



## Original Research Article

Maternal supplementation with konjac glucomannan and  $\kappa$ -carrageenan promotes sow performance and benefits the gut barrier in offspring

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## ABSTRACT

This research aims to investigate the effects of dietary konjac glucomannan and  $\kappa$ -carrageenan (SF) on sow performance and suckling piglet gut barrier. Thirty-four sows in late gestation (parity 2–5) were selected at random and grouped into two treatments. The control group (Con group;  $n = 17$ ) was fed the basal diet; the SF group was fed the same diet supplemented with 0.25% konjac glucomannan + 0.25%  $\kappa$ -carrageenan (SF group;  $n = 17$ ). The results showed that sows fed the SF diet had a higher feed intake during lactation than the Con group ( $P < 0.05$ ), and the levels of neuropeptide tyrosine (NPY) ( $P = 0.006$ ) and acetylcholine enzyme (AChE) ( $P < 0.05$ ) significantly increased. The fecal microbial analysis indicated that the SF diet had a higher abundance of *Subdoligranulum*, *Holdemanella*, and *Succinivibrio* at the genus level, and the acetate level was significantly increased ( $P < 0.05$ ). Moreover, SF lowered the level of interleukin-6 (IL-6) in milk ( $P < 0.05$ ). Regarding suckling piglets, maternal supplementation with SF reduced jejunal IL-6 protein levels in suckling piglets ( $P < 0.05$ ). In the colon of the piglet, the SF group up-regulated protein levels of occludin ( $P < 0.05$ ), and the nuclear factor erythroid 2-related factor 2 (Nrf2) ( $0.05 \leq P < 0.1$ ), and claudin 4 (CLDN4) ( $0.05 \leq P < 0.1$ ) protein levels tended to be up-regulated. Consequently, supplementation of SF in sow diets positively affects lactation feed intake and maternal microflora. Furthermore, the maternal effect improves the jejunum and colon barriers of suckling piglets. © 2024 The Authors. Publishing services by Elsevier B.V. on behalf of KeAi Communications Co. Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

## 1. Introduction

During lactation, nutrients are required for the maintenance of sow health and the growth of suckling piglets (Gourley et al., 2017). Moreover, lactation is a high-energy process that relies on the sow's capability to expend enough energy to maintain milk yield (Zhang et al., 2020). Thus, adequate nutrition input during lactation positively affects the performance and body condition of the sow and

facilitates rapid recovery from the effects of lactation (Luise et al., 2023). However, the feed intake of lactating sows does not always meet the energy requirements for maintaining and producing milk (Hu et al., 2019). This is further illustrated that feed consumption directly impacts domestic animal performance (Han et al., 2019). Therefore, the main aims of lactating sow management are to improve the feed intake to increase milk production voluntarily, to prevent excess loss of body weight, and to achieve a timely weaning-to-estrus interval (Kim et al., 2020). However, we have limited knowledge of the link between milk production, feed intake, and body mobilization of sows (Strathe et al., 2017). It remains a challenge in animal nutrition, especially during lactation.

Sows often suffer from negative energy balance due to the high metabolic demands of milk production when feed intake is insufficient to meet the energy requirements for milk production (Costermans et al., 2020). Therefore, addressing the factors that potentially limit feed intake in lactation will decrease weight loss,

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enhance litter gain, and the sow's future reproductive performance (Kiarie et al., 2021). In a balanced diet, dietary fiber plays a crucial role (Suresh et al., 2020). Dietary fiber is widely applied to sow diets (Xu et al., 2022). Intestinal bacteria can degrade dietary fiber, and soluble dietary fiber (SDF) ferments more quickly than insoluble dietary fiber (IDF) (Huang and Hsieh, 2019). From functional and physiological perspectives, SDF is more critical than IDF (Bader UI Ain et al., 2019). Konjac glucomannan is a natural neutral polysaccharide extracted from konjac tubers (Zhu et al., 2019). Over the decades, diets rich in konjac flour or konjac glucomannan have been favored to be healthier and purified konjac glucomannan has been used as a food additive and dietary supplement in many countries (Devaraj et al., 2019). Studies have shown that supplementing a low-fiber diet with konjac glucomannan promotes frequent bowel movements and increases fecal volume in healthy adults (Chen et al., 2006). Carrageenan is an anionic polysaccharide isolated from seaweeds (Tsitsos et al., 2023) and is widely applied in the food industry (Mei et al., 2022). Konjac glucomannan synergizes favorably with  $\kappa$ -carrageenan (Wu et al., 2021). Carrageenan oligomers and konjac oligo-glucomannans are particularly interesting for their apparent health benefits and consumer acceptance (Zhu et al., 2022a). Currently, the application of konjac flour in sows mainly focuses on the gestation period (Tan et al., 2015, 2016). Maternal nutrition during lactation is essential for the health of the offspring (Hsu and Tain, 2018). However, there are few reports on applying konjac glucomannan and  $\kappa$ -carrageenan (SF) in sows during late gestation and lactation. Our findings provide some inspiration and ideas for improving maternal microbiota and enhancing the performance of lactating sows through SF supplementation during late gestation and lactation.

## 2. Materials and methods

### 2.1. Animal ethics statement

All animal experiments complied with the guidelines for the care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Northeast Agricultural University (NEAU - [2013]-9).

### 2.2. Experimental design

Animal experiments were conducted at Harbin Sanyuan Livestock Industry Co. (Harbin, China). A total of 34 sows (Large White  $\times$  Landrace) were randomly assigned to two treatment groups which were as follows: sows in the control group were fed a basal diet (Con group;  $n = 17$ ); sows in the SF group were fed with a diet enriched with 0.5% supplementation (0.25% konjac glucomannan + 0.25%  $\kappa$ -carrageenan, SF group;  $n = 17$ ), with the equivalent proportions of corn in the basal diet replaced by food-grade konjac glucomannan and  $\kappa$ -carrageenan. Approximately seven days before the expected farrowing date, the sows were moved into individual farrowing stalls (1.5 m  $\times$  2.1 m). The temperature of the room was kept at  $23 \pm 2$  °C with a relative humidity from 65% to 75%. The veterinarian examined each sow to ensure that it was healthy and pregnant. Cross-fostering of suckling piglets from the same group was completed within 24 h after farrowing. The experimental diet was consumed by sows from farrowing until weaning and their litters were weaned at 25 d of lactation. During rearing, all sows were healthy. The experimental diets were based on National Research Council guidelines (NRC, 2012). Throughout the experimental period, sows were allowed to drink water freely. The ingredients of the diet are listed in Table 1. From d  $108 \pm 1$  of gestation, the lactation diet for all sows was 3 kg/d. On the day of delivery, sows were fed 0.5 kg and the feed intake was gradually

**Table 1**  
Ingredients and nutrient composition of experimental diets (% air-dry basis)<sup>1</sup>.

Item	Treatment <sup>2</sup>	
	Con	SF
<b>Ingredients</b>		
Corn	64.50	64.00
Soybean meal	16.00	16.00
Puffed soybean	5.70	5.70
Wheat bran	5.00	5.00
Soybean oil	3.00	3.00
Fish meal	2.00	2.00
Dicalcium phosphate	1.20	1.20
Limestone	0.90	0.90
Sodium chloride	0.40	0.40
Choline chloride	0.25	0.25
L-Lysine HCl (78%)	0.05	0.05
Konjac glucomannan + $\kappa$ -carrageenan		0.50
Vitamin and mineral premix <sup>3</sup>	1.00	1.00
<b>Nutrient composition<sup>4</sup></b>		
Digestible energy, kcal/kg	3446	3307
Crude protein	16.13	16.04
Crude fat	7.26	7.30
Lysine	0.93	0.92
Threonine	0.63	0.63
Ash	8.34	8.02
Neutral detergent fiber	20.40	21.02
Calcium	1.20	1.23
Total phosphorus	0.85	0.88

<sup>1</sup> The diet was formulated based on the National Research Council's recommended requirements (NRC, 2012).

<sup>2</sup> Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan + 0.25%  $\kappa$ -carrageenan to replace the same percentage of corn in the basal diet ( $n = 17$ ).

<sup>3</sup> Provided the following per kilogram of diet: Cu, 20 mg; Fe, 100 mg; Zn, 100 mg; Mn, 25 mg; Se, 0.3 mg; I, 0.4 mg; vitamin A, 14,000 IU; vitamin D<sub>3</sub>, 2000 IU; vitamin E, 140 mg; vitamin K<sub>3</sub>, 2 mg; vitamin B<sub>1</sub>, 2.4 mg; vitamin B<sub>2</sub>, 8.5 mg; vitamin B<sub>6</sub>, 4.5 mg; vitamin B<sub>12</sub>, 0.03 mg; pantothenic acid, 20 mg; biotin, 0.5 mg; niacin, 28 mg; folic acid, 3.5 mg.

<sup>4</sup> Digestible energy (DE) value was calculated according to the prediction equation of Noblet and Perez (1993), all others were analyzed values.

increased by approximately 0.5 to 1 kg per day to the maximum intake.

### 2.3. Sample and data collection

During parturition and weaning, the backfat of sows was measured with an ultrasonic scanner (Renco Lean-Meater, MN, USA). The backfat thickness of the sow was recorded on the left and right of the P2 position (left side of the 10th rib and 6 cm away from the spine). Colostrum samples were collected from sows of each group on the day of piglet delivery, within 4 h after the first piglet was born. The udder was washed, dried, and sterilized before collection. On the 25th d of lactation, the feces from six sows were obtained from each group, and milk was obtained from each sow of each group. The fasted blood samples were collected early in the morning from the anterior vena cava of the sows. Six suckling piglets (half male and half female, each from a different sow, with similar weight) of each group were randomly selected and euthanized at the end of the experiment to collect the blood, jejunum, colon, and colon contents. The blood samples of sows and piglets were allowed to stand at room temperature for 30 min and centrifuged at  $3500 \times g$  for 10 min to obtain serum. Serum samples were stored in a refrigerator at  $-80$  °C. Tissue samples and contents were collected from the proximal and distal colon, with the jejunum in the middle. A portion of the jejunum and colon samples were quickly frozen in tanks containing liquid nitrogen and finally stored in the refrigerator at  $-80$  °C for subsequent RNA and protein extract. The remaining jejunum and colon tissue were frozen

at  $-20^{\circ}\text{C}$  and used to analyze the immune and antioxidant indexes. The colon content of piglets and the fecal content of sows were frozen in liquid nitrogen for later microbiome analysis.

