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Effects of galacto-oligosaccharide on growth performance, faecal microbiota, immune response and antioxidant capability in weaned piglets

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ABSTRACT
The present experiment investigated the effects of galacto-oligosaccharide (GOS) on growth performance, faecal microbiota, immune response and antioxidant capability in weaned piglets. Ninety 28-day-old weaned piglets were randomly allocated into 5 treatments with 6 replicate pens per treatment and 3 piglets per pen (n = 18). These five diets were formulated by adding 0, 500, 1000, 1500 and 2000 mg/kg GOS to the basal diet, respectively. The experiment was lasted for 28 days. Body weight and feed intake were determined. Faeces samples were collected to detect the amount of microbiota. Blood samples were used to determine antioxidative and immune parameters. The results indicated that GOS supplementation improved the growth performance, increased the number of Lactobacillus and Bifidobacteria and decreased the number of Escherichia coli in a linear or quadratic dose-dependent manner. Dietary GOS decreased serum concentration of pro-inflammatory cytokines in a quadratic dose-dependent manner, but increased anti-inflammatory cytokines in a linear or quadratic dose-dependent manner. Moreover, GOS supplementation promoted the activities of antioxidant enzyme in a linear or quadratic dose-dependent manner during the experiment. The levels of MDA quadratically decreased on d 28. These results suggested that GOS exhibited positive effects on growth performance, immune function and antioxidant capability in weaned piglets.

1. Introduction
Weaning period is a critical phase for piglets due to some factors such as underdeveloped immune function and changed microbiota composition in gut (Lallès et al. 2007), when the piglets suffered from a range of disorders including anorexia, diarrhoea, growth suppression, intestinal microbe imbalance and increased susceptibility to diseases (Che et al. 2012). Consequently, many functional oligosaccharides have been developed and applied in diets to relieve piglet stress, improve immunoglobulins and reduce proinflammatory interleukins. Oligosaccharides, a class of prebiotics, have different healthy properties, including prebiotic activity, immunoregulation, and inhibition of pathogen adhesion in the gastrointestinal tract (Hajiaghapour and Rezaei-pour, 2018; Verardo et al. 2018). Among the various oligosaccharides tested for potential prebiotic application, galacto-oligosaccharide (GOS), mainly found in animal milk (Boehm et al. 2004; Martinez-Ferez et al. 2006), is one of the functional oligosaccharides with high safety, wide acceptance and excellent feeding effect (Cardellecobas et al. 2011). Moreover, a variety of beneficial health effects of GOS have been reported including modulating immune function and improving intestinal health (Dai et al. 2014; Weaver et al. 2011). In addition, much attention has been focused on growth regulating effects of GOS. The most extensively used animal models to examine growth-promoting effect of GOS included rats (Sakaguchi et al. 1998), broilers (Jung et al. 2008), common carps (Hoseinifar et al. 2017) and to a lesser extent pigs (Tian et al. 2018); however, the results were inconsistent. No enhancing effects were observed on growth performance of rats and broilers fed diets containing GOS, whereas improvement in growth performance of pigs and common carps were observed.

Adding antioxidants in diets is one of the effective means to improve the antioxidative capacity of livestock (Sen et al. 2010). Previous study had proved the efficiency of GOS in decreasing reactive oxygen species (ROS) production in meat of finisher pigs (Rajauria et al. 2016). Moreover, some researches provided scientific evidences that GOS regulated immune system through selectively promoting the population and growth of some intestinal bacteria such as Bifidobacteria and Lactobacilli (Tuohy et al. 2005; Fanaro et al. 2009; Jeurink et al. 2013). Lactobacillus and Bifidobacteria are thought to contribute to the health of animals and humans through mechanisms such as competitively excluding potential pathogenic bacteria, enhancing the production of short-chain fatty acids, controlling intestinal function and stimulating immune function (Alizadeh et al. 2016; Matsu ki et al. 2016; Monteagudomera et al. 2016). Given these features, we put forward the hypothesis that the application of GOS as prebiotic in diets to mitigate the weaning stress response of piglets is feasible. However, the roles of GOS as immune regulators and antioxidants via inducing changes of inflammatory cytokines and antioxidant enzymes in piglets have not been studied in detail.

