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Research article

Effects of galacto-oligosaccharides on growth performance and gut health in broiler chickens

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Abstract

This study investigated the effects of prebiotic galacto-oligosaccharides (GOS) derived from beta-galactosidase from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081, on growth performance and gut health in broilers. A total of 100-day-old ROSS-308 male broilers were randomly allocated into two groups, five replications per group. The control group (CON) received only a basal diet, while the experimental group (GOS) received a basal diet supplemented with 1% functional GOS for the first three weeks, then a basal diet until week five. Body weight, feed intake, average daily gain, and feed conversion ratio were recorded. Short-chain fatty acids (SCFAs) and blood malondialdehyde (MDA) levels in intestinal contents were analyzed on weeks 1, 2, 3, and 5. Internal organ weights and intestinal morphology were determined on weeks 3 and 5. The results showed no effect of the GOS on growth performance indicators. However, the GOS group revealed FCR improvement during weeks 4-5 (P <0.05). Higher total SCFAs levels in the duodenum and ileum of the GOS group were observed in week 1. Whereas only propionic acid in the cecum on week 2 and acetic acid and total SCFAs in the ileum on week 3 were higher in the GOS group than in the CON group (P <0.05). In the ileum, shorter crypt depth and higher villi height per crypt depth ratio were demonstrated in week 3 (P <0.05). The GOS group had lower liver and bursa weights than the CON group (P <0.05). Additionally, the GOS group had lower MDA levels than the CON group on weeks 1 and 3 (P <0.05). These findings suggest that GOS supplementation could promote gut health in broilers.

Keywords: Broiler, Growth performance, GOS, Gut health, Short-chain fatty acids

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INTRODUCTION

In the agricultural industry, a wide variety of substances have been used as feed additives to enhance the health and productivity of livestock (Gadde et al., 2017). Prebiotics are non-digestible food ingredients that play an important role in promoting gut health. They function as food for one or more types of beneficial bacteria in the intestinal tract (Gibson et al., 2010). Prebiotics, which are designed to resist hydrolysis and absorption in the upper part of the intestinal tract, have been identified as a promising alternative to antibiotics for the poultry industry. Their unique properties allow them to support metabolic activity and/or the growth of selective bacteria in the intestinal tract, thereby promoting a healthy gut microbiome. This can lead to numerous benefits, including enhanced growth and effective pathogen prevention (Ricke et al., 2020). Therefore, the incorporation of prebiotics into poultry feed could represent a significant advancement in the poultry industry, offering a sustainable and effective solution for improving poultry health and productivity. Non-digestible oligosaccharides (NDOs) are often used as an alternative to antibiotics (Swennen et al., 2006). Various types of NDOs, including galacto-oligosaccharides (GOS), xylo-oligosaccharides (XOS), mannan-oligosaccharides (MOS), and fructo-oligosaccharides (FOS), have been researched for their potential prebiotic effects in broilers (Jung et al., 2008a; Oliveira et al., 2008; Baurhoo et al., 2009; Ao and Choct, 2013; De Maesschalck et al., 2015).

Galacto-oligosaccharides (GOS) are a mixture of substances derived from lactose, consisting of 2 to 8 saccharide units. One of these units is terminal glucose, while the rest are made up of galactose and disaccharides with two galactose units (Charalampopoulos and Rastall, 2009). The GOS is produced through a transgalactosylation reaction, which is catalyzed by the enzyme β-galactosidase using lactose as substrate (Intanon et al., 2014). Various microbes produce the GOS through the action of glycoside hydrolase (GH). The sources of GH influence the characteristics of the resulting GOS, such as the degree of polymerization (DP), the type of monomeric sugar (galactose and glucose), and the amount of unreacted lactose (Torres et al., 2010). The variation of the structure of GOS directly affects the efficiency of the microbiome in metabolism and promoting gut health. Therefore, the GOS from different sources can influence the selectivity and metabolic activity of different microbial genera in the intestinal tract, potentially enhancing the effects of prebiotics (Intanon et al., 2014). For example, the GOS from *Pichia pastoris* X-33 does not affect growth performance but can enhance the population of the beneficial bacteria in broiler chickens (Jung et al., 2008a). The GOS from *Bifidobacterium bifidum* 41171 can improve overall growth performance and reduce the harmful effects of hyperthermia (Slawinska et al., 2019). The GOS from *Papiliotrema terrestris* can promote intestinal microbiota, maintain intestinal integrity, and prevent all heat-stress-induced changes in the jejunum (Varasteh et al., 2015). Furthermore, the supplementation of the GOS, which is produced from a variety of enzymes, has been reported in broiler chicken (Jung et al., 2008a; Pruszynska-Oszmalek et al., 2015; Hughes et al., 2017; Slawinska et al., 2019; Richards et al., 2020).

