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Effects of supplementing sow diets during late gestation with *Pennisetum purpureum* on antioxidant indices, immune parameters and faecal microbiota

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Abstract

The purpose of this study was to investigate the effects of adding *Pennisetum purpureum* (*P. purpureum*, also known as Napier grass or elephant grass) to the diets of late gestation on the antioxidant indexes, immune indexes and faecal microbiota of sows. At the 90 days of gestation, 300 healthy sows were randomly divided into three groups, and they received the basic commercial diet or added 5% *P. purpureum* and 10% *P. purpureum*, respectively. The experiment started from 90 days of gestation to parturition. The results showed that the total antioxidant capacity, immunoglobulins and serum equol concentrations of sows on 100 days of gestation and at parturition increased linearly ($p < .05$) with the increase of the content of *P. purpureum* in the gestation diet. The 5% *P. purpureum* increased the relative abundance of Bacteroidetes ($p = .027$) and Actinobacteria ($p < .001$) at phylum level, Coriobacteriaceae ($p < .001$) at family level and Prevotellaceae_UCG_001 ($p = .004$) at genus level, and decreased the relative abundance of Escherichia_Shigella ($p < .001$) at genus level. In summary, this study shows that the additive of *P. purpureum* can increase the concentration of serum equol, improve the antioxidant capacity and immune function of sow in late gestation. In addition, the additive of 5% *P. purpureum* in the diet might change the composition of intestinal microbiota of sows, particularly the relative abundance of *Coriobacteriaceae* ($p < .001$) increased.

KEYWORDS

antioxidant capacity, fiber, gestation diet, immune function, Napier grass

1 | INTRODUCTION

Pregnant sows suffer from a variety of physiological stress conditions (Li et al., 2019; Merlot et al., 2019), which can adversely effect on the sow (Hu et al., 2019; Verdon et al., 2016; Wang, Ji, et al., 2018). Recent studies reported that the metabolites of intestinal microbiota can reduce the oxidative stress experienced by sows during pregnancy (Tan et al., 2016; Wang, Hu, et al., 2019; Zhou, Xiong, et al., 2019). Many studies have shown that forage grasses application can affect sows gut microbiota composition and

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improve sows health (Castagnino et al., 2016; Liu et al., 2016; Martin et al., 2016).

Forage grasses are high-quality feeds for herbivorous livestock, and they are common supplemental raw materials for proteins and vitamins in various livestock and poultry compound feeds. Adding forage grasses to the diets of livestock and poultry helps to improve the antioxidant capacity, immune function and intestinal microbial balance (Al-Sagheer et al., 2019; He et al., 2017; Huang et al., 2018; Jiang et al., 2014). Fibre has a positive role in maintaining the intestinal microbiota of pigs (Jha et al., 2010; Knudsen, 2001). Forage grasses is rich in fibre, and adding high fibre forage grasses to the feed can affect the composition of pig intestinal microbiota (Chen et al., 2013; Varel, 1987). Such as alfalfa, many studies have reported that alfalfa is beneficial for the growth of essential bacteria and promote the production of metabolites, and inhibit the proliferation of harmful bacteria (Liu, Wang, et al., 2018; Liu, Yuan, et al., 2018; Mattioli et al., 2019; Zheng et al., 2019). There have been many studies on the effects of adding alfalfa to diets during gestation on sows (Aube et al., 2019; Calvert et al., 1985; Holzgraefe et al., 1985, 1986; Krogh et al., 2017). *P. purpureum* is fertilizer-resistant, moisture-resistant and palatable. Its composition is similar to alfalfa. The planting area is wide, the cost is low, and it has a broad development prospects in feed. However, there are few reports on the effect of adding *P. purpureum* to diets during pregnancy on sows.

Therefore, we hypothesized for the first time that the appropriate additive of *P. purpureum* in the diet of sows in late gestation can improve the antioxidant capacity and immune function, as well as alter the composition of the intestinal microbiota. The purpose of this study was to investigate the effects of *P. purpureum* on the antioxidant index, immune parameters and faecal microbiota of the sows during gestation.

2 | MATERIALS AND METHODS

The experimental animals were raised in pig farms in Hubei Province, China. Animal management and sampling procedures have been approved by the Animal Care and Use Committee of the Hunan Normal University (1907017).

2.1 | Experimental design and animals

This experiment selected 300 Landrace × Yorkshire mixed sows (parity 2.37 ± 1.22; back fat 14.57 ± 1.46 mm, mean ± *SD*). At 90 days of gestation, sows were randomly divided into three groups based on back fat and parity, with 100 in each group. Dietary treatment included basic commercial pregnancy diet (control group, 0% *P. purpureum*; Table 1) or a diet that was supplemented with 5% *P. purpureum* and 10% *P. purpureum* from 90 days of gestation to parturition, respectively. The *P. purpureum* was provided by Changsha Haishang Environmental Technology Co. Ltd. All the sows were restrictively fed with diets from 90 days of gestation to delivery. At 90 to 95 days of gestation, the feed intake was controlled at 2.5 kg; at 95 to 112 days of gestation, feeding to 3.5 kg; 2 days before delivery, the feed was reduced to 2.0 kg. The design of the experiment is presented in Figure 1.

