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Effects of isomaltooligosaccharide and *Bacillus* supplementation on sow performance, serum metabolites, and serum and placental oxidative status

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ABSTRACT

This study investigated the effects of isomaltooligosaccharide (IMO) and *Bacillus* supplementation on sow performance, serum metabolites, and serum and placental oxidative status. Multiparous gestating sows (n = 130) with similar body conditions were randomly allocated to five groups (n = 26) receiving a basal diet (CON group) or a basal diet supplemented with 0.5% IMO (IMO group); 0.5% IMO and 0.02% *Bacillus subtilis* (IMO + S group); 0.5% IMO and 0.02% *Bacillus licheniformis* (IMO + L group); or 0.5% IMO, 0.02% *Bacillus subtilis*, and 0.02% *Bacillus licheniformis* (IMO + S + L group). There were no significant differences in the litter sizes among all dietary groups. The average piglet birth weight was improved in all treatment groups, and the placental efficiency was greater in the IMO + S and IMO + S + L groups than in the CON group ($P < 0.05$). The IMO + S + L group had increased the low-density lipoprotein cholesterol and reduced the total cholesterol in umbilical venous serum ($P < 0.05$). Additionally, the malondialdehyde concentrations were greater in umbilical venous serum of piglets in all treatment groups relative to that in the CON piglets ($P < 0.05$). The placental total antioxidant capacity was increased in the IMO + L and IMO + S + L groups ($P < 0.05$). Furthermore, the growth hormone concentration in umbilical venous serum was greater ($P < 0.05$) in all treatment groups. Overall, IMO and *Bacillus* supplementation during late gestation resulted in a changed metabolism of sows, improved the placental antioxidant capacity, and increased the growth hormone concentrations in umbilical venous serum, which ultimately improved the piglet birth weight and placental efficiency.

1. Introduction

Oxidative stress in pregnancy arises from an increase in placental mitochondrial activity and production of reactive oxygen species (including nitric oxide, carbon monoxide, and peroxynitrite), which have pronounced effects on placental function, including trophoblast proliferation and differentiation and vascular reactivity (Myatt and Cui, 2004). Placental oxidative stress is a major contributing factor to preeclampsia and abortion (Burton and Jauniaux, 2004). Oxidative stress also contributes to a reduction in reproductive performance of female animals (Lipkoprzybylska and Kankofer, 2012; Kim et al., 2013), such as litter performance, offspring survival rate, and milk yield (Yoon and Mcmillan, 2006; Albera and Kankofer, 2010). Although results of numerous studies

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indicate that there is systemic oxidative stress of sows during late gestation, the underlying mechanism involved and the nutritional regulatory strategy required to prevent this condition are still unclear (Albera and Kankofer, 2010; Berchieri-ronchi et al., 2011).

According to Fuller's definition (Fuller, 1989), probiotics are "live active ingredients used as food supplements that are composed of live microorganisms that react favorably on the host by improving the intestinal microbial equilibrium". These microbes also have a pivotal role in regulating the health condition of sows owing to the capacity to selectively promote the growth and/or metabolic functions of one or more bacterial species in the colon (Roberfroid, 1999). Isomaltooligosaccharide (IMO), a functional oligosaccharide (Patel and Goyal, 2011), is known for its potential to activate the immune system (Mizubuchi et al., 2005), thereby enhancing the host's resistance to diseases and oxidation (Mao et al., 2015). There was also improvement in lipid metabolism and the functions of the liver and kidneys (Wang et al., 2001; Li et al., 2009). The placental tissue is the only site connecting the fetus and sow during pregnancy, and placental oxidation is closely related to the growth of the fetus (Zhou et al., 2018). Chitosan oligosaccharide supplementation in the diet of sows during late gestation also enhanced the antioxidant defense capacity of the animals and the placentas (Xie et al., 2016; Wan et al., 2017), which improved their reproductive performance (Xie et al., 2015; Wan et al., 2016). Species of the genus *Bacillus*, which are gram-positive, spore-forming, facultative, anaerobic bacteria, can also provide effective protection against oxidative stress damage (Tang et al., 2018). Supplementation of *Bacillus* to the diet in late pregnancy was improved reproductive performance, such as piglet birth weight and reduced the rate of stillbirths and number of weak piglets (Kritas et al., 2015). The synergistic effects between probiotics and prebiotics create an optimal micro-environment for the growth of many bacteria, thereby improving the gastrointestinal function of animals and enhancing health and performance (Shinja et al., 2009; Ipek et al., 2016). The effects of IMO on the reproductive performance of sows, however, are unknown, and there is no research on the synergistic effects of IMO and *Bacillus* species in these animals.

