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RESEARCH ARTICLE

Effects of Co-Supplementation of β-Galacto-oligosaccharides and Methionine on Production Performance, Blood Metabolites, and Gut Histomorphometry in Broilers

Sohrab Ahmad[1], Muhammad Shahbaz Yousaf[1*], Sajid Khan Tahir[1], Ghulam Mustafa[2], Khalid Abdul Majeed[1], Muhammad Afzal Rashid[3], Hafsa Zaneb[4], Riaz Mustafa[5] and Habib Rehman[1]

[1]Department of Physiology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[2]Department of Pathology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[3]Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Pakistan
[4]Department of Anatomy and Histology, University of Veterinary and Animal Sciences, Lahore, Pakistan
[5]University of Agriculture, Faisalabad, Sub-Campus Toba Tek Singh, Pakistan
*Corresponding author: drmshahbaz@uvas.edu.pk

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A B S T R A C T

The study was conducted to investigate the role of β-Galacto-oligosaccharides (β-GOS) and methionine on production performance, blood metabolites, and intestinal microarchitecture in broilers. A total of 288, one-day-old, chicks were distributed in a 3×2 factorial arrangement (6 replicates/group) based on three dietary levels of β-GOS (0, 0.2, and 0.5%) and two levels of methionine (0.5 and 1%). Birds were kept under standard husbandry conditions till day 35. In the end, two birds from each replicate were exsanguinated to collect the blood samples, viscera, and portions of each segment of the small intestine for histology. Results of the main effects revealed that FCR was improved ($P<0.05$) in the β-GOS supplementation groups during the third week. A dose-dependent increase ($P<0.05$) in globulins levels with an increase in β-GOS concentrations was observed. A high level of methionine (1%), however, increased ($P<0.05$) the creatinine concentration. The findings of interaction effects between β-GOS and methionine revealed increased ($P<0.05$) body weights during the third, fourth, and fifth weeks along with higher feed intake during the fifth week only in the birds supplemented with 0.2% β-GOS and 0.5% methionine. Moreover, the birds in 0.5% of β-GOS and 0.5% of methionine showed increased ($P<0.05$) crypt depth and reduced ($P<0.05$) VH:CD in the jejunum. In conclusion, 0.2% β-GOS supplementation in the diets containing a basal level of methionine (0.5%) confers some benefits to the broilers. Moreover, the extra topping of methionine did not show any benefits.

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INTRODUCTION

In the recent past, consumers' concern about the safety and nutritional value of food and its ingredients has increased greatly across the globe. This urges the need to develop such practices that enhance both the quantity and quality of poultry products (Kamel and Mohamed, 2016). These targets could be met by optimizing certain key factors including genetics, feed quality, housing environment, and health status of broilers. Among the health-associated factors, gut health is of prime importance and received greater attention from scientists working in the field of poultry production (Saki *et al.,* 2011). The gut is mainly responsible for the digestion, absorption, and metabolism of nutrients, therefore, any physiological or pathological impairment in the gut compromises the performance of birds (Yousaf *et al.,* 2016). To ensure gut health and consequently promote growth, sub-therapeutic levels of antibiotics were added into poultry feed. However, after being banned by the European Commission in 2006, antibiotic growth promoters were replaced by non-antibiotic feed supplements, including vitamins, minerals, enzymes, amino acids, organic acids, phytobiotics, probiotics, prebiotics, and synbiotics (Yousaf *et al.,* 2016).

Prebiotics are non-digestible oligosaccharides that, upon reaching the hindgut, are fermented by the gut microbiota to produce short-chain fatty acids (Rehman *et al.,* 2007). The short-chain fatty acids act as anti-inflammatory substances and also repair the intestinal, liver, and kidney histological attributes (Rashid *et al.,* 2019). β-Galactooligosaccharide (β-GOS) is produced by the action of galactosidases originated from *Lactobacillus* and contain β (1-6) and β (1-3) glycosidic linkages (Ashraf *et al.,* 2017). β-GOS exhibits positive effects on the growth performance (Yousaf *et al.,* 2016), intestinal microbial ecology, immune response (Yousaf *et al.,* 2017), and gut microarchitecture (Ashraf *et al.,* 2017, Varasteh *et al.,* 2015) in broilers. Methionine, an essential amino acid, is responsible for protein synthesis and affects lipid metabolism (Bouyeh and Gevorgyan, 2011). Methionine supplementation enhances protein utilization, feed efficiency, and ultimately growth performance, and intestinal histology of broilers (Zhang *et al.,* 2017; Nazem *et al.,* 2019).