TABLE 1 Ingredient and nutrient composition of basal commercial and experimental gestation diets

Items	C[a]	5%[b]	10%[c]
Ingredient (%)			
Corn	59.18	61.44	63.72
Rice bran	10.00	10.00	10.00
Wheat bran	18.02	11.05	4.04
Soybean meal	6.83	6.71	6.61
Pennisetum purpureum	—	5.00	10.00
Dicalcium phosphate	1.20	1.22	1.28
Zeolite powder	2.00	2.00	2.00
Calcium carbonate	1.43	1.24	1.02
Salt	0.29	0.29	0.29
Lysine hydrochloride	0.05	0.05	0.04
Premix[c]	1.00	1.00	1.00
Calculated composition[d]			
GE (Mcal/kg)	2.25	2.21	2.26
SID Lys (%)	0.51	0.52	0.55
SID Met (%)	0.22	0.21	0.22
SID Thr (%)	0.41	0.41	0.41
SID Trp (%)	0.09	0.10	0.09
Ca (%)	0.81	0.83	0.81
STTD phosphorus (%)	0.38	0.38	0.38
Analysed composition			
CP (%)	12.50	12.50	12.5
EE (%)	4.55	4.47	4.40
NDF (%)	15.96	16.46	16.93
ADF (%)	5.64	7.12	8.61

Abbreviations: CF, Crude fiber; CP, Crude protein; EE, ether extract; GE, gross energy;SID, standardized ileal digestible; STTD, standardized total tract digestible.

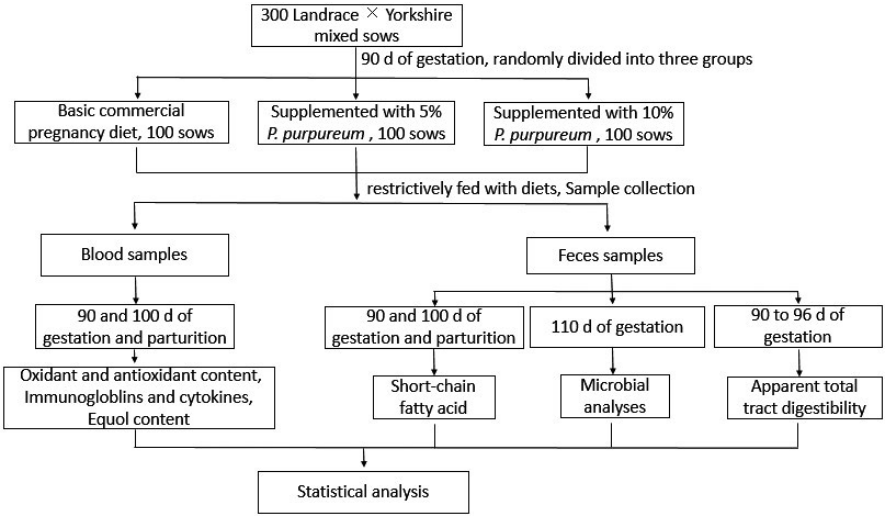
[a]C = control diet group; 5% = 5% *P. purpureum* diet group; 10% = 10% *P. purpureum* diet group; NDF = neutral detergent fibre; ADF = acid detergent fiber.

[b]*P. purpureum* contains 19.17% crude protein, 2.37% crude fat and 22.77% crude fiber, and GE = 1.66 Mcal/kg.

[c]Provided per kilogram of diet: Cu, 10.0 mg; Fe 100 mg; Mn 30 mg; Zn 60 mg; I 0.6 mg; Se 0.3 mg; Vitamin A 7,500 IU; Vitamin D_3 1,500 IU; Vitamin E 30 mg; Vitamin K 2 mg; 2 mg thiamin; 2 mg riboflavin; 2 mg pyridoxine; 0.02 mg cobalamin.

[d]Calculated chemical concentrations using values for feed ingredients from National Research Council (NRC 2012).

FIGURE 1 Experimental design



2.2 | Sample collection

2.2.1 | Blood samples

At 90 and 100 days of gestation and parturition, fasting blood samples (10 ml) were collected from the veins of the ear marginal veins. All blood samples were collected into vacuum tubes. Samples were immediately placed on ice, then centrifuged at 3,000*g* for 10 min at room temperature and the serum was stored at −20°C.

2.2.2 | Faeces samples

Faecal samples of 10 healthy sows in each treatment group with no disease and diarrhoea were collected in sterile test tubes at 90, 100 and 110 days of gestation and at parturition, the samples were collected for 12 hr from 8:00 to 12:00 for three consecutive days each time. Then immediately stored in liquid nitrogen until it is transferred to the refrigerator at −80°C.

2.3 | Sample analysis

2.3.1 | Apparent total tract digestibility

The apparent total tract digestibility (ATTD) including gross energy (GE), contents of dry matter (DM), crude protein (CP), ether extract (EE), crude ash (Ash), Ca and total P from the 90 to 96 days of gestation was determined by adding 0.3% chromium oxide to sow feed. On 94 to 96 days of gestation, the uncontaminated faeces were collected continuously from 08:00 to 20:00 in each sow for 12 hr.

2.3.2 | Oxidant and antioxidant content

Specific detection kits (Nanjing Institute of Jiancheng Biological Engineering, Nanjing, China) were used to determine the contents of Catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GSH-PX), malondialdehyde (MDA) and total antioxidant capacity (T-AOC) in the serum of sows (Barbato et al., 2019; Gabai et al., 2019).

2.3.3 | Immunogloblins and cytokines

The concentrations of IgA, IgG and IgM in serum were measured by ELISA kit (Beyotime Biotechnology Company, Shanghai, China). For the analytical method, refer to the kit instructions. All samples were tested in triplicates.