The aim of this present study was to explore the effects of IMO, alone and in combination with *Bacillus* species, on the reproductive performance and serum biochemical indexes of sows. Whether the dietary supplementation could regulate the placenta and umbilical venous blood contents to improve the growth and development of the fetus was also evaluated.

2. Materials and methods

2.1. Prebiotic and probiotics

The protocol of this study was approved by the Institution Animal Care and Use Committee of the College of Animal Science and Technology, Hunan Agricultural University (Changsha, China), and was conducted in accordance with the National Institutes of Health (Changsha, China) guidelines for the care and use of experimental animals. The IMO (IMO-900; Purity ≥ 90%, with total isomaltose, panose, and isomaltotriose contents > 45%) was provided by Baolingbao Biology Company (Shandong, China). The *Bacillus subtilis* (2.0×10^{11} cfu/g) and *Bacillus licheniformis* (2.0×10^{11} cfu/g) cultures were provided by Shandong Kangdien Biotechnology Company (Shandong, China).

2.2. Animals, diets, and experimental design

In total, 130 sows in late pregnancy (gestation day 85, Large White × Landrace), with an initial body weight (BW) of 236.2 ± 23.7 kg and mean parity of 3.2 ± 1.2, were randomly allocated to 1 of 5 dietary treatments (with 26 replicates) on the bases of BW, parity, and back fat. The five groups were as follows: 1) CON group, fed a basal diet from late gestation to farrowing; 2) IMO group, fed a basal diet plus 0.5% IMO; 3) IMO + S group, fed a basal diet plus 0.5% IMO and 0.02% *Bacillus subtilis*; 4) IMO + L Lgroup, fed a basal diet plus 0.5% IMO and 0.02% *Bacillus licheniformis*; and 5) IMO + S + L group, fed a basal diet plus 0.5% IMO, 0.02% *Bacillus subtilis*, and 0.02% *Bacillus licheniformis*. The composition of the basal diet (Table 1) was formulated in compliance with National Research Council (NRC, 1998) nutrient requirements.

The sows were housed in concrete-floored farrowing pens (2.0 m × 0.6 m) during gestation days 85–110. The sows in each group were fed a total of 2.6–2.8 kg of food daily, where approximately half the amount was provided at each feeding twice a day (07:30 AM and 14:30 PM). During gestation days 110 to parturition, the sows were housed indoors in concrete-floored delivery room pens (2.13 m × 0.66 m). Again, approximately half the daily total of 3.2 kg of feed was provided to the sows at each feeding, twice a day (08:00 AM and 16:30 PM). Before farrowing on days 1–2, the average feeding amount was reduced to 2.0 kg/d. The sows were provided *ad libitum* access to water throughout the experimental period. The experiment was conducted at a pig farm (Zhenghong Inc., Hunan, China), where the feeding management and immunization procedures were performed in accordance with the company's standard of breeding management. The feeding trial lasted for 30 days with seven sows in each group randomly selected for sample collection.

2.3. Sample collection

During farrowing, a 10 ml sample of blood was collected from the ear vein of the sows. Additionally, using a disinfected disposable syringe, a 10 ml blood sample was extracted from the umbilical vein of piglets at the time of birth (from the largest umbilical cord that could be located). After storage of the blood samples for 1 h at 4 °C, these were centrifuged at 3000 × *g* for 15 min at 4 °C, whereupon the plasma samples obtained were immediately stored at −80 °C for biochemistry and antioxidant analyses. Meanwhile, after the placenta was expelled from the sow, 1 to 2 *g* of placental tissue was cut with sterilized scissors (two portions for each sow), immediately frozen in liquid nitrogen, and stored at −80 °C for analysis.

Table 1
Composition and nutrient levels of basal diets(Based on dry matter).