However, there are limited studies regarding the effects of GOS produced by *Lactobacillus delbrueckii* subsp. *bulgaricus* on growth performance and gut health promotion in broilers, especially the GOS that produced by β-galactosidase from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081, a source that has not been previously studied in broiler chicken before. This study hypothesized that the GOS produced from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 could improve the metabolic activity of beneficial microbes in the broiler gut and enhance broiler growth performance and gut health. This study investigated the effects of the GOS on growth performance, feed efficiency, short-chain fatty acids production, small intestinal morphology, internal organ weight, and serum malondialdehyde levels in the gut of broiler chickens.

MATERIALS AND METHODS

Ethical approval

The experiment procedures involving animals were approved by the Faculty of Veterinary Medicine Chiang Mai University Animal Care and Use Committee (FVM-ACUC) (No. S39/2561).

Galacto-oligosaccharides preparation

The GOS were produced using beta- galactosidase from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081, with lactose as a substrate (Nguyen et al., 2012). In preparation of the basal diet supplemented with 1% functional GOS (2% w/w GOS), the dry GOS were dissolved in warm water (30-40°C) at the ratio of 1 g GOS per 4 ml water. The dissolved GOS were sprayed onto the basal feed, after which the mixed feed was dried in a hot air oven for 3-4 hours before being fed to the GOS group.

Diet and bird management

One hundred 1-day-old Ross-308 broiler chicks, vaccinated for Newcastle disease and Infectious bronchitis, were obtained from a commercial broiler hatchery (Pasang hatchery CPF (Thailand) Public Co., Ltd). All birds were housed in a closed environment, with a controlled temperature and regulated light according to ROSS-308 management guidelines. Birds were individually weighed and randomly allocated into two groups. Each group was divided into five replicates, with ten birds per replicate. Birds were fed with a commercial basal diet, a starter diet from day 1 to 21, and a finisher diet from day 22 to 35 (Table 1) with ad libitum drinking water. The control group (CON) was fed with a basal diet from days 1-35. The GOS group (GOS) was fed with a basal diet supplemented with 1% functional GOS from days 1-21 and only a basal diet from days 22-35.

Table 1 Feed analytical composition of basal diet for broiler chickens (NRC, 1994)

Variable component	Starter phase	Finisher phase
Metabolizable Energy (kcal/kg)	3150.00	3200.00
Crude protein (minimum), %	21.00	19.00
Lysine, %	1.24	1.09
Methionine, %	0.45	0.41
Methionine + cysteine, %	0.95	0.86
Tryptophan, %	0.20	0.18
Threonine, %	0.83	0.74
Isoleucine, %	0.84	0.75
Leucine, %	1.36	1.20
Arginine, %	1.31	1.11
Tryptophan + thionine, %	1.49	1.31
Histidine, %	0.42	0.37
Valine, %	0.96	0.81
Crude fat (minimum), %	4.00	4.00
Linoleic acid (minimum), %	1.50	1.50
Calcium, %	0.90	0.80
Phosphorus, %	0.45	0.40
Crude fiber (maximum), %	5.00	5.00