2.3.4 | Equol content

The equol concentration in serum was measured by an ELISA kit (Nanjing Institute of Jiancheng Biological Engineering, Nanjing, China). For the analytical method, refer to the kit instructions. All samples were tested in triplicates.

2.3.5 | Short-chain fatty acid

Short-Chain Fatty Acid (SCFAs) including acetic acid, propionic acid and butyric acid in faeces samples were analysed by 7890B gas chromatography (Agilent, Inc.) as described previously (Cao et al., 2019).

2.3.6 | Microbial analyses

Using the Qubit dsDNA Assay Kit (Cat. No. Q328520, Life Technologies) based on the manufacturer's protocols, total bacterial DNA was extracted from faeces of sows in the control group (n = 10) and the group with 5% *P. purpureum* (n = 10) were collected for three consecutive days from the 110 days of gestation,

this analysis was conducted using faeces from one period of time. To perform amplicon pyrosequencing on the Illumina MiSeq PE300 platform, DNA samples were sent to OE Biotech Company (Shanghai, China) and amplified using 515F and 806R primers to obtain the V3–V4 hypervariable region of the 16S rRNA gene (Bolger et al., 2014). The splicing sequences were called paired end data (total). Further denoising on the paired end data was performed to obtain the clean tags (Bolger et al., 2014; Reyon et al., 2012). Then chimeric filtering to get high-quality effective labels (Figure 2a; Caporaso et al., 2010). Using Vsearch software (v2.4.2) for analysis, valid tags with sequence similarity of 97% are classified as operating classification units (OUT; Edgar, 2013; Edgar et al., 2011). The sequence with the most grace in each OUT was screened as a representative sequence. The Silva database (v123) was used to compare the representative sequences to obtain a phylogenetic tree and OTU classification table, which ultimately performed downstream alpha and beta diversity analysis (Qiong et al., 2007).

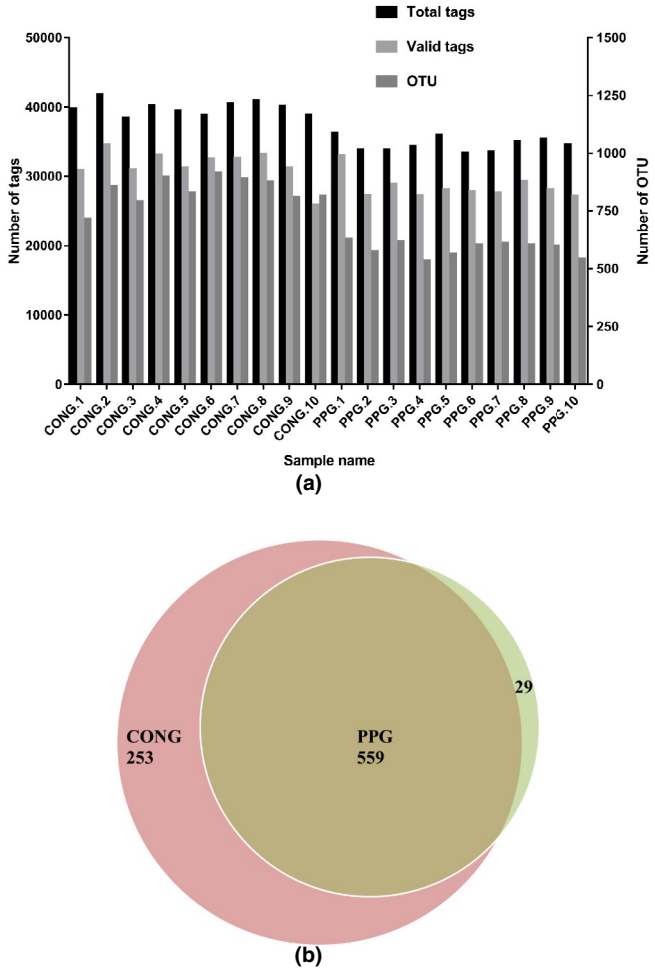


FIGURE 2 Tags number, OTUs number and Venn diagrams. (a) Total tags are equivalent to clean tags. Taxon tags refer to the number of tags used to build OTUs. OTUs, operational taxonomic units. (b) CONG, the control group at gestation; PPG, the 5% *Pennisetum purpureum* group at gestation

2.4 | Statistical analysis

Excel 2016 was used to sort out the experimental data, ANOVA software was used to further analyse the data, and GLM program of SPSS 20.0 software was used to design the random block. The results are represented by the mean value, and its standard error or the standard error of the mean value.

when $p < .05$, it means that there is a significant difference between the data; when $.05 \leq p < .10$, it means that there is a trend of difference.

3 | RESULTS

3.1 | Apparent total tract digestibility

The results in Table 2 showed that the 5% *P. purpureum* inclusion had no effects on the ATTD of sows during late gestation. However, the 10% *P. purpureum* treatment significantly reduced ($p < .05$) the GE, EE, Ca and total P digestibility compared with sows fed the control diet, but DM, CP and ash had no effect.

3.2 | Serum immunoglobulins concentrations

The serum IgA and IgM concentrations of sows increased linearly ($p < .05$) with the increase of *P. purpureum* at 100 days of gestation and at parturition. The serum IgG concentrations of sows increased linearly ($p < .05$) with the increase of *P. purpureum* at 100 days of gestation (Table 3).

