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Effects of Feed Restriction and Prebiotic Supplementation on Growth Performance, Immune Responses, Microbial Population, and Intestinal Morphology of Broiler Chickens

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Abstract

This study examined the effects of feed restriction and prebiotic supplementation on broiler chickens' performance, immune response, gut microbiota, and intestinal morphology. A total of 240 one-day-old male Ross 308 broilers were randomly assigned to four treatments: control (C), feed restriction (FR), prebiotic supplementation (P), and a combination of prebiotic supplementation with feed restriction (FR+P). The prebiotic contained mannan-oligosaccharides (MOSs) and β-glucans at 0.1% of the diet. Feed restriction was set at 80% ad libitum intake during the second week. The findings indicated that prebiotic supplementation increased the feed intake and weight gain, particularly during the early growth phase (P<0.05). Feed restriction resulted in low daily weight gain and impaired cellular immune response (P<0.05); however, these detrimental effects were partially mitigated by prebiotic supplementation. The FR+P group at 21 days had higher counts of *Lactobacillus* with lower coliform counts in the cecum (P<0.05). Moreover, prebiotic supplementation improved intestinal morphology as indicated by an increase in villus height and crypt depth of the duodenum and jejunum with a significant effect in the FR+P and P groups (P<0.05). The FR+P group recorded the highest villus height of the duodenum at 21 days, whereas the highest jejunal villus height and duodenal crypt depth were observed in the P group at 42 days (P<0.05). The cell-mediated immunity, as determined by footpad swelling following the injection of PHA, was greatly enhanced in birds fed prebiotics compared to birds with feed restriction alone (P<0.05). Overall, prebiotics, particularly with feed restriction, enhanced gut health, immune function, and intestinal morphology, suggesting their potential in broiler production.

Introduction

The broiler chicken industry is one of the most important sectors of modern agriculture, playing a vital role in providing animal protein for the growing global population. However, this industry faces numerous challenges, including health issues such as sudden death syndrome, ascites, skeletal abnormalities, and excessive fat deposition at slaughter ages. These problems not only reduce flock performance but also impose significant economic costs on producers (Sahraei, 2014). One proposed solution to mitigate these issues is the implementation of feed restriction during the early life of broiler chickens. Studies have shown that feed restriction can improve feed efficiency, reduce losses of sudden death syndrome, decrease the incidence of ascites and skeletal abnormalities, and reduce abdominal and carcass fat at slaughter ages (Sahraei, 2014; Tumova *et al.*, 2022). However, feed restriction also has disadvantages. This method induces stress in birds

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due to reduced growth rates, which can negatively impact the gut microbiota (Rahimi-Ratki *et al.,* 2012). The gut microbiota plays a crucial role in the health and nutritional status of poultry. This microbial community directly and indirectly influences gastrointestinal morphology, nutrient absorption, control of intestinal pathogens, and the immune system. However, the gut microbiota is relatively unstable and can easily be disrupted by stress factors (Mandal *et al.,* 2020). Therefore, finding solutions to alleviate the negative effects of feed restriction on gut microbiota has great importance.

Prebiotics have been suggested as one possible approach to address this problem and are increasingly applied in poultry production to alleviate environmental stressors. These compounds can enhance growth performance and overall poultry health by improving gut health through modulation of the microbiota, enhancing digestion and nutrient absorption, and inhibiting the activity and proliferation of pathogens (Yaqoob *et al.,* 2021). Mannan-oligosaccharides (MOS) are one of the important groups of prebiotics that function by binding and removing pathogens from the gastrointestinal tract and stimulating the immune system. These compounds provide specific binding sites (such as D-mannose) for intestinal pathogens, reducing their likelihood of attaching to the gut wall and thereby improving gut health (Griggs and Jacob, 2005).

Given the importance of feed restriction in reducing health issues in broiler chickens and the role of prebiotics in improving gut health and immune responses, we hypothesized that supplementing prebiotics containing MOS and β-glucans would mitigate the negative effects of early feed restriction in broilers. To the best of our knowledge, while numerous studies have separately examined either feed restriction or prebiotic supplementation, little is known about their combined effects and how prebiotics may serve as a compensatory nutritional strategy during feed restriction. Thus, this study investigates the effects of a prebiotic combination based on mannan-oligosaccharides and β-glucans on performance, immune responses, gut microbial population, and intestinal morphology of broiler chickens under feed restriction conditions.