Consequently, before the benefits of GOS can be successfully used in weaned piglets, a better understanding of its...
mechanism of action is needed, and so the further exploration is imperative. The objective of this study was to investigate the effects of GOS on growth performance, faecal microorganism, immunity and antioxidant capability in weaned piglets.

2. Materials and methods

The experiment procedures involving animals were approved by the Animal Research and Ethics Committee of College of Animal Science, Inner Mongolia Agricultural University, Hohhot, China.

2.1. Animals, experiment design and treatments

A total of 90 piglets (Duroc × Landrace × Large Yorkshire, 45 males and 45 females) weaned at the age of 28-day-old were selected with an average initial bodyweight of 7.78 ± 1.66 kg, and randomly allotted into five treatment groups with six replicate pens per treatment and 3 piglets per pen (n = 18) with a space allowance of 0.85 m² per pig in a randomized complete block design according to the body weight, sex and litter. The piglets from one litter were evenly distributed to each treatment group. All piglets were housed in an environmentally controlled nursery with the hard-plastic slatted floor. Each pen was equipped with a self-feeder and a nipple drinker to allow the piglets ad libitum access to feed and water. The temperature was controlled between 25 and 29°C. The lighting programme was maintained at 16 h light and 8 h dark in each day. These five diets were formulated by adding 9, 500, 1000, 1500 and 2000 mg/kg of GOS to the basal diet, respectively. The animal feeding trial was lasted 28 days. The basal diet was formulated to meet the nutrient needs for the pigs proposed by the National Research Council (2012), and the ingredient compositions are tabulated in Table 1. Commercial GOS was provided by a commercial company (Jinan Gerun Bioengineering Institute, Jinan, China).

2.2. Growth performance and sample collection

After 12 h of fasting, all piglets were individually weighed on d 0, 14 and 28, respectively. Meanwhile, orts were collected and weighed daily, and feed intake of each pen was recorded. Average daily body weight gain (ADG), average daily feed intake (ADFI) and the ratio of feed to gain (F/G) were calculated from the values described above. After 12 h fasting, pigs from each treatment were anaesthetized with pentobarbital sodium (30 mg/kg, i.v.). Under sedation, approximately 10 mL of blood sample for each head was collected aseptically from the precaval vein into non-anticoagulant tubes. Then, the blood samples were allowed to coagulate for 20 min and centrifuged at 1200×g for 15 min at 4°C to obtain serum. Serum was stored at −20°C until analysis of immune and antioxidant indicators.

2.3. Culture-based bacterial count analysis

On d 14 and 28, fresh faecal samples were collected from each pen and placed into the sterilized centrifuge tube, then stored at −20°C for analyses. Selective culture medium counting method was used to detect the amount of microorganism in the faeces (Hu et al. 2012). One gram of the fresh sample was diluted with 9.0 mL sterile saline solution and homogenized for analyses. Selective culture medium counting dilution gradients for this experiment, 10⁻¹, 10⁻², and 10⁻³ dilution ratios. According to the number of colonies at culturing, three suitable dilution gradients were chosen (in this experiment, 10⁻², 10⁻³, and 10⁻⁴) were chosen as the suitable dilution gradients for Lactobacillus and Bifidobacteria, but for Escherichia coli, the suitable dilution gradients were 10⁻², 10⁻³ and 10⁻⁴. Two parallels were measured for each sample, and 0.3 mL of each dilution was daubed on a petri dish. Lactobacillus and Bifidobacteria were, respectively, cultured on MRS medium and bifidobacterium agar under an anaerobic condition at 37°C for 24 h. Escherichia coli was cultured on eosin methylene blue agar under an aerobic condition at 37°C for 24 h. All these operations were done aseptically in an ultra clean cabinet and completed within 15 min. After 24 h of culture at 37°C in an incubator, bacterial colony number was recorded, and the results were expressed as the logarithm value of colony-forming units per gram faeces (Log CFU/g).

