mannanoligosaccharide) + 0.5 g/kg probiotic (Bacillus subtilis)

Abdel-Fattah and Fararh [28] who reported the low FCR in the synbiotic treatment group, followed by the probiotic treatment group. These improved performance indices that arise from synbiotic supplementation are likely the result of the stimulation of beneficial gut microbials. This synergistic effect stems from enhanced gut microbial fermentation and host nutrient utilization [33]. With the prospects of improving growth performance, synbiotics are a promising alternative to antibiotics [34].

Our study demonstrated that the synbiotic and probiotic groups achieved the highest slaughter weight, hot carcass weight, and cold carcass weight, respectively. Previous studies have also found that synbiotics positively affect carcass characteristics in broilers [28,35,36]. Cheng et al.[25] reported no synbiotic effect on carcass weight, although some studies have reported improvements in breast yield in the synbiotic group [25,28,37]. In our study, there were no significant differences in the percentages of cold and hot carcass, gizzard weight, or liver weight, consistent with the work by Sarangi et al.[16] In addition, Chumpawadee et al.[38] reported no probiotic effect on these parameters. Notably, gizzard weight was significantly higher in the synbiotic group, and the maximum heart weight was in the probiotic and synbiotic groups, consistent with the work by Tayeri et al.[39] who reported increased heart weight after synbiotic supplementation. This increased gizzard and heart weights can be attributed to the improved gut functionality and metabolic activity of the broiler chickens. Gizzard play a major role in the mechanical digestion of feed. The synergistic effect of the probiotic and prebiotic promoted higher FI through gut microflora and enzymes modulation, resulting in prolonged retention time of the feed in the upper digestive tract and greater muscle development of the gizzard for improved gastrointestinal functioning [40]. The increased heart weight in the synbiotic group likely indicates improved metabolic performance and the associated increase in circulatory demand. Birds in the supplemented groups showed higher FI, ADG and improved FCR and this improvement were highest in the synbiotic supplemented group. Birds exhibiting superior growth and feed efficiency require increased oxygen and nutrient supply, which may stimulate the cardiac muscle development as a physiological response.

Intestinal integrity and function in broilers are greatly affected by villus structure, specifically villus length and crypt depth, which characteristically regulate the capacity to absorb nutrients. A greater V/C ratio is highly correlated with increased intestinal integrity and efficiency of nutrient utilization [41]. Increased villus height enhances feed efficiency by increasing the area for nutrient uptake [42]. Synbiotics, probiotics, and prebiotics have been reported to increase villus length and the V/C ratio, mainly by reducing crypt depth [43]. The intestinal crypts are the places at the base of the villi where stem cells proliferate to replace the enterocytes. A shallow crypt indicated longer lifespan and a slower turnover rate of epithelial cells. Thus, lower crypt depth is considered as a positive indicator of intestinal health and functional maturity. In the present study, the addition of probiotic, prebiotic, and synbiotic supplements also resulted in the reduction in the depth of the crypts following the previous trend. Contrary to previous reports, the present study noted a decline in villus length with supplementation with these additives. Therefore, villus height alone is insufficient for evaluating the effects of probiotics, prebiotics, and synbiotics on intestinal health; it must be considered alongside other intestinal morphology and performance parameters. Extensive studies have shown that dietary supplementation with probiotics and synbiotics improves intestinal morphology as seen by significant increments in the V/C ratio [44,45]. In this study, the V/C ratio increased in the probiotic and synbiotic treatment groups. The performance improvements observed can be attributed to the ability of biological feed additives to modulate microbial populations and gastrointestinal pH, thereby enhancing nutrient absorption and feed utilization efficiency [46]. In our study, the V/C ratio was lowered in the prebiotic group. Although prebiotics generally enhance the V/C ratio [45,47], exceptions can occur depending on specific conditions. This underscores the fact that the effects of prebiotics and probiotics can vary based on species, dosage, and application methods. These findings suggest that probiotics, prebiotics, and synbiotics optimize gut health and mucosal efficiency rather than just only increasing villus height.