	Basal diet
Corn	47.90
Barley	30.00
Bran	5.00
Soybean meal, 43%	12.10
Fish meal	1.00
Limestone	1.00
Ca HPO4	1.20
Salt	0.50
Lys	0.15
DL-Met	0.15
Premix[1]	1.00
Total	100
Calculated nutrient content[2]	
DE MJ/kg	3, 150.00
CP %	13.50
EE %	2.50
CF %	3.24
Ash %	5.18
Ca %	0.95
TP %	0.60
AP %	0.35
Lys %	0.70

[1] The premix provided the following per kg of diets: VA 10, 000 IU, VD_3 2, 100 IU, VE 45 IU, VK_3 2.0 mg, thiamine 3.0 mg, riboflavin 5.0 mg, VB_6 1.8 mg, VB_{12} 0.03 mg, cholinechloride 1 000 mg, nicotinic acid 25.0 mg, pantothenic acid 15.0 mg, biotin 0.08 mg, folic acid 1.0 mg, Mn20.0 mg, Zn 80.0 mg, Fe 80 mg, Cu 6.0 mg, I 0.15 mg, Se 0.25 mg.
[2] Nutrient amounts were calculated value.

2.4. Reproductive performance

The total number of births, number of live births, number of robust births (weight > 800 g), number of stillbirth piglets, number of mummies, average piglet birth weight (BW), placental weight, and placental efficiency (piglet birth weight/placental weight, average placental efficiency, not individual efficiencies) (Wilson et al., 1999) were recorded at farrowing. The backfat thickness of the sows at point P2 (in line with the last rib, 65 mm from the midline) at the beginning of the trial and at the time of parturition were recorded as well.

2.5. Antioxidant analysis

Frozen placental tissue (2 mg) in 2 ml of phosphate-buffered saline was homogenized on ice with an Ultra-Turrax homogenizer (Bioblock Scientific, Illkirch, France) for 10 s at 6800 rpm. The homogenate was centrifuged at 950 × *g* for 10 min at 4 °C, and the supernatant was stored in a 2-mL centrifuge tube at −80 °C until analysis. The glutathione peroxidase (GSH-Px) and catalase (CAT) activities, total antioxidant capacity (T-AOC), and malondialdehyde (MDA) concentrations in the placental and umbilical venous sera were assayed using a UV/visible spectrophotometer (UV-2450, Shimadzu, Kyoto, Japan). The assays were conducted using assay kits purchased from Nanjing Jiancheng Institute of Bioengineering (Nanjing, Jiangsu, China) and conducted according to the manufacturer's instructions. All samples were measured in triplicate, at appropriate dilutions, and the activities of the enzymes were estimated from the linear range of standard curves constructed with the pure enzymes. The protein concentration of the supernatants was determined using Coomassie Brilliant Blue G250 (BlueGene, Shanghai, China).

2.6. Serum biochemistry

The total protein (TP), albumin (ALB), and urea nitrogen (urea N) concentrations; alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities; and total cholesterol (TC), triacylglycerol (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) concentrations in the blood plasma samples were determined using an automatic biochemical analyzer (BS-200, Shenzhen Mindray Bio-medical Electronics Co., Ltd., Shenzhen, China). The assay kits were purchased from Nanjing Jiancheng Institute of Bioengineering and the assays conducted according to the manufacturer's protocols.

Serum insulin (INS) and growth hormone (GH) concentrations were quantified using a radioimmunoassay. Serum concentrations

Table 2
Effects of Isomaltooligosaccharide and *Bacillus* on the reproductive performance of sows during late gestation.

Items	Maternal treatment[1]					SEM	*P*-value		
	CON	IMO	IMO + S	IMO + L	IMO + S+L		Diet	Parity	D*P
Total pigs born, *n*	12.81	12.59	12.82	12.83	12.88	0.204	0.993	0.705	0.724
Pigs born alive, *n*	12.44	12.24	12.12	12.22	12.63	0.192	0.957	0.424	0.384
Pigs born robust, *n*	11.69	11.88	11.65	12.11	12.25	0.184	0.852	0.489	0.396
Stillbirth number, *n*	0.48	0.43	0.46	0.48	0.21	0.066	0.844	0.706	0.869
Mummy number, *n*	0.21	0.09	0.08	0.11	0.21	0.039	0.688	0.676	0.378
Average piglet BW[2], kg	1.39[b]	1.53[a]	1.52[a]	1.54[a]	1.59[a]	0.020	0.033	0.010	0.683
Placental weight, kg	3.76	3.06	2.52	3.13	2.77	0.162	0.111	0.124	0.141
Placental efficiency	4.68[b]	5.96[ab]	6.37[a]	5.61[ab]	6.33[a]	0.210	0.026	0.297	0.272
Initial backfat, mm	16.41	17.20	16.12	16.29	18.21	0.330	0.490	0.118	0.030
Farrowing backfat, mm	17.26	17.57	17.84	18.27	18.93	0.343	0.699	0.412	0.151