3.3 | Oxidative and antioxidative indicators in the serum

The serum MDA concentrations of sows decreased linearly and quadratically ($p < .05$) with the increase of *P. purpureum* at 100 days

TABLE 2 Effect of inclusion of *Pennisetum purpureum* in gestation diet on ATTD of energy and nutrients of sows*

Item	C[1]	5%	10%	*SEM*	*p* value
GE	81.72[a]	81.31[a]	80.45[b]	0.25	.014
DM	82.23	81.79	81.72	0.31	.191
CP	83.66	83.14	83.77	0.44	.837
EE	56.61[a]	55.05[a]	50.99[b]	1.56	.004
Ash	36.33	36.93	33.74	1.96	.152
Ca	41.26[a]	41.01[a]	40.75[b]	0.17	.001
Total P	35.77[a]	34.20[a]	37.62[b]	1.52	.018

Note: Different letters in the same line indicate significant statistical differences ($p < .05$).

C = control diet group; 5% = 5% *P. purpureum* diet group; 10% = 10% *P. purpureum* diet group. Values were considered statistically significant when $p < .05$ and as a trend to significance when $.05 \leq p < .10$.

*Values are standard error of the mean (*SEM*).

TABLE 3 Effect of *Pennisetum purpureum* additive in late gestation on serum immunoglobulin concentrations of sows*

	P. purpureum, % of diet			*p* value		
Item	0	5	10	Con versus *P. purpureum*	Linear	Quadratic
90 days, μg/ml						
IgA	65.34 ± 4.78	65.99 ± 6.63	70.34 ± 7.54	.186	.093	.463
IgG	419.93 ± 31.62	414.43 ± 20.87	411.39 ± 20.39	.740	.449	.899
IgM	80.79 ± 9.21	75.15 ± 5.46	76.59 ± 6.20	.206	.200	.213
100 days, μg/ml						
IgA	101.18 ± 8.26	111.61 ± 12.90	112.95 ± 9.77	.036	.018	.272
IgG	461.29 ± 25.59	478.30 ± 36.80	502.78 ± 22.41	.012	.003	.741
IgM	146.79 ± 8.91	156.97 ± 8.75	161.69 ± 14.34	.016	.005	.526
At parturition, μg/ml						
IgA	136.10 ± 15.40	142.34 ± 13.33	149.38 ± 11.99	.113	.039	.939
IgG	809.47 ± 30.89	803.28 ± 38.22	820.47 ± 41.33	.583	.513	.423
IgM	175.99 ± 19.26	179.71 ± 8.53	188.47 ± 11.88	.141	.056	.645

Note: Values were considered statistically significant when $p < .05$ and as a trend to significance when $.05 \le p < .10$.
*Values are means ± *SE*, $n = 10$ per treatment.

TABLE 4 Effect of *Pennisetum purpureum* additive in late gestation on serum MDA concentrations of sows*

	P. purpureum, % of diet			*p* value		
Item	0	5	10	Con versus *P. purpureum*	Linear	Quadratic
90 days, nmol/ml	2.75 ± 0.21	2.82 ± 0.23	2.60 ± 0.29	.122	.223	.094
100 days, nmol/ml	5.53 ± 0.67	2.60 ± 0.26	1.62 ± 0.17	<.001	<.001	<.001
At parturition, nmol/ml	7.48 ± 0.67	6.50 ± 0.55	4.23 ± 0.32	<.001	<.001	.013

Note: Values were considered statistically significant when $p < .05$ and as a trend to significance when $.05 \le p < .10$.
*Values are means ± *SE*, $n = 10$ per treatment.

of gestation and at parturition (Table 4). The serum T-AOC concentrations of sows increased linearly ($p < .05$) with the increase of *P.purpureum* at 100 days of gestation and at parturition. The serum total superoxide dismutase (T-SOD) and CAT concentrations of sows increased linearly ($p < .05$) with the increase of *P. purpureum* at 100 days of gestation and at parturition, The serum GSH-PX concentration of sows has no effect with the increase of *P. purpureum* at 100 days of gestation and at parturition (Table 5).

3.4 | The equol concentration in the serum and feces

The serum equol concentration of sows increased linearly and quadratically ($p < .05$) with the increase of *P. purpureum* at 100 days of gestation and at parturition (Table 6).

3.5 | The SCFAs concentration in faeces samples

The butyric acid concentration of sows faeces increased linearly ($p < .05$) with the increase of *P. purpureum* at 100 days of gestation and at parturition, The acetic acid and propionic acid concentration of sows has no effect with the increase of *P. purpureum* at 100 days of gestation and at parturition (Table 7).

3.6 | Faecal microbiota

A total of 745,198 tags were obtained from the faeces samples range of 33,405 to 41,868, and a total of 14,289 OTUs from 97% of the same level were available for downstream analysis (Figure 2a). The Venn diagram was used to analyse the distribution of OTUs between the two groups. The results showed that there were a total of 559 identical OTUs in the two groups, and the OTUs in the 5% *P. purpureum* were significantly fewer compared to the control group (5,883 vs. 8,406; Figure 2b). The richness and diversity of microbial community can be reflected by α diversity and β diversity. α diversity is calculated by the observed species index (Figure 3a), and β diversity by principal component analysis (PCA; Figure 3b). Compared with the control group, the species index of sow faeces fed with 5% *P. purpureum* was significantly higher ($p < .05$). In the PCA diagram, the faeces microbiota of the control group and the 5% *P. purpureum* group were divided into two different clusters. In

TABLE 5 Effect of *Pennisetum purpureum* additive in late gestation on serum antioxidative indicators of sows*