#### 2.4. Determination of the nutrients in feed

Crude protein (CP) in the diet was determined by GB/T 6432–2018 (China National Standard, 2018a) using a Kjeltac 8400 (FOSS Inc., Eden Prairie, MN). Crude protein was calculated as  $N \times 6.25$ . Crude fat (EE) was detected using the China National Standard GB/T 6433–2006 (China National Standard, 2006) and analyzed using an ANKOM<sup>XT15</sup> Extractor (ANKOM Technology, USA). Neutral detergent fiber (NDF) was measured in compliance with GB/T 20806–2022 (China National Standard, 2022) using an ANKOM<sup>200</sup> Fiber Analyzer (ANKOM Technology, Macedo, NY, USA). Calcium was assayed in compliance with GB/T 6436–2018 (China National Standard, 2018b). Total phosphorus in diets was analyzed with GB/T 6437–2018 (China National Standard, 2018c). The absorbance was measured with a spectrophotometer (UV-2401, Shimadzu, Kyoto, Japan). Crude ash was determined with GB/T 6438–2007 (China National Standard, 2007). Amino acids were determined using a previous experimental method (Teng et al., 2023a). In brief, 100 mg of feed sample was pretreated with 6 mol/L HCl and then 1 mL of hydrolysate was dried in a lyophilizer (Lyoquest-85, Telstar, Spain). The samples were homogenized with 1 mL of 0.02 mol/L HCl and centrifuged at  $14,000 \times g$  at  $4^{\circ}\text{C}$  for 15 min. The supernatant was analyzed by a high-speed analyzer for amino acids (Hitachi L-8900, Tokyo, Japan). Digestible energy (DE) was calculated according to the prediction equation of Noblet and Perez (1993):  $\text{DE} = 4168 - (9.1 \times \text{Ash}) + (1.9 \times \text{CP}) + (3.9 \times \text{EE}) - (3.6 \times \text{NDF})$ .

#### 2.5. Determination of serum biochemical parameters of sows

The levels of total triglycerides (TG), glucose (Glu), total cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alanine transaminase (ALT), and aspartate transaminase (AST) were measured using an automated biochemical analyzer (Hitachi 7160, Japan).

#### 2.6. Composition of colostrum and milk

Frozen samples of colostrum and milk components were thawed at  $4^{\circ}\text{C}$  and then analyzed for milk components using 5 mL of each sample. Fully automated milk composition analyzers (Milkoscan Mars, Foss) were used to analyze colostrum and milk samples as part of the analytical process. Milk fat, protein, lactose, and solids-not-fat contents were analyzed.

#### 2.7. Antioxidant enzyme activity assessment

Serum superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities were assayed with corresponding kits, and purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

#### 2.8. Assessment of cytokine levels

The serum, colostrum, and milk were analyzed for cytokine content. Interleukin (IL)-6, IL-10, and tumor necrosis factor-alpha (TNF- $\alpha$ ) levels were detected by corresponding commercial enzyme-linked immunosorbent assay (ELISA) kits. The ELISA kit

was obtained from the Shanghai Enzyme-linked Biotechnology Company (Shanghai, China).

#### 2.9. Detection of serum indexes

The serum 5-hydroxytryptamine (5-HT), insulin (INS), adenosine monophosphate-activated kinase (AMPK), free fatty acid (FFA), adenosine triphosphate (ATP), motilin (MTL), adenosine monophosphate (AMP), agouti-related peptide (AgRP), ghrelin, endothelin-1 (ET-1), substance P (SP), neuropeptide tyrosine (NPY), acetylcholine enzyme (AChE), and leptin (LEP) were determined by the corresponding ELISA kit (Shanghai Enzyme-linked Biotechnology Company, Shanghai, China). The operation steps were strictly carried out according to the instructions of the kit.

#### 2.10. RNA extraction and real-time quantitative PCR (RT-qPCR)

Total RNA was extracted from jejunum and colon samples using the TRIZOL method (RNAiso Plus TaKaRa, Dalian, China). An ultramicro spectrophotometer (Implen GmbH, Munich, Germany) was used to detect the RNA concentration, and the  $A_{260}/A_{280}$  ratio ranged between 1.8 and 2.0, demonstrating high RNA purity. Subsequently, 1  $\mu\text{g}$  of total RNA was transcribed to cDNA using the Prime-Script RT kit and gDNA Eraser (RR047A, Takara, China) according to the manufacturer's instructions. ABI QuantStudio 3 (America, Applied Biosystems) was used for the RT-qPCR. SYBR Premix Ex Taq (RR420A; TaKaRa, China) was used to determine the mRNA level of the gene. The data were analyzed according to the  $2^{-\Delta\Delta\text{Ct}}$  method, and  $\beta$ -actin was used as the reference gene (Teng et al., 2023b). All primer sequences (Table S1) were synthesized by Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China).

#### 2.11. 16S rRNA sequencing and analysis

Using the specific primers 341F (5'-CCTACGGGNGGCWGCAG-3') and 805R (5'-GACTACHVGGGTAAATCC-3'), the 16S rRNA gene targeting the V3–V4 region was amplified for bacteria. The Agilent 2100 Bioanalyzer DNA kit (Agilent, USA) was used to assess library size and quality. Using the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA), the amplicon pool was quantified. The bioinformatics analysis was performed as described in our previous study (Gao et al., 2024).

#### 2.12. Short-chain fatty acid (SCFA) analysis of sow feces and piglet colon contents

Sow feces and piglet colon contents were mixed with phosphoric acid solution (0.5%, v/v), and ultrasonicated for 5 min on ice for each sample. Afterward, samples were centrifuged at  $13,400 \times g$  for 10 min at  $4^{\circ}\text{C}$ . The collected supernatant was vortexed for 3 to 5 min in methyl tert-butyl ether (MTBE; internal standards) and ultrasonicated for 5 min. The supernatant obtained after centrifugation ( $13,400 \times g$  for 10 min at  $4^{\circ}\text{C}$ ) was used for GC–MS/MS analysis.

#### 2.13. Western blot analysis

Frozen jejunum and colon tissue were lysed in an ice-cold immunoprecipitation assay (RIPA) buffer containing phenylmethylsulfonyl fluoride (PMSF) (Beyotime, Shanghai, China). The protein concentrations were quantified with a BCA kit (#P0010S, Beyotime, Shanghai, China). The proteins were resolved with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-

PAGE), and then membranes were transferred. Membranes with 5% skimmed milk were blocked for 2 h at room temperature, followed by primary antibody incubation at 4 °C overnight. The information related to antibodies is as follows:  $\beta$ -actin (#AC026, ABclonal, Wuhan, Hubei, China), Nrf2 (#A0674, ABclonal, Wuhan, Hubei, China), CLDN4 (#WL05091, WANLEIBIO, Shenyang, Liaoning, China), SOD1 (#A0274, ABclonal, Wuhan, Hubei, China), occludin (#A2601, ABclonal, Wuhan, Hubei, China), NQO1 (#A0047, ABclonal, Wuhan, Hubei, China), IL-6 (#WL02841, WANLEIBIO, Shenyang, Liaoning, China), IL-10 (#WL03088, WANLEIBIO, Shenyang, Liaoning, China), and TNF- $\alpha$  (#WL01581, WANLEIBIO, Shenyang, Liaoning, China). They were then incubated with secondary antibodies (HRP Goat Anti-Rabbit IgG (H + L)). To visualize the immunoblots, the BeyoECL Star chemiluminescence reagent kit (Beyotime, Shanghai, China) and a Uvitec Cambridge Imaging system (UVITEC, Cambridge, UK) were used to visualize the results. The band intensity was quantified with software Image J and the band density for each target protein was normalized by  $\beta$ -actin.

### 2.14. Statistical analyses

Statistical analyses were conducted using SPSS software (version 23; IBM), we performed two-tailed, unpaired Student's *t*-test on data with normal distributions and similar variances to determine whether they were statistically significant. The results are means  $\pm$  standard error of the mean (SEM).  $P < 0.05$  was considered statistically significant. Microflora were compared using the Kruskal-Wallis followed by Wilcoxon tests, and then a linear discriminant analysis was performed to evaluate the effect size. The graphs were created using Graph Pad Prism 8.0 (Graph Pad Software), Pixelmator Pro (Pixelmator Team), and Figdraw.

## 3. Result

### 3.1. Sow and piglet performance

Table 2 shows the performance of sows and suckling piglets. The SF and Con groups did not significantly differ in backfat thickness during lactation ( $P > 0.05$ ). The weaning-to-estrus interval of sows in the SF group was not remarkably changed compared to the Con group ( $P > 0.05$ ). Regarding the performance of sows, the two treatments did not affect the litter size, live births, litter weight, and individual body weights at birth ( $P > 0.05$ ). There was no effect of SF on litter weight at weaning, the average daily gain of piglets, and survival at weaning ( $P > 0.05$ ). The weight of weaned piglets tended to increase compared with the Con group ( $P = 0.056$ ). The feeding intake of sows during lactation is displayed in Fig. 1. The SF group of sows had higher feed intake during lactation than the Con group ( $P < 0.05$ ). Specifically, the sows of the SF group showed significantly higher feed intake than those of the Con group from 15 to 21 d ( $P < 0.05$ ) and 22 to 25 d ( $P < 0.05$ ) of lactation.

### 3.2. Biochemical indicators of sow serum

The effect of SF on the serum biochemical parameters in sows is shown in Table 3. The level of AST, LDL, Glu, AST/ALT ratio, ALT, TC, TG, and HDL did not differ between the groups ( $P > 0.05$ ).

### 3.3. Serum hormone concentrations of sows

Table 4 shows the serum hormone concentrations in the sows. The level of NPY was remarkably enhanced ( $P = 0.006$ ). There were no remarkable differences between the two groups in terms of the levels of ghrelin, AMPK, AgRP, INS, LEP, FFA, ATP, and AMP ( $P > 0.05$ ).

**Table 2**

Effects of konjac glucomannan and  $\kappa$ -carrageenan supplementation of sows during late gestation and lactation on the performance of lactating sows and piglets<sup>1</sup>.