2.4. Assay of immune and antioxidant indicators

Serum concentrations of IgG, IgA, IgM, interleukin-1(IL-1), IL-2, IL-4, IL-6, TNF-α, IFN-γ, soluble CD3 (sCD3), sCD4 and sCD8 were proposed as indicators of immune response in piglets.
and all of the immune indicators were determined with commercial ELISA kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Serum samples were thawed at 4°C. Standard substances and samples (four-fold dilution) were added into 96-well microplate which was precoated with specific antibodies, and then secondary HRP-labelled antibody was added and incubated at 37°C for 60 min. After washing the plate, substrate solutions A and B were added to the wells separately for colorimetric detection and then the 96-well microplate was incubated in dark at 37°C for 15 min. Finally, the reaction was stopped by adding the stop solution. Optical density was read at 450 nm within 15 min using an ELASA microplate reader Synergy H4 (BioTek, Winooski, USA). The coefficient of variation within the microplate was less than 10% and between the microplates was less than 15%. All measurements were done at least in triplicate according to the manufacturer’s instructions.

The activities of catalase (CAT), glutathione peroxidase (GSH-Px), total superoxide dismutase (T-SOD), contents of malondialdehyde (MDA) and total antioxidant capacity (T-AOC) were measured with commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Serum samples were thawed at 4°C. Standard solutions A and B were added to the wells separately for colorimetric detection and then the 96-well microplate which was precoated with specific antibodies solutions were added to the wells separately for colorimetric detection and then the 96-well microplate was incubated in dark at 37°C for 15 min. Finally, the reaction was stopped by adding the stop solution. Optical density was read at 450 nm within 15 min using an ELASA microplate reader Synergy H4 (BioTek, Winooski, USA). The coefficient of variation within the microplate was less than 10% and between the microplates was less than 15%. All measurements were done at least in triplicate according to the manufacturer’s instructions.

2.5. Statistical analysis

Normality of all data was confirmed by PROC UNIVARIATE of Statistical Analysis Systems Institute (SAS Institute Inc., 2004). All data were analysed of the linear and quadratic responses to increasing dietary GOS levels (0, 500, 1000, 1500 and 2000 mg/kg of GOS in diet) using the REG Procedure of SAS. The model included the effect of treatment. Results were expressed as means and the standard error of the mean (SEM). A P-value less than 0.05 was considered as the criterion for statistical significance.

3. Results

As shown in Table 2, from d 14 to 28, with increased dietary supplementation of GOS, the ADG significantly increased in a quadratic dose-dependent manner (P = 0.04). Dietary supplementation with GOS tended to improve the ADG in a linear or quadratic dose-dependent manner during the whole experiment period (P = 0.06, P = 0.07). In addition, from d 0 to 14, the ADFI tended to increase in a quadratic dose-dependent manner with an increasing amount of GOS inclusion (P = 0.07).

As shown in Table 3, with increased dietary supplementation of GOS, the number of *Escherichia coli* significantly decreased in a quadratic dose-dependent manner on d 14 (P < 0.01), and a linear dose-dependent manner on d 28 (P = 0.03). Meanwhile, the number of *Lactobacillus* and *Bifidobacteria* increased in a linear (P = 0.01, P < 0.01) or quadratic (P = 0.04, P < 0.01) dose-dependent manner on d 28.

As indicated in Table 4, on d 14 of the experiment, serum IL-4 concentrations increased in a linear or quadratic dose-dependent manner (P = 0.01, P = 0.04), but TNF-α level decreased in a quadratic dose-dependent manner (P = 0.03) with the increasing amount of GOS inclusion on d 14. Moreover, IFN-γ concentrations tended to reduce in a linear dose-dependent manner on d 14 (P = 0.07).

