

Association of differential meat quality traits with gut microbiota in Angus cattle and Xinjiang Brown cattle

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ABSTRACT. While most meat quality traits (MQTs) are investigated in Angus cattle (AG), the differences in MQTs and the relationship of MQTs with the gut microbiota between AG and Xinjiang brown cattle (XBC) have not yet been well elucidated. Fourteen heads of 24-month-old uncastrated AG and XBC males (7 in each group) reared under identical feeding condition were selected for MQT testing. The composition and structure of the gut microbiota were analysed by 16S rRNA gene sequencing of rectal faecal samples. The correlation between MQTs and the gut microbiota was analysed. The results showed that backfat thickness of XBC was significantly lower compared to AG, and the muscular fibre sectional area was significantly higher than that of AG. The composition of the gut microbiota showed that the relative abundance of the genus *rc4_4* was significantly lower in XBC in relation to AG cattle ($P < 0.05$) and Lefse analysis demonstrated that the family *Peptostreptococcaceae* were characteristic of the XBC gut. Thirteen genera of the gut microbiota significantly correlated with MQTs. The differential MQTs between Angus cattle and Xinjiang brown cattle related to fat metabolism/deposition were found to be associated with the relative abundance of certain gut microbial genera, which could serve as potential gut microbial biomarkers to assist in improving meat quality of Xinjiang brown cattle.

Introduction

Nowadays, consumers are paying increasing attention to meat quality. Although a large number of studies concerning meat quality traits (MQTs) have been conducted in Angus cattle (AG), few studies have systematically compared MQTs in AG and Xinjiang brown cattle (XBC), an indigenous dual-purpose beef and dairy cattle breed with excellent adaptability, strong disease resistance, good grazing and tolerance to extreme weather conditions in China (Zhou et al., 2017).

Most MQTs, such as backfat thickness, eye muscle area, intramuscular fat (IMF) content,

marbling, shear force, muscle fibre sectional area, etc., are related to body fat metabolism/deposition (Schumacher et al., 2022). The gut microbiota has been dubbed the second set of the host genome, closely associated with host metabolism and health (Noel et al., 2019). Studies have shown that there is a close relationship between the gut microbiota and fat metabolism (Zierer et al., 2018; Kuno et al., 2018). Tang et al. (2020) sequenced the 16S rRNA gene in the intestinal microbiota from separate gut segments of pigs and analysed the correlation with MQTs showing that the ceecal, colon and jejunal microbiota played more important roles in determining traits associated with fat deposition in pigs.

Whon et al. (2021) compared the gut microbiota and MQTs of males and castrated Holstein cattle and found that castrated Holstein cattle had a higher relative abundance of the family *Gastrostreptococcus* and increased extra- and IMF storage. Another study compared the gut microbiota between grazing and feedlot Angus cattle and the authors speculated that the significant difference in gut microbiota composition in Angus cattle could affect the meat quality of Angus beef (Zhang et al., 2021). Zheng et al. (2022) studied the association of the gut microbiota with differentially expressed intramuscular genes of the hosts and metabolites in Angus and Chinese Simmental cattle, and revealed different relationships of the gut microbiota and meat quality in these two breeds. However, the relationship between the gut microbiota and MQTs related to lipid metabolism/fat deposition in cattle has not been fully elucidated. Therefore, backfat thickness, muscle fibre sectional area, muscle fibre number, muscle shear force, intramuscular fat content, moisture content and ash content were assessed in 24-month-old XBC and AG cattle. The composition and structure of the gut microbiota were determined by sequencing the 16S rRNA gene, and correlation analysis was carried out to explore the possible associations of differential MQTs with the gut microbiota in AG and XBC to improve XBC meat quality.

Material and methods

Animals, housing, and feeding

A total of 14 24-month-old uncastrated AG (n = 7) and XBC males (n = 7) reared under identical feeding regime, management and conditions were selected from a beef cattle breeding farm in Xinjiang (Table 1). The study protocol was approved by the Animal Ethics Committee of the Xinjiang Agricultural University (2017015).

Table 1. Diet composition and nutrient levels

Feed ingredients	Weight, kg	Content, %	Nutritional ingredient	Content
Straws	1.30	5.78	Metabolic energy, MJ/kg	5.82
Alfalfas	0.50	2.22	Crude protein, %	16.2
Cossettes	2.00	8.89	Crude fat, %	2.32
Wheat straws	1.50	6.67	Calcium, %	0.54
Ensilings	10.00	44.46	Phosphorus, %	0.49
Molasses	0.70	3.11	Acid detergent fibre, %	5.9
Concentrates*	6.49	28.86	Neutral detergent fibre, %	12.98
Total	22.49	100.00	Total digestible nutrient, %	61.57

* Concentrate: %: corn 53.16, cotton meal 11.56, bran 7.40, magnesium oxide 3.08, concentrate 24.81