Item	Treatment <sup>2</sup>		P-value
	Con	SF	
Backfat thickness, mm			
At birth	18.47 $\pm$ 0.376	17.73 $\pm$ 0.371	0.176
At weaning	16.80 $\pm$ 0.380	16.46 $\pm$ 0.336	0.517
Backfat loss	1.67 $\pm$ 0.159	1.27 $\pm$ 0.153	0.081
Weaning-to-estrus interval, d	5.00 $\pm$ 0.378	4.38 $\pm$ 0.263	0.199
Litter size (number of born piglets)			
Total born	11.93 $\pm$ 1.285	12.87 $\pm$ 0.990	0.565
Born alive	9.86 $\pm$ 1.108	10.40 $\pm$ 0.660	0.672
After cross-foster	9.79 $\pm$ 0.681	10.07 $\pm$ 0.511	0.742
At weaning	9.36 $\pm$ 0.589	9.67 $\pm$ 0.475	0.684
Litter weight, kg			
At birth	12.44 $\pm$ 1.378	13.89 $\pm$ 0.815	0.366
After cross-foster	12.47 $\pm$ 0.896	13.53 $\pm$ 0.783	0.375
At weaning	67.08 $\pm$ 4.019	73.14 $\pm$ 3.099	0.239
Piglets weight, kg			
At birth	1.29 $\pm$ 0.037	1.37 $\pm$ 0.055	0.262
After cross-foster	1.28 $\pm$ 0.034	1.36 $\pm$ 0.054	0.217
At weaning	7.22 $\pm$ 0.105	7.63 $\pm$ 0.177	0.056
Average daily gain, g	247.25 $\pm$ 4.230	261.14 $\pm$ 7.467	0.120
Survival at weaning, %	96.47 $\pm$ 1.644	96.46 $\pm$ 1.711	0.997

<sup>1</sup> The data are expressed as the mean  $\pm$  SEM.

<sup>2</sup> Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25  $\kappa$ -carrageenan to replace the same percentage of corn in the basal diet ( $n = 17$ ).

### 3.4. Serum gastrointestinal regulatory peptides and neurotransmitters of sows

Serum gastrointestinal regulatory peptides and neurotransmitters were measured to investigate potential changes in intestinal motility. The level of 5-TH was measured (Fig. 2A) and it was found that there was no marked difference between the two groups. Fig. 2B–D displays the levels of three gastrointestinal regulatory peptides. The SF group showed a substantial increase in the level of AChE compared to the Con group ( $P < 0.05$ ) and a trend toward an increase in MTL ( $0.05 \leq P < 0.1$ ) and SP ( $0.05 \leq P < 0.1$ ) in the SF group.

### 3.5. Serum inflammatory cytokines

Table 5 displays the serum cytokine concentrations in sows and suckling piglets. The IL-6 concentration in the serum of sows was significantly reduced by the SF diet ( $P = 0.016$ ), whereas the levels of IL-10 and TNF- $\alpha$  showed no significant change ( $P > 0.05$ ). Regarding inflammatory factors in the serum of suckling piglets, the levels of IL-6, IL-10, and TNF- $\alpha$  were not significantly changed in both groups ( $P > 0.05$ ).

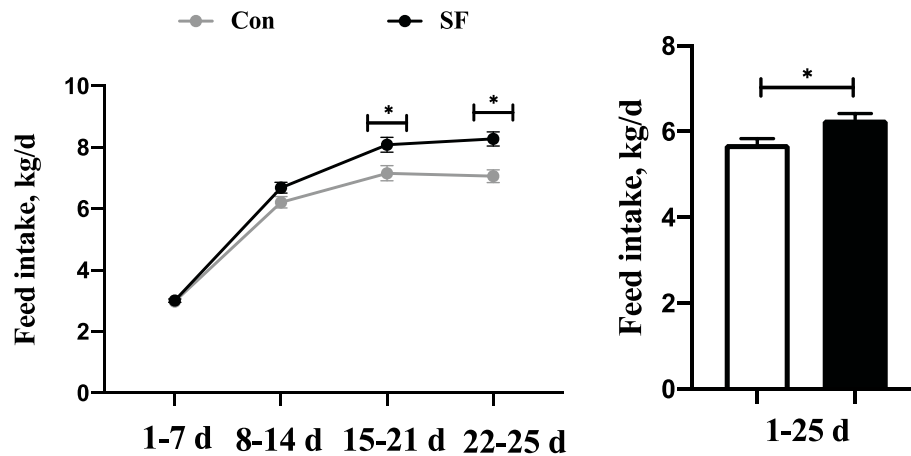
### 3.6. Serum antioxidant status

Serum antioxidant capacity in sows and suckling piglets is presented in Table 5. The activity of T-SOD in the SF group was significantly increased compared to the Con group ( $P = 0.007$ ), and the activity of GSH-Px was not changed in the two groups ( $P > 0.05$ ). For suckling piglets, compared to the Con group, the activity of GSH-Px was markedly increased ( $P = 0.026$ ) in the SF group, but the difference in T-SOD activity in each group was not significant ( $P > 0.05$ ).

### 3.7. Colostrum and milk composition and inflammatory cytokines

As shown in Fig. 3, the colostrum and milk composition of sows (including fat, protein, lactose, and solids-not-fat) were not





**Fig. 1.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on feed intake of sows during lactation. Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet. The data are presented as mean  $\pm$  SEM ( $n = 17$ ). \* represents  $P < 0.05$ .

**Table 3**

Effect of konjac glucomannan and  $\kappa$ -carrageenan supplementation on serum parameters in lactating sows during late gestation and lactation<sup>1</sup>.

Item	Treatment <sup>2</sup>		P-value
	Con	SF	
GLU, mmol/L	4.63 $\pm$ 0.179	3.93 $\pm$ 0.344	0.101
ALT, U/L	63.35 $\pm$ 11.928	55.70 $\pm$ 6.066	0.580
AST, U/L	22.70 $\pm$ 2.803	27.08 $\pm$ 1.339	0.189
AST/ALT ratio	0.41 $\pm$ 0.086	0.53 $\pm$ 0.070	0.331
TG, mmol/L	0.26 $\pm$ 0.046	0.22 $\pm$ 0.013	0.428
TC, mmol/L	2.51 $\pm$ 0.367	2.35 $\pm$ 0.168	0.689
HDL, mmol/L	1.40 $\pm$ 0.169	1.27 $\pm$ 0.053	0.485
LDL, mmol/L	1.00 $\pm$ 0.132	0.94 $\pm$ 0.085	0.719

GLU = glucose; ALT = alanine aminotransferase; AST = aspartate aminotransferase; TG = triglycerides; TC = cholesterol; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

<sup>1</sup> The data are expressed as the mean  $\pm$  SEM.

<sup>2</sup> Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan to replace the same percentage of corn in the basal diet ( $n = 6$ ).

**Table 4**

Effect of konjac glucomannan and  $\kappa$ -carrageenan supplementation on serum feeding-related hormones in lactating sows during late gestation and lactation<sup>1</sup>.

Item	Treatment <sup>2</sup>		P-value
	Con	SF	
AgRP, ng/mL	13.92 $\pm$ 2.186	13.03 $\pm$ 1.360	0.729
NPY, pg/mL	3194.17 $\pm$ 198.679 <sup>b</sup>	4466.39 $\pm$ 241.996 <sup>a</sup>	0.006
Ghrelin, ng/mL	5.16 $\pm$ 0.817	4.43 $\pm$ 0.530	0.459
AMPK, ng/mL	127.29 $\pm$ 11.272	114.81 $\pm$ 7.748	0.373
INS, mIU/L	27.86 $\pm$ 2.738	24.25 $\pm$ 3.440	0.446
FFA, $\mu$ mol/L	574.72 $\pm$ 79.447	735.00 $\pm$ 44.358	0.098
LEP, ng/mL	13.89 $\pm$ 1.226	15.01 $\pm$ 0.829	0.457
ATP, nmoL/L	6277.50 $\pm$ 773.638	5994.17 $\pm$ 635.083	0.781
AMP, nmoL/L	21.30 $\pm$ 0.636	18.16 $\pm$ 2.012	0.187

AgRP = agouti-related peptide; NPY = neuropeptide tyrosine; AMPK = adenosine monophosphate-activated kinase; INS = insulin; FFA = free fatty acid; LEP = leptin; ATP = adenosine triphosphate; AMP = adenosine monophosphate.

Different superscript letters indicate significant differences ( $P < 0.05$ ).

<sup>1</sup> The data are expressed as the mean  $\pm$  SEM.

<sup>2</sup> Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet ( $n = 6$ ).

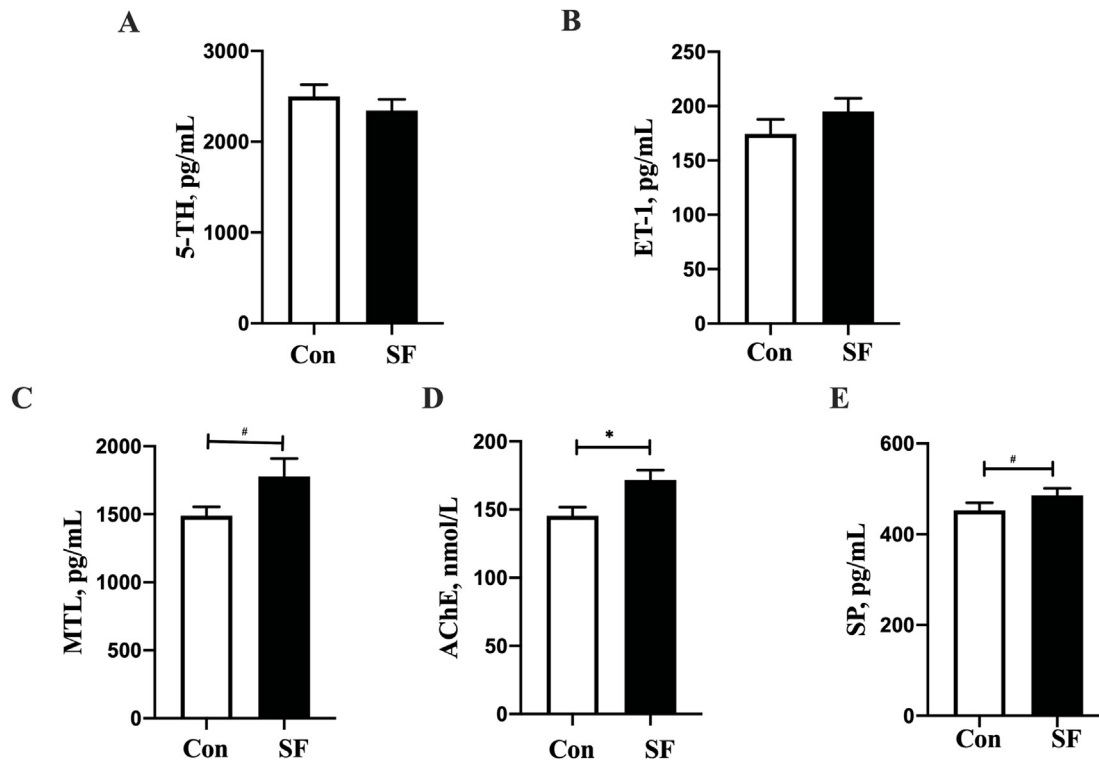
markedly different between the two groups ( $P > 0.05$ ). The milk content of IL-6 was remarkably reduced in the SF group ( $P < 0.05$ ). There were no significant changes in IL-6 level in colostrum ( $P > 0.05$ ). The levels of IL-10 were not markedly different between the two treatments in the colostrum and milk ( $P > 0.05$ ).