As indicated in Table 5, dietary addition of GOS increased serum concentration of sCD3 in a linear or quadratic dose-dependent manner on d 14 (P < 0.01, P < 0.01) and d 28 (P = 0.04, P = 0.02).

In addition, as indicated in Tables 4–6, no significant dose-dependent effects on the serum levels of IL-1, IL-2, IL-6, sCD4, sCD8, IgA, IgG and IgM were observed (P > 0.05).

### Table 2. Effects of GOS on growth performance of weaned piglets<sup>a</sup>

| Items<sup>d</sup> | GOS levels (mg/kg) | <sup>P-value</sup><sup>b</sup> | SEM<sup>c</sup> |
|-------------------|--------------------|----------------|----------------|
|                   | 0      | 500   | 1000  | 1500  | 2000  | Linear | Quadratic |
| ADG (g/d)         |        |       |       |       |       |        |            |
| d 0–14            | 253    | 294   | 282   | 204   | 263   | 20.7   | 0.62    | 0.62 |
| d 14–28           | 397    | 458   | 480   | 518   | 442   | 26.8   | 0.20    | 0.04 |
| d 0–28            | 350    | 354   | 381   | 312   | 287   | 28.6   | 0.06    | 0.07 |
| ADFI (g/d)        |        |       |       |       |       |        |            |
| d 0–14            | 509    | 566   | 591   | 541   | 512   | 29.2   | 0.85    | 0.07 |
| d 14–28           | 621    | 701   | 694   | 599   | 566   | 54.9   | 0.23    | 0.15 |
| d 0–28            | 589    | 633   | 643   | 570   | 539   | 38.9   | 0.19    | 0.11 |
| F/G (g/g)         |        |       |       |       |       |        |            |
| d 0–14            | 1.95   | 1.87  | 2.18  | 2.25  | 2.15  | 0.161  | 0.19    | 0.41 |
| d 14–28           | 1.49   | 1.61  | 1.63  | 1.41  | 1.52  | 0.097  | 0.64    | 0.70 |
| d 0–28            | 1.69   | 1.79  | 1.80  | 1.88  | 1.82  | 0.081  | 0.18    | 0.29 |

<sup>a</sup>Values are means of six pens representing each dietary treatment.

<sup>b</sup>SEM: standard error of mean.

<sup>c</sup>P < 0.05 means different, P < 0.01 means significantly different, 0.05 < P < 0.10 means tending to be different.

<sup>d</sup>ADG: average daily gain; ADFI: average daily feed intake; F/G: feed/gain.
As indicated in Table 7, higher activities of CAT were observed in GOS treatment groups compared with control group, and with the increased amount of GOS inclusion, CAT level increased quadratically \((P = 0.03)\) on d 14 and also increased linearly \((P < 0.03)\) or quadratically \((P < 0.01)\) on d 28. MDA level tended to quadratically decrease \((P = 0.09)\) on d 14 and significantly quadratically decreased \((P = 0.02)\) on d 28 with the increased amount of GOS inclusion.

