



Original Research Article

Effects of sex on fat deposition through gut microbiota and short-chain fatty acids in weaned pigs

Linfang Yao, Bo Wang, Yu Wang, Jie Bai, Yiming Gao, Xintong Ru, Chongpeng Bi, Jianping Li*, Anshan Shan*

College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China

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ABSTRACT

Nitrogen pollution resulting from excessive feed consumption poses a significant challenge for modern swine production. Precision nutrition technology seems to be an effective way to solve this problem; therefore, understanding the law of pig body composition deposition is a prerequisite. This study investigated the sex effects on growth performance, body composition, nutrient deposition, gut microbiota, and short-chain fatty acids (SCFA) in weaned piglets. Eighty weaned pigs were randomly allocated to 2 treatments according to the sex of pigs. An individual pig was considered as a treatment replicate. Six body weights (BW 5, 7, 11, 15, 20, and 25 kg) were chosen as experimental points; for each point 10 piglets close to the average BW (5 males and 5 females) were slaughtered, and there was one growth phase between each 2 BW points. Results indicated that the males had higher average daily gain (ADG) and average daily feed intake (ADFI) compared to the females ($P < 0.05$) at growth phases 15 to 20 kg BW and 20 to 25 kg BW. Meanwhile, males at 20 kg BW had higher body fat content than females ($P < 0.10$). Males showed a higher body fat ($P < 0.05$) deposition rate at phase 15 to 20 kg BW ($P < 0.05$) than females. For pigs at 20 kg BW, the relative abundance of *Ruminococcaceae* UCG-005, *Clostridium*, *Christensenellaceae*_R-7_group, and *Peptostreptococcaceae* was significantly increased in males ($P < 0.05$) but that of *Bifidobacterium* was decreased ($P < 0.05$). At 25 kg BW, the relative abundance of *Ruminococcaceae*_NK4A214_group, *Fibrobacter*, *Ruminococcaceae* UCG-009, *Ralstonia*, *Klebsiella*, and *Christensenellaceae*_R-7_group in males was higher when compared with females ($P < 0.05$). In terms of SCFA, females exhibited higher concentrations of propionate compared to males ($P < 0.05$). The results of the current study indicated that sex influenced fat deposition through changes in the composition of gut microbiota and the content of SCFA, which has significant implications for the realization of precision nutrition in modern swine production.

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1. Introduction

The traditional feeding approach aims to maximize pig growth by providing uniform feed to all pigs throughout the feeding phase

(Andretta et al., 2016). However, this results in over-consumption of feed, raising the cost of feeding. To solve these problems, the concept of precision nutrition was put forward (Wathes et al., 2008) to improve the efficiency of feed utilization and the performance of animal production, eventually reducing the cost of feeding (Moss et al., 2021). To realize precision nutrition, fitting a body composition deposition model is indispensable; according to this model, a dynamic model of nutrition of pigs can be established, which is necessary for precision nutrition.

A lot of factors, such as sex, seasons, litter size, gut microbiota, and short-chain fatty acids (SCFA) can influence the livestock body's nutrient deposition rates. For example, wolves' fat content in the spring is higher than in summer and autumn (Hilderbrand and Golden, 2013). Further, when the litter size reduces from 17 to 14,

* Corresponding authors.

E-mail addresses: ljpneau@neau.edu.cn (J. Li), asshan@neau.edu.cn (A. Shan).

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weaned piglets' ADG and body fat content increase (Kobek-Kjeldager et al., 2021). In addition, gut microbiota and its byproducts, SCFA, are thought to play an essential role in body nutrient deposition. *Olsenella* and *Slackia* are considered beneficial for depositing abdominal fat (Mejicanos et al., 2020). Meanwhile, research has shown that individuals with more *Blautia* in their feces tend to have a lower fat consumption rate in the cecum than those with a lower abundance of *Blautia* (Nielsen et al., 2014). Sex is a vital factor for body composition and nutrient deposition rates. Compared with females, males displayed a higher fat content and deposition rate between 60 and 90 kg BW (Dunsha et al., 1993). In Nellore bulls, bulls exhibited a higher protein deposition rate than steers (Rodrigues et al., 2009). Meanwhile, sex is also regarded as a factor that influences the gut microbiota. Female mice in good health had been found to have a more significant presence of *Ruminococcus*, *Coproccoccus*, and *Dorea* but a lower presence of *Allobaculum*, *Anaeroplasm*, Lactobacillaceae Veillonellaceae (Rizzetto et al., 2018).

However, the effects of sex on body composition and nutrient deposition rates in weaned piglets were reported less, including the relationship of gut microbiota and SCFA to this effect. Thus, this study examined how sex influences body composition and nutrient deposition rates through a comparative slaughter method and explored the impact of gut microbiota and short-chain fatty acids on appearance.

2. Materials and methods

2.1. Animal ethics statement

The present experiment underwent a review and approval process by the Northeast Agricultural University Laboratory Animal Ethics Committee (NEAUEC 20190241).

2.2. Animal experimental design

Eighty-weaned piglets (21 d of age; 5.48 ± 0.35 kg BW; Duroc \times Landrace \times Yorkshire) were divided into 2 treatments based on sex, with 40 males and 40 females in each treatment. Each individual was considered one replicate and fed in a separate metabolic cage. Pigs were weighed at 6 BW points during the experiment (5, 7, 11, 15, 20, and 25 kg). Thus, the complete trial was segmented into 5 growth phases according to BW (5–7 kg, 7–11 kg, 11–15 kg, 15–20 kg, and 20–25 kg), the same diet was fed to 2 sexes at each growth phase, and piglets were individually weighed at the end of each growth phase. If the piglet weight reached the target weight points, it would go to next grow phase and change the diet at the same time. Table 1 presents the ingredient and nutrient values of the diets at each growth phase, meeting the necessary amino acid requirements and net energy (NE) for weaned piglets weighing between 5 and 25 kg according to the Nutrient Requirements of Swine (NRC, 2012).