### 3.8. SCFA in the feces of sows and colon contents of suckling piglets

The results of SCFA in sow feces are depicted in Fig. 4A. The acetate concentration in the SF group was significantly increased ( $P < 0.05$ ), and the total SCFA in the SF group tended to increase ( $0.05 \leq P < 0.01$ ) compared with the Con group. There were no significant differences in the contents of propionate, butyrate, valerate, isobutyrate, and isovalerate between the two groups ( $P > 0.05$ ). The content of SCFA in the colon of the suckling piglet is presented in Fig. 4B. There are no significant changes in the levels of acetate, propionate, butyrate, valerate, isobutyrate, isovalerate, and total SCFA in the colon contents of suckling piglets ( $P > 0.05$ ).

### 3.9. Microbial diversity analysis of sow feces

The diversity of sow feces was assessed using high-throughput sequencing. This study used four indexes to analyze  $\alpha$  variety: Chao1, Observed operational taxonomic units (OTUs), Shannon, and Simpson (Fig. 5A). The Observed OTUs, Shannon, Simpson, and Chao1 analyses showed no notable changes in the two groups ( $P > 0.05$ ). The variation of the bacterial flora at the level of the phylum is shown in Fig. 5B: Firmicutes, Actinobacteria, Bacteroidota, Proteobacteria, Spirochaetota, Patescibacteria, Verrucomicrobiota, Cyanobacteria, Planctomycetota, and Campylobacterota were the major bacterial in sow feces. At the genus level, the abundant taxa in the top 20 are shown in Fig. 5C. The linear discriminant analysis effect size (LEfSe) analysis was employed to uncover biomarkers. It exhibited significant changes between SF and Con treatments (Fig. 5D and E). The results indicated that the SF group enriched *Subdoligranulum*, *Holdemanella* and *Succinivibrio* at the genus level. The relative abundance of *Coriobacteriaceae\_unclassified*, *Phenylobacterium*, and *Lachnospiraceae\_XPB1014\_group* in the Con group was significantly higher than that in the SF group.



**Fig. 2.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on serum gastrointestinal regulatory peptides and neurotransmitter factors of sows. (A) The serum level of 5-HT. (B to D) The serum levels of gastrointestinal regulatory peptides. (E) The serum level of SP. Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet. 5-HT = serotonin; ET-1 = endothelin-1; MTL = motilin; AChE = acetylcholine enzyme; SP = substance P. The data are presented as mean  $\pm$  SEM ( $n = 6$ ). \*,  $P < 0.05$  and #,  $0.05 \leq P < 0.1$ .

**Table 5**

Effect of maternal konjac glucomannan and  $\kappa$ -carrageenan supplementation during late gestation and lactation on serum inflammatory factor levels and antioxidant capacity in lactating sows and piglets<sup>1</sup>.

Item	Treatment <sup>2</sup>		P-value
	Con	SF	
<b>Sow</b>			
IL-10, pg/mL	182.51 $\pm$ 9.117	180.23 $\pm$ 2.394	0.819
IL-6, pg/mL	668.50 $\pm$ 43.455 <sup>a</sup>	494.00 $\pm$ 28.289 <sup>b</sup>	0.016
TNF- $\alpha$ , pg/mL	143.64 $\pm$ 13.716	150.74 $\pm$ 9.752	0.676
T-SOD, U/mL	114.09 $\pm$ 1.068 <sup>b</sup>	135.13 $\pm$ 4.862 <sup>a</sup>	0.007
GSH-Px, U/mL	292.05 $\pm$ 13.350	275.66 $\pm$ 15.503	0.442
<b>Piglet</b>			
IL-6, pg/mL	472.50 $\pm$ 41.264	555.75 $\pm$ 32.359	0.151
IL-10, pg/mL	183.11 $\pm$ 3.582	179.66 $\pm$ 7.540	0.691
TNF- $\alpha$ , pg/mL	119.40 $\pm$ 10.081	129.64 $\pm$ 6.893	0.411
T-SOD, U/mL	232.26 $\pm$ 15.559	218.41 $\pm$ 9.553	0.465
GSH-Px, U/mL	269.16 $\pm$ 2.785 <sup>b</sup>	296.75 $\pm$ 8.988 <sup>a</sup>	0.026

IL-10 = interleukin 10; T-SOD = total superoxide dismutase; IL-6 = interleukin 6; GSH-Px = glutathione peroxidase; TNF- $\alpha$  = tumor necrosis factor-alpha. Different superscript letters indicate significant differences ( $P < 0.05$ ).

<sup>1</sup> The data are expressed as the mean  $\pm$  SEM.

<sup>2</sup> Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet ( $n = 6$ ).

### 3.10. Effect of maternal SF supplementation on mRNA levels of the Nrf2/Keap1 signaling pathway in the jejunum of suckling piglets

The effect of SF on mRNA levels of the Nrf2/Keap1 signaling pathway in the jejunum of suckling piglets is shown in Fig. 6A. According to the results, the mRNA levels of nuclear factor erythroid 2-related factor 2 (Nrf2) ( $P < 0.05$ ) and superoxide

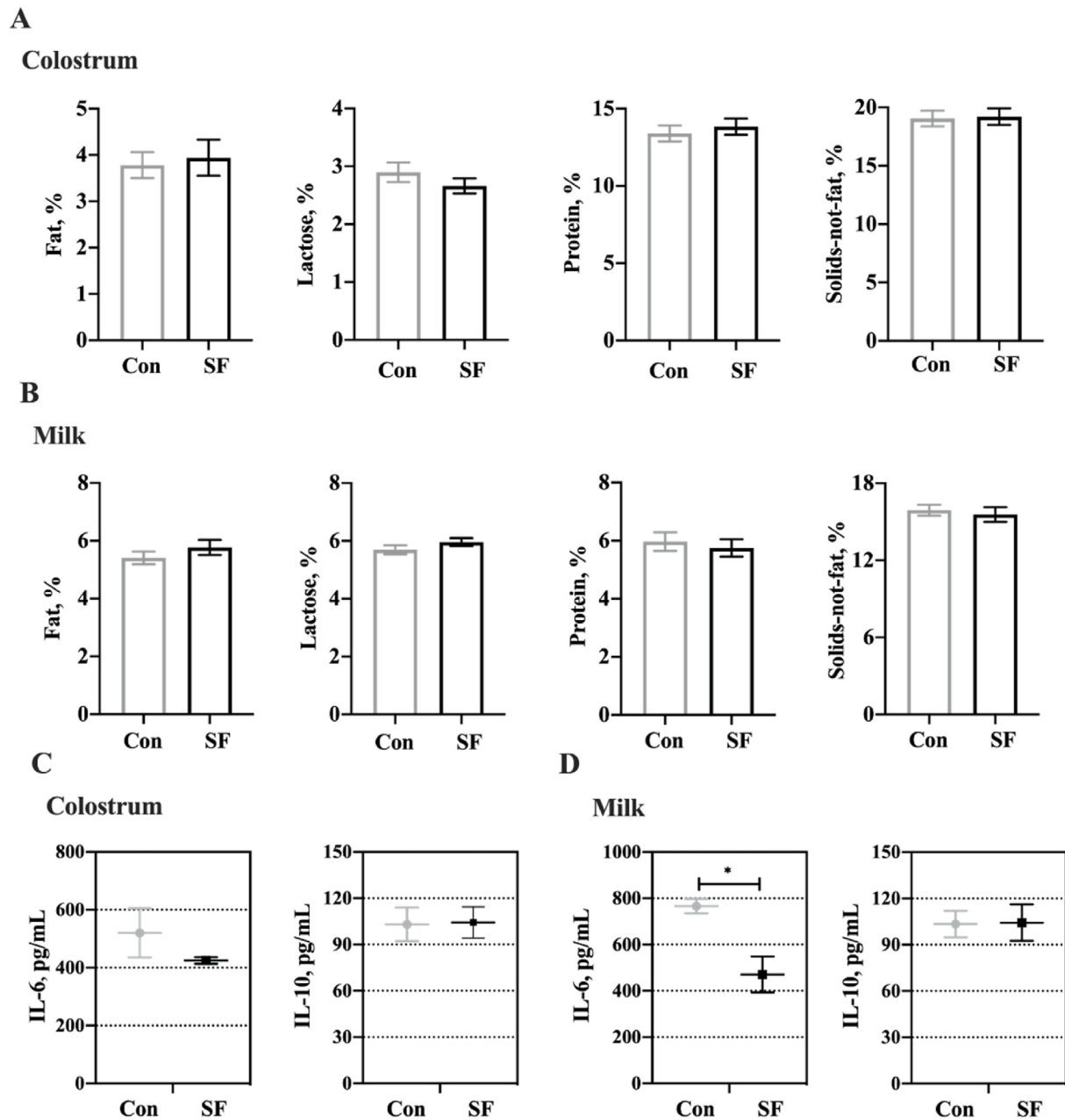
dismutase 1 (SOD1) ( $P < 0.05$ ) were dramatically up-regulated in SF group compared to Con group. However, there was no significant difference in the level of Kelch-like ECH-associated protein 1 (Keap1), SOD2, glutathione peroxidase (GPX2), glutamate-cysteine ligase catalytic subunit (GCLC), glutamic acid cysteine ligase modified subunit (GCLM), heme oxygenase-1 (HO-1), and NAD(P)H quinone oxidoreductase 1 (NQO1) between the two groups ( $P > 0.05$ ).