Meat color is the first sensory trait that the customer perceives and the most significant factor in product acceptance or rejection since meat color often relates to other quality factors such as freshness, nutritional value, maturity, or spoilage [48]. Modifying the dietary pattern has been the strategic approach towards meat quality improvement, particularly for broilers [24]. Myoglobin content and muscle tissue pH are two significant factors that determine meat color and color defects [49]. The L* value indicates meat brightness, with higher values representing paler colors, while higher a* and b* values reflect consumer desirable redness (freshness) and healthier pigmentation, respectively [50]. In this study, probiotic and synbiotic supplementation reduced breast muscle L* values (darker color) while increasing a* (redness) across all treated groups. Synbiotics further enhanced b* (yellowness) compared to controls. Consistent with the findings of the present study, it has also been reported that supplementation with *B. subtilis* increased the a* and b* values of meat color [4,24]. These changes in the meat color parameters can be explained

by the modulatory effects of the probiotic, prebiotic and synbiotic on the gut health, antioxidant status and muscle metabolism. The probiotic and prebiotic supplementation enhances the intestinal integrity and nutrient absorption including pigments such as carotenoids and xanthophylls that contribute to b* values of the meat [51]. moreover, improved a* values of meat color can be attributed to the higher blood circulation, antioxidant enzyme activity, and myoglobin stability. Probiotic, prebiotics and synbiotics are known to decrease the antioxidant levels, reducing lipid peroxidation in the muscle tissue, and prevent oxidation of myoglobin in the muscles resulting in a higher a* values of meat [52]. The lower lightness in the supplemented groups suggests that meat retained better muscle pigment integrity that resulted in darker and more natural color. Tavaniello et al.[53] reported decreased a* values following prebiotic supplementation in broilers, whereas conflicting results were observed by other studies, which reported increased L* values [4, 24]. However, no differences among meat color parameters were observed in some studies [54-56].

Meat quality is based to a great extent on the pH level of the rigor mortis process that encompasses the biochemical reactions occurring as the muscle tissue is converted to meat following slaughter [49]. In the current research, the pH level of the meat was significantly higher in the prebiotic, probiotic, and synbiotic supplemented groups. Meat pH findings are extremely variable in the previously reported literature. While there have been researchers who have observed the trend to be decreasing [4,24], some researchers observed the trend to be increasing [55], and a few have observed no significant impact [54,56]. In broilers, muscles with pH values above 6.0 contain minimal protein denaturation that manifests as low light scattering and translucency. In contrast, muscles with pH values below 6.0 contain increased protein denaturation that causes opaque appearance and increased light scattering [49]. Optical properties of meat that depend on pH influence the light reflected by internal and external surfaces. Light scattering has minimal influence on color properties such as redness (a*) and yellowness (b*) but has large impacts on meat brightness (L*) and pigment concentration [57]. Low pH poultry meat has also been associated with low water holding capacity that causes increased shelf life but reduced tenderness [58].

This research tested the impact of probiotic, prebiotic, and synbiotic supplementation on broiler growth performance, meat quality, and intestinal health. Results indicated that probiotic, prebiotic, and synbiotic supplementation improved growth performance in the broilers, with maximum improvement observed in the synbiotic groups. Generally, synbiotic supplementation resulted in higher increases in BW, BWG, and ADFI, as well as improving the feed conversion ratio. Intestinal morphology, as measured in terms of the V/C ratio, improved in the probiotic group as well as in the synbiotic group. In addition, breast meat color and pH was high in prebiotic, probiotic, and synbiotic supplemented broilers relative to the unsupplemented group. In conclusion, based on the findings, synbiotic supplementation can be an effective management system for improving broiler growth performance, intestinal health, as well as increasing the quality of the meat, in meeting the increasing demand for high quality chicken meats.

Declarations

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