2.3. Sample collection

Each piglet was weighed at the start and end of each growth phase to measure ADG, ADFI, and feed-to-gain ratio (F:G). At the beginning of the experiment and when the average weight of the weaned piglet group reached 7, 11, 15, 20 and 25 kg BW, ten piglets close to the average BW (5 males and 5 females) were selected. The difference between the weight of the selected piglets and the target BW did not exceed 5% of the target BW. The selected piglets were subjected to anesthesia using sodium pentobarbital and were then slaughtered following a 12 h fasting period. Colon digesta were quickly gathered and saved at -80 °C to extract bacterial DNA and analyze metabolites. The piglet carcasses were emptied of all

substances in the internal organs prior to evaluation of body composition. Subsequently, all carcasses were kept at a temperature of -20 °C until they were ready for further processing. The empty body was transformed into small pieces using a double-shaft crusher (L-SP380-2, LiWill Co. Ltd., Zhengzhou, China). These pieces were then ground in a bone mash machine (Model 130, Shenghong Co. Ltd., Weifang, China) after homogenizing in a mixer (YBX60, Hengjie Co. Ltd., Zibo, China). Finally, samples were subjected to a quartering procedure to obtain approximately 2 kg of sample to measure the body composition.

2.4. Chemical analysis

Duplicate analyses were conducted on the entire body carcass samples to measure their body water, protein, fat, and ash content, following the methods provided by AOAC (2006). The crude protein content was measured using a Kjeltec 2300 analyzer (FOSS Analytical AB, Höganäs, Sweden). Crude fat was measured using an automated extractor analyzer (XT 15i, Ankom Technology Co., Macedon, NY, USA). The same methods were also used for dietary crude protein and crude fat analysis. The body composition deposition rate was calculated by dividing the body composition deposition total amount by the feed days in each growth phase; the deposition total amount was obtained by the difference between the end and the beginning of the phase (Leal Ribeiro et al., 2016).

2.5. Colon microbiota analysis

DNA from each sample of colonic digesta was extracted using the Cetyltrimethylammonium Bromide (CTAB) method, following the instructions provided by the manufacturer. To amplify the 16S rDNA Bacterial V3–V4 region, the forward primer 338F (5'-ACTCTRCGGGAGGCAGCAG-3') and the reverse primer 806R (5'-GGACTACCVGGGTATCTAAT-3') were utilized. Following PCR amplification, the resultant products were isolated from a 2% agarose gel and cleansed using AMPure XT beads (Beckman Coulter Genomics, Danvers, MA, USA) and quantified by Qubit (Invitrogen, USA). The amplicon pools were prepared for sequencing, and the size and quantity of the amplicon library were assessed using the Agilent 2100 Bioanalyzer (Agilent, USA) and the Library Quantification Kit for Illumina (Kapa Biosciences, Woburn, MA, USA). After passing the quality assessment, the library was sequenced on an Illumina NovaSeq platform. The sequencing was performed using a paired-end approach, generating 250 base pair reads in both the forward and reverse directions. Following the sequencing step, bioinformatics analysis was conducted, and the specific details of the bioinformatics analysis were described previously (Liu et al., 2021a).

2.6. Colon short-chain fatty acid concentration analysis

The concentrations of SCFA were examined using the following procedure: approximately 2 g of each sample was combined with 2 mL of distilled water, the mixture was centrifuged at $12,000 \times g$ at 4 °C for 10 min, and the resulting liquid was filtered through a 0.22 μ m filter. The samples were collected after repeating the above operation 3 times. Finally, the samples were analyzed using a gas chromatograph (Agilent) equipped with an HP-INNOWAX column A to determine the concentration of SCFA in the colon.

2.7. Statistical analysis

SPSS software version 25.0 (SPSS Inc., Chicago, IL, USA) was used for data analysis. GraphPad Prism software version 9.4.1 (GraphPad Software Inc., San Diego, CA, USA) was used for model fit:

Table 1
Ingredients and nutrient levels of the diets at each growth phase (% as-fed basis).

Item	Phase				
	5–7 kg	7–11 kg	11–15 kg	15–20 kg	20–25 kg
Ingredients					
Corn	25.76	27.42	37.50	42.05	42.50
Expanded corn	15.80	20.04	14.56	11.34	10.01
Wheat bran	1.20	1.20	1.44	1.40	1.40
Low-protein whey powder	9.47	8.60	9.20	8.60	9.49
Whole fat soybean	8.80	4.90	1.86	2.08	2.40
Fermented soybean meal	9.52	10.54	15.44	14.62	13.34
Soybean meal	9.02	9.08	5.80	6.70	7.95
Soybean oil	1.62	1.56	1.50	1.50	1.36
Rice bran meal	2.95	2.74	3.20	3.20	3.36
Fish meal	3.86	3.20	2.20	1.90	2.20
Soy protein concentrate	4.60	3.60	2.16	1.26	0.60
Sucrose	1.50	1.50	–	–	–
Limestone	0.86	0.67	0.59	0.66	0.60
Calcium formate	0.30	0.45	0.45	0.40	0.40
Dicalcium phosphate	0.45	0.45	0.40	0.32	0.40
Mono-dicalcium phosphate	0.50	0.45	0.35	0.40	0.40
Sodium chloride	0.30	0.20	0.20	0.20	0.20
L-Lys HCl	0.57	0.51	0.46	0.52	0.54
DL-Met	0.25	0.24	0.10	0.22	0.22
L-Thr	0.21	0.19	0.14	0.17	0.17
L-Trp	0.06	0.06	0.05	0.06	0.06
Titanium dioxide	0.40	0.40	0.40	0.40	0.40
Premix ¹	2.00	2.00	2.00	2.00	2.00
Total	100.00	100.00	100.00	100.00	100.00
Energy and nutrient content					
Net energy ² , kcal/kg	2450	2450	2400	2400	2400
Crude protein	22.34	20.72	19.29	18.88	18.58
Calcium	0.87	0.83	0.75	0.73	0.73
Total phosphorus	0.71	0.67	0.62	0.62	0.64
SID ³ lysine phosphorus	1.49	1.36	1.22	1.23	1.23
Analyzed nutrient composition					
Crude protein	21.97	20.83	19.23	18.90	18.46
Crude fat	5.70	5.28	4.45	4.34	4.16

¹ Provided per kilogram of diet, 13,000 IU vitamin A, 3500 IU vitamin D₃, 30 mg vitamin E, 4 mg vitamin K₃, 3 mg thiamin, 8 mg riboflavin, 35 mg niacin, 7 mg pyridoxine, 20 mg pantothenic acid, 80 mg Zn, 110 mg Fe, 60 mg Mn, 10 mg Cu, 0.7 mg I, 0.35 mg Se.