### 3.11. Effect of maternal SF supplementation on mRNA levels of inflammatory factors and intestinal barrier in the jejunum of suckling piglets

The levels of inflammatory factors in the jejunum of suckling piglets are presented in Fig. 6B. There are no significant differences in the mRNA level of IL-6, IL-1 $\beta$ , IL-8, and TNF- $\alpha$  ( $P > 0.05$ ) in the SF group compared with the Con group. The mRNA levels of claudin 2 (CLDN2), CLDN4, zonula occludens-1 (ZO-1), and occludin in the jejunum can be found in Fig. 6C. The mRNA level of CLDN4 ( $P < 0.05$ ) and occludin ( $P < 0.05$ ) are significantly up-regulated in the jejunum of suckling piglets in the SF group compared with the Con group. No changes were observed for CLDN2 and ZO-1 mRNA levels ( $P > 0.05$ ).

### 3.12. Effect of maternal SF supplementation on mRNA levels of the Nrf2/Keap1 signaling pathway in the colon of suckling piglets

Furthermore, mRNA levels of Nrf2/Keap1 signaling pathway-related genes in the colon of the suckling piglets were measured (Fig. 7A). The mRNA levels of Nrf2, GCLC, SOD2, and GCLM were markedly increased in the SF group ( $P < 0.05$ ). The mRNA levels of



**Fig. 3.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on colostrum and milk composition and inflammatory factor levels of sows. (A and B) The colostrum and milk composition of sows. (C and D) Inflammatory cytokines in colostrum and milk of sows. Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet. The data of fat, lactose, protein, and solids-not-fat are presented as mean  $\pm$  SEM ( $n = 12$ ). The data of interleukin (IL)-6 and IL-10 are presented as mean  $\pm$  SEM ( $n = 6$ ). \*,  $P < 0.05$ .

*SOD1*, *GPX2*, *HO-1*, and *NQO1* did not show significant changes in the two groups ( $P > 0.05$ ).

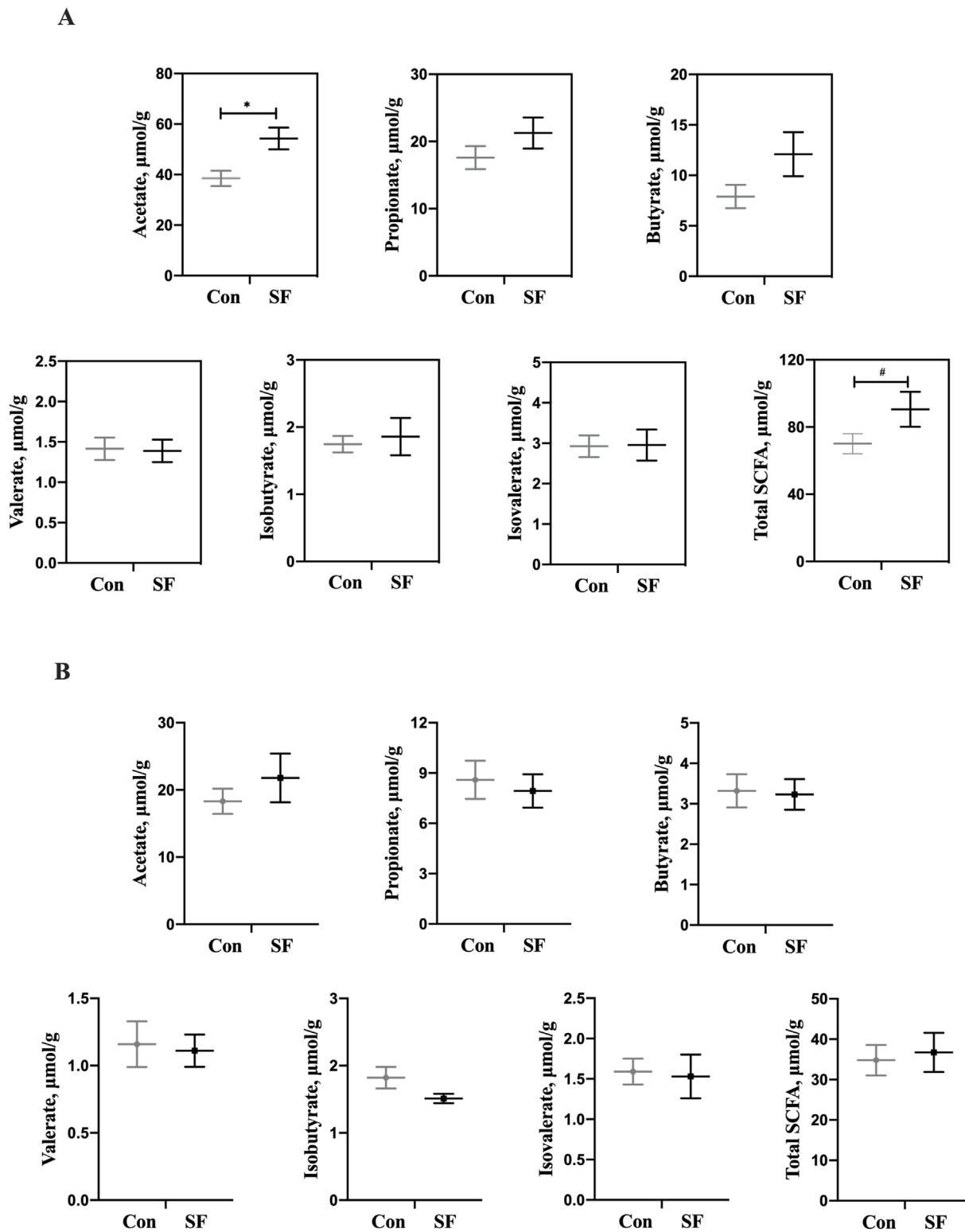
### 3.13. Effect of maternal SF supplementation on mRNA levels of inflammatory factors and intestinal barrier in the colon of suckling piglets

The piglets' colon mRNA levels of inflammation factor-related genes are presented in Fig. 7B. The mRNA levels of *IL-6* and *TNF- $\alpha$*  were significantly down-regulated and *IL-10* was up-regulated in the SF group compared with the Con group ( $P < 0.05$ ). There were no significant differences in *IL-8* mRNA levels between the two groups ( $P > 0.05$ ). Fig. 7C illustrates the mRNA levels of colonic tight

junction proteins in suckling piglets. The mRNA level of *CLDN4* was significantly up-regulated in the SF group ( $P < 0.05$ ) compared with the Con group and the mRNA levels of *ZO-1*, *CLDN2*, and occludin did not significantly change between the two groups ( $P > 0.05$ ).

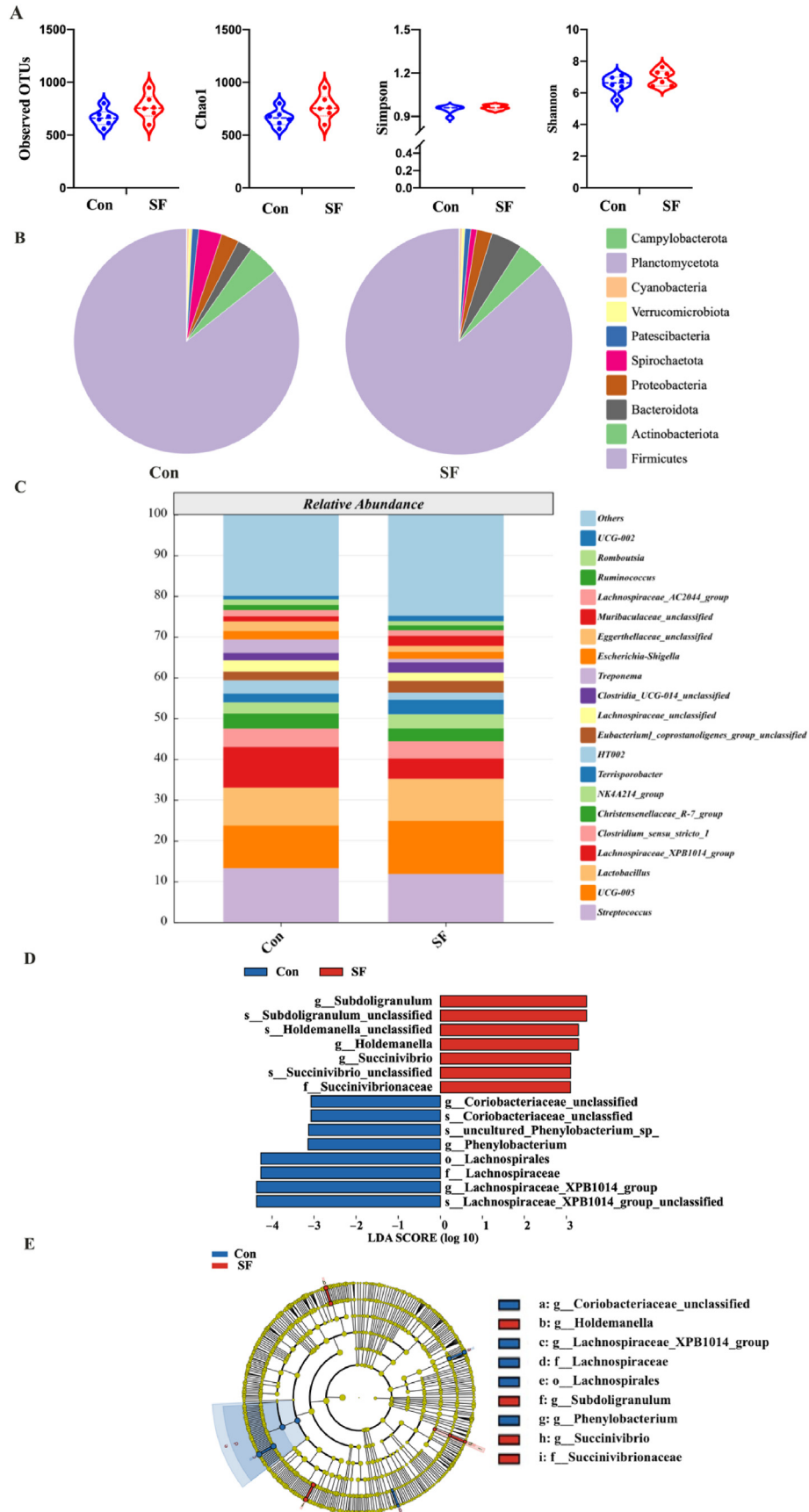
### 3.14. Effect of maternal SF on the expression of Nrf2 pathway and intestinal barrier-related proteins in the jejunum of suckling piglets