Sample collection

A bird from each replicate was randomly selected for sample collection on weeks 1, 2, 3, and 5. Blood samples were collected before birds were euthanized by the cervical dislocation method, following AVMA Guidelines for the Euthanasia of Animals: 2013 Edition. The liver, spleen, and bursa from each bird were prepared and weighed. The intestinal contents of the duodenum (from the pylorus to the distal portion of the duodenum loop), jejunum (from the distal portion of the duodenum loop to Meckel's diverticulum), ileum (from Meckel's diverticulum to cecum openings) and cecum (from cecum junction of both cecum) were also collected. On weeks 3 and 5, the tissues of the duodenum, jejunum, and ileum were collected and immediately frozen in liquid nitrogen. The samples were then stored at -80 °C until further analysis. For morphological analysis, the small intestinal samples were flushed with 0.9% normal saline and fixed in 10% formalin for 24 hours.

Growth performance

The body weight (BW) and feed intake (FI) of each replicate were recorded weekly. At the end of the study, body weight, average daily gain (ADG), and feed conversion ratio (FCR) were calculated and recorded. Average daily gain (ADG) was calculated from the weight of the broiler chicken divided by the number of days on feed to represent the weight in grams gained by that broiler chicken each day. The feed conversion ratio (FCR) was calculated from the amount of feed consumed (in kilograms) required to produce a one-kilogram bodyweight of a bird.

Short-chain Fatty Acids

The intestinal contents were thawed and weighed 0.5 g. The contents were diluted with triple-distilled water (0.5 g:1 ml) in 2.0 ml microcentrifuge tubes. The intestinal contents were homogenized by using a vortex mixture and centrifuged at 10,000 rpm for 30 minutes at 4°C. One milliliter of supernatants was transferred and mixed with 0.2 ml of ice-cold 25% metaphosphoric acid solution. The sample tubes were placed in an ice bath for 30 minutes, and the samples were centrifuged at 12,000 rpm for 30 minutes at 4°C. The supernatants were filtered using a 0.45 µm syringe filter into a vial tube (Calik and Ergün, 2015). Supernatants were analyzed using a gas chromatograph (Nexis GC-2030, Shimadzu's premier gas chromatograph) coupled with a Restek GC Column Temp. Limits: Rtx-1 -60 to 270/290 °C, Length: 15 m, Internal Diameter: 0.53 mm, df (µm):5.00 µm Cat. #10177 Serial #1473061) and a flame ionization detector to determine SCFA concentrations in cecal digesta. The injector-port and flame ionization detector temperatures were set at 250°C, splitters. In the temperature program, the initial temperature was held at 60°C after injection and then increased from 20°C/min to 200°C for 5 min. Hydrogen and helium were used as the carrier gas. Total SCFAs represented the total of acetate, propionate, butyrate, and valerate.

Small Intestinal Histology

The tissues were sliced into 5 mm sections, embedded in paraffin wax, and then stained with hematoxylin and eosin stain before being fixed in a slide. The villus height was measured from the villus's distal tip to the region where it meets the crypt. Additionally, the depth of the invagination between two villi was used to define the crypt depth. A microscopic (Olympus) was used to view the slide, and measurements were taken using ImageJ software. The average values were determined for each intestinal segment of each bird, and the villus height to crypt depth ratio (VH: CD) was then calculated (Fernandes et al., 2014; Kareem et al., 2016).

Blood Malondialdehyde level analysis

The method is as follows: 180 µl of plasma samples were added in 400 µl of 10% trichloroacetic acid (TCA) and heated at 90°C for 30 minutes in a water bath, then cooled down to room temperature. The samples were then centrifuged at 6,000 rpm for 30 minutes. Next, 180 µl of supernatants were mixed with 540 µl of 0.44M phosphoric acid and 360 µl of 0.67M 2-thiobarbituric acid. The samples were heated at 90°C for 30 minutes in a water bath, and after that, they were cooled down to room temperature. Then, the samples were filtered via a 0.45 µm polysulfone membrane syringe filter. The pink-colored sample mixture results from the reaction of thiobarbituric acid reactive substances (TBARS) end products (malondialdehyde) with TBA. TBARS in the blood samples were measured using high-performance liquid chromatography (HPLC) Shimadzu LC-10. TBARS concentrations of samples were determined from a standard curve and calculated as nmol MDA/ml blood samples (Pongkan et al., 2022).