	P. purpureum, % of diet			*p* value		
Item	0	5	10	Con versus *P. purpureum*	Linear	Quadratic
90 days, U/ml						
T-AOC	10.52 ± 1.47	11.04 ± 1.39	10.79 ± 1.08	.518	.259	.893
T-SOD	41.67 ± 1.32	42.36 ± 2.09	40.12 ± 1.92	.080	.105	.109
CAT	1.76 ± 0.19	1.58 ± 0.17	1.94 ± 0.33	.248	.174	.329
GSH-Px	850.6 ± 62.1	859.4 ± 30.4	862.5 ± 60.1	.149	.349	.086
100 days, U/ml						
T-AOC	12.58 ± 1.64	14.41 ± 2.19	15.66 ± 2.16	.012	.008	.135
T-SOD	65.26 ± 3.75	65.87 ± 2.72	69.22 ± 4.10	.039	.014	.044
CAT	1.95 ± 0.21	2.46 ± 0.15	2.62 ± 0.43	<.001	<.001	.302
GSH-Px	970.5 ± 43.5	955.8 ± 60.2	991.6 ± 51.2	.512	.707	.278
At parturition, U/ml						
T-AOC	8.25 ± 1.42	10.16 ± 1.61	11.12 ± 1.07	<.001	<.001	.299
T-SOD	68.41 ± 6.89	70.27 ± 6.52	72.14 ± 7.59	<.001	<.001	.552
CAT	2.15 ± 0.23	2.55 ± 0.42	2.69 ± 0.37	.001	<.001	.720
GSH-Px	807.4 ± 48.9	799.3 ± 33.7	815.7 ± 45.2	.088	.029	.957

Note: Values were considered statistically significant when $p < .05$ and as a trend to significance when $.05 \le p < .10$.
*Values are means ± *SE*, $n = 10$ per treatment.

TABLE 6 Effect of *Pennisetum purpureum* additive in late gestation on serum equol of sows*

	P. purpureum, % of diet			*p* value		
Item	0	5	10	Con versus *P. purpureum*	Linear	Quadratic
90 days, U/ml	86.27 ± 6.89	89.92 ± 6.11	83.56 ± 8.42	.247	.156	.374
100 days, U/ml	97.55 ± 5.41	327.51 ± 27.92	350.13 ± 29.42	<.001	<.001	<.001
At parturition, U/ml	90.22 ± 7.31	412.21 ± 36.14	444.12 ± 42.14	<.001	<.001	<.001

Note: Values were considered statistically significant when $p < .05$ and as a trend to significance when $.05 \le p < .10$.
*Values are means ± *SE*, $n = 10$ per treatment.

TABLE 7 Effect of *Pennisetum purpureum* additive in gestation on SCFAs concentration in faeces of sows*

	P. purpureum, % of diet			*p* value		
Item	0	5	10	Con versus *P. purpureum*	Linear	Quadratic
90 days, μmol/g						
Acetic acid	48.39 ± 5.52	47.64 ± 6.12	49.25 ± 5.26	.052	.018	.576
Propionic acid	20.65 ± 2.17	22.14 ± 2.58	20.18 ± 2.72	.058	.071	.107
Butyric acid	10.87 ± 1.44	11.24 ± 1.35	10.52 ± 1.07	.673	.609	.470
100 days, μmol/g						
Acetic acid	49.13 ± 4.31	48.45 ± 4.24	50.16 ± 5.04	.423	.889	.196
Propionic acid	22.95 ± 2.56	23.14 ± 2.79	21.22 ± 2.76	.338	.158	.700
Butyric acid	11.29 ± 1.45	14.37 ± 1.72	16.42 ± 1.88	<.001	<.001	.301
At parturition, μmol/g						
Acetic acid	46.41 ± 6.28	44.52 ± 5.29	45.24 ± 5.31	.229	.145	.361
Propionic acid	20.42 ± 3.24	21.74 ± 2.98	21.85 ± 2.49	.632	.343	.964
Butyric acid	11.80 ± 2.01	16.52 ± 2.35	18.64 ± 2.75	<.001	<.001	.063

Note: Values were considered statistically significant when $p < .05$ and as a trend to significance when $.05 \le p < .10$.
*Values are means ± *SE*, $n = 10$ per treatment.