Numerous studies are conducted on dietary supplementation of β-GOS and methionine that showed improvement in production parameters and intestinal attributes of broilers. However, studies regarding the combined use of β-GOS and methionine are limited. Therefore, the present study was conducted to evaluate the co-supplementation of β-GOS and methionine at different dietary levels on the growth performance, blood metabolites, and gut histomorphometry in broilers. We hypothesized that co-supplementation of β-GOS and methionine may help in improving the gut histology and liver protein anabolic functions, leading to higher growth performances in broilers under standard managemental conditions.

MATERIALS AND METHODS

This study was approved by the Ethical Review Committee of the University of Veterinary and Animal Sciences, Lahore-Pakistan (DR/324/02/04/2018).

Experimental diet, design, and husbandry: Methionine was purchased from the commercial market (Sumitomo Chemicals, Japan). The β-GOS was prepared at the Department of Food Science and Human Nutrition, UVAS, Lahore by the method described elsewhere (Splechtna *et al.,* 2007). In this process, β-galactosidases originating from *Lactobacillus reuteri L103* were used for the transgalactosylation of lactose.

One-day-old, broiler chicks (n=288) were procured from a local hatchery. The experiment was arranged in a 3×2 factorial arrangement. Birds were randomly divided into six groups (n=48/group) and 6 replicates (n=8/ replicate). The dietary treatments are comprised of three levels of β-GOS (0, 0.2, and 0.5%) and two levels of methionine (0.5% and 1%). Birds were provided with a corn-based diet in mash form (Table 1). Feed and water were provided *ad libitum*. Birds were reared under standard husbandry conditions.

Growth performance: Performance traits were recorded in terms of body weight (BWT), feed intake (FI), and feed conversion ratio (FCR). BWT and FCR were recorded weekly, while FI was determined daily by subtracting the feed leftover from the feed offered.

Sample collection: On day 35, two birds per replicate were killed by exsanguination and blood samples were collected. After centrifugation, serum samples were stored at -40°C for biochemical analyses. The viscera were collected to determine relative weights or lengths. Intestinal segments were preserved in 10% neutral buffered formalin for histological studies.

Biochemical analysis: The serum glucose, total cholesterol, HDL-C, triglycerides, ALT, AST, ALP, BUN, creatinine, total proteins, and albumin were determined by commercial kits (DiaSys, GmBH, Germany). Serum globulins were determined by subtracting the albumin concentrations from the total proteins concentrations. The readings were taken with the Epoch™ micro-plate spectrophotometer (Biotek Instruments Inc., Winooski, USA).

Intestinal histomorphometry: The samples preserved in buffered formalin were processed and stained according to the conventional hematoxylin and eosin method. The tissue slides were observed under a light microscope (Olympus CX31, Olympus, Center Valley, Pennsylvania, USA), equipped with a digital camera (Olympus DP20, Olympus USA). Five well-oriented and intact villi were observed. The area from the tip of the villus to the crypt junction was recorded as villus height, while the region from the base of the villi up to the transition between the villus and the crypt was recorded as crypt depth. The villus surface area was determined by applying the formula $(2\pi)(VW/2)(VH)$, where VW is villus width and VH is the villus height. VH, VW, and CD were expressed as µm, while VSA was expressed as mm^2.

Statistical analysis: For statistical analysis, the data were arranged in a 3×2 factorial arrangement and subjected to the ANOVA under the GLM procedure (SPSS version 20.0, IBM Inc. USA). The main and interaction effects of the treatments were determined. Duncan's post hoc test was applied to measure the group differences at $P<0.05$.