Statistical analysis

The data were described using means with standard error of means (SEM). The effects of the GOS or CON group on growth performance parameters, including body weight (BW), average daily gain (ADG), feed intake (FI), and feed conversion ratio (FCR), were investigated by repeated measure analyses using the mixed procedure (SAS. 2018. SAS University Edition: Statistics, 6th ed), the significance differences between groups were determined by the least square means calculations. Student's t-test or analysis of variance was used to compare the means of short-chain fatty acids (SCFAs) concentration, small intestinal morphology measurement, internal organ weight, and blood malondialdehyde (MDA) level between the GOS and CON groups, respectively. The data were examined for normality before statistical analysis, and nonnormally distributed data were logarithmically transformed. Significance was defined at $P \leq 0.05$, and trends were defined at $P > 0.05$ and ≤ 0.10.

RESULTS

Growth performance

There was no effect of GOS supplementation on BW at different weeks of age (Table 2). Additionally, the GOS supplementation did not affect the FI, ADG, and FCR of broilers in weeks 1-3. The same result was observed for the overall period of weeks 1-5. However, the FCR of GOS supplementation on weeks 4-5 was significantly lower than the CON group ($P < 0.05$).

Short-Chain Fatty Acids

On week 1, the SCFAs concentration in the duodenum of the GOS group was significantly greater than the SCFAs concentration of the CON group ($P < 0.05$). Similarly, the concentrations of acetic acid, propionic acid, butyric acid, and total SCFAs in the ileum were significantly higher ($P < 0.05$) in the GOS group compared to the CON group. Additionally, the concentration of propionic acid in the cecum was significantly higher ($P < 0.05$) in the GOS group compared to the CON group in week 2. The GOS group also showed a significantly higher ($P < 0.05$) concentration of acetic acid and total SCFAs in the ileum when compared with the CON group on week 3. However, there was no significant difference in the concentration of SCFAs between the two groups in week 5 (Table 3).

Table 2 The effects of galacto-oligosaccharides (GOS) on BW (kg), FI, ADG (g/d), and FCR values of broiler chickens. Data were presented in Mean±SE, and the P-value was calculated by the comparison of the least-square means.

	Week	Group		*P*-value
		CON	GOS	
BW	1	160.79±2.77	156.64±2.94	0.88
	2	427.77±6.80	417.24±7.46	0.70
	3	868.39±12.52	853.08±14.03	0.57
	4	1468.28±23.20	1446.43±26.66	0.42
	5	2189.68±37.51	2170.79±37.27	0.49
FI	1-3	1019.38±7.61	987.52±13.48	0.26
	4-5	1969.60±48.54	1941.98±29.02	0.33
	1-5	2988.97±47.27	2929.49±40.63	0.37
ADG	1-3	41.48±0.62	40.70±0.70	0.70
	4-5	94.37±2.01	94.12±1.88	0.90
	1-5	63.26±1.10	62.70±1.09	0.71
FCR	1-3	1.41±0.03	1.41±0.03	0.99
	4-5	2.56±0.10	2.33±0.49	0.01
	1-5	1.65±0.04	1.60±0.02	0.56

GOS: GOS group (galacto-oligosaccharide 1% functional+basal diet); CON: Control group (basal diet). Significant differences are indicated at $P < 0.05$.

Small Intestinal Morphology

On week 3, there were no significant differences in the villi height, crypt depth, and VH: CD ratio in the duodenum and jejunum between the GOS and CON groups (Table 4). However, in the ileum, the crypt depth length of the GOS group (74.56±2.04) was significantly shorter (P <0.05) than that of the CON group (95.57±5.34). Additionally, the VH: CD ratio of the GOS group (8.68±0.55) was significantly higher (P <0.05) than that of the CON group (6.19±0.62). The GOS group also showed a trend of increasing ileum villi height (P =0.08) when compared with the CON group. There were no significant differences in small intestinal morphology between both groups on week 5.