### Table 4. Effects of GOS on the contents of cytokines in serum of weaned piglets.\(^a\)

| Items         | GOS levels (mg/kg) | P-value\(^c\) | SEM\(^b\) | Linear | Quadratic |
|---------------|--------------------|---------------|-----------|--------|-----------|
|               | 0                  | 500           | 1000      | 1500   | 2000      |         |
| IL-1, ng/L    | 361.1              | 220.8         | 382.5     | 290.8  | 392.9     | 44.6    | 0.49    | 0.23    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 290.0              | 389.3         | 367.5     | 394.2  | 262.6     | 48.8    | 0.80    | 0.18    |
| IL-2, ng/L    | 111.1              | 206.7         | 142.7     | 107.5  | 159.4     | 24.4    | 0.85    | 0.84    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 141.0              | 159.2         | 234.5     | 148.7  | 194.4     | 26.1    | 0.45    | 0.46    |
| IL-4, ng/L    | 20.7               | 23.5          | 26.7      | 32.5   | 34.4      | 3.4     | 0.01    | 0.04    |
| IL-6, ng/L    | 16.3               | 19.9          | 20.9      | 17.2   | 1.9       | 1.9     | 0.83    | 0.85    |
| TNF-\(\alpha\), ng/L | 29.1              | 24.6          | 28.9      | 26.0   | 32.6      | 2.2     | 0.48    | 0.26    |
| IFN-\(\gamma\), pg/mL | 186.2             | 142.6         | 118.5     | 129.0  | 184.3     | 22.9    | 0.89    | 0.03    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 134.2              | 121.2         | 176.8     | 110.1  | 133.8     | 24.4    | 0.93    | 0.97    |
| IL-1, ng/L    | 579.6              | 457.2         | 653.9     | 365.5  | 437.3     | 57.3    | 0.07    | 0.19    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 340.8              | 451.8         | 469.1     | 334.6  | 419.5     | 33.3    | 0.56    | 0.30    |

\(^a\)Values are means of six pigs representing each dietary treatment.  
\(^b\)SEM: standard error of mean.  
\(^c\)\(P < 0.05\) means different, \(P < 0.01\) means significantly different, 0.05 < \(P < 0.10\) means tending to be different.

### Table 5. Effects of GOS on serum sCD3, sCD4 and sCD8 concentrations of weaned piglets.\(^a\)

| Items         | GOS levels (mg/kg) | P-value\(^c\) | SEM\(^b\) | Linear | Quadratic |
|---------------|--------------------|---------------|-----------|--------|-----------|
|               | 0                  | 500           | 1000      | 1500   | 2000      |         |
| sCD3, U/mL    | 6.56               | 5.65          | 8.87      | 9.38   | 11.21     | 1.00    | <0.01   | <0.01   |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 10.24              | 8.90          | 10.76     | 10.50  | 14.26     | 1.08    | 0.04    | 0.02    |
| sCD4, U/mL    | 4.40               | 5.23          | 4.19      | 4.83   | 1.83      | 0.90    | 0.16    | 0.12    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 4.70               | 5.13          | 7.17      | 3.31   | 6.54      | 0.92    | 0.47    | 0.77    |
| sCD8, U/mL    | 35.5               | 85.0          | 61.2      | 85.8   | 66.3      | 11.7    | 0.17    | 0.12    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 83.6               | 69.6          | 115.8     | 65.5   | 79.8      | 17.2    | 0.81    | 0.81    |

\(^a\)Values are means of six pigs representing each dietary treatment.  
\(^b\)SEM: standard error of mean.  
\(^c\)\(P < 0.05\) means different, \(P < 0.01\) means significantly different, 0.05 < \(P < 0.10\) means tending to be different.

### Table 6. Effects of GOS on the level of immunoglobulins in serum of weaned piglets.\(^a\)

| Items         | GOS levels (mg/kg) | P-value\(^c\) | SEM\(^b\) | Linear | Quadratic |
|---------------|--------------------|---------------|-----------|--------|-----------|
|               | 0                  | 500           | 1000      | 1500   | 2000      |         |
| IgA, g/L      | 39.2               | 48.4          | 42.1      | 30.0   | 44.7      | 3.9     | 0.60    | 0.87    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 49.6               | 55.7          | 65.7      | 64.0   | 51.5      | 6.8     | 0.71    | 0.19    |
| IgG, g/L      | 216.3              | 293.6         | 177.8     | 253.7  | 168.5     | 30.1    | 0.12    | 0.23    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 296.1              | 190.8         | 234.3     | 228.5  | 331.9     | 38.2    | 0.51    | 0.23    |
| IgM, g/L      | 31.5               | 39.3          | 28.8      | 32.2   | 22.9      | 5.8     | 0.27    | 0.43    |
| d 14          |                    |               |           |        |           |         |         |         |
| d 28          | 75.2               | 53.7          | 96.1      | 36.8   | 54.2      | 12.3    | 0.18    | 0.37    |

\(^a\)Values are means of six pigs representing each dietary treatment.  
\(^b\)SEM: standard error of mean.  
\(^c\)\(P < 0.05\) means different, \(P < 0.01\) means significantly different, 0.05 < \(P < 0.10\) means tending to be different.