Table 1: Feed composition

Ingredients %	0.5% Met	1% Met
Corn	46.5	46.5
Rapeseed cake	5	5
Sunflower meal	3.5	3.5
Maize gluten (30%)	10	10
Soybean meal	15	15
Mineral mixture	0.28	0.28
DCP	1	1
Limestone	1.51	1.51
Oil	1	1
Molasses	6	5.5
Lysine	0.12	0.12
Methionine	0.18	0.69
Canola meal	10	10
CP	20.51	20.79
M.E	2703 Kcal/kg	2722 Kcal/kg
Methionine	0.5	1
Lysine	0.85	0.85
Calcium	0.972	0.972
Phosphorous	0.37	0.37

RESULTS

Production performance: The main effects of the β-GOS and methionine on body weight (BWT) and feed intake (FI) were non-significant compared to the control group (Table 2 & 3). However, the interaction between the two treatments on the BWT and FI was significant. It was observed that 0.2% β-GOS and 0.5% methionine improved (P<0.05) BWT during the third, fourth, and fifth weeks of age (Table 2) and FI during the last week only (Table 3). The main effects of supplementations on FCR were significant and it was observed that FCR was improved (P<0.05) in birds supplemented with 0.2% and 0.5% β-GOS during the third week, whereas methionine supplementation did not affect FCR (Table 4).

Viscera development: The relative weight of the spleen was higher (P<0.05) in the 0.5% β-GOS group (Main effects) compared with the control and 0.2% β-GOS groups. Moreover, the relative weight of the proventriculus was lower (P<0.05) in the 0.2% β-GOS group (Main effects) compared with the control group. However, other studied organs were not affected by any treatment (Table 5).

Blood metabolites: The results demonstrated that β-GOS supplementation (Main effects) significantly affected the concentrations of albumin and globulins along with the A/G ratio. The concentrations of globulins were increased with an increase in the concentration of β-GOS, whereas the reverse was true for the albumin concentration and A/G ratio. All other studied metabolites remained unchanged in all the treatment groups (Table 6).

Intestinal histomorphometry: The results of the main effects of treatments indicated that neither β-GOS nor methionine affected the histological attributes of different segments of the small intestine (Table 7). However, in the jejunum, a significant interaction between the β-GOS and methionine was observed on crypt depth and VH:CD ratio (Table 7). The birds in the 0.5% of β-GOS and 0.5% of methionine showed increased (P<0.05) crypt depth and reduced (P<0.05) VH:CD.

DISCUSSION

Growth performance is mainly attributed to a healthy and efficient gastrointestinal tract that helps in the absorption of nutrients and protein deposition as muscles. At present we are reporting the effects of a prebiotic and methionine at different concentrations in broilers. In the present experiment, it was observed that dietary supplementation of 0.2% β-GOS and 0.5% methionine had significant interaction and increased the body weight

Table 2: Effects of β-GOS and methionine supplementation on body weights

Age	β-GOS 0%		β-GOS 0.2%		β-GOS 0.5%		P-Value		
	MET 0.5%	MET 1%	MET 0.5%	MET 1%	MET 0.5%	MET 1%	β-GOS	MET	β-GOS×MET
Week 1	138±6	144±3	147±2	144±2	138±3	137±1	0.083	0.710	0.387
Week 2	382±12	408±31	399±6	362±8	382±12	358±5	0.294	0.365	0.114
Week 3	763±34^{b}	808±19^{ab}	849±12^{a}	767±13^{b}	784±25^{b}	764±13^{b}	0.271	0.280	0.021
Week 4	1260±58^{b}	1320±38^{ab}	1374±15^{a}	1255±19^{b}	1285±29^{ab}	1245±25^{b}	0.369	0.248	0.047
Week 5	1774±74^{ab}	1876±62^{ab}	1909±23^{a}	1738±29^{b}	1753±37^{b}	1753±43^{b}	0.252	0.571	0.030