Internal organ weight

The weights of the liver and bursa of Fabricius in the GOS group were significantly lower (P<0.05) than those of the CON group on week 3. However, there were no differences between spleen weight on week 3 and all internal organ weight on week 5 (Table 5).

Table 3 Effects of dietary supplementation with GOS on short-chain fatty acids (SCFAs) levels (μmol/g) in the gastrointestinal tract of broiler chickens

Week		Duodenum			Jejunum			Ileum			Cecum		
	SCFAs	CON	GOS	*P*-value	CON	GOS	*P*-value	CON	GOS	*P*-value	CON	GOS	*P*-value
1	Acetic acid	1.17±0.09	1.77±0.13	0.01	2.87±0.34	3.04±0.23	0.68	2.56±0.36	6.27±0.52	0.01	30.40±2.78	34.95±4.98	0.45
	Propionic acid	1.15±0.06	1.45±0.05	0.01	1.23±0.14	1.12±0.03	0.47	0.78±0.03	1.09±0.10	0.02	1.62±0.31	1.49±0.22	0.74
	Butyric acid	0.11±0.01	0.16±0.01	0.01	0.16±0.02	0.21±0.04	0.35	0.15±0.03	0.36±0.05	0.01	2.23±0.38	3.35±0.56	0.13
	Valeric acid	0.36±0.01	0.81±0.13	0.01	1.38±0.30	1.46±0.05	0.79	1.82±0.42	1.64±0.13	0.68	3.17±0.50	2.13±0.71	0.26
	Total SCFAs	2.79±0.15	4.20±0.11	0.01	5.64±0.72	5.84±0.29	0.80	5.32±0.42	9.35±0.65	0.01	37.42±3.15	41.93±5.64	0.50
2	Acetic acid	1.04±0.07	1.22±0.20	0.41	3.21±0.44	3.28±0.20	0.88	4.37±0.78	5.41±0.46	0.28	55.74±1.53	53.66±3.67	0.61
	Propionic acid	1.24±0.06	1.22±0.11	0.87	1.52±0.14	1.45±0.17	0.76	1.01±0.11	0.94±0.06	0.60	2.66±0.43	6.16±1.12	0.03
	Butyric acid	0.11±0.01	0.12±0.01	0.31	0.18±0.01	0.19±0.02	0.58	0.28±0.03	0.27±0.02	0.72	6.42±0.57	5.75±0.44	0.37
	Valeric acid	0.35±0.03	0.39±0.07	0.66	1.34±0.22	1.25±0.07	0.71	1.88±0.19	1.89±0.11	0.97	0.72±0.13	0.59±0.02	0.38
	Total SCFAs	2.75±0.15	2.95±0.37	0.63	6.26±0.72	6.19±0.32	0.92	7.55±1.06	8.52±0.60	0.45	65.54±1.78	66.17±4.64	0.90
3	Acetic acid	0.99±0.08	1.07±0.20	0.71	3.46±0.40	3.94±0.31	0.37	5.16±0.56	7.55±0.82	0.04	55.2±5.32	60.21±3.00	0.43
	Propionic acid	1.18±0.07	1.03±0.10	0.23	1.23±0.09	1.29±0.06	0.63	0.87±0.10	0.99±0.05	0.29	6.56±1.29	5.11±1.19	0.43
	Butyric acid	0.11±0.01	0.10±0.01	0.70	0.28±0.07	0.22±0.02	0.43	0.27±0.02	0.32±0.05	0.39	8.03±1.34	8.49±0.92	0.78
	Valeric acid	0.29±0.01	0.29±0.03	0.95	1.00±0.16	1.09±0.11	0.65	1.82±0.20	1.88±0.17	0.81	0.59±0.07	0.65±0.07	0.57
	Total SCFAs	2.58±0.16	2.50±0.34	0.83	5.98±0.47	6.54±0.36	0.36	8.11±0.64	10.74±0.97	0.05	70.38±7.22	74.46±4.34	0.64
5	Acetic acid	0.54±0.09	0.54±0.07	0.99	1.53±0.10	1.93±0.33	0.28	4.49±0.61	3.99±0.75	0.61	29.22±3.53	26.10±3.06	0.53
	Propionic acid	0.59±0.04	0.69±0.07	0.23	0.76±0.05	0.74±0.06	0.88	1.06±0.13	1.01±0.09	0.74	4.06±0.90	4.01±0.34	0.96
	Butyric acid	0.07±0.01	0.07±0.01	0.82	0.10±0.01	0.11±0.01	0.77	0.13±0.02	0.16±0.04	0.58	5.16±0.69	5.00±0.68	0.87
	Valeric acid	0.50±0.04	0.44±0.04	0.30	1.59±0.11	1.49±0.23	0.70	1.52±0.05	2.25±0.79	0.38	0.94±0.09	0.87±0.07	0.49
	Total SCFAs	1.70±0.14	1.74±0.14	0.85	3.97±0.23	4.28±0.59	0.64	7.22±0.58	7.42±1.50	0.90	39.38±4.76	35.98±3.55	0.58