[a, b]Mean values within each row with different superscripts differ ($P < 0.05$); There is no difference unless superscript lettering indicates otherwise ($P > 0.05$).

[1] CON = sows fed a basal diet from late gestation to farrowing, IMO = basal diet + 0.5%. Isomaltooligosaccharide, IMO + S = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis*, IMO + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus licheniformis*, IMO + S+L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis* + 0.02% *Bacillus licheniformis*.

[2] BW: Brith weight.

of immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) were determined by the radial immunodiffusion method, using a commercial kit (Wuhan Biological Engineering Co., Ltd., Wuhan, China) according to the manufacturers' instructions.

2.7. Statistical analysis

SPSS 21.0 (SPSS, Chicago, IL, USA) was used for all data analyses. Individual sows served as the experimental units. Statistical analysis was performed with analysis of covariance and one-way analysis of variance, followed by Duncan's multiple range test. All data are expressed as the mean ± standard error of the mean. Differences were considered significant at $P < 0.05$.

3. Results

3.1. Sow reproductive performance

There were no observed differences in the numbers of total piglets born, piglets born alive, stillborn piglets, and mummified piglets, as well as in the backfat thickness of the sows in late pregnancy, among the various dietary treatment groups, and there was also no effect on these indexes of parity ($P > 0.05$; Table 2). There, however, was an increase in the average piglet BW in all treatment groups and in the placental efficiency of the IMO + S and IMO + S+L groups ($P < 0.05$) compared with those in the CON group (Table 2).

3.2. Sow serum biochemistry

The effects of IMO alone and in combination with *Bacillus subtilis* and/or *Bacillus licheniformis* on the serum biochemistry of sows in late gestation were determined and the results are presented in Table 3. The IMO supplementation alone did not affect the serum metabolite contents in the sows ($P > 0.05$) except the serum TG concentration ($P < 0.05$), whereas supplementation with IMO+S decreased the urea N concentration ($P < 0.05$) and also tended to increase the HDL-C and TP concentrations compared with the other treatments ($P = 0.056$; $P = 0.050$)). Furthermore, dietary supplementation with IMO + S+L increased the TG concentrations ($P < 0.05$).

3.3. Umbilical venous serum biochemistry

Data for supplementation with IMO and its combination with *Bacillus subtilis* and/or *Bacillus licheniformis* on biochemical indexes of umbilical venous serum are included in Table 4. There were no differences in the contents of metabolites in umbilical venous serum of the piglets between the CON and IMO groups ($P > 0.05$). In contrast, the HDL-C concentration in the IMO+S group was greater ($P < 0.01$), and there was also a trend for greater TP concentrations in all treatment groups ($P = 0.069$). In addition, the concentrations of TC and urea N were less in the IMO + L group ($P < 0.01$). The concentrations of AST, ALT, and ALP in the IMO + S+L group and those of ALT and ALP in the IMO + L group, however, were increased ($P < 0.01$). Furthermore, IMO + S+L supplementation increased the concentrations of LDL-C and HDL-C ($P < 0.01$) but decreased the concentration of TC ($P < 0.001$).

Table 3
Effect of Isomaltooligosaccharide and *Bacillus* on serum biochemistry of sows in late gestation.