Table 3: Effects of β-GOS and methionine supplementation on feed intake

Age	β-GOS 0%		β-GOS 0.2%		β-GOS 0.5%		P-Value		
	MET 0.5%	MET 1%	MET 0.5%	MET 1%	MET 0.5%	MET 1%	β-GOS	MET	β-GOS×MET
Week 1	111±6	117±4	123±2	118±3	111±5	107±5	0.045	0.664	0.385
Week 2	352±22	359±13	372±10	330±6	342±12	325±9	0.229	0.111	0.199
Week 3	631±25	654±21	682±12	618±9	617±19	612±17	0.145	0.305	0.073
Week 4	839±49	859±37	903±24	817±13	836±23	844±37	0.837	0.474	0.229
Week 5	948±39^{b}	1033±44^{ab}	1074±20^{a}	951±11^{b}	991±26^{b}	983±7^{b}	0.625	0.519	0.004

Table 4: Effects of β-GOS and methionine supplementation on FCR

Age	β-GOS 0%		β-GOS 0.2%		β-GOS 0.5%		P-Value		
	MET 0.5%	MET 1%	MET 0.5%	MET 1%	MET 0.5%	MET 1%	β-GOS	MET	β-GOS×MET
Week 1	1.16±0.02	1.14±0.04	1.22±0.02	1.18±0.02	1.18±0.04	1.12±0.04	0.200	0.128	0.815
Week 2	1.46±0.07	1.42±0.09	1.48±0.02	1.52±0.02	1.40±0.08	1.46±0.04	0.459	0.685	0.680
Week 3	1.66±0.07	1.67±0.11	1.50±0.03	1.50±0.03	1.54±0.05	1.52±0.02	0.034	0.894	0.982
Week 4	1.70±0.03	1.66±0.02	1.72±0.04	1.68±0.02	1.68±0.04	1.72±0.02	0.738	0.584	0.310
Week 5	1.88±0.15	1.88±0.04	2.00±0.03	1.96±0.09	2.16±0.02	1.94±0.07	0.261	0.305	0.522

Table 5: Effects of β-GOS and methionine supplementation on relative viscera index

Organ	β-GOS 0%		β-GOS 0.2%		β-GOS 0.5%		P-Value		
	MET 0.5%	MET 1%	MET 0.5%	MET 1%	MET 0.5%	MET 1%	β-GOS	MET	β-GOS×MET
Heart	0.46±0.02	0.46±0.01	0.45±0.03	0.45±0.03	0.44±0.02	0.42±0.02	0.419	0.632	0.828
Liver	2.57±0.12	2.66±0.15	2.62±0.11	2.73±0.11	2.51±0.13	2.66±0.10	0.765	0.252	0.974
Pancreas	0.23±0.01	0.22±0.01	0.22±0.01	0.22±0.02	0.65±0.41	0.22±0.01	0.350	0.302	0.343
Spleen	0.13±0.02	0.15±0.02	0.12±0.01	0.12±0.01	0.18±0.02	0.15±0.02	0.029	0.998	0.277
Bursa	0.19±0.02	0.20±0.02	0.19±0.02	0.17±0.02	0.18±0.02	0.18±0.02	0.402	0.299	0.580
Gizzard	1.92±0.11	1.91±0.09	1.85±0.05	1.84±0.07	1.82±0.19	1.84±0.10	0.743	1.000	0.984
Proventriculus	0.33±0.01	0.36±0.02	0.32±0.01	0.30±0.01	0.36±0.02	0.35±0.01	0.009	0.981	0.234
Small Intestine	1.84±0.10	1.90±0.08	1.98±0.09	1.92±0.10	2.08±0.09	2.06±0.06	0.087	0.923	0.789
Caecum	0.27±0.05	0.24±0.03	0.27±0.03	0.26±0.04	0.31±0.02	0.28±0.05	0.491	0.495	0.920
				Intestinal Lengths					
Small Intestine	0.45±0.07	0.37±0.04	0.37±0.05	0.33±0.01	0.35±0.01	0.34±0.01	0.206	0.186	0.592
Caecum	0.55±0.09	0.40±0.05	0.42±0.06	0.35±0.02	0.38±0.01	0.36±0.02	0.099	0.064	0.463