² Calculated according to NRC (2012): NE (kcal/kg) = (0.700 × digestive energy) + (1.61 × crude fat) + (0.48 × farina) - (0.91 × crude protein) - (0.87 × acid detergent fiber).

³ SID, standardized ileal digestibility.

$$Y = aX^b, \quad (1)$$

where Y is the weight (g) of each body component (total body water, protein, fat, and ash), X is the BW (g), a is the intercept of the logarithm of the linear regression on Y , and b is the relative growth coefficient or coefficient of allometry.

Statistical analysis utilized a significance level of $P < 0.05$ to establish statistical significance. For P -values ranging from 0.05 to 0.10, tendencies or trends were identified. The information is displayed as averages \pm SEM, where SEM represents the standard error of the mean.

3. Results

3.1. Growth performance of weaned piglets

Compared to females, males had notably greater ADG and ADFI at the last 2 growth phases, 15 to 20 kg BW and 20 to 25 kg BW ($P < 0.05$) (Table 2).

3.2. Body composition content of weaned piglets

The weaned piglets' body composition of the 2 sexes is shown in Table 3. The ash content in the bodies of males was significantly greater than that of females at 5, 15, 20 kg BW ($P < 0.05$). Males weighing 20 kg had a noticeable inclination towards higher body fat content compared to females ($P < 0.10$). Body protein in females

tended to be higher at 5 kg BW ($P < 0.10$). Furthermore, no varied differences were found in body composition in the weaned piglets of 7 kg BW, 11 kg BW and 25 kg BW. Table 4 showed the anisotropic growth equation (Eq. 1: $Y = aX^b$) established between body weight and body composition, which was provided by Henn (Henn et al., 2014), the equations were well fitted. In addition, the actual measurements of body energy in weaned piglets were generally higher than the values calculated according to NRC (2012).

3.3. Body composition deposition rates of weaned piglets

Compared with females, the males showed significantly decreased body ash deposition rates at phase 5 to 7 kg BW ($P < 0.05$) and increased at phase 11 to 15 kg BW ($P < 0.05$) (Table 5). There was a trend for body energy deposition rates to be significantly higher at phase 7 to 11 kg BW in males than in females ($P < 0.10$). Males showed a higher body fat ($P < 0.05$) deposition rate at phase 15 to 20 kg BW than females, as well as at phase 20 to 25 kg BW ($P < 0.05$). Apart from fat, at phase 20 to 25 BW, the rates at which piglets retained protein and energy varied significantly across the 2 sexes ($P < 0.05$), with males being higher.

3.4. Colonic bacterial community structure of weaned piglets

Compared with the males, the females had higher Observed species, Chao1, Shannon, and Simpson indices at 5 kg BW ($P < 0.05$) (Fig. 1).

Table 2
Effects of sex on growth performance of weaned piglets.

Item	Sex		SEM	P-value
	Male	Female		
Phase 5–7 kg BW				
ADFI, g/d	207.90	212.77	9.637	0.615
ADG, g/d	143.30	155.17	9.490	0.216
F:G	1.45	1.37	0.091	0.366
Phase 7–11 kg BW				
ADFI, g/d	538.60	558.47	25.193	0.434
ADG, g/d	400.70	406.82	22.016	0.782
F:G	1.36	1.37	0.055	0.614
Phase 11–15 kg BW				
ADFI, g/d	809.02	829.95	47.860	0.664
ADG, g/d	553.71	557.36	37.963	0.924
F:G	1.46	1.49	0.073	0.582
Phase 15–20 kg BW				
ADFI, g/d	1128.45 ^a	968.21 ^b	58.340	0.010
ADG, g/d	707.15 ^a	560.96 ^b	42.816	0.002
F:G	1.60	1.73	0.115	0.265
Phase 20–25 kg BW				
ADFI, g/d	1448.47 ^a	1186.38 ^b	71.478	0.001
ADG, g/d	833.91 ^a	669.83 ^b	44.238	0.001
F:G	1.74	1.77	0.105	0.823
Total				
Initial BW, kg	5.48	5.51	0.132	0.755
Final BW, kg	25.23	24.98	0.171	0.156
ADFI, g/d	722.89	707.73	28.838	0.605
ADG, g/d	468.76	450.02	13.753	0.187
F:G	1.54	1.57	0.034	0.409

BW = body weight; ADG = average daily gain; ADFI = average daily feed intake; F:G = feed-to-gain ratio.

^{a,b} Different superscripts within a row indicate a significant difference ($P < 0.05$).

Figure 2 shows the form of colonic flora phyla in weaned piglets between the 2 sexes. The most common phyla in male and female piglets were Bacteroidetes and Firmicutes, with Proteobacteria and Desulfobacteria following closely (Fig. 2A). Meanwhile, no differences ($P > 0.05$) were observed in the ratio of Firmicutes to Bacteroidetes between the 2 sexes (Fig. 2B). Figure 3 displays the top 30 genera in the relative abundance of the colonic bacteria found in weaned piglets. At the genus level, the predominant components of the colonic microbiota were *Prevotella*, *Muribaculaceae_unclassified*, *Prevotellaceae_NK3B31_group*, *Ruminococcaceae_UCG-005*, and *Rikenellaceae_RC9_gut_group*.

Figure 4 demonstrates the impact of sex on the colonic microbiota genera of weaned piglets. Compared with the males, the females showed a significant increase in relative abundance of *Treponema*, *Oscillibacter*, *F082_unclassified*, *Christensenellaceae_R-7_group*, *Pseudoflavonifractor*, *Ruminococcaceae_UCG-009*, and *Incertae_Sedis* at 5 kg BW ($P < 0.05$), and *Ruminococcaceae_unclassified* relative abundance decreased ($P < 0.05$). Females weighing 7 kg had a notably higher occurrence of *Muribaculaceae_unclassified*, *Rikenellaceae_RC9_gut_group*, *Ruminococcus*, and *Barnesiella* ($P < 0.05$), and conversely, *Escherichia-Shigella* and *Megamonas* significantly declined ($P < 0.05$). Three genera, *Terrisporobacter*, *Lachnospiraceae_UCG-004*, and *Romboutsia*, had a considerably lower relative abundance in males at 11 kg BW ($P < 0.05$). On the contrary, *Pseudoflavonifractor* and *Devosia* relative abundance significantly reduced ($P < 0.05$). At 15 kg BW, *Rikenellaceae_RC9_gut_group*, *Pseudoflavonifractor*, and *Family_XIII_AD3011_group* exhibited significantly increased relative abundances in females ($P < 0.05$), and tended to have a higher *Ruminococcaceae_UCG-009* relative abundance ($P < 0.10$). Meanwhile, *Acidaminococcus* and *Lachnospira* were significantly promoted in males ($P < 0.05$). When comparing the relative abundance at 20 kg BW, the relative abundance of *Ruminococcaceae_UCG-005*, *Clostridium*, *Christensenellaceae_R-7_group*, and *Peptostreptococcaceae* were significantly decreased in males ($P < 0.05$), whereas *Prevotella* showed a tendency towards

Table 3
Effects of sex on body composition content of weaned piglets.