Items[2]	Maternal treatment[1]					SEM	*P*-value
	CON	IMO	IMO + S	IMO + L	IMO + S+L		
LDL-C, mmol/L	0.59	0.57	0.66	0.58	0.51	0.025	0.615
HDL-C, mmol/L	0.46	0.43	0.61	0.52	0.44	0.020	0.056
TC, mmol/L	1.32	1.19	1.55	1.36	1.19	0.047	0.169
TG, mmol/L	0.16^{c}	0.23^{ab}	0.24^{ab}	0.19^{bc}	0.28^{a}	0.012	0.002
TP, g/L	63.21	68.46	70.01	67.11	67.47	0.813	0.050
ALB, g/L	36.40	35.93	40.79	37.84	38.25	0.601	0.113
Urea N, mmol/L	4.39^{ab}	4.40^{ab}	2.82^{c}	5.22^{a}	4.11^{b}	0.196	< 0.001
ALT, u/L	32.60^{ab}	27.38^{b}	29.12^{b}	35.17^{a}	27.68^{b}	0.999	0.020
ALP, u/L	44.86	39.92	40.63	38.13	48.72	1.687	0.388
AST, u/L	29.75	25.10	25.40	28.02	22.88	1.022	0.231

[a, b, c]Mean values within each row with different superscripts differ ($P < 0.05$) There is no difference unless superscript letters indicate otherwise ($P > 0.05$).

[1] CON = sows fed a basal diet from late gestation to farrowing, IMO = basal diet + 0.5% Isomaltooligosaccharide, IMO + S = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis*, IMO + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus licheniformis*, IMO + S +L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis* + 0.02% *Bacillus licheniformis*.

[2] LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TC: total cholesterol, TG: triacyloglycerol, TP: total protein, ALB: albumin, ALT: aminotransferase alanine, ALP: alkaline phosphatase, AST: aminotransferase aspartate.

Table 4
Effect of Isomaltooligosaccharide and *Bacillus* on umbilical venous serum biochemistry.

Items[2]	Maternal treatment[1]					SEM	*P*-value
	CON	IMO	IMO + S	IMO + L	IMO + S+L		
LDL-C, mmol/L	0.46^{bc}	0.52^{b}	0.55^{b}	0.37^{c}	0.76^{a}	0.035	< 0.001
HDL-C, mmol/L	0.37^{b}	0.47^{ab}	0.50^{a}	0.37^{b}	0.50^{a}	0.017	0.005
TC, mmol/L	1.12^{a}	1.32^{a}	1.25^{a}	0.85^{b}	0.74^{b}	0.059	< 0.001
TG, mmol/L	0.28	0.33	0.24	0.30	0.32	0.014	0.261
TP, g/L	17.07	21.25	22.27	23.77	25.12	0.774	0.069
ALB, g/L	6.21	7.20	7.30	7.66	8.48	0.237	0.141
Urea N, mmol/L	5.87^{a}	5.82^{a}	4.07^{b}	6.82^{a}	6.08^{a}	0.283	0.001
ALT, u/L	9.42^{b}	9.45^{b}	10.21^{b}	11.94^{b}	17.79^{a}	0.755	0.001
ALP, u/L	$1, 603.89^{b}$	$2, 089.09^{b}$	$3, 384.83^{b}$	$2, 250.68^{a}$	$2, 242.62^{b}$	178.854	0.005
AST, u/L	19.00^{c}	24.97^{bc}	27.17^{b}	26.014^{bc}	37.11^{a}	1.429	0.006

[a, b, c]Mean values within each row with different superscripts differ ($P < 0.05$); There is no significant difference unless superscript letters indicate otherwise ($P > 0.05$).

[1] CON = sows fed a basal diet from late gestation to farrowing, IMO = basal diet + 0.5% Isomaltooligosaccharide, IMO + S = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis*, IMO + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus licheniformis*, IMO + S + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis* + 0.02% *Bacillus licheniformis*.

[2] LDL-C: low density lipoprotein cholesterol, HDL-C: high density lipoprotein cholesterol, TC: total cholesterol, TG: triacyloglycerol, TP: total protein, ALB: albumin, ALT: aminotransferase alanine, ALP: alkaline phosphatase, AST: aminotransferase aspartate.

3.4. Placental antioxidation

To investigate the effects of IMO and its combination with *Bacillus subtilis* and/or *Bacillus licheniformis* on the antioxidant capacity during late gestation, the placental GSH-Px, T-AOC, and MDA concentrations and CAT activities were analyzed (Table 5). The results indicated the GSH-Px concentrations were decreased in both the IMO and IMO + S+L groups ($P < 0.05$). The T-AOC concentration was improved by adding IMO + L and IMO + S+L to the diet ($P < 0.05$). Furthermore, IMO + S+L supplementation reduced the MDA concentration ($P < 0.05$). The CAT concentrations were less, however in all the treatment groups as compared with the CON group ($P < 0.001$).