Table 6: Effects of β-GOS and methionine supplementation on blood metabolites

Parameter	β-GOS 0%		β-GOS 0.2%		β-GOS 0.5%		P-Value		
	MET 0.5%	MET 1%	MET 0.5%	MET 1%	MET 0.5%	MET 1%	β-GOS	MET	β-GOS×MET
Glucose (mg/dl)	296±14.3	319±24.2	329 ±13.6	342±11.9	290±19.4	317±15.7	0.132	0.142	0.923
Cholesterol (mg/dl)	98±2.87	89±4.44	102±3.07	103±4.99	100±6.91	112±6.57	0.060	0.716	0.121
HDL (mg/dl	66.6±4.05	64.1±2.42	67.4±3.38	63.9±2.38	66.3±3.50	63.2±3.78	0.963	0.266	0.988
TGs (mg/dl)	112±3.73	109±5.55	113±4.10	111±5.03	110±2.73	110±4.43	0.889	0.695	0.594
ALT (U/L)	7.03±0.94	8.32±0.57	6.05±0.50	5.88±0.23	6.32±0.84	8.10±1.04	0.067	0.116	0.394
AST (U/L)	56.8±5.47	51.0±3.71	59.0±6.98	41.8±5.36	52.7±3.54	51.5±3.62	0.778	0.510	0.261
ALP (U/L)	552±25.7	657±77.7	630±77.1	556±36.6	749±58.6	652±98.5	0.229	0.692	0.268
Total Proteins (g/dl)	4.78±0.14	4.81±0.10	4.93±0.08	4.76±0.08	4.98±0.21	5.02±0.15	0.432	1.000	0.512
Albumin (g/dl)	3.84±0.15	3.70±0.09	3.38±0.05	3.35±0.05	3.21±0.06	3.12±0.03	<0.001	0.187	0.772
Globulin (g/dl)	0.94±0.12	1.12±0.09	1.55±0.08	1.41±0.09	1.69±0.21	1.90±0.16	<0.001	0.448	0.390
A/G Ratio	2.61±0.03	2.84±0.12	2.26±0.15	2.46±0.16	2.14±0.23	1.76±0.16	0.001	0.903	0.155
BUN (mg/dl)	8.57±0.15	8.27±0.11	8.53±0.13	8.33±0.10	8.35±0.14	8.52±0.07	0.989	0.291	0.130
Creatinine (mg/dl)	0.21±0.03	0.33±0.02	0.25±0.03	0.31±0.03	0.27±0.02	0.27±0.02	0.916	0.010	0.097

Table 7: Effects of β-GOS and methionine supplementation on intestinal histomorphometry

Parameter	β-GOS 0%		β-GOS 0.2%		β-GOS 0.5%		P-Value		
	MET 0.5%	MET 1%	MET 0.5%	MET 1%	MET 0.5%	MET 1%	β-GOS	MET	β-GOS×MET
				Duodenum					
VH (µm)	1296±28	1364±101	1370±19	1265±27	1378±32	1236±76	0.890	0.131	0.091
VW (µm)	140±10.6	157±34.5	153±11.2	133±12.5	166±17.7	185±11	0.120	0.666	0.324
CD (µm)	135±3.54	175±13.0	206±24.1	137±21.1	196±35.5	180±21	0.391	0.406	0.063
VSA (mm^2)	0.63±0.09	0.65±0.23	0.77±0.10	0.53±0.04	0.77±0.01	0.71±0.01	0.558	0.264	0.372
VH:CD	9.63±0.44	7.90±1.17	7.03±1.07	9.85±1.28	7.23±1.14	6.98±0.53	0.332	0.749	0.102
				Jejunum					
VH (µm)	995±106	975±51.3	923±132	1059±250	745±38	1038±113	0.682	0.212	0.475
VW (µm)	132±17.7	119±14.2	159±2.39	126±7.06	127±17.2	126±11.0	0.400	0.159	0.507
CD (µm)	$130±15.3^{bc}$	$159±14.2^{bc}$	$151±3.22^{bc}$	$205±42.3^{ab}$	$250±47.9^{a}$	$107±5.35^{c}$	0.327	0.337	0.003
VSA (mm^2)	0.40±0.01	0.37±0.06	0.46±0.07	0.41±0.08	0.29±0.03	0.41±0.04	0.332	0.809	0.292
VH:CD	$7.66±0.49^{ab}$	$6.20±0.33^{b}$	$6.08±0.76^{bc}$	$5.49±1.20^{bc}$	$3.20±0.61^{c}$	$9.87±1.35^{a}$	0.478	0.059	0.001
				Ileum					
VH (µm)	820±91	645±7.67	725±67.8	804±104	621±60	637±95.6	0.311	0.732	0.425
VW (µm)	128±11.3	155±14.9	133±45.5	113±13.8	108±6.9	132±9.90	0.260	0.350	0.174
CD (µm)	141±7.97	279±14	200±37.1	204±29.1	161±16.7	148±19.9	0.367	0.245	0.210
VSA (mm^2)	0.34±0.06	0.31±0.05	0.29±0.05	0.28±0.05	0.21±0.02	0.27±0.06	0.213	0.851	0.654
VH:CD	5.82±0.60	3.13±1.59	3.68±0.34	4.14±0.65	3.91±0.40	4.67±1.37	0.821	0.508	0.151