Material and methods

All animal-related procedures in this study were performed in compliance with the general ethical guidelines established by the Iranian Council of Animal Care (1995). Ethical standards concerning animal welfare were rigorously maintained throughout the duration of the experiment.

A total of 240 one-day-old male Ross 308 broiler chicks were used in a completely randomized design with four treatments and six replicates per treatment. The experimental treatments included the following: 1) control group (C), 2) feed restriction (FR), 3) prebiotic supplementation without feed restriction (P), and 4) combined prebiotic supplementation and feed restriction (FR+P). The diets ingredients and nutrients are presented in Table 1. The prebiotic used in the study was obtained from TechnoMOS (Biochem, Lohne, Germany). It is a combination of mannan-oligosaccharides (MOSs) and β-glucans, which were added to the diet at a rate of 0.1%. During the second week of rearing, the feed intake of the designated groups was restricted to 80% of the intake of the ad libitum fed groups.

Weekly measurements of weight gain and feed intake were performed from weeks 2 to 6. Feed consumption was recorded starting in week 2 due to feed restriction, and the feed conversion ratio (FCR) was calculated. The average final body weight of each replicate was recorded weekly.

On days 21 and 42, one chick per replicate was euthanized for cecal microbial and intestinal morphology analyses. Cecal contents were serially diluted (10^{-1} to 10^{-6}) in quarter-strength Ringer's solution. Lactobacilli were enumerated using the pour plate method with 20 mL of sterilized MRS-A medium, incubated at 33°C for 3 days, and CFU/g calculated considering dilution and sample volume. For coliforms, 1 mL of each dilution was mixed with 20 mL of sterilized VRB-A medium in sterile Petri dishes, incubated at 37°C for 24h.

For intestinal morphology assessment, a 2.5 cm segment was excised from the midpoint of each small intestine section (duodenum, jejunum, and ileum). These segments underwent fixation in 10% formalin, dehydration, xylene clarification, paraffin embedding, molding, microtome sectioning, slide preparation, and staining. Villus dimensions were then measured using a light microscope.

To evaluate humoral immunity, two chicks per replicate were given Newcastle disease vaccine via eye drops and a 5% sheep red blood cell (SRBC) suspension (0.2 mL) via intramuscular injection on day 7. Antibody titers against the vaccine and SRBCs were measured on days 17 and 27. For cellular immunity assessment, two chicks per cage were randomly selected at 15 and 30 days of age. The webbing thickness between the second and third toes of their right foot was measured with a calliper. Then, 0.1 mL of Phytohemagglutinin-P was injected into the foot web using an insulin syringe to assess basophilic sensitivity. After 24 hours, the thickness increase was remeasured.

Statistical analysis was performed using the General Linear Models (GLM) of SAS (SAS Institute, 2003), with mean comparisons conducted via Duncan's multiple range test.

Table 1: Composition and calculated nutrient content of diets fed in this experiment

Ingredient	Starter (0 to 21 d)	Grower (21 to 42 d)
Yellow corn (%)	54.7	62.25
Soybean meal (%)	35.5	29.73
Fish meal (%)	3.35	2.90
Soybean oil (%)	3.00	2.00
Dicalcium phosphate (%)	1.12	0.90
Limestone (%)	1.20	1.25
Common salt (%)	0.39	0.30
DL-Methionine (%)	0.14	0.07
Vitamin and Mineral premix* (%)	0.50	0.50
Prebiotic** (or Sand) (%)	0.10	0.10
Nutrients		
ME (Kcal/Kg)	3000	3075
CP (%)	21.68	19.26
Lys (%)	1.037	0.963
Met (%)	0.471	0.366
Met + Cys (%)	0.848	0.693
Ca (%)	0.943	0.867
$P_{av.}$ (%)	0.424	0.337
Na (%)	0.189	0.144