### 4. Discussion

Tian et al. (2018) showed that piglets fed the diet supplemented with GOS had greater ADG than those fed the basal diet in the third week. In the present study, ADG of the weaned piglets increased during d 0–14 and d 0–28 of the experiment. This could be explained that, first, these effects were attributed to the improvement effect of GOS on nutrients availability.
Mountzouris et al. (2006) reported that increased digestibility of carbohydrate components, such as nitrogen free extract and NDF, of pigs consuming the trans-galactooligosaccharides diet might be due to the improving effect of GOS on the morphology and function of gut (Lefebure et al. 2009; Akbari et al. 2015; Bhatia et al. 2015; Tian et al. 2018). Second, other research also found that dietary GOS enhanced net absorption of mineral elements and improved their retention time (Weaver et al. 2011; Takasugi et al. 2013). Longer mean retention time of chyme can support more complete decomposition and utilization of indigestible oligosaccharides by microbes in the large intestine. In addition, GOS increased the viscosity of the chyme (Torres et al. 2010), which led to slower chyme passage rate, better fermentative capacity and longer chyme transit time in the large intestine. Third, short-chain fatty acids, for another key reason, are the main end products of the fermentation of non-digestible carbohydrates (Gibson et al. 2000; Montagne et al. 2003) and metabolized by hepatocytes, thereby contributing to energy utilization of the host. In general, pigs can digest soluble non-digestible carbohydrates better (Choct and Cadogan, 2001); however, the net efficiency of energy utilization via fermentation is still low in pigs.

In the present study, non-digestible GOS was considered as one of the potential prebiotics (Weaver et al. 2011). Traditionally, the main health effect of GOS on the intestinal microbiota is selectively stimulating the growth and activity of saccharolytic Bifidobacteria and Lactobacilli species, while inhibiting the activation of harmful bacteria such as Enterobacteriaceae, Clostridium sensu strictu, Streptococcus faecalis and Proteus sp. (Gopal et al. 2001; Tzortzis et al. 2005; Vulevic et al. 2015; Matsuki et al. 2016). In our study, dietary addition of GOS inhibited the number of Escherichia coli and increased the number of Lactobacillus and Bifidobacteria on d 28, which is consistent with the previous reports. This is maybe due to Bifidobacteria’s having a broad and rapid enzyme response to the presence of GOS (Tzortzis et al. 2009). In addition, emerging evidences suggested that Bifidobacteria and Lactobacilli produced organic acids and other antagonistic agents, which directly suppressed opportunistic pathogens, promoted gut mucosal immunity and attenuated the inflammation (Inan et al. 2000; Scheppach et al. 2004; Nurmi et al. 2005). Hence, we thought that GOS administration reduced pro-inflammatory responses (Akbari et al. 2015), however, so far, no study has assessed the effects of dietary supplementation with GOS on cytokines of piglets. Fructooligosaccharides appeared to elicit immunological effects via bacterial changes in the intestinal environment (Hosono et al. 2003). Bacterial stimulation on the mucosal immune system has been shown to involve TLRs expression in macrophages and dendritic cells through regulating production of proinflammatory cytokines such as TNF-α (Kaiho et al. 2002). In the present study, TNF-α level decreased with increasing GOS supplementation, and this was probably related to that GOS could act as antiadhesives against enteropathogenic Escherichia coli adherence to intestinal epithelial cells (Shoa et al. 2006). Hence, we thought that GOS administration reduced production of proinflammatory cytokines via modification of the intestinal microbiota, especially Escherichia coli. Moreover, our finding demonstrated for the first time that dietary supplementation of GOS improved serum IL-4 concentration, which implied better protection against potential leading to inflammation. Oligosaccharides have been shown to interfere with T helper Th1/Th2 skewing in mononuclear cells and to affect the Th2-type immune response (Eiwegger et al. 2010; Eiwegger et al. 2004). The Th2 immune response is essential for antibody production and the elimination of some viruses and extracellular pathogens, which can be characterized by the production of IL-4 (Wills-Karp et al. 1999). It was suggested that GOS stimulated systemic immunity via a shifting in the Th1/Th2 balance toward Th2 dominant immunity. However, it is still unclear how GOS modulates the activity of Th1/Th2 cells. In addition, our results showed that dietary addition of GOS linearly or quadratically elevated serum concentration of sCD3. The CD3 molecule reacts with all mature T-cells and it is a common surface marker for T lymphocytes (Youakim et al. 1999). Thus, it was deduced that the increasing concentrations of sCD3 in the GOS supplementation group reflected the rising in total number of peripheral mature T lymphocytes, and then, the