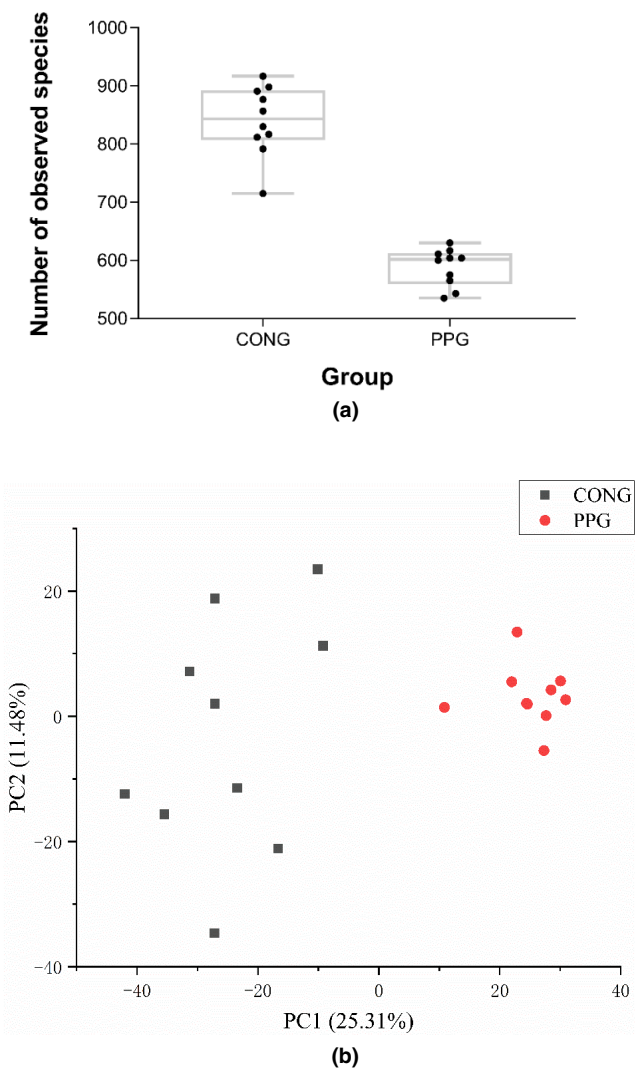


FIGURE 3 Alpha diversity and beta diversity analyses of microbial community structure. (a) The observed species index analyses, $p < .001$; (b) principal component analysis; ANOSIM analyses show the difference of microbial community structure. *R* value is >0, indicating significant differences between groups. The reliability of statistical analysis is expressed by *p* value

addition, the change of faeces microbiota composition of the 5% *P. purpureum* group were lighter than that in the control group. The results showed that 5% *P. purpureum* had an important effect on the diversity of intestinal flora. The significance analysis was performed according to the top seven phyla (relative abundance >0.1% in at least one sample) and the *Firmicutes*/*Bacteroidetes* ratio (Figure 4a). The results showed that the relative abundances of *Bacteroidetes* ($p = .027$), *Fibrobacteres* ($p = .048$) and *Actinobacteria* ($p < .001$) were increased, and the relative abundances of *Firmicutes* ($p < .001$) and *Firmicutes*/*Bacteroidetes* ratio ($p = .021$) were decreased by 5% of *P. purpureum*. Thirty-two families with relative abundance >0.1% in at least one sample were selected for significance analysis. There were eight families with significant difference (Figure 4b). The results showed that the additive of 5% *P. purpureum* increased the relative abundance of *Ruminococcaceae* ($p = .037$), *Lachnospiraceae* ($p = .043$) and *Coriobacteriaceae* ($p < .001$), and decreased the relative abundance of *Prevotellaceae* ($p = .041$), *Rikenellaceae* ($p = .045$), *Spirochaetaceae* ($p = .045$), *Porphyromonadaceae* ($p = .009$) and *Enterobacteriaceae* ($p = .009$). Fifty-five genera with relative abundance >0.1% in at least one sample were selected for significance analysis. There were 14 genera with significant difference (Figure 4c). The results showed that the additive of 5% *P. purpureum* increased the relative abundance of *Oscillibacter* ($p < .001$), *Streptococcus* ($p < .001$), *Oscillospira* ($p = .001$), *Prevotellaceae_UCG_001* ($p = .004$), and *Lachnospiraceae_UCG_005* ($p = .036$), and decreased the relative abundance of *Romboutsia* ($p < .001$), *Lachnospiraceae_AC2044_group* ($p < .001$), *Erysipelotrichaceae_UCG_004* ($p = .007$), *Desulfovibrio* ($p = .008$), *Turicibacter* ($p = .015$), *Terrisporobacter* ($p = .023$), *Escherichia_Shigella* ($p < .001$), *Succinivibrio* ($p = .043$) and *Fibrobacter* ($p = .048$).

4 | DISCUSSION

In this study, we investigated the effects of adding *P. purpureum* to sow diets on antioxidant indices, immune parameters and faecal microbiota of sows during late gestation. The results showed that the additive of *P. purpureum* improved the antioxidant ability and immune function, enhanced the serum equol content and changed the composition of the intestinal microbiota of sows. In addition, in consideration of nutrient digestibility, the additive of 5% *P. purpureum* shows the best effect.

In this study, we provided equal amounts of net energy and standard ileal digestible amino acids to the diets of different treatment groups. Through composition analysis, it was found that the contents of NDF and ADF in different treatment groups differed greatly. We infer that the effects of *P. purpureum* on antioxidant ability, immune function and intestinal microbiota of sows in late pregnancy may be due to the fibre content of different treatment groups.

Studies have shown that the metabolic burden of sows will increase the oxidation of lipids and proteins in late pregnancy, leading to increased oxidative stress (Berchieri-Ronchi et al., 2011). MDA, T-AOC, GSH-Px, CAT and SOD can be used to determine the redox state of sow serum. MDA is a sign of oxidative stress, and its increased level indicates that the animal is in a strong oxidative stress state (Cao et al., 2019). SOD can catalyse the degradation of superoxide radicals into oxygen and hydrogen peroxide, which are then degraded by CAT. T-AOC is an important comprehensive index reflecting the total antioxidant capacity of animals. GSH PX can act as a reducing agent to prevent the production of lipid peroxide (Berchieri-Ronchi et al., 2011; Mou et al., 2020; Xie et al., 2016). It is well known that due to the special physiological metabolism during late gestation, a large amount of free radicals are produced (Alanazi et al., 2018; Gitto et al., 2002; Peter Stein et al., 2008; Zimmer & Spencer, 2015). Oxidative stress occurs if excess free radicals are not removed in time, which leads to metabolic disorders and impaired the health of sows (Kim et al., 2013; Zhao et al., 2013; Zhou, Xu, et al., 2019). Oxidative stress might be an important reasons leading to lower reproduction