* Vitamin and Mineral premix provided per kilogram of diet: vitamin A, 360000 IU; vitamin D3, 800000 IU; vitamin E, 7200 IU; vitamin K3, 800 mg; vitamin B1, 720 mg; vitamin B9, 400 mg; vitamin H2, 40 mg; vitamin B2, 2640 mg; vitamin B3, 4000 mg; vitamin B5, 12000 mg; vitamin B6, 1200 mg; vitamin B12, 6 mg; choline chloride, 200000 mg; manganese, 40000 mg; iron, 20000 mg; zinc, 40000 mg; copper, 4000 mg; iodine, 400 mg; selenium, 80 mg.
** The prebiotic (TechnoMOS) has the same amounts of mannan-oligosaccharide and β-1,3-Glucan.

Results

The results of the weekly feed intake (Table 2) indicated that during the second week, as anticipated, there was a significant difference between the ad libitum fed group and the feed-restricted groups (P<0.05). The group receiving prebiotic (P) had the highest feed intake, and its difference from the other treatments was significant (P<0.05). The control group (C) also significantly differed from the FR and FR+P groups (P<0.05). Among the feed-restricted groups, the FR+P group had significantly greater feed intake than the FR group did (P<0.05). Throughout the following weeks, the P and FR+P groups consistently presented the highest feed intake. Throughout the entire experiment (from 7-42 days of age), the P group had greater feed intake than the other groups did, and the difference between the P and FR groups was significant (P<0.05). However, there was no significant difference between the P group and the FR+P or control groups. The other groups did not demonstrate significant differences compared to one another.

Table 2: Effects of experimental treatments on the weekly feed intake of broiler chickens (g/bird/day)

Treatment	Week 2	Week 3	Week 4	Week 5	Week 6	7-42 days
C	36.02^{b}	68.81	113.27^{ab}	130.30^{b}	160.93	101.87^{ab}
FR	26.70^{d}	68.69	106.71^{b}	129.69^{b}	159.01	98.15^{b}
P	28.28^{a}	73.94	117.57^{a}	142.34^{a}	159.51	106.33^{a}
FR+P	29.25^{c}	71.14	122.22^{ab}	140.50^{ab}	159.25	104.47^{ab}
P-value	< 0.001	0.596	0.022	0.040	0.993	0.047
SEM	0.458	2.891	3.483	3.778	4.982	2.089

C: Control (no feed restriction or prebiotic); FR: feed restriction; P: prebiotic supplementation; FR+P: prebiotic supplementation with feed restriction; SEM: standard error of the mean
$^{a-d}$: Within each column, means with different letters are significantly different (P<0.05).

The influence of experimental treatments on weekly body weight gain of broiler chickens is shown in Table 3. In the second week, the prebiotic-supplemented group (P) gained the highest weight that was significantly different from that of the control (C), feed restriction (FR), and combined (FR+P) groups (P<0.05). The FR group had the lowest weight gain, revealing the suppressing effect of feed restriction during this phase. No statistically significant treatment differences were observed during the third week, although the P group still had numerically higher weight gain than the other groups.

This pattern persisted from weeks 4 to 6, where, especially in week 5, the P group recorded higher weight gains compared to the rest of the treatments. Over the entire study period (7-42 days), the P group had the highest cumulative weight gain, which was significantly greater than that of the FR group ($P<0.05$). The FR+P and control groups gained intermediate weights with no significant differences between each other or the other groups.

Table 4 shows the effects of the various treatments on the weekly feed conversion ratio (FCR) of the broilers in the experimental groups. Significant differences were detected between treatments in weeks 2 and 4 ($P<0.05$), but no significant differences were detected in other weeks or over the entire period of 7–42 days. In week 2, the FCR of the feed-restricted groups was greater than that of the ad libitum-fed groups ($P<0.05$). In week 4, the FR+P group presented the highest FCR, and its difference from those of the control and FR groups was significant ($P<0.05$). However, throughout the entire period from 7-42 days, the experimental treatments did not significantly affect the feed conversion ratio (FCR).