3.5. Umbilical venous serum antioxidation

Along with the effects of IMO and its combination with *Bacillus subtilis* and/or *Bacillus licheniformis* on antioxidant defense in the placenta, there was also assessments of the effects on umbilical venous serum antioxidation (Table 6). The concentration of MDA in umbilical venous serum was greater in all the treatment groups relative to the CON group ($P < 0.01$). Furthermore, the CAT activity was less in the IMO group than in the other treatment groups ($P < 0.001$), but was greater in the IMO + L group than in the CON and other treatment groups ($P < 0.001$).

Table 5
Effect of Isomaltooligosaccharide and *Bacillus* on placental antioxidation.

Items[2]	Maternal treatment[1]					SEM	*P*-value
	CON	IMO	IMO + S	IMO + L	IMO + S+L		
MDA, nmol/mg prot	17.60^{a}	18.87^{a}	16.81^{a}	13.26^{ab}	9.74^{b}	1.177	0.035
T-AOC, U/mg prot	3.59^{c}	2.98^{c}	3.74^{c}	6.33^{b}	9.93^{a}	0.703	< 0.001
GSH-Px, U/mg prot	0.47^{a}	0.30^{bc}	0.44^{ab}	0.34^{abc}	0.25^{c}	0.025	0.027
CAT, U/mg prot	0.20^{a}	0.10^{b}	0.12^{b}	0.11^{b}	0.09^{b}	0.014	0.004

$^{a, b, c}$Mean values within each row with different superscripts differ ($P < 0.05$) There is no difference unless superscript letters indicate otherwise ($P > 0.05$).

[1] CON = sows fed a basal diet from late gestation to farrowing, IMO = basal diet + 0.5% Isomaltooligosaccharide, IMO + S = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis*, IMO + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus licheniformis*, IMO + S + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis* + 0.02% *Bacillus licheniformis*.

[2] MDA: malondialdehyde, T-AOC: total antioxidant capability, GSH-Px: glutathione peroxidase, CAT: catalase.

Table 6
Effect of Isomaltooligosaccharide and *Bacillus* on umbilical venous serum antioxidation.

Items[2]	Maternal treatment[1]					SEM	*P*-value
	CON	IMO	IMO + S	IMO + L	IMO + S+L		
MDA, nmol/ml	3.87^{b}	6.10^{a}	7.35^{a}	6.40^{a}	6.57^{a}	0.381	0.004
T-AOC, U/ml	2.22	2.24	2.51	1.95	2.92	0.148	0.371
GSH-Px, U/ml	331.74	377.83	359.13	330.87	350.43	7.889	0.238
CAT, U/ml	20.70^{b}	6.78^{c}	19.42^{b}	36.13^{a}	24.99^{b}	2.591	< 0.001

$^{a, b, c}$Mean values within each row with different superscripts differ ($P < 0.05$); There is no difference unless superscript letters indicate otherwise ($P > 0.05$).

[1] CON = sows fed a basal diet from late gestation to farrowing, IMO = basal diet + 0.5% Isomaltooligosaccharide, IMO + S = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis*, IMO + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus licheniformis*, IMO + S + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis* + 0.02% *Bacillus licheniformis*.

[2] MDA: malondialdehyde, T-AOC: total antioxidant capability, GSH-Px: glutathione peroxidase, CAT: catalase.

3.6. Umbilical venous serum hormones and immunity

The results of the umbilical venous serum hormone concentrations and immunity assessments are presented in Table 7. The supplementations of IMO and its combination with *Bacillus subtilis* and/or *Bacillus licheniformis* to the sow increased the GH concentration in umbilical venous serum ($P < 0.001$). Dietary supplementation of IMO + L reduced the concentration of IgM ($P < 0.01$). In addition, the concentrations of INS, IgG, and IgM ($P < 0.05$) were reduced by IMO + S+L supplementation.

4. Discussion

Gestating sows are generally fed a restricted intake diet and there is frequently severe oxidative stress during late pregnancy, which could reduce reproductive performance (Göransson, 1989; Berchieri-ronchi et al., 2011). In addition, the fetuses grow rapidly

Table 7
Effect of Isomaltooligosaccharide and *Bacillus* on umbilical venous serum hormones and immunity.