Item	Sex		SEM	P-value
	Male	Female		
5 kg BW				
Water, g/kg	697.44	690.08	11.460	0.545
Protein, g/kg	141.43	145.47	2.100	0.067
Fat, g/kg	101.00	100.05	4.181	0.822
Ash, g/kg	29.84 ^a	27.22 ^b	0.594	0.001
Analyzed gross energy ¹ , MJ/kg	7.84	7.85	0.252	0.971
Calculated gross energy ² , MJ/kg	7.35	7.41	0.132	0.665
7 kg BW				
Water, g/kg	731.66	723.53	9.543	0.419
Protein, g/kg	140.37	143.26	1.941	0.148
Fat, g/kg	80.96	83.38	4.129	0.562
Ash, g/kg	27.11	27.77	0.506	0.201
Analyzed gross energy, MJ/kg	6.61	6.89	0.191	0.156
Calculated gross energy, MJ/kg	6.53	6.70	0.155	0.297
11 kg BW				
Water, g/kg	730.06	731.00	6.101	0.881
Protein, g/kg	142.99	142.58	1.925	0.833
Fat, g/kg	78.71	77.55	3.739	0.758
Ash, g/kg	25.73	25.92	0.521	0.717
Analyzed gross energy, MJ/kg	6.79	6.72	0.124	0.579
Calculated gross energy, MJ/kg	6.51	6.45	0.131	0.675
15 kg BW				
Water, g/kg	712.72	728.37	21.608	0.489
Protein, g/kg	158.56	152.62	5.372	0.278
Fat, g/kg	72.28	70.57	2.302	0.464
Ash, g/kg	27.86 ^a	25.25 ^b	0.874	0.006
Analyzed gross energy, MJ/kg	6.62	6.64	0.140	0.822
Calculated gross energy, MJ/kg	6.48	6.41	0.140	0.633
20 kg BW				
Water, g/kg	719.57	721.34	7.411	0.817
Protein, g/kg	151.08	154.88	2.794	0.185
Fat, g/kg	83.65	81.52	1.218	0.091
Ash, g/kg	24.82 ^a	23.82 ^b	0.485	0.049
Analyzed gross energy, MJ/kg	6.98	7.08	0.086	0.246
Calculated gross energy, MJ/kg	6.89	6.90	0.087	0.950
25 kg BW				
Water, g/kg	710.69	701.07	5.42	0.110
Protein, g/kg	158.55	159.37	2.080	0.696
Fat, g/kg	85.91	86.04	1.559	0.935
Ash, g/kg	26.66	27.80	0.846	0.190
Analyzed gross energy, MJ/kg	7.21	7.27	0.076	0.452
Calculated gross energy, MJ/kg	7.12	7.18	0.074	0.397

^{a, b} Different superscripts within a row indicate a significant difference ($P < 0.05$).

¹ Analyzed by using an oxygen bomb calorimeter.

² Calculated from analyzed body protein and fat content using 0.0237 and 0.0396 MJ/g for retained protein and fat energies (Nutrition and Agriculture, 2012).

Table 4
Effects of sex on the allometric growth coefficients of weaned piglets (Eq. 1: $Y = aX^b$).

Item	Male			Female		
	a	b	R ²	a	b	R ²
Water	0.8513	0.9832	0.9965	0.9259	0.9739	0.9946
Protein	0.0675	1.0835	0.9894	0.0611	1.0946	0.9854
Fat	0.0391	1.0764	0.9621	0.0385	1.0766	0.9524
Ash	0.0299	0.9884	0.9670	0.0171	1.0430	0.9464

a, the intercept of the logarithm of the linear regression on Y; b, the relative growth coefficient or coefficient of allometry; R², related coefficient.

higher relative abundance ($P < 0.10$). Meanwhile, *Bifidobacterium* had a significantly lower ($P < 0.05$), and *Butyricoccus* tended to have a lower ($P < 0.10$) relative abundance in males than females. At 25 kg BW, males had significantly higher levels of *Ruminococcaceae_NK4A214_group*, *Fibrobacter*, *Ruminococcaceae_UCG-009*, *Ralstonia*, *Klebsiella* and *Christensenellaceae_R-7_group* than females ($P < 0.05$); meanwhile, the relative abundance of *Phascolarctobacterium* tended to have a lower and *Collinsella* tended to have a higher relative abundance in males than females ($P < 0.10$).

Table 5
Effects of sex on body composition deposition rates of weaned piglets.