Table 5 shows the effects of the experimental treatments on the cecal bacterial counts of the broilers. At 21 days of age, the FR+P group had the highest *Lactobacillus* count and the lowest coliform count ($P<0.05$), indicating the positive effect of the prebiotic used on the gut microbiota under feed restriction conditions. However, at 42 days of age, there were no significant differences in the cecal bacterial counts among the experimental groups.

Table 3: Effects of experimental treatments on the weekly weight gain of broiler chickens(g/bird/day)

Treatment	Week 2	Week 3	Week 4	Week 5	Week 6	7-42 days
C	26.08^{b}	52.70	79.29	64.62	85.11	61.56^{ab}
FR	24.93^{b}	51.38	73.99	63.14	78.17	58.32^{b}
P	27.88^{a}	55.40	76.38	70.91	85.13	63.14^{a}
FR+P	25.99^{b}	53.43	76.19	63.31	83.25	60.50^{ab}
P-value	0.039	0.295	0.431	0.200	0.316	0.030
SEM	0.603	1.314	1.757	2.623	2.977	1.238

C: Control (no feed restriction or prebiotic); FR: Feed restriction; P: Prebiotic supplementation; FR+P: Prebiotic supplementation with feed restriction; SEM: Standard error of the mean.
$^{a, b}$: Within each column, means with different letters are significantly different ($P<0.05$).

Table 4: Effects of experimental treatments on the weekly feed conversion ratio (FCR) of broiler chickens

Treatment	Week 2	Week 3	Week 4	Week 5	Week 6	7-42 days
C	1.39^{a}	1.31	1.44^{b}	2.03	1.91	1.66
FR	1.08^{b}	1.34	1.45^{b}	2.10	2.08	1.69
P	1.38^{a}	1.33	1.54^{ab}	2.04	1.87	1.68
FR+P	1.13^{b}	1.34	1.61^{a}	2.22	1.91	1.73
P-value	< 0.001	0.977	0.041	0.557	0.395	0.803
SEM	0.034	0.071	0.046	0.101	0.090	0.052

C: Control (no feed restriction and no prebiotic); FR: feed restriction; P: prebiotic supplementation; FR+P: prebiotic supplementation with feed restriction
$^{a, b}$: Within each column, means with different letters are significantly different ($P<0.05$).

Table 5: Effects of experimental treatments on the cecal bacterial counts of broilers at 21 and 42 days of age (log CFU/gr).

Treatment	*Lactobacillus*		Coliforms	
	21 days	42 days	21 days	42 days
C	7.99^{b}	7.97	7.82^{ab}	7.52
FR	8.07^{b}	7.85	8.18^{a}	7.94
P	8.20^{ab}	8.18	7.91^{a}	7.75
FR+P	8.34^{a}	7.92	7.41^{b}	7.80
P-value	0.048	0.635	0.016	0.624
SEM	0.833	0.147	0.161	0.188

C: Control (no feed restriction or prebiotic); FR: Feed restriction; P: Prebiotic supplementation; FR+P: Prebiotic supplementation with feed restriction
$^{a, b}$: Within each column, means with different letters are significantly different ($P<0.05$).

Tables 6 and 7 present the effects of the experimental treatments on the morphological characteristics of the small intestine in broilers. At 21 days of age, the FR+P group presented the greatest villus length in the duodenum, which was significantly different from that of the control group ($P<0.05$). Additionally, the

control group presented the greatest crypt depth and the lowest villus height–crypt depth ratio in the duodenum at 21 days of age, which was significantly greater than that of the FR group ($P<0.05$). In the groups receiving prebiotics and undergoing feed restriction, the crypt depth in the jejunum at 21 days of age was significantly greater than that of the control group ($P<0.05$). The morphometric characteristics of the ileal villi did not significantly vary between experimental groups at day 21. At day 42, the highest duodenum crypt depth was observed in the P group; however, its superiority was only significant compared to the FR group ($P<0.05$). Alternatively, jejunum villus length was significantly greater in the P group than in the FR group ($P<0.05$). Also, the prebiotic- and feed restriction-associated group (FR+P) had the largest ileum villus height to crypt depth ratio and differed significantly from the control group ($P<0.05$).