Items[2]	Maternal treatment[1]					SEM	*P*-value
	CON	IMO	IMO + S	IMO + L	IMO + S+L		
INS, u IU/ml	231.38^{ab}	203.32^{ab}	180.02^{bc}	261.43^{a}	153.38^{c}	13.209	0.005
GH, u IU/ml	60.04^{c}	80.73^{b}	79.43^{b}	80.91^{b}	92.15^{a}	2.443	< 0.001
IgA, ug/ml	319.63	310.61	304.00	314.01	305.13	2.105	0.125
IgG, ug/ml	1, 662.20^{a}	1, 651.20^{a}	1, 663.06^{a}	1, 634.15^{a}	1, 539.96^{b}	13.128	0.019
IgM, ug/ml	203.79^{a}	172.51^{ab}	193.03^{ab}	163.19^{bc}	135.02^{c}	7.142	0.003

$^{a, b, c}$Mean values within each row with different superscripts differ ($P < 0.05$); There is no difference unless. superscript letters indicate otherwise ($P > 0.05$).

[1] CON = sows fed a basal diet from late gestation to farrowing, IMO = basal diet + 0.5% Isomaltooligosaccharide, IMO + S = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis*, IMO + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus licheniformis*, IMO + S + L = basal diet + 0.5% Isomaltooligosaccharide + 0.02% *Bacillus subtilis* + 0.02% *Bacillus licheniformis*.

[2] INS: insulin, GH: growth hormone, Ig A: Immunoglobulin A, Ig G: Immunoglobulin G, Ig M: Immunoglobulin M.

in late pregnancy, which can easily lead to dysfunction of the gastrointestinal tract of the sow (Chuanshang et al., 2018), thereby further exacerbating the extent of oxidative stress in sows. Results from numerous studies indicate the reproductive performance of the sow and the growth performance of the offspring can be improved by feeding probiotics (Sen et al., 2012; Jinsuk et al., 2015). *Bacillus* intake may improve the immune function of pregnant sows by altering the proliferative response of lymphocytes (Schierack et al., 2009). In addition, IMO can enhance resistance to diseases and oxidation by activating the immune system (Mao et al., 2015; Mizubuchi et al., 2005). Furthermore, by improving the health of sows, the growth and development of their fetuses can also be improved (Hayakawa et al., 2016). From previous studies, there have been reports that *Bacillus subtilis* and *Bacillus licheniformis* serve as safe and effective feed additives and can improve the reproductive performance of sows (Mizubuchi et al., 2005; Mao et al., 2015). Furthermore, Wan et al. (2016) reported that dietary chitosan oligosaccharide supplementation enhanced the survival rate and size (crown-to-rump length) of fetuses after 35 days of gestation and there was also an increase in the number of viable piglets born per litter as well as the average weight of piglets born alive. In the present study, supplementation with IMO also improved the reproductive performance of sows, including the average piglet BW. Furthermore, the combination of feeding IMO with *Bacillus subtilis* and *Bacillus licheniformis* had a synergistic effect on placental efficiency. As the ratio of fetal weight to placental weight, the placental efficiency is generally related to the growth and development of the fetus (Molteni et al., 1978; Vonnahme et al., 2002). In the present study, the placental efficiency was improved by IMO supplementation when fed in combination with *Bacillus subtilis* and *Bacillus licheniformis*. The possible reason for this outcome is that the addition of a functional oligosaccharide to the diet of sows at the later stage of pregnancy stimulates the mammalian target of rapamycin (mTOR) signaling pathway and increases the placental amino acid transporters, which may contribute to fetal development during gestation (Xie et al., 2016). In addition, supplementation with chitosan oligosaccharide resulted in an upregulation of the expression of leptin and vascular endothelial growth factor A (VEGFA) genes in the placenta and enhanced the expression of genes that are important to fetal development. Additionally, there was an induction of the antioxidant milieu in the amniotic fluid. The resulting modification of the metabolic profiles also improved fetal survival and growth, which may be another reason for the increased placental efficiency (Wan et al., 2016, 2017).