To further investigate whether SF stimulates the Nrf2 signaling pathway to enhance its antioxidant effects, we detected Nrf2 protein expression by Western blot. The results showed that there was no significant change in jejunal Nrf2 protein levels ( $P > 0.05$ ) (Fig. 8H). Following this, we performed Western blot analyses to

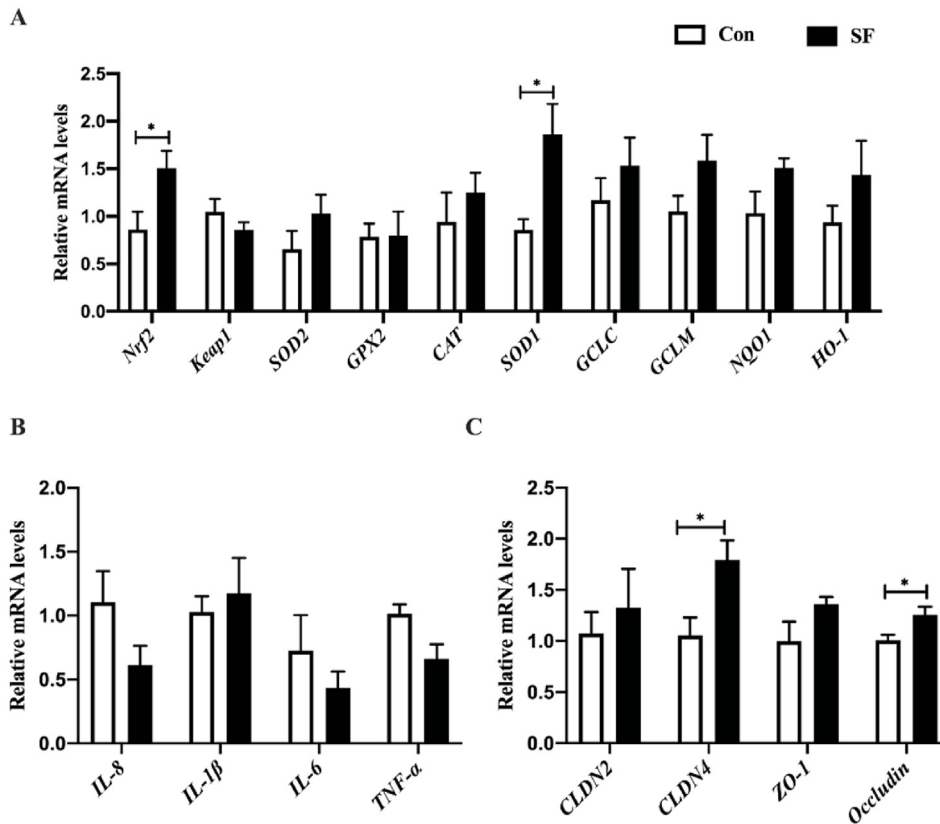


**Fig. 4.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on fecal short-chain fatty acid (SCFA) content of sows and piglets. (A) SCFA contents in the feces of sows. (B) SCFA contents in the colon of suckling piglets. Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan + 0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet. Total SCFA = total short-chain fatty acids. The data are presented as mean  $\pm$  SEM ( $n = 6$ ). \*,  $P < 0.05$  and #,  $0.05 \leq P < 0.1$ .





**Fig. 5.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on the fecal microbiota of sows. Observed operational taxonomic units (OTUs), Chao1, Simpson, and Shannon (A) indexes of the fecal microbiota of sows from the two treatments, and the differential bacteria at the phylum level (B) and genus level (C). LefSe analysis (D and E) of fecal microbiota of sows at 25 d. Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet ( $n = 6$ ). LefSe = linear discriminant analysis effect size.



**Fig. 6.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on the mRNA levels of Nrf2 pathway-related genes (A), inflammatory factors (B), and intestinal barrier (C) related genes in the jejunum of piglets. Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet. Nrf2 = nuclear factor erythroid 2-related factor 2; HO-1 = heme oxygenase-1; Keap1 = kelch-like ECH-associated protein 1; GPX2 = glutathione peroxidase 2; SOD1 = superoxide dismutase 1; GCLC = glutamate cysteine ligase catalyzes subunits; GCLM = glutamic acid cysteine ligase modified subunit; NQO1 = NAD(P)H quinone oxidoreductase 1; SOD2 = superoxide dismutase 2; IL-8 = interleukin-8; IL-1 $\beta$  = interleukin-1 $\beta$ ; IL-6 = interleukin-6; TNF- $\alpha$  = tumor necrosis factor-alpha; CLDN4 = claudin 4; CLDN2 = claudin 2; ZO-1 = zonula occludens-1. The data are presented as mean  $\pm$  SEM ( $n = 6$ ). \*,  $P < 0.05$ .

determine whether SF affects the expression of CLDN4, IL-10, SOD1, NQO1, and occludin in the jejunum of piglets (Fig. 8). The results showed that IL-6 protein levels were significantly lower in the SF group ( $P < 0.05$ ). The protein levels of CLDN4, IL-10, SOD1, NQO1, and occludin were not found to be remarkably changed between the two groups ( $P > 0.05$ ).

### 3.15. Effect of maternal SF on the expression of Nrf2 pathway and intestinal barrier-related proteins in the colon of suckling piglets

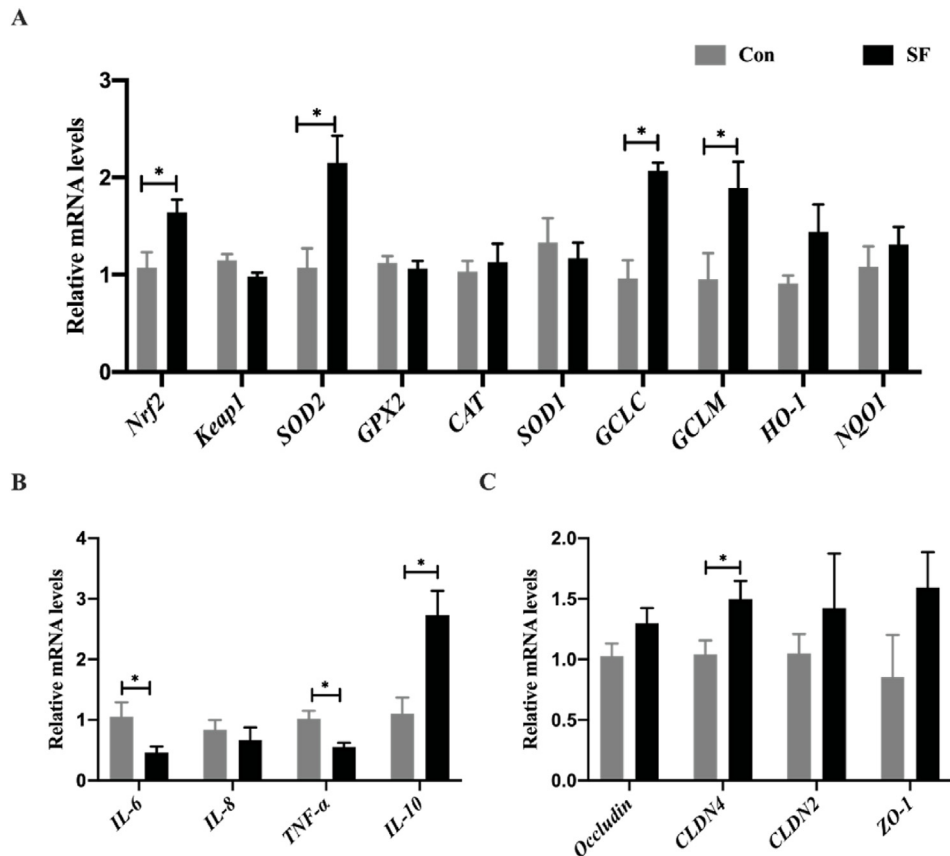
For the colon (Fig. 9), the SF group significantly increased the protein level of occludin ( $P < 0.05$ ) and showed a trend towards up-regulation of Nrf2 and CLDN4 ( $0.05 \leq P < 0.1$ ) protein levels compared to the Con group. The protein levels of IL-10, SOD1, NQO1, and TNF- $\alpha$  were not found to be remarkably changed between the two groups ( $P > 0.05$ ).

## 4. Discussion

Lactation is an essential period in a sow's reproductive cycle (Li et al., 2023), during which maternal feed intake is strongly related to suckling piglet growth (Sulabo et al., 2010). Sow feed intake during lactation has been highlighted as a potential solution for supporting sufficient growth of piglets reared in a large litter (Hawe et al., 2020). This study aimed to explore the effect of SF supplementation in diets on sow performance and the gut barrier of suckling piglets.

In previous studies, fiber diets have been shown to favor lactation feed intake in sows (Piao et al., 2019; Li et al., 2023). In this study, we observed that sow feed intake exhibited a significant increase during lactation with SF supplementation in late gestation and lactation. Many factors regulate feed intake, including the hypothalamus which contains many peptides and other neurotransmitters that affect feed intake (Wilding, 2002). For example, the NPY/AgRP neuron releases NPY and AgRP (Kettner et al., 2015). NPY is one of the most potent physiological appetite stimulators known in mammals (Lu et al., 2013; Zhang and van den Pol, 2016). In this study, we found that SF significantly increased the serum levels of NPY in sows. NPY activity is functionally required for resistance to hunger status (Zou et al., 2022). As NPY increases, further feed intake increases (Hussain et al., 2015). Moreover, AgRP/NPY neuronal activity is tightly regulated by nutritional status (He et al., 2016). In this study, we found that sows in the SF group had significantly higher feed intake than the Con group during lactation. This was similar to the study results of Dube et al. (1992), who found that NPY increased feed intake. This is also confirmed by our previous research that konjac glucomannan improves sow feed intake during lactation and is inseparably associated with elevated NPY levels (Gao et al., 2024).