Table 6: Effects of experimental treatments on the morphology of the small intestine of 21-day-old broilers

Treatment	Duodenum			Jejunum			Ileum		
	Villus height (µm)	Crypt depth (µm)	Villus height/Crypt depth	Villus height (µm)	Crypt depth (µm)	Villus height/Crypt depth	Villus height (µm)	Crypt depth (µm)	Villus height/Crypt depth
C	1146.4^{b}	258.03^{a}	5.11^{b}	1074.7	167.09^{b}	6.69	806.51	173.49	5.22
FR	1344.1^{ab}	173.14^{b}	8.21^{a}	1152.4	233.09^{a}	5.28	750.80	168.36	5.05
P	1276.5^{ab}	214.59^{ab}	6.21^{ab}	1302.6	264.72^{a}	4.97	814.63	195.28	4.52
FR+P	1462.2^{a}	214.56^{ab}	7.25^{ab}	1200.8	232.53^{a}	5.56	834.34	179.58	4.70
P-value	0.038	0.045	0.038	0.258	0.009	0.209	0.190	0.777	0.828
SEM	94.766	22.791	0.904	80.430	19.628	0.612	27.214	19.129	0.591

C: Control (no feed restriction and no prebiotic); FR: feed restriction; P: prebiotic supplementation; FR+P: prebiotic supplementation with feed restriction

$^{a, b}$: Within each column, means with different letters are significantly different ($P<0.05$).

Table 7: Effects of experimental treatments on the morphology of the small intestine of 42-day-old broilers

Treatment	Duodenum			Jejunum			Ileum		
	Villus height (µm)	Crypt depth (µm)	Villus height/Crypt depth	Villus height (µm)	Crypt depth (µm)	Villus height/ Crypt depth	Villus height (µm)	Crypt depth (µm)	Villus height/Crypt depth
C	1549.4	201.18^{ab}	7.69	1204.9^{ab}	202.32	6.25	768.34	192.43^{a}	4.13^{b}
FR	1394.0	193.44^{b}	7.68	1147.6^{b}	216.32	5.54	707.29	135.33^{b}	5.40^{ab}
P	1712.7	255.24^{a}	7.07	1344.5^{a}	242.28	5.93	820.06	167.30^{ab}	5.09^{ab}
FR+P	1513.9	217.51^{ab}	7.21	1255.6^{ab}	211.62	6.33	834.56	149.48^{ab}	6.10^{a}
P-value	0.259	0.025	0.877	0.047	0.564	0.761	0.161	0.043	0.034
SEM	112.268	18.212	0.663	53.586	21.146	0.592	43.624	14.419	0.503

C: Control (no feed restriction or prebiotic); FR: feed restriction; P: prebiotic supplementation; FR+P: Prebiotic supplementation with feed restriction

$^{a, b}$: Within each column, means with different letters are significantly different ($P<0.05$).

Table 8: Effects of experimental treatments on the humoral and cellular immune parameters of broilers

Treatment	Antibody titer against the Newcastle vaccine		Antibody titer against SRBC		Increase in footpad thickness after Phytohemagglutinin injection (mm)	
	17 days	27 days	17 days	27 days	16 days	31 days
C	3.50	5.12	1.62^{b}	3.00	0.71a^{b}	0.73^{ab}
FR	2.87	4.25	2.12^{ab}	3.19	0.59^{b}	0.51^{c}
P	3.12	4.87	2.00^{ab}	3.06	0.87^{a}	0.78^{a}
FR+P	3.00	4.69	2.50^{a}	3.56	0.79^{a}	0.60^{bc}
P-value	0.490	0.380	0.030	0.775	0.009	0.002
SEM	0.287	0.374	0.228	0.408	0.058	0.053

C: Control (no feed restriction or prebiotic); FR: feed restriction; P: prebiotic supplementation; FR+P: Prebiotic supplementation with feed restriction

$^{a, b, c}$: Within each column, means with different letters are significantly different ($P<0.05$).