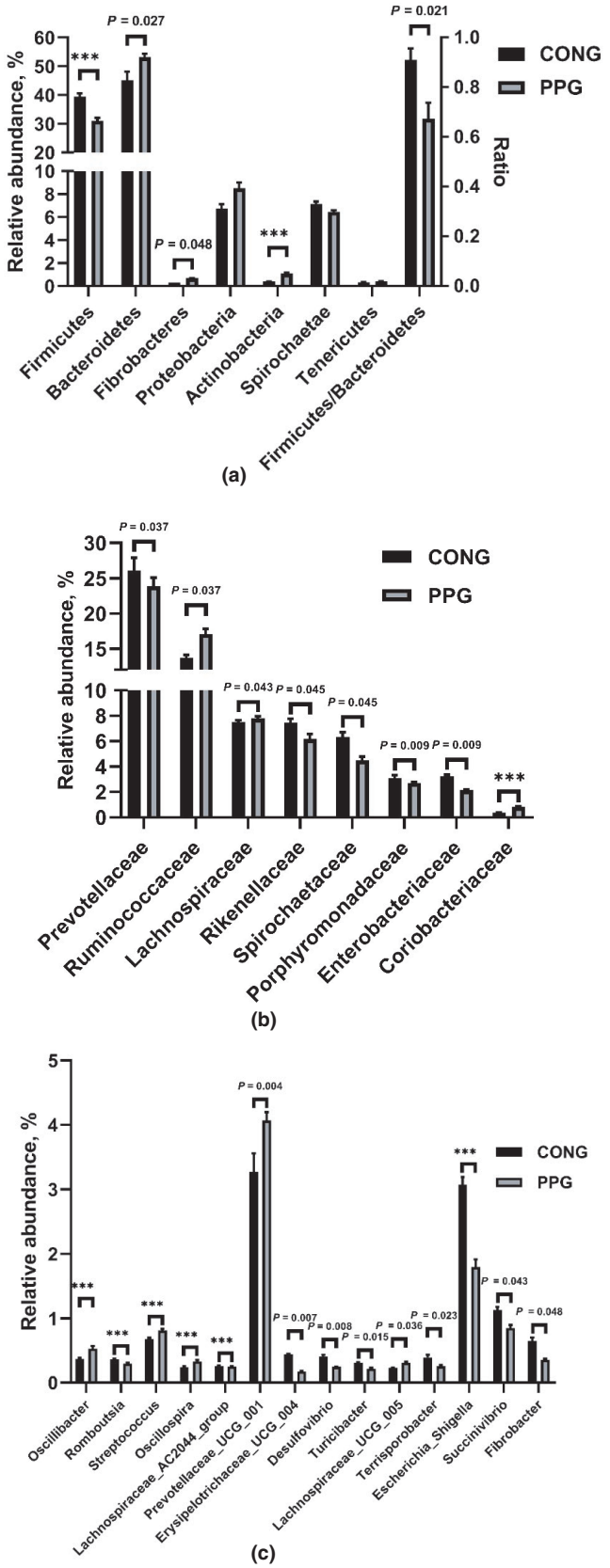


FIGURE 4 Relative abundances of faecal microbiota composition at the phylum level (a), family level (b), and the genus level (c). (a) The relative abundances of top seven phyla of faecal microbiota composition. (b) The relative abundances of eight family (%, >0.1% in at least one sample) with significant difference. (c) The relative abundances of 14 genera (%, >0.1% in at least one sample) with significant difference. Data were expressed as means ± *SE*, $n = 10$ for each treatment. CONG, the control group; PPG, the 5% *Pennisetum purpureum* group

performance of sows (Cools et al., 2011; Gaykwad et al., 2019; Meng et al., 2018; Su et al., 2017; Tan et al., 2015; Wang et al., 2016). In this study, with the increase of *P. purpureum*, the ability of scavenging free radicals was improved, the content of MDA in serum of sows in late gestation was reduced, and the content of T-AOC, T-SOD and CAT was increased, which indicated that the antioxidant capacity of sows was improved. The study of Shah et al. showed that betaine supplementation can improve the digestibility of NDF and ADF, and improve the antioxidant capacity of dairy cows (Shah et al., 2020). The study of Pillai et al. also showed that dietary fiber can control the level of free radicals and prevent oxidative stress (Pillai et al., 1999). In addition, we found that the abundance of IgG and IgM in the serum of the *P. purpureum* inclusion diet sows were significantly increased, which were involved in humoral immunity and enhances immune function (Kielland et al., 2015; Osterlundh et al., 1998). This is consistent with previous studies (Jiang et al., 2014; Xie et al., 2019). Adding *P. purpureum* can enhance the immune function of sows, which may be because *P. purpureum* rich in fibre can regulate the intestinal microbiota interacting with the immune system (Chen et al., 2013; Wang, Qin, et al., 2018), and the colonization of intestinal microbiota is closely related to the animal immune function (Cerf-Bensussan & Gaboriau-Routhiau, 2010).