Item	Sex		SEM	P-value
	Male	Female		
Phase 5–7 kg BW				
Water, g/d	147.49	142.05	5.506	0.332
Protein, g/d	23.92	23.53	1.728	0.823
Fat, g/d	6.43	5.22	2.829	0.429
Ash, g/d	3.14 ^b	5.02 ^a	0.362	0.001
Gross energy, MJ/d	0.63	0.70	0.134	0.628
Phase 7–11 kg BW				
Water, g/d	336.25	344.65	20.243	0.681
Protein, g/d	68.53	66.35	5.644	0.701
Fat, g/d	34.63	29.89	4.734	0.326
Ash, g/d	10.77	10.62	0.966	0.884
Gross energy, MJ/d	3.27	2.89	0.237	0.084
Phase 11–15 kg BW				
Water, g/d	334.09	357.83	19.588	0.243
Protein, g/d	90.21	96.68	7.025	0.365
Fat, g/d	24.64	27.83	4.164	0.449
Ash, g/d	16.58 ^a	12.59 ^b	1.739	0.003
Gross energy, MJ/d	3.00	3.45	0.313	0.167
Phase 15–20 kg BW				
Water, g/d	494.30	493.52	43.174	0.986
Protein, g/d	97.84	100.73	8.423	0.734
Fat, g/d	83.10 ^a	71.00 ^b	4.299	0.009
Ash, g/d	10.58	12.04	1.129	0.206
Gross energy, MJ/d	5.60	5.23	0.512	0.461
Phase 20–25 kg BW				
Water, g/d	478.51	489.94	46.121	0.807
Protein, g/d	160.35 ^a	135.81 ^b	7.051	0.002
Fat, g/d	84.46	76.49	4.167	0.066
Ash, g/d	32.07	32.77	2.676	0.796
Gross energy, MJ/d	7.22 ^a	5.89 ^b	0.257	0.002
Total				
Water, g/d	338.53	332.32	4.628	0.194
Protein, g/d	76.21	76.76	1.068	0.608
Fat, g/d	39.46	38.66	0.660	0.234
Ash, g/d	12.47	13.14	0.438	0.135
Gross energy, MJ/d	3.36	3.34	0.038	0.593

^{a, b} Different superscripts within a row indicate a significant difference ($P < 0.05$).

3.5. SCFA in colonic digesta of weaned piglets

Figure 5 exhibits the impact of sex on SCFA in the colonic digesta of weaned piglets. Compared with the males, females tended to have higher acetate concentrations at 20 and 25 kg BW ($P < 0.10$) (Fig. 5A). Females displayed higher propionate concentrations at 20 and 25 kg BW ($P < 0.05$) (Fig. 5B). Besides, this study identified that the isobutyrate of females leaned toward a significant increase in 11 kg BW ($P < 0.10$) (Fig. 5D). Nevertheless, the acetate concentration of males was higher at 11 kg BW ($P < 0.10$) (Fig. 5A), and valerate tended toward a substantial increase at 20 kg BW ($P < 0.10$) (Fig. 5E). No significant statistical difference was observed in the concentrations of butyrate and isovalerate in the colonic digesta between the 2 sexes ($P > 0.05$), as indicated by Fig. 5C and F.

4. Discussion

In general, males have a higher growth potential relative to females, not only in studies about pigs as this phenomenon has also been found in studies of other animals. A survey about piglets suggested that females exhibited lower growth performance than males (Vazquez-Gomez et al., 2020), which was similarly proved in other research (Magnoli et al., 2022; Ruczizka et al., 2020). Another study discovered that Japanese eel *Anguilla japonica*'s average growth rates were lower in females than males (Yoshikawa, 2013). Similarly, in the present study, compared with females, males showed higher growth performance at the growth phase 15 to

20 kg BW and 20 to 25 kg BW. Despite the absence of any notable disparity in piglet growth performance during the preceding 3 growth phases, the females exhibited a slightly superior rate of growth performance compared to the males. That is, the impact of sex on the growth performance of weaned piglets might manifest when they reach approximately 15 kg BW.

Animal growth is manifested, on the one hand, by the gain and increase in weight and volume of body tissues and organs, and on the other hand, by the increase in the sum of body composition deposited, such as body protein, lipid, water, and ash (Henn et al., 2014).

Numerous factors influence the body composition and nutrient deposition rates of animals. The daily-phase feeding (DPF) method demonstrated superior growth performance, higher lipid retention, and lower nitrogen excretion compared to the three-phase feeding (3PF) system when feeding pigs (Pomar et al., 2014). Compensatory growth, also known as the phenomenon of rapid growth after a period of malnutrition or artificial restriction, can occur when pigs are provided with adequate nutrients in a subsequent stage (Yu et al., 2019). Nutrient restriction is considered a factor that can impact body composition and nutrient deposition rates. While a lack of dietary nutrients can decrease animal performance, it has been observed that piglets can still exhibit rapid growth when given sufficient nutrients after experiencing previous malnutrition or artificial restriction (Zhao et al., 2020). It was demonstrated that the piglets in the low protein (LP) group exhibited a significant decrease in body protein content at d 14 compared to the normal protein (NP) group (Hou et al., 2021). However, at the conclusion of the study, the statistical disparity in body protein content between the 2 groups disappeared. A similar result was also shown in another experiment (Shi et al., 2019). Meanwhile, nutrient levels influence body composition and nutrient deposition rates. When the diet's metabolizable energy (ME) was increased, it resulted in higher daily lipid deposition in the piglets' carcasses. Additionally, ME significantly influenced the lipid-protein deposition ratio (Leal Ribeiro et al., 2016). Otherwise, it was found that the piglets on the low-protein diet exhibited lower growth performance and nitrogen deposition compared to the normal-protein diet-fed groups, despite the fact that nitrogen digestion and utilization improved (Hu et al., 2022c). Compared with high birth weight group piglets, low birth weight group piglets presented a higher protein deposition efficiency (Van der Peet-Schwering et al., 2021; van der Peet-Schwering et al., 2020). One study suggested that a smaller litter size benefited fat and protein deposition (Kobek-Kjeldager et al., 2021). A different study demonstrated that protein deposition occurred regardless of the surrounding temperature, while fat deposition was influenced by the temperature of the environment (Le Dovidich et al., 1980).

In addition to the above factors, sex has an essential effect on animals' body composition and nutrient deposition rates. Males tended to have a higher deposition than females, similar to the relationship of growth potential between the 2 sexes. Liver lipid content in males was higher than in females (Vazquez-Gomez et al., 2020). It has been shown that males retained more lipids than females at 70, 100, and 120 kg BW (Zomen et al., 2023). However, the protein deposited was slightly less. Similar results occurred in our study. Males retained nutrients more efficiently. These results suggested that males have a more efficient nutrient utilization and growth rate than females.