Table 8 shows the effects of the experimental treatments on the humoral and cellular immune parameters of the broilers. According to the results, there was no significant difference among the experimental treatments in terms of the antibody titer against the Newcastle vaccine. However, the antibody titer against SRBC in the FR+P group at 17 days of age was significantly different from that in the control group ($P<0.05$), whereas the other treatments did not significantly differ. At 27 days of age, no significant

differences were observed among the treatments in terms of the antibody titer against SRBC. However, the experimental treatments resulted in significant differences in hypersensitivity to Phytohemagglutinin injection at both time points ($P<0.05$). At 16 days of age, the groups receiving prebiotics presented greater increases in footpad thickness than the FR group did. At 31 days of age, the feed-restricted groups presented the lowest increase in footpad thickness, and their difference from that of the P group was significant. Additionally, the control group also showed a significant difference compared with the FR group.

Discussion

Our findings suggest that prebiotic supplementation, whether alone or in combination with feed restriction, positively influences feed intake. The greater feed intake in the P and FR+P groups may be attributed to the beneficial effects of prebiotics on gut health and nutrient absorption, which could increase appetite and feed utilization. The absence of significant differences between the P group and the control or FR+P groups indicates that prebiotics alone can effectively improve feed intake without the need for feed restriction. In agreement with the findings of this study, others reported a slight improvement in final weight without a significant effect on the feed conversion ratio when 0.5% mannan-oligosaccharides (MOSs) were used in the diet (Iji *et al.,* 2001). Similarly, Waldroup *et al.* (2003) reported no significant differences in body weight or the feed conversion ratio when MOS was used over 42 days. In contrast, Yang *et al.* (2007) reported that different levels of MOS (0.05%, 0.1%, and 0.2% of the diet) improved the growth performance of broilers, although their effect diminished with age. Additionally, Mohamed *et al.* (2008) compared the use of MOS at 0.1% for up to 28 days and 0.05% for up to 42 days with a control diet and reported that MOS improved the weight and feed conversion ratio. The discrepancies observed in various experiments may be due to differences in the type of prebiotic used, dosage, rearing conditions, and other influencing factors. However, in most cases, prebiotics did not significantly affect poultry performance when stress factors were not present in the experiment. In addition, Novele *et al.* (2009) reported that limiting feed intake to 75% of ad libitum levels during the early phase adversely affects the final weight of broilers. Rincon (2000) reported reductions in body weight of 7%, 14%, and 17% with feed restrictions of 95%, 90%, and 85%, respectively. In contrast, Onbaşılar *et al.* (2009) noted that although the weight of feed-restricted chicks was lower at 21 days of age, no significant differences were observed between the feed-restricted and ad libitum-fed groups at the conclusion of the study. Also, Karar *et al.* (2023) reported that adding prebiotic (MOS and beta-glucan) can partially alleviate the negative effects of high stocking density on broiler production performance. Another study demonstrated that dietary prebiotic supplementation significantly enhances production performance in heat-stressed broilers due to the greater metabolic activity in the intestine (Islam *et al.*, 2024). It seems that prebiotics could improve the performance of broilers by reducing the harmful effects of stress on the intestinal microbial population and gut morphology (Mandal *et al.*, 2020; Islam *et al.*, 2024).

Several studies in broilers have reported that the use of MOS increases the population of *Lactobacillus* and Bifidobacterium in the cecum (Fernandez *et al.*, 2002; Baurhoo *et al.*, 2007). The prebiotic used in this study, which contains MOSs, likely facilitates the elimination of harmful gut bacteria. Reducing the populations of these detrimental bacteria creates a favorable environment for beneficial bacteria, such as *Lactobacillus*, to thrive and proliferate (Griggs and Jacob, 2005). Furthermore, reports indicate that feed restriction can negatively impact the immune system, particularly cellular immunity (Hangalapura *et al.*, 2005; Savino *et al.*, 2010). This may explain the observed increase in coliform counts at 21 days of age. However, by 42 days of age, the adverse effects of early feed restriction on the immune system likely diminished, resulting in no significant differences in coliform counts among the experimental groups.