Oxidative stress adversely influences the feed intake of lactating sows (Li et al., 2022). Multiple studies have demonstrated that sows suffer from heightened systemic oxidative stress during late gestation and lactation (Berchieri-Ronchi et al., 2011; Huang et al., 2022). The antioxidant enzymes in tissues are an essential



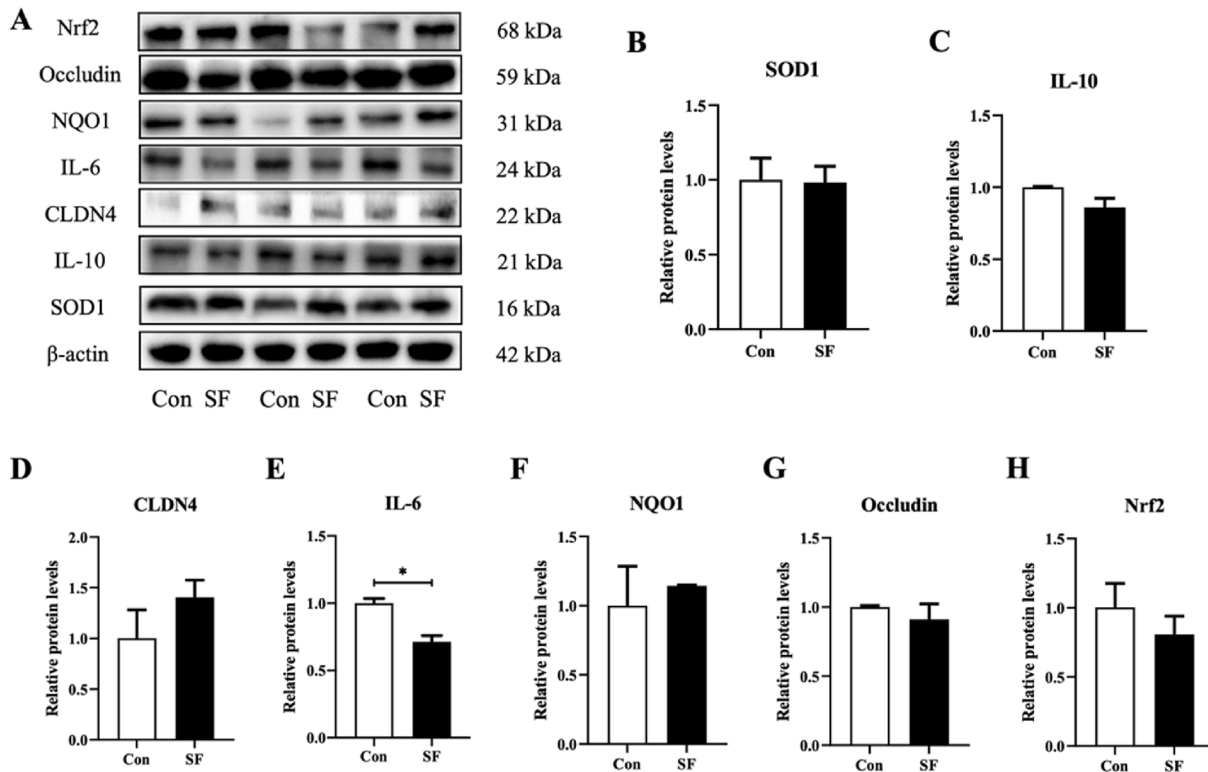
**Fig. 7.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on the mRNA levels of Nrf2 pathway-related genes (A), inflammatory factors (B), intestinal barrier (C) related genes in the colon of piglets. Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan + 0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet. *Nrf2* = nuclear factor erythroid 2-related factor 2; *HO-1* = heme oxygenase 1; *Keap1* = kelch-like ECH-associated protein 1; *SOD2* = superoxide dismutase, mitochondrial; *GPX2* = glutathione peroxidase 2; *SOD1* = superoxide dismutase, soluble; *GCLC* = glutamate cysteine ligase catalyzes subunits; *NQO1* = NAD(P)H quinone oxidoreductase 1; *GCLM* = glutamic acid cysteine ligase modified subunit; *IL-6* = interleukin-6; *IL-8* = interleukin-8; *TNF- $\alpha$*  = tumor necrosis factor-alpha; *IL-10* = interleukin-10; *CLDN4* = claudin 4; *CLDN2* = claudin 2; *ZO-1* = zonula occludens-1. The data are presented as mean  $\pm$  SEM ( $n = 6$ ). \*,  $P < 0.05$ .

indicator of their antioxidant status, including SOD and GSH-Px (Dou et al., 2023). In the current research, our results indicated that SF significantly increased T-SOD activity in sow serum. Oxidative stress and inflammatory factors are inseparable (Gao et al., 2023). Thus, the levels of proinflammatory factor IL-6 and anti-inflammatory factor IL-10 in the sow serum were measured. In parallel, our findings suggested that sows in the SF group had significantly lower levels of IL-6 than the Con group. Therefore, we speculated that SF might benefit lactation feed intake by increasing antioxidant enzyme activities and decreasing the levels of inflammatory factors.

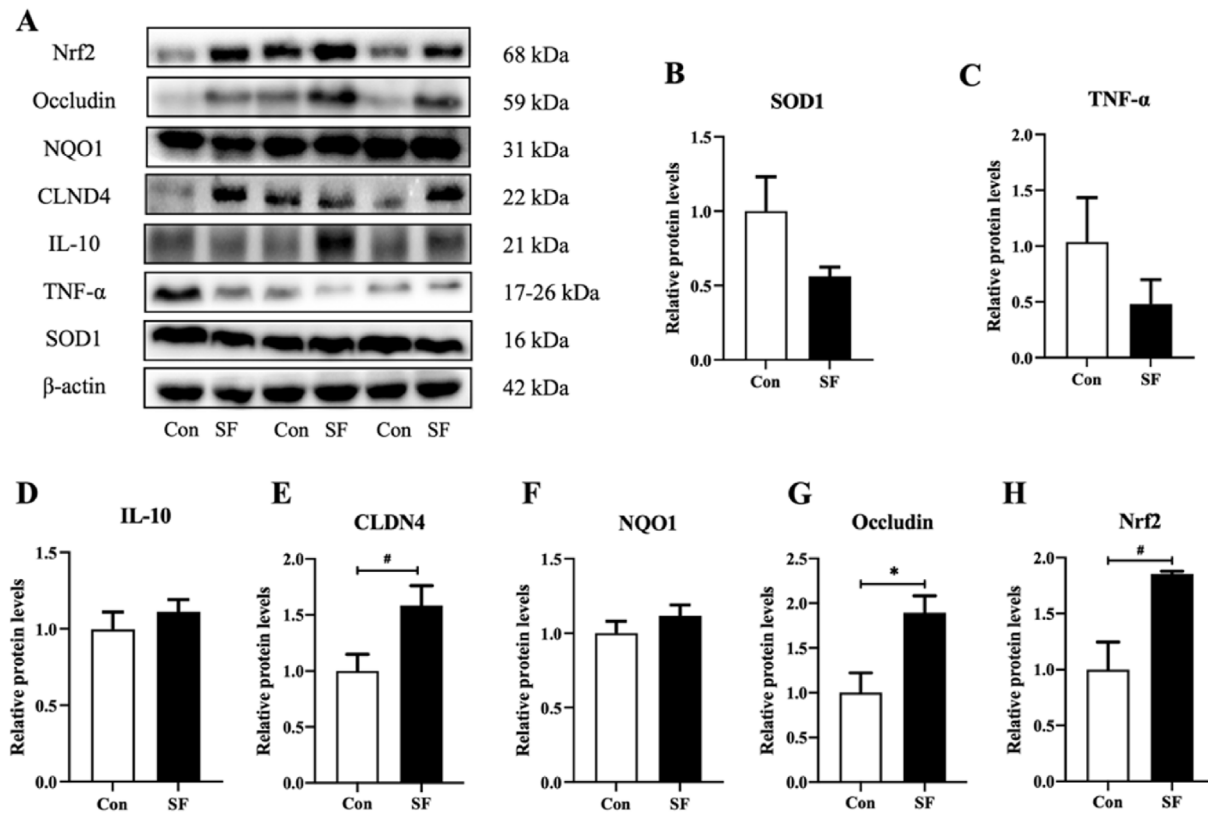
Constipation is harmful to sow performance. Late gestation and lactation are typical times for constipation in sows, especially during the summer (Yu et al., 2021). Gastrointestinal motility directly affects feed intake and gastric emptying (Mugambi et al., 2012). Several gastrointestinal hormones regulate gastrointestinal motility (Zhao et al., 2020). For example, AChE promotes the movement of feces by regulating muscle contraction in the intestine and mucus secretion in the large intestine (Suo et al., 2014). MTL stimulates pepsin production and increases the migrating myoelectric complex component of gastrointestinal motility (Suo et al., 2014) and stimulates intestinal motility in sows (Lu et al., 2022), which was confirmed by the present study. The SF group exhibited a significant increase in serum AChE levels in sows and there was a tendency to increase serum MTL levels in the SF group. Accordingly, our findings support the hypothesis that SF intake by

sows during late gestation and lactation stimulates the release of intestinal motility regulators. More importantly, it might also increase sows' feed intake during lactation.

The gut microbiome is increasingly fundamental to host health (Petersen et al., 2017; Fang et al., 2024). The gut microbiota has recently become a novel, ingenious, and non-negligible way of regulating host health due to an increase in understanding about the interactions between microbes and hosts (Zhang et al., 2022). Therefore, we further investigated the effect of SF on the fecal microflora of sows to explore the underlying mechanisms. In the present study, we found that SF enriched *Subdoligranulum*, *Holdemanella*, and *Succinivibrio*; whereas the Con group enriched *Coriobacteriaceae\_unclassified*, *Phenylobacterium*, and *Lachnospiraceae\_XPB1014\_group* at the genus level. Studies indicate that *Succinivibrio* is involved in the production of acetate and lactate (Lv et al., 2019), and *Holdemanella* exhibited anti-inflammatory activity in patients with colitis (Pujo et al., 2021). *Subdoligranulum* is a potentially beneficial bacterium with anti-inflammatory properties (Sun et al., 2022). Therefore, it may have a positive effect on improving sow performance during lactation. Conversely, *Coriobacteriaceae\_unclassified* is a pro-inflammatory bacteria (Bi et al., 2022). The results showed that SF supplementation in sows in late gestation and lactation improved the microbiota of sows. Intriguingly, the SF group reduced the abundance of *Lachnospiraceae\_XPB1014\_group* and *Phenylobacterium*. Consequently, further research is required to investigate the evolution of intestinal flora in



**Fig. 8.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on the levels of proteins in the jejunum of piglets. Protein band (A), SOD1 (B), IL-10 (C), CLDN4 (D), IL-6 (E), NQO1 (F), occludin (G), and Nrf2 (H). Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet. Nrf2 = nuclear factor erythroid 2-related factor 2; IL-10 = interleukin-10; CLDN4 = claudin 4; IL-6 = interleukin-6; SOD1 = superoxide dismutase 1; NQO1 = NAD(P)H quinone oxidoreductase 1. The data are presented as mean  $\pm$  SEM ( $n = 3$ ). \*,  $P < 0.05$ .