Nowadays, more and more research has proved that gut microbiota affects body composition (Mathew et al., 1997). Generally, Firmicutes and Bacteroidetes have the highest phyla relative abundance of gut microbiota. It's worth mentioning that improving the ratio of Firmicutes and Bacteroidetes has been found to be beneficial for fat deposition (Grigor'eva, 2021; Ijaz et al., 2018),

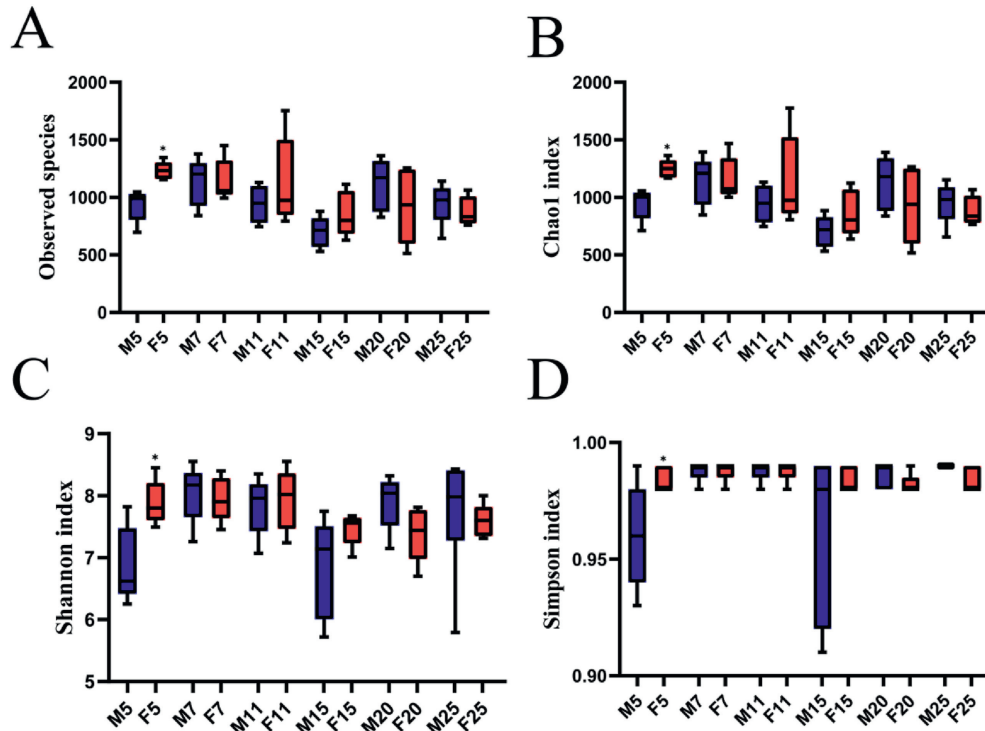


Fig. 1. Effects of sex on the alpha-diversity of colonic microbiota of piglets at different BW. M5 to M25, males from 5 to 25 kg BW; F5 to M25, females from 5 to 25 kg BW. (A) Observed species. (B) Chao1 indexes. (C) Shannon index. (D) Simpson index. * P -value < 0.05.

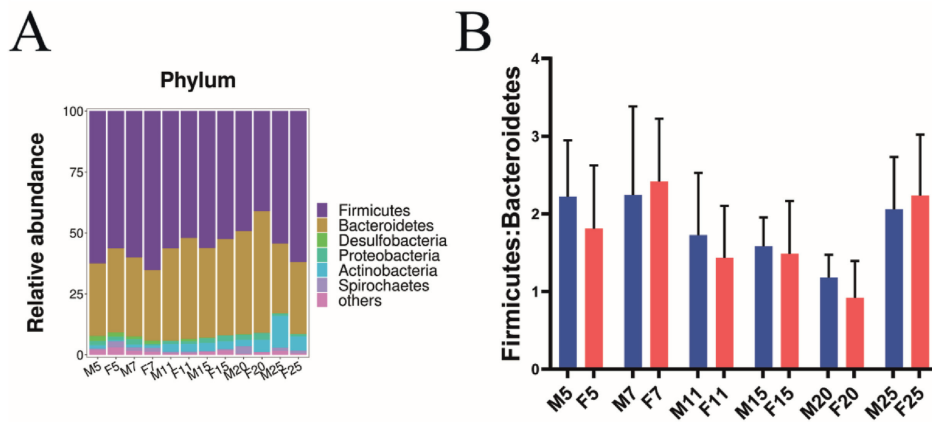


Fig. 2. Effects of sex on phylum of colonic microbiota of weaned piglets. M5 to M25, males from 5 to 25 kg BW; F5 to F25, females from 5 to 25 kg BW. (A) The relative abundance of the top 6 phyla between 2 sexes. (B) The ratio of Firmicutes to Bacteroidetes.

which has been confirmed in numerous studies. In our study, the ratio of Firmicutes and Bacteroidetes in males was higher than in females at 20 kg BW. The body composition and nutrient deposition influenced by gut microbiota were primarily focused on fat (Kang et al., 2019). Some specific types of gut bacteria have been found to promote fat accumulation, for example, a study found that obese people generally had notably elevated levels of *Faecalibacterium prausnitzii* in comparison to non-obese people (Balamurugan et al., 2010). *Blautia* has been discovered to hinder the accumulation of fat in adipocytes influenced by insulin while enhancing the breakdown of free lipids and glucose in various tissues. As a result, it ultimately led to a decrease in the deposition of fat (Liu et al., 2021b). Furthermore, Ozato et al. (2022) found an inverse relationship between *Blautia* and the accumulation of visceral fat area.

Diet is considered the most critical factor influencing the composition of gut microbiota. It is widely recognized that a high-fat diet (HFD) significantly impacts gut microbiota composition (Ijaz et al., 2018; Wu et al., 2019). Moreover, sex is a crucial element that must not be overlooked, which has a giant impact on gut microbiota composition. One study found that at 97 d of age, females had higher diversity in their gut microbiota compared to males; the relative abundance of Firmicutes was also higher in females, while the relative abundance of Proteobacteria and Bacteroidetes was lower compared to boars (Ruczizka et al., 2020). In another study conducted on mice, it was observed that specific genera of gut microbiota had higher abundance in males compared to females, which included *Allobaculum*, *Akkermansia*, *Clostridiaceae*, and *Ruminococcus*. However, the genera *Dorea*,