of the birds in the third, fourth, and fifth weeks of the experiment along with feed intake during the fifth week only. These results are in line with various studies conducted on β-GOS or methionine supplementation. The prebiotic β-GOS has been reported to improve the growth performance of broilers in various research trials (Yousaf *et al.,* 2016; Ashraf *et al.,* 2017; Flaujac Lafontaine *et al.,* 2020). These experiments suggested that improved body weights could be attributed to the ability of β-GOS to increase the population of beneficial bacteria like *Bifidobacteria* and *Lactobacilli* as well as immunomodulation (Yousaf *et al.,* 2017) that may improve intestinal growth and integrity (Varasteh *et al.,* 2015). Similarly, dietary inclusion of methionine has also been found to increase the performance traits of broilers (Zhang *et al.,* 2017; Sigolo *et al.,* 2019). These findings could be explained by the role of methionine as a proteinogenic amino acid that leads to muscle development and an increase in the expression of brush border membrane transporters (Zhang *et al.,* 2017; Zeitz *et al.,* 2019). It has also been reported that deficiency or excess of methionine results in a declined performance of broilers (Wang *et al.,* 2019). In our study, methionine supplemented at 1% of the diet failed to show promising effects on the performance traits. It has been reported earlier that supplementing various levels of methionine higher than the NRC recommendations (1994) did not enhance the performance of birds (Bouyeh, 2012; Sigolo *et al.,* 2019). In another study, it was also found that methionine supplemented at the levels of 6.28 and 10.08 g/kg did not influence the production performance but reduced the abdominal fat pad in birds. The lack of response to the dietary supplementation of amino acids was attributed to the imbalance in amino acids ratios because these studies focused on the inclusion of only lysin and methionine (Cengiz *et al.,* 2008; Bouyeh, 2012; Jankowski *et al.,* 2020). Moreover, during metabolism, methionine is converted into homocysteine which is a toxic metabolite and is believed to be responsible for the decreased growth in broilers supplemented with an excess of methionine in their diet (Xie *et al.,* 2007).

In the present study, 0.5% β-GOS increased ($P<0.05$) the relative weights of the proventriculus and spleen. The results of organs development are inconsistent and have many contradictions among different studies. Our results agreed with the results reported previously (Cengiz *et al.,* 2008; Yousaf *et al.,* 2016; Ashraf *et al.,* 2017; Sigolo *et al.,* 2019). These studies could not find a valid biological role of β-GOS in enhancing the relative weights of the proventriculus.