### Table 7. Effects of GOS on antioxidative indexes in serum of weaned piglets.a

| Items      | GOS levels (mg/kg) | SEMb | Linear | Quadratic |
|------------|--------------------|------|--------|-----------|
| T-AOC, U/mL|                    |      |        |           |
| d 14       | 2.31               | 0.30 | 0.48   | 0.42      |
| d 28       | 2.96               | 0.41 | 0.80   | 0.95      |
| CAT, U/mL  |                    |      |        |           |
| d 14       | 2.29               | 0.54 | 0.60   | 0.03      |
| d 28       | 0.97               | 0.70 | <0.01  | <0.01     |
| GSH-Px, U/mL|                   |      |        |           |
| d 14       | 608                | 35   | 0.37   | 0.39      |
| d 28       | 618                | 549  | 0.84   | 0.72      |
| MDA, nmol/mL|                  |      |        |           |
| d 14       | 2.07               | 0.25 | 0.73   | 0.09      |
| d 28       | 2.85               | 0.26 | 0.36   | 0.02      |
| T-SOD, U/mL|                    |      |        |           |
| d 14       | 93                 | 73   | 0.11   | 0.28      |
| d 28       | 88                 | 103  | 0.46   | 0.76      |

*Values are means of six pigs representing each dietary treatment.*

bSEM: standard error of mean.

*P < 0.05 means different, P < 0.01 means significantly different, 0.05 < P < 0.10 means tending to be different.
immunity was improved. Dietary supplementation of GOS had a positive effect on the immune system. In spite of the above-mentioned improvements, the exact mechanism of GOS’s modulating immune response is still unclear and deserves further research. Furthermore, previous study has proved the efficiency of GOS in decreasing ROS production in meat (Rajauria et al. 2016). Other research indicated that GOS protected the intestinal epithelial barrier against heat stress by a suppression of the heat-induced oxidative stress response (Varasteh et al. 2015). Several antioxidative enzymes in cells, including CAT, SOD and GPx, act as the defense system to prevent the excessive production of ROS (Sanders et al. 2004). The level of MDA is generally used as a biomarker of ROS-mediated damages and the degree of endogenous lipid peroxidation (Sehirli et al. 2008; Yousef et al. 2009). The results of our experiment showed that GOS concentration in the later period was decreased. It was assumed that GOS could maintain a proper oxidative status. Moreover, we noticed that CAT activity was promoted, which suggested that GOS might possess the ability to eliminate \( \text{H}_2\text{O}_2 \) primarily by enhancing CAT.

5. Conclusion

In summary, the results observed in this study indicated that dietary supplementation with GOS contributed to better growth performance in weaned piglets, inhibited the proliferation of \textit{Escherichia coli} and increased the population of \textit{Bifidobacteria} and \textit{Lactobacillus} and was effective in improving the immune and antioxidant function of weaned piglets. The health benefits of GOS were attributed to elevating the gut probiotics (\textit{Lactobacillus} and \textit{Bifidobacteria}), improving immune response and antioxidant capability.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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