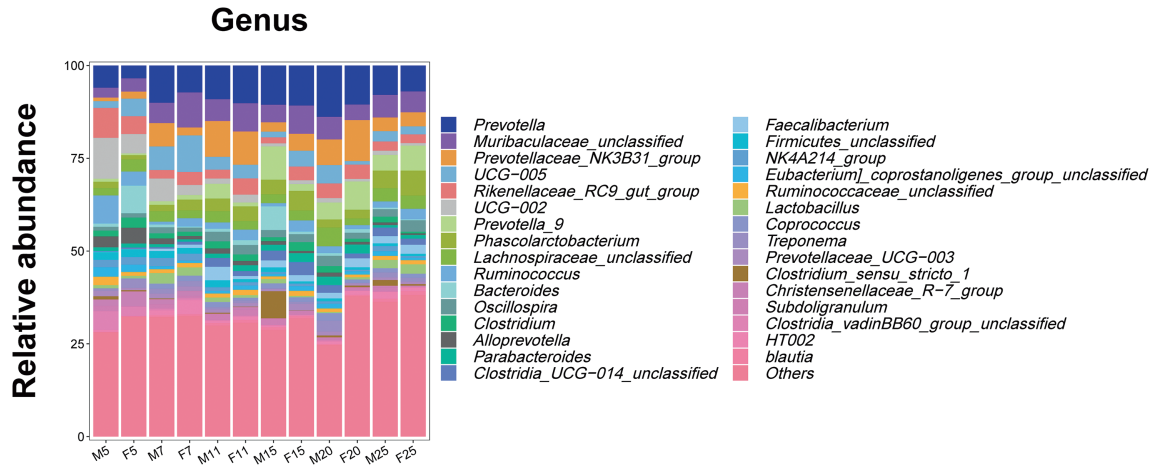


Fig. 3. Effects of sex on genus of colonic microbiota of weaned piglets. M5 to M25, males from 5 to 25 kg BW; F5 to F25, females from 5 to 25 kg BW.

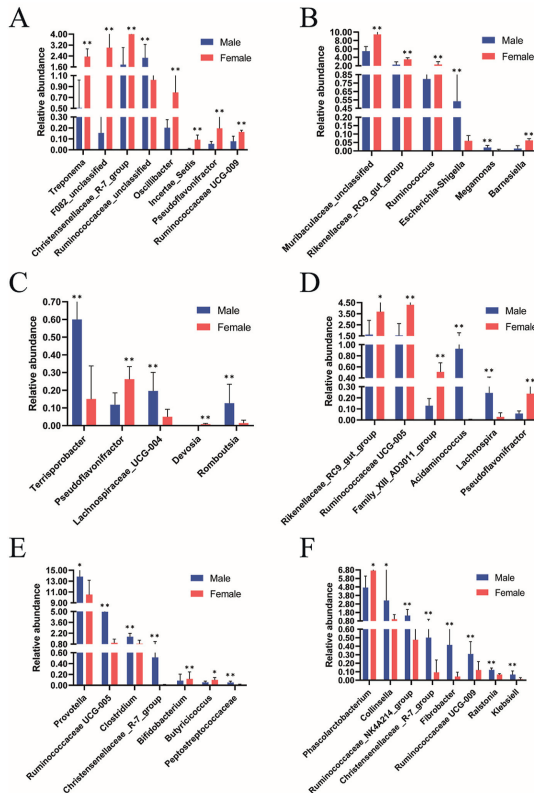


Fig. 4. Effect of sex on genus of colonic microbiota of weaned piglets. (A) The changed genera at 5 kg BW. (B) The changed genera at 7 kg BW. (C) The changed genera at 11 kg BW. (D) The changed genera at 15 kg BW. (E) The changed genera at 20 kg BW. (F) The changed genera at 25 kg BW. *0.05 < P-value < 0.10, **P-value < 0.05.

Coprococcus, and *Bacteroides* were found to be more abundant in female mice (Org et al., 2016).

Fat deposition rates were higher in males than in females during the last two growth phases of the experiment, and there were also differences in the relative abundance of some microbiota genera in the colons of piglets between the two sexes. Thus, it was necessary to explore whether the differences in fat deposition were microbiota-related.

In male colons weighing 20 kg, there was a higher proportion of *Ruminococcaceae* UCG-005, *Peptostreptococcaceae*, *Clostridium*,

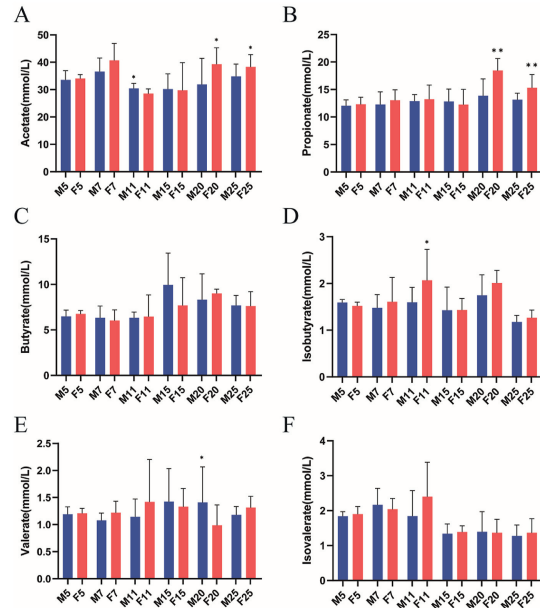


Fig. 5. The concentrations of short-chain fatty acids (SCFA) of 2 sexes at different BW. M5 to M25, males from 5 to 25 kg BW; F5 to F25, females from 5 to 25 kg BW. (A) Acetate concentration. (B) Propionate concentration. (C) Butyrate concentration. (D) Isobutyrate concentration. (E) Valerate concentration. (F) Isovalerate concentration. *0.05 < P-value < 0.10, **P-value < 0.05.

Christensenellaceae_R-7_group, and *Prevotella*. The males' proportion of *Ralstonia*, *Ruminococcaceae_NK4A214_group*, *Fibrobacter*, *Ruminococcaceae_UCG-009*, *Klebsiella*, *Collinsella*, and *Christensenellaceae_R-7_group* were higher compared to females at 25 kg BW. The high-fat diet (HFD) group in 2 studies exhibited a higher relative abundance of *Ruminococcaceae_UCG-005* and *Christensenellaceae_R-7_group* compared to the normal group (Qin et al., 2022; Zhang et al., 2023). *Prevotella* is an anaerobic bacteria that breaks down polysaccharides and produces SCFA (Christensen et al., 2022). Studies have shown that individuals with higher body fat had a higher relative abundance of *Prevotella* (Zou, 2022). Similarly, the relative abundance of *Clostridium* has been noticed to be positively connected with body fat concentration (Wang et al., 2021). In addition, the relative abundance of *Peptostreptococcaceae* has been significantly and positively correlated with back-fat thickness (Soares et al., 2021). In a study by Hu et al.