The β-GOS supplementation did not influence serum glucose, ALT, ALP, total proteins, total cholesterol, HDL-cholesterol, and LDL-cholesterol. These results are in agreement with Yousaf *et al.* (2017), who reported that β-GOS supplemented broilers did not affect serum metabolites. Ajdar *et al.* (2016) and Wang *et al.* (2015) also reported that dietary supplementation of prebiotics and isoleucine did not influence serum biochemical parameters. However, serum globulins were significantly increased in birds supplemented with 0.5% β-GOS. It is

expected that high levels of β-GOS might have immuno-potentiating effects. This notion may be further strengthened while observing the improved weight of the spleen in the 0.5% β-GOS group in our present study. Yousaf *et al.* (2017) have reported that β-GOS enhanced the cell-mediated immunity in broilers, however, the effect was observed at 0.1% of β-GOS. Methionine supplemented at the levels of 100, 110, and 120% of the NRC recommendations in broilers was reported to be ineffective in influencing the various serum metabolites (Sigolo *et al.,* 2019). It was suggested that being the precursor of L-carnitine, lysine, and methionine can play a vital role in the metabolism of lipids. L-carnitine possesses hypolipidemic properties and decreases the circulating levels of cholesterol, triglycerides, and very-low-density lipoproteins (VLDL), while increasing HDL and LDL concentrations in the blood (Bouyeh and Gevorgyan, 2011; Sigolo *et al.,* 2019). However, in our experimental conditions, we could not observe such effects of methionine on lipid metabolism which might be due to the differences in the source of the methionine or experimental conditions. In the present study, we found that serum albumin concentration was significantly lower in broilers receiving 0.5% β-GOS. Our results of serum albumin agree with the results of Wang *et al.* (2015), who reported that serum albumin was lower in the 0.13% prebiotic supplemented group in comparison with the control group. Serum creatinine level was higher in 1% methionine supplemented birds. Creatinine is the converted form of creatine and creatine phosphate which are present in the skeletal and cardiac muscles and the brain. Methionine acts as a methyl donor in the formation of S-adenosylhomocysteine and ultimately creatine. Hence, increasing the dietary levels of methionine may increase the creatinine levels in the blood (Brosnan *et al.,* 2011). This hypothesis is further supported by the findings of Cooke *et al.* (2018), who suggested that the creatinine levels are decreased in mice fed with a methionine-restricted diet. Moreover, dietary increment of methionine from 0.25 to 0.5% also resulted in a higher excretion rate of creatinine in broilers (Hasegawa *et al.,* 2016).

Intestinal histological attributes including villus height, crypt depth, villus surface area, and villus crypt to depth ratio reflect the gut health of broilers. Longer villi represent increased luminal area resulting in maximum digestion and absorption of the nutrients required for animal development (Abudabos *et al.,* 2015). In the present study, it was observed that β-GOS and methionine were unable to show significant effects on histomorphological parameters of the duodenum and ileum. However, an increase in crypt depth and reduction in VH:CD were observed in the jejunum of the birds supplemented with 0.5% β-GOS and 0.5% methionine. Our results partially agreed with the findings of Richards *et al.* (2020), who did not observe any significant changes in the gut morphology of broilers supplemented with 3.37% and 1.68% GOS in the starter and finisher phases, respectively. GOS supplementation at the dose rates of 3.37% and 1.68%, however, improved the ileal histology in the early days of the experiment (days 8 and 15) but could not sustain the effects in the later stages of the trial in broilers challenged with *Campylobacter jejuni* (Flaujac Lafontaine *et al.,* 2020). Contrary to this, some studies reported that the dietary inclusions of β-GOS and other prebiotics improved the intestinal morphometric parameters in broilers subjected to cyclic heat stress, and it was further suggested that the prebiotic effects are more pronounced in stressful conditions compared with the normal physiological and environmental conditions (Varasteh *et al.,* 2015; Ashraf *et al.,* 2017). Regarding the methionine, it was evident from an earlier study that the *in-ovo* supplementation of methionine improved the gut histological parameters of broiler embryos (Nazem *et al.,* 2019). However, in our study, we observed that increasing the methionine inclusion rate did not show any promising effects on intestinal morphology.

Conclusions: It is presumed that supplementation of 0.2% β-GOS in the basal diet containing 0.5% methionine partially improved the production performance of the broilers. Moreover, dietary methionine supplementation at a level of 1% had no additional benefits on the studied parameters in the broilers.

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