Our results also show that adding *P. purpureum* can increase the amount of equol in serum. The daidzein is metabolized by the intestinal microbiota to equol in the gastrointestinal tract (Setchell & Clerici, 2010). Equol has anti-inflammatory effect and is more antioxidant than its precursor, daidzein (Atkinson et al., 2005; Danciu et al., 2018). Moreover in human serum, equol has higher effective free fraction and lower plasma clearance than daidzein (Lampe, 2003). However, intestinal microbiota are key determinants of the formation of metabolites and variable between individuals (Levy et al., 2016; Ma et al., 2017). In order to improve the beneficial effect of soybeans, it has been tested that the intestinal microbiota can regulate the production of equol (Clavel et al., 2005; Hazim et al., 2016; Lambert et al., 2017; Tousen et al., 2013).

Therefore, we hypothesis that the additive of *P. purpureum* in the diet may change the composition of intestinal microbiota to produce equol, and then enter the circulatory system by absorption. To validate the correlation between equol content and intestinal microbiota, we analysed the faecal microbiota in sows with 5% *P. purpureum* inclusion ($n = 10$) and controls ($n = 10$). Faecal samples were collected from 110 days of gestation for three consecutive days, and the diversity analysis of 16srrna was performed. In this 16srrna analysis, we have some limitations in the sampling of faecal. Considering that the concentration of equol in serum of sows increased linearly with the increase of *P. purpureum* at 100 days of gestation and at parturition, we only selected faecal samples from the last three

consecutive days of late gestation, and did not analyse faecal microbiota in different periods. There are similar sampling analysis before (Hasan et al., 2018). Therefore, our study can provide some research basis for the influence of *P. purpureum* in diets on the faecal microbiota of sows in late gestation, but further research is needed. The results showed that the relative abundance of *Bacteroidetes*, *Fibrobacteres* and *Actinobacteria* could be increased and the relative abundance of *Firmicutes* and *Firmicutes*/*Bacteroidetes* ratio could be reduced by adding 5% *P. purpureum*. At family level, the 5% *P. purpureum* mainly increased the relative abundance of *Ruminococcaceae*, *Lachnospiraceae* and *Coriobacteriaceae*; and decreased the relative abundance of *Prevotellaceae*, *Rikenellaceae*, *Spirochaetaceae*, *Porphyromonadaceae* and *Enterobacteriaceae*. At genus level, the 5% *P. purpureum* mainly increased the relative abundance of *Oscillibacter*, *Streptococcus*, *Oscillospira*, *Prevotellaceae_UCG_001* and *Lachnospiraceae_UCG_005*; and decreased the relative abundance of *Romboutsia*, *Lachnospiraceae_AC2044*_group, *Erysipelotrichaceae_UCG_004*, *Desulfovibrio*, *Turicibacter*, *Terrisporobacter*, *Escherichia_Shigella*, *Succi*nivibrio and Fibr*obacter*. We infer that the additive of *P. purpureum* to the feed causes differences in the gut microbiota, which may be due to the different fibre content. Fibre digestion is a process of microbial fermentation, most of which occurs in the cecum and large intestine of pigs (Farrell & Johnson, 1972; Imoto & Namioka, 1978; Kass et al., 1980). Fibre can affect the diversity of intestinal microbial community and play a positive role in maintaining intestinal health (Jha et al., 2010; Knudsen, 2001). Feeding high fibre diet to growing pigs can increase the activity of bacteria related to fibre degradation in large intestine (Chen et al., 2013; Wang, Ji, et al., 2018), and fiber degrading bacteria are beneficial to intestinal health (Williams et al., 2002). SCFAs concentration can be used as an important index to evaluate the fermentation intensity of hindgut (Serena et al., 2008). Research shows that bacteria of *Bacteroidetes* phylum, particularly *Prevotellaceae_UCG_001*, are effective in fermenting fibers into SCFAs (Qi et al., 2019; Wang, Martin, et al., 2019). SCFAs play an important role in the fermentation of equol, especially butyrate (Decroos et al., 2005; Dey, 2019). In addition, SCFA can effectively restrain the reproduction of harmful bacteria (for example Escherichia_Shigella) and promote the reproduction of beneficial bacteria. It is known that many bacteria capable of fermenting daidzein and its metabolites into equol are mainly concentrated in the *Actinobacteria* phylum, especially in the *Coriobacteriaceae* (Abiru et al., 2013; Guadamuro et al., 2019; Kawada et al., 2016; Matthies et al., 2008; Tsuji et al., 2010). Above all, the antioxidant capacity and immune function of the sows from the 5% *P. purpureum* group were increased, and the mechanism may be due to the increased abundance of *coriobacteriaceae* and *Prevotellaceae_UCG_001*, and increased the equol and SCFAs contents, which played a central role in antioxidant capacity and immune function.

5 | CONCLUSIONS

This study shows that the additive of *P. purpureum* can enhance the antioxidant ability and immune function of sow in late gestation. In addition, the additive of 5% *P. purpureum* in the diet might change the composition of intestinal microbiota of sows, particularly the relative abundance of Coriobacteriaceae increased; however, this warrants further investigation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Peng-Fei Huang: Conceptualization; Supervision; Writing-original draft; Writing-review & editing. Qi Mou: Conceptualization; Supervision; Writing-original draft; Writing-review & editing. Ying Yang: Investigation. Jiaming Li: Investigation. Minglang Xu: Investigation. Jing Huang: Data curation. Jianzhong Li: Conceptualization. Huansheng Yang: Conceptualization. Xiaoxiao Liang: Data curation. Yulong Yin: Conceptualization.

ETHICAL STATEMENT

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate Ethical Review Committee approval has been received. All procedures and the use of animals in this experiment were carried out in accordance with the Hunan Normal University Animal Care and Use Committee guidelines (1907017).

PEER REVIEW

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