CON: Control group (basal diet), GOS: GOS group (galacto-oligosaccharide 1% functional+basal diet). Significant differences are indicated by $P < 0.05$ in comparison with the control group. SCFAs concentration (μmol/g digesta)

Table 4 Effect of GOS supplementation on small intestinal morphology at weeks 3 and 5

Week 3	Parameters	Group		*P*-value
		CON	GOS	
Duodenum	Villi Height (μm)	1327.80±23.05	1295.20±7.62	0.21
	Crypt Depth (μm)	105.85±5.22	97.51±2.99	0.20
	VH: CD Ratio	13.34±0.77	13.69±0.3	0.70
Jejunum	Villi Height (μm)	919.23±15.12	901.74±36.03	0.66
	Crypt Depth (μm)	93.42±4.47	92.03±8.19	0.88
	VH: CD Ratio	10.17±0.58	10.40±0.75	0.81
Ileum	Villi Height (μm)	562.05±20.03	630.12±27.76	0.08
	Crypt Depth (μm)	95.57±5.34	74.56±2.04	0.01
	VH: CD Ratio	6.19±0.62	8.68±0.55	0.01
Duodenum	Villi Height (μm)	1673.42±92.65	1626.70±58.05	0.68
	Crypt Depth (μm)	95.32±7.59	98.20±6.04	0.77
	VH: CD Ratio	18.42±2.16	17.16±1.29	0.63
Jejunum	Villi Height (μm)	1088.04±32.08	1096.78±36.16	0.86
	Crypt Depth (μm)	93.47±4.03	94.13±7.27	0.94
	VH: CD Ratio	12.08±0.73	12.25±1.13	0.90
Ileum	Villi Height (μm)	651.84±9.78	658.86±15.41	0.71
	Crypt Depth (μm)	95.93±3.41	95.73±3.22	0.96
	VH: CD Ratio	6.97±0.23	6.98±0.0.09	0.97

Value is reported as the least square mean ± standard error of villi height, crypt depth, and Villi height: Crypt depth Ratio, n = 5. CON: Control group (basal diet), GOS: GOS group (galacto-oligosaccharide 1% functional + basal diet). Significant differences are indicated by $P < 0.05$ in comparison with the control group. Villi height: Crypt depth ratio (VH: CD ratio)

Table 5 Effect of GOS supplementation on absolute internal organ weight in weeks 3 and 5

Internal organ (% per body weight)				
Week	Organs	Group		*P*-value
		CON	GOS	
3	Spleen	0.09±0.01	0.10±0.01	0.31
	Liver	2.58±0.11	2.25±0.04	0.02
	Bursa	0.31±0.01	0.24±0.02	0.03
5	Spleen	0.11±0.01	0.10±0.01	0.26
	Liver	2.15±0.12	2.09±0.07	0.73
	Bursa	0.10±0.01	0.08±0.01	0.31

Value is least square mean ± standard error; n = 5
GOS: GOS group (galacto-oligosaccharide 1% functional+basal diet); CON: Control group (basal diet). Significant differences are indicated by $P<0.05$ in comparison with the control group.