(2022b), the high-fat group exhibited a greater abundance of *Colinsella* in the gut compared to the control group (Hu et al., 2022b). Another experiment revealed that relative weights of mesenteric white adipose tissue (mWAT), epididymal white adipose tissue (eWAT), and renal white adipose tissue (kWAT) were increased in mice related with low relative abundance of *Ralstonia*. It was supposed that *Ruminococcaceae* had a close relationship with fat deposition (Kaminska et al., 2022). In the previous discussion, it was noticed that the high body fat group's gut exhibited a higher abundance of *Ruminococcaceae* UCG-005. Similarly, other researchers also found that the relative abundance of *Ruminococcus_NK4A214_group*, along with *Ruminococcaceae* UCG-009, was higher in the gut of the increased body fat group (He et al., 2020; van Trijp et al., 2021). Interestingly, all these genera, except *Fibrobacter* (Jeong et al., 2022) and *Klebsiella* (Yao et al., 2020), were positively correlated with fat deposition, indicating that a rise in their abundance may contribute to increased fat deposition.

On the other hand, the relative abundance of *Bifidobacterium* and *Butyricoccus* was higher in females than in males at 20 kg BW. There was a tendency for *Phascolarctobacterium* to have a significantly higher abundance in females. *Bifidobacterium* was negatively associated with fat deposition (Xu et al., 2012). Another study revealed that *Butyricoccus* was significantly negatively correlated with abdominal fat area, myofiber density, and adipose triglyceride lipase (ATGL) mRNA expression. Furthermore, *Phascolarctobacterium* was negatively correlated with fat deposition (Qi et al., 2019). However, Lecomte et al. (2015) proposed that *Phascolarctobacterium* was correlated positively with fat metabolic parameters, and the opposite conclusion was reached. These 3 studies suggested that a decrease in *Bifidobacterium*, *Butyricoccus*, and *Phascolarctobacterium* may be related to increased fat deposition. In conclusion, our findings indicate that males exhibited a higher fat deposition rate than females during the last 2 growth phases analyzed in this study. This disparity in fat accumulation is closely linked to the composition and abundance of gut microbiota.

The significant role of the gut microbiota in breaking down indigestible carbohydrates, such as polyacrylamide and oligonucleotides, within the fore-gut is widely recognized. These complex carbohydrates are broken down into simple metabolites, and SCFA are one of the prominent byproducts (Beaumont et al., 2021; Hu et al., 2019). Acetate, propionate, and butyrate are the most abundant SCFA produced in this process. Interestingly, a research showed that the contents of SCFA, such as acetate and butyrate, coincided with the pattern of change in certain bacterial populations, including *Bifidobacterium*, *Enterococcus*, *Weissella*, and *Streptococcus* (Yao et al., 2022). Various factors influence the production of SCFA (Pylkas et al., 2005; Venema, 2013), including the dietary fiber composition, specific bacterial species, and the composition of the gut microbiota. In the available reports, sex seems to influence the composition of SCFA (Song et al., 2021). However, the impact of sex on porcine SCFA has been relatively understudied in animals, especially pigs. Thus, more research is needed to understand how sex may affect SCFA concentrations in pigs. SCFA, like gut microbes, are indeed closely associated with fat deposition. Studies have demonstrated that the activation of free fatty acid receptor 2 (FFAR2) by SCFA was vital in controlling the accumulation of fat; when SCFA activate FFAR2 in adipose tissue, it inhibits the phosphorylation of protein kinase B (Akt) and the insulin signaling pathway, leading to a reduction in fat deposition (Kimura et al., 2013). Acetate, one of the SCFA, is a substrate for fatty acid synthesis; it has been shown to decrease fat deposition in the liver and improve glucose tolerance (Khalaf et al., 2023). Propionate, another SCFA, has been found to reduce energy intake,

reducing abdominal fat deposition (Li et al., 2021). In the current experiment, the contents of acetate and propionate in females were higher than in males, which could potentially explain why females exhibited lower fat deposition compared to males. Butyrate, the third major SCFA, has several beneficial effects. Some of its benefits include enhancing insulin sensitivity, boosting energy expenditure, and facilitating the development of slow or oxidative muscle fibers (Gao et al., 2009). Gavage of butyric acid has been shown to inhibit the expression of hypothalamic neurons that stimulate appetite, thereby reducing feed intake (Wang et al., 2018). Additionally, butyrate activates the Akt signaling pathway, promoting protein synthesis. It also enhances the breakdown of branched-chain amino acids, leading to accelerated protein deposition; these effects contribute to the positive role of butyric acid in skeletal muscle development and protein deposition (Duan et al., 2019).

The previous discussion demonstrated that sex influences the gut microbiota and SCFA in weaned piglets, ultimately impacting fat deposition. However, the underlying reasons for this phenomenon were not discussed. Upon reviewing published studies, it appears that sex hormones may significantly influence this relationship (Hu et al., 2022a). The relationship between sex hormones and gut microbiota is reciprocal, with estrogen influencing the composition of gut microbiota, and in turn, the gut microbiota can influence estrogen concentrations (Rizzetto et al., 2018). Notably, studies have demonstrated that decreased testosterone secretion in mice leads to increased fat content (Sebo and Rodeheffer, 2021), accompanied by an elevation in estradiol secretion, which reduces sugar metabolism and enhances fat metabolism, favoring fat synthesis. Additionally, lower levels of androgens have been associated with higher adipose tissue deposition in animals.

5. Conclusion

Our study found that males can deposit fat faster than females, which is influenced by changes in microbiota composition and the content of SCFA. Sex has effects on body composition and nutrient deposition. This study created the groundwork for subsequent modeling of dynamic nutrient requirements, laying the foundation for precision nutrition.

Author contributions

Linfang Yao: Conceptualization, Validation, Data curation, Writing—original draft; **Bo Wang:** Methodology, Formal analysis, Writing—review and editing; **Yu Wang:** Validation, Writing—review and editing; **Jie Bai:** Software; **Yiming Gao:** Formal analysis; **Xintong Ru:** Software; **Chongpeng Bi:** Validation; **Jianping Li:** Conceptualization, Project administration; **Anshan Shan:** Project administration.

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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