The placenta is a highly efficient multifunctional organ, mediating the exchange of nutrients, gases, and waste products between the dam and fetus (Osgerby et al., 2004). The lipid metabolism in the sow at late pregnancy affects the lipid concentrations in the placenta (Zhou et al., 2018). The serum TC and TG contents can reflect the absorption of lipids by the animal and its nutrition status (Wheeler et al., 1987). In the present study, the TG concentration in sows was enhanced in all the treatment groups. The lipids in the placenta are transported to the fetuses mainly as a result of maternal lipid transport and placental lipid metabolism processes (Herrera and Ortegasenovilla, 2014). The maternal cholesterol, LDL-C, and free fatty acids (FFAs) are transported to the placenta as a result of actions of placental receptors, whereas TG enters the blood in the form of lipoprotein and is hydrolyzed into FFAs that can then be transported to the placenta (Herrera and Ortegasenovilla, 2014). The maternal serum TG concentrations are positively correlated with the neonatal birth weight. Furthermore, IMO has gastrointestinal functions (Yang et al., 2012; Jiao et al., 2015) and reduces oxidative stress (Wan et al., 2016; Xie et al., 2016), thereby removing some additional barriers to lipid catabolism or mobilization for fetal growth. The excessive decomposition of fat can lead to an excessive deposition of placental lipids, which may result in lipid oxidation damage in the placenta. In the present study, the TC concentration was reduced and the MDA concentration was increased by adding IMO + S + L to the diet of sows in late gestation, which means that placental lipid metabolism may have been improved.

Fetal development increases the oxygen and nutrient demands of the sow in late pregnancy (Kim et al., 2013), resulting in a severe metabolic burden for the sow and leading to oxidative stress (Berchieri-ronchi et al., 2011). The oxygen free radicals produced by metabolic processes can damage the placental villi, resulting in villi cell membrane, mitochondria membrane, and placental dysfunction. Such damage can affect the growth of the fetus *in utero* and lead to a decrease in the sow's lactation performance and immune function, thereby seriously affecting the health and longevity of sows and development of offspring (Serenius and Stalder, 2006). In the present study, the concentration of placental T-AOC was increased and the MDA concentration was decreased with IMO + S + L supplementation to the diet of sows at late pregnancy. The IMO and *Bacillus* species can enhance the concentrations of antioxidant enzymes (Lei et al., 2013). Furthermore, probiotics can improve the antioxidant capacity of piglets by improving the antioxidant capacity and health of the sows (Xie et al., 2016). Improvement of the placental antioxidant capacity may be an important reason for the improvement in placental efficiency (Richter et al., 2009). Furthermore, the increase in placental efficiency can improve the average BW of the piglet (Wilson et al., 1999).

The umbilical cord is the only link between the sow and the fetus. There is transfer of nutrients from the sow to the fetus through the umbilical vein, and waste is delivered to the sow through the umbilical artery. In the present study, the concentration of umbilical venous serum GH was increased by the supplementations. Jin et al. (2017) reported that alginate oligosaccharide supplementation could enhance the serum hormone concentrations, including those of GH, INS and INS-like growth factor-1. Results of the present study indicate that the increase in the GH concentration may be one reason for the growth of the fetus. In addition, the TP and ALB concentrations reflect the amount of protein deposition, whereas the urea N concentration reflects the amount of protein degradation. In the present experiment, the amount of protein metabolites in umbilical venous blood was greater in all the treatments groups as compared with the CON group, which perhaps was associated with the enhanced development of the fetuses. The IMO and *Bacillus* supplementation resulted in a lesser immune status of the fetus, which may be related to the reduction of fetal infection and immune response (Cederqvist et al., 1978; Sarfati and Delespesse, 1996). Unexpectedly, the MDA concentrations in umbilical venous serum was greater in the treatment groups, which was perhaps due to a greater metabolism of the fetus; however, the exact mechanism remains to be clarified.

5. Conclusions

In conclusion, IMO supplementation and its combination with *Bacillus subtilis* and/or *Bacillus licheniformis* changed the lipid and protein metabolism profiles of the sows and improved the placental antioxidant capacity, perhaps by improving protein metabolism. Furthermore, the dietary supplementation increased the GH concentrations in umbilical venous serum, thereby there was a greater piglet birth weight and placental efficiency. Taken together, the results indicate the synergistic effects of *Bacillus* species and IMO may improve the placental efficiency of sows.

Conflict of interest

The authors declare that there are no conflicts of interest that could affect the integrity of the currently reported results.

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