Serum Malondialdehyde

The MDA level in the GOS group was significantly lower than the MDA level in the CON group on week 1 (4.61±0.10 and 5.04±0.15) and week 3 (3.71±0.05 and 4.19±0.02), respectively (Figure 1.). However, there were no significant differences in MDA levels in both groups on weeks 2 and 5.

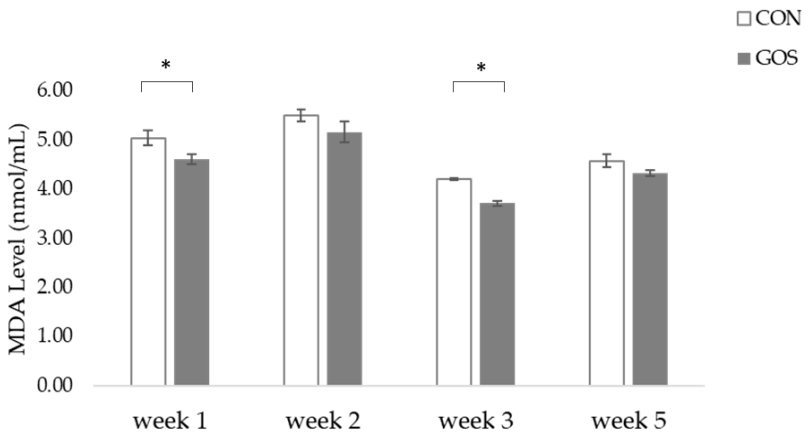

Figure 1 Effects of galacto-oligosaccharides (GOS) supplementation on MDA levels.

DISCUSSION

This study determined the effects of GOS originating from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 on broilers growth performance and gut health. The GOS supplementation did not enhance BW and ADG in the GOS group, similar to many previous studies that GOS supplementation does not alter the BW and ADG. However, the broilers remained healthy with no significant mortality throughout the study period (Jung et al., 2008a). This study found that the FCR of broilers in the GOS group was lowered compared to those in the CON group on weeks 4-5, similar to another study (Richards et al., 2020). GOS supplementation can lead to a lower FCR for several reasons, including improved gut health, enhanced gut microbial metabolism, enhanced nutrient absorption, and increased production of SCFAs (Jha et al., 2021; Rafiq et al., 2022). These SCFAs, through morphological differentiation, can improve intestinal digestion and nutritional absorption. Improvements in intestinal morphology, such as a higher VH and VH: CD ratio, are expected to improve nutrient digestion and absorption capacity in the small intestine (Pluske et al., 1996; Montagne et al., 2003). Prebiotics like GOS can stimulate the growth of beneficial bacteria and improve digestion and feed efficiency (Mookiah et al., 2014).

Many studies suggest that GOS can selectively promote microbes and increase SCFA levels. SCFAs, beneficial organic acids, are the by-product of oligosaccharide utilization by intestinal microbes. They promote intestinal health, with acetic acid modifying the acidic environment to inhibit pathogenic bacteria (Gibson et al., 2005), propionic acid boosting the immune system, and butyric acid serving as an energy source for intestinal wall development (Sakata and Yajima, 1984; Frye et al., 2017). The GOS used in this study was produced using β-galactosidase from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 and contains β-D-Galp-(1→3)-D-Gal and β-D-Galp-(1→6)-D-Gal as major components. These main GOS components have been found to provide a better bifidogenic impact than the GOS mixture, mainly including β(1→4) (Depeint et al., 2008; Kittibunchakul et al., 2020). The result of this study demonstrated a higher level of total SCFAs in the duodenum and ileum of the GOS group than the CON group on week 1. This suggests that GOS from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081, due to its effective composition for bacterial metabolism, could increase certain beneficial bacteria during the early stage of life and provide more gut protection against pathogenic bacterial invasion. *Lactobacillus*, a