**Fig. 9.** Effects of maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan on the levels of proteins in the colon of piglets. Protein band (A), SOD1 (B), TNF- $\alpha$  (C), IL-10 (D), CLDN4 (E), NQO1 (F), occludin (G), and Nrf2 (H). Con, sows were fed a basal diet; SF, sows were fed 0.25% konjac glucomannan +0.25%  $\kappa$ -carrageenan replacements of the equivalent proportion of corn in the basal diet. SOD1 = superoxide dismutase 1; NQO1 = NAD(P)H quinone oxidoreductase 1; TNF- $\alpha$  = tumor necrosis factor-alpha; Nrf2 = nuclear factor erythroid 2-related factor 2; IL-10 = interleukin-10; CLDN4 = claudin 4. The data are presented as mean  $\pm$  SEM ( $n = 3$ ). \*,  $P < 0.05$  and #,  $0.05 \leq P < 0.1$ .

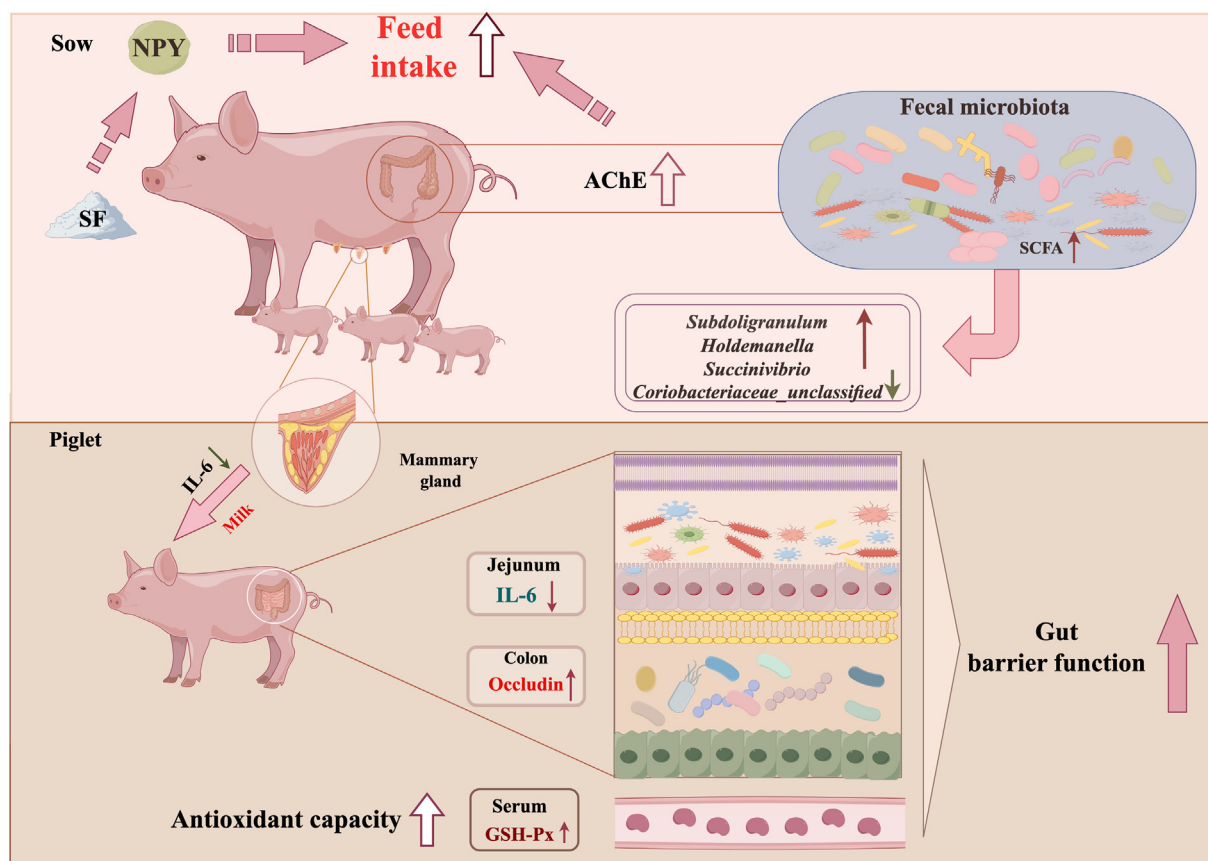
sows supplemented with SF. Moreover, SCFA are fermentation end products produced by the intestinal microbiota from the fermentation of undigested carbohydrates and proteins in the diet and have multiple effects on human health, including improved bowel movements (Rios-Covian et al., 2020; Mizutani et al., 2021). SCFA, specifically butyrate, propionate, and acetate, are crucial for maintaining gut barrier integrity (Arzani et al., 2020). In the current study, we found that the fecal acetate content of sows in the SF group significantly increased. It was further demonstrated that *Succinivibrio* was involved in acetate synthesis. Acetate also enhances motility by affecting ileal contractions (Zhu et al., 2022b). Therefore, this is beneficial for improving gastrointestinal motility in sows.

Improvement of sow performance during lactation directly benefits suckling piglet health (Kim et al., 2020). This phenomenon was also revealed in our study. During lactation, sows influence the piglets through colostrum and milk (Reyes-Camacho et al., 2020). Despite no significant changes in milk composition in both colostrum and milk, milk IL-6 was markedly reduced in the SF group. It is generally thought that IL-6 is a biomarker of inflammation (Szymanska et al., 2023). Thus, we speculated that SF has the benefit of improving sow milk quality. Suckling piglets' performance and health depend on sow nutrition management (Lu et al., 2019). According to Wang et al. (2016), the increased fiber intake of mothers during gestation improves the antioxidant capacity of mothers and their offspring by increasing T-SOD activity and decreasing serum MDA concentration. This research found that

serum GSH-Px activity significantly increased SF group piglets. The results showed that SF supplementation in sows during late gestation and lactation improved the antioxidant capacity of suckling piglets.

The intestinal tract of piglets develops fastest in the early stages of lactation (Tian et al., 2019). Suckling piglets obtain most of their nutrients from milk, and the intestine plays a crucial role in digestion, absorption, and metabolism (Zhong et al., 2017). Therefore, we further measured the level of genes and proteins associated with the jejunal and colonic Nrf2 signaling pathway and intestinal barrier in suckling piglets. In the antioxidant defense system, Nrf2 plays a vital role in antioxidative signaling (Wang et al., 2023). SOD1 is a critically important antioxidant downstream of Nrf2 (Gao et al., 2022). This study found that *Nrf2* and *SOD1* mRNA levels were remarkably up-regulated in the jejunum of suckling piglets in the SF group. However, the protein levels of Nrf2 and SOD1 were not found to be considerably changed as a consequence of SF.

Furthermore, Nrf2 regulates the integrity of the intestinal barrier (Xiao et al., 2022). In this study, we found that the SF group showed a significant down-regulation of the protein levels of jejunal IL-6 in suckling piglets. Reducing inflammation makes the intestinal barrier more secure, contributing to suckling piglets' growth (Cui et al., 2020). Furthermore, IL-6 may affect the integrity and tight junction status of the mucosal barrier (Mabbott, 2015). This view was also confirmed in our study. We found that the jejunal *CLDN4* and occludin



**Fig. 10.** In the late gestation and lactation period of sows, maternal supplementation of konjac glucomannan and  $\kappa$ -carrageenan (SF) could have a potential mechanism of gut barrier effect on suckling piglets. AChE = acetylcholine enzyme; MTL = motilin; NPY = neuropeptide tyrosine; IL-6 = interleukin-6; GSH-Px = glutathione peroxidase; SCFAs = short-chain fatty acid.



mRNA levels were significantly upregulated in suckling piglets of the SF group. These results suggested that SF supplementation for sows enhanced the jejunal barrier function of suckling piglets.

In colon tissue, we found that *Nrf2*, *SOD2*, *GCLC*, and *GCLM* mRNA levels were notably up-regulated in the colon of suckling piglets in the SF group. *NQO-1*, *GCLM*, and *GCLC* were identified as Nrf2-regulated target genes (Myung et al., 2019). Furthermore, there was a trend of increasing Nrf2 protein expression in the colon of suckling piglets in the SF group. We further compared the intestinal barrier function between the two groups based on the level of intestinal inflammatory factors and tight junction proteins. We observed that administration of SF reduced the mRNA levels of *IL-6* and *TNF- $\alpha$*  in the colon of suckling piglets, and the mRNA levels of *IL-10* were markedly up-regulated. *IL-6* and *TNF- $\alpha$*  are markers of inflammation in the intestine, whereas *IL-10* is an essential anti-inflammatory regulator in the intestinal system (Xie et al., 2021). Moreover, ZO-1, occludin, and claudin-1 are essential tight junction proteins that enhance barrier function, and decreased levels of these proteins may lead to increased intestinal mucosal barrier permeability (Xu et al., 2021). Our results found that the SF group significantly up-regulated the protein level of occludin in the colon of suckling piglets, and there was an increasing trend in the CLDN4 protein content. These results demonstrated that adding SF to the diet of sows during the latter stages of gestation and lactation enhances the colonic antioxidant capacity and barrier function of suckling piglets. Supplementation of SF during late gestation and lactation in sows' diet benefits the gut barrier of suckling piglets, as indicated in Fig. 10.

## 5. Conclusion

Dietary SF supplementing in sows' diets from late gestation to lactation improves sow feed intake during lactation. Moreover, this benefit is not limited to the maternal generation but is confirmed for the offspring. The antioxidant capacity and intestinal barrier function of both the jejunum and colon were improved in suckling piglets. More interestingly, there was a tendency to enhance the growth performance of suckling piglets.

## Credit Author Statement

**Feng Gao:** Investigation, performed experiments and analyzed data, Data curation and writing - original draft. **Yongqing Du** and **Haiyang Liu:** Investigation, formal analysis and data curation. **Hongwei Ding:** Software and visualization. **Wentao Zhang:** Methodology and software. **Zhongyu Li:** writing - review and editing. **Baoming Shi:** Funding acquisition, project administration, conceptualization, resources, supervision, writing - review and editing.

## Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aninu.2024.05.011>.

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