The morphological alterations in intestinal architecture caused by prebiotic supplementation are likely to be mediated by enterocyte-gut microbiota interactions. Earlier research demonstrated that some groups of gut microbes have significant impacts on the growth patterns of intestinal villi. The enhanced villus length noted with prebiotic treatment in this study may be due to enhanced proliferation or hypertrophy in intestinal epithelial cells (Rahimi *et al.*, 2009). Increased villus height is also seen in the increased surface area, which has potentially greater ability for absorption of nutrients (Awad *et al.*, 2009). Consistent with this, Pourian *et al.* (2025) found greater villus height and surface area at 42 days of age following probiotic supplementation. Similar to probiotics, prebiotics are known for their ability to inhibit the growth of pathogenic and nonpathogenic bacteria in the intestines, and in doing so, may contribute to the increase in villus surface area (Ebeid *et al.*, 2022).

In summary, prebiotic fermentation to short-chain fatty acids (SCFAs), including butyric acid, promotes enterocyte growth, increases villus height, enhances villus height/crypt depth ratio, and fortifies the intestinal epithelial barrier via improved tight junction integrity (Swaggerty *et al.*, 2019; Yaqoob *et al.*, 2021). Such gastrointestinal tract morphological promotion maximizes the efficiency of feed intake

and delivers a protective barrier to intestinal pathogens. These benefits are realized with preservation of epithelial cell integrity, reduction of endotoxin permeability, and reduction of susceptibility to infection by pathogens.

Feed restriction during the early growth period led to an increase in the VH/crypt depth ratio in the duodenum at 21 days of age and a reduction in crypt depth in the ileum at 42 days of age. The effect of feed restriction on the VH/crypt depth ratio may be due primarily to a reduction in crypt depth rather than a significant effect on villus length. Overall, the intensity of feed restriction applied in this study did not significantly affect intestinal morphological characteristics. This result may be due to the low intensity of restriction being insufficient to impact intestinal traits or the intestinal adaptive response to the feed restriction method used in this study. Previous histomorphological research (Metzler-Zebeli *et al.*, 2019) has generally indicated that feed restriction (FR) does not adversely impact most intestinal histomorphological parameters. In contrast, Bentley *et al.* (2020) observed that early feed restriction in Pekin ducks resulted in decreased villus height, villus width, villus surface area, muscle thickness, and the villus height-to-crypt depth ratio at 14 days of age. Additionally, nutrient density appears to influence intestinal development, as reduced nutrient density has been associated with a decline in jejunal epithelial cell numbers and diminished expression of digestive enzymes and nutrient transporters (Amoozmehr *et al.*, 2023).

In this study, prebiotics had a positive effect, whereas feed restriction had a negative effect on the cellular immune response to phytohemagglutinin injection. Hangalapura *et al.* (2005) reported that feed restriction reduces the cellular immune response because the cellular components of the immune system require more energy than the humoral components do. On the other hand, Hooge (2004) noted that the positive effects of mannan-oligosaccharides are more pronounced under stressful conditions. In this study, the use of prebiotics also led to an increase in the cellular immune response against the stress factor of feed restriction. The humoral immune response in this study was not significantly affected by the experimental treatments. Silva *et al.* (2009) also reported no increase in the titer of antibodies against the Newcastle vaccine as a result of the use of prebiotics. Additionally, Fanoosi and Torky (2010) reported no reduction in the antibody titer against the Newcastle vaccine with 90% feed restriction, whereas Jahanpour (2012) reported that 75% feed restriction during the second week reduced the antibody titer against the Newcastle vaccine. These differences may be due to the intensity of the feed restriction applied.

Conclusion

Overall, the results of this study showed that feed restriction, despite its numerous benefits, can weaken the immune system, particularly its cellular components. Therefore, under such conditions, the use of immune-boosting compounds such as prebiotics has beneficial effects. The prebiotic used in this study had some effect on stimulating appetite, increasing feed intake, and consequently improving daily weight gain, but it did not improve the feed conversion ratio. Additionally, the feed-restricted groups, despite consuming less feed, presented lower daily weight gain and a similar feed conversion ratio than the ad libitum-fed groups. Furthermore, the use of prebiotics under feed restriction conditions had beneficial effects on the gut microbiota and gastrointestinal morphology of broilers.

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