dominant genus in the duodenum of the small intestine in poultry, can utilize the GOS and produce SCFAs (Liao et al., 2020; Yang et al., 2020). Increasing of SCFAs in the ileum of the GOS group in this study is similar to the previous piglet model study, which reported significantly higher concentrations of propionate and butyrate in the ileum of the piglet supplemented with GOS compared to the control group at day 8 (Tian et al., 2019). These results indicated a significant impact of GOS in the early life of broilers. On week 2, the GOS group had a significantly higher propionic acid concentration in the cecum than the CON group, similar to the result from Pan et al. (2009). The propionic acid in the cecum of broilers plays several roles, such as inhibiting the pathogenic bacteria, enhancing broiler performance by altering the pH of the gastrointestinal tract, and decreasing the invasion of the intestinal epithelial cells (Khan and Iqbal, 2016; Dittoe et al., 2018). On week 3, an increase in acetic acid and total SCFAs in the ileum of the GOS group could related to *Lactobacillus* species enhancement via GOS supplementation (Jung et al., 2008b; Richards et al., 2020). Selective bacteria can utilize GOS to produce acetic acids and other SCFAs. *Lactobacillus* can ferment the GOS into SCFAs, mainly acetic acid (Zhai et al., 2019; Markowiak-Kopeć and Śliżewska, 2020). Significantly higher acetic acid in the ileum, together with an increase in villi height, decrease in crypt depth, and increase in VH:CD ratio in ileum on week 3, suggest that GOS, derived from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 can promote acetic acid production and improve ileum epithelium, similar to previous studies (Monaco et al., 2011; Richards et al. 2020).

The GOS group resulted in a lower bursa of Fabricius weight on week 3, potentially due to the high acetic acid concentration suppressing harmful bacteria (Gibson et al., 2005). Furthermore, the GOS group also showed a significant decrease in relative liver weight. This finding is similar to the results of several previous studies by Bozkurt et al. (2008), Pournazari et al. (2017), Zhou et al. (2017), Askri et al. (2020), Rehman et al. (2020) which have similarly reported a reduction in liver weight in response to the GOS supplementation. This study also suggests that the effect of GOS supplementation on internal organ weight was not persistent since no significant difference was observed in week five after withdrawal from GOS supplementation.

The present study also observed that the blood MDA level, a marker of oxidative stress, was significantly reduced in the GOS group on weeks 1 and 3. Although no significant differences were observed in weeks 2 and 5, these periods demonstrated a similar trend. This suggests a healthier oxidative status in the GOS group, similar to the previous study by Tian et al. (2018) and Xing et al. (2020). SCFAs are known to play a role in maintaining gut health. They can activate cellular antioxidant systems, which can help combat oxidative stress and promote overall cellular health. Additionally, SCFAs can stimulate mucosal blood flow, thereby enhancing nutrient absorption and oxygen supply to the gut tissues (Mann and Forman, 2015; González-Bosch et al., 2021).

CONCLUSIONS

The GOS derived from *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 did not significantly enhance the growth performance of broilers. However, it was observed to provide some gut health benefits in the broiler, including increased ileum villi height per crypt depth ratio, increase in SCFAs during the early GOS supplementation period, especially in the duodenum, ileum, and cecum, and reduced stress as suggested by the reduction in the blood malondialdehyde levels, a marker of oxidative stress. However, the limited sample size in this study could have influenced the results. Further research with a larger sample size is recommended.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: NA
Ethical process: NA, SF
Experiment: NA, SF, MI
Performed the laboratory analysis: NA, SF, MI
Analyzed the data: NA, WS, MI
Contributed reagents/materials/analysis tools: NA
Wrote the paper: NA, SF

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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