Sample collection

AG and XBC animals were fasted for 24 h and water drinker was removed 12 h before slaughter, and body weight was determined just before slaughter. Backfat thickness and eye muscle area were measured *in vivo* using veterinary B-ultrasound (Pyle Co. LTD, Aquila Vet, Maastricht, the Netherlands). Fresh faeces were collected from the rectum of the animals with aseptic gloves 6 h prior to slaughter; the samples were immediately transferred into cryopreservation tubes and stored in liquid nitrogen until gut microbiota analysis. After slaughter, half a kilogram of *longissimus dorsi* muscle was collected from each animal, of which approximately 1 cm³ of the sample was cut out and fixed in picric acid solution, with the remainder stored at 4 °C for further muscle shear force and other MQT analyses.

MQT detection

Muscle shear force was measured using a computer-coupled muscle tenderness tester (Brad Technology Development Co. LTD, c-lm4, Beijing, China), as previously reported (Bai et al., 2022). Muscle moisture content was measured by drying at 101–105 °C for 24 h (Luo et al., 2019). Ash content was measured after high temperature burning of the sample for 6 h (Luo et al., 2019). Intramuscular fat content was measured using the Soxhlet extraction method (Chen et al., 2021). Fixed muscle tissue was dehydrated and embedded in paraffin; paraffin sections were stained with hematoxylin-eosin (H&E) stain, and observed under an optical microscope (Nikon Instruments Co. LTD, 55I-1000, Shanghai, China). Muscle fibre cross-sectional area was measured using a microscopic imaging system (Motic Advanced 3.5, Hong Kong, China). Three non-consecutive muscle sections from each muscle sample were used to measure muscle fibre cross-sectional area. the diameter of muscle fibre was measured in each section, and the number of muscle fibres was counted in 3 separate fields of view. The muscle fibre cross-sectional area was calculated according to the method published elsewhere (Ding et al., 2021).

Bacterial diversity assessment

Sequencing of the 16S rRNA gene was performed as previously described by Zhang et al. (2021). Briefly, total DNA was extracted from faecal samples using the OMEGA Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA), DNA was quantified with Nanodrop (Thermo Fisher Scientific, Wilmington, DE, USA), and its extraction quality was analysed by running a 1.2% agarose gel electrophoresis.

(2) PCR amplification was carried out using universal primers for the V3-V4 region (5'-ACTC-CTACGGGAGGCAGCAG-3' and 5'-GGAC-TACHVGGGTWTCTAAT-3') of 16S rRNA (Sangon Biotech, Shanghai, China). The amplified products were purified and subsequently detected by agarose gel electrophoresis and fluorescence quantitative detection. (3) DNA fragments were sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA), and original sequencing data were acquired. (4) QIIME2 software was used to control the original data, operational taxonomic units (OTUs), classification statistics, diversity analysis, microbiota structure and abundance analysis.

Statistical analysis

GraphPad Prism software (GraphPad Software v 9.0, San Diego, CA, USA) was applied to perform statistical analysis; the data were expressed as mean \pm standard error (mean \pm SE). $P < 0.05$ indicated a significant difference, and $P < 0.01$ indicated an extremely significant difference. Measured indices were analysed by unpaired Student's t-test, and a Spearman's correlation analysis was performed to correlate MQTs with the gut microbiota of AG and XBC.

Results and discussion

Meat quality detection

The results presented in Table 2 and Figure 1 indicated that backfat thickness was significantly higher ($P < 0.05$), while muscle fibre cross-sectional area was significantly smaller ($P < 0.05$) in AG compared to XBC. Fu et al. (2018) compared the biceps femoris of 12-month-old Angus cattle with that of Japanese black cattle, and found that muscle satellite cell density in Angus cattle was markedly

Table 2. Comparison of MQTs

Characteristics	AG	XBC	P-value
Body weight, kg	784.48 \pm 46.56	704.29 \pm 28.93	0.160
Backfat thickness, cm	1.67 \pm 0.22	1.02 \pm 0.09	0.021
Eye muscle area, cm ²	98.10 \pm 3.35	92.72 \pm 3.09	0.261
Muscle fibre sectional area, μm^2	927.8 \pm 55.38	1534.15 \pm 285.23	0.033
Number of muscle fibres, n	220.4 \pm 7.73	200.60 \pm 16.64	0.260
Shear force, N	5.75 \pm 0.72	5.66 \pm 0.58	0.922
Intramuscular fat, %	1.81 \pm 0.06	1.81 \pm 0.14	0.993
Moisture content, %	73.18 \pm 1.02	74.15 \pm 0.99	0.505
Ash content, %	1.05 \pm 0.11	1.56 \pm 0.29	0.131

MQTs – meat quality traits, AG – Angus cattle, XBC – Xinjiang brown cattle; data are presented as mean \pm SEM (standard error of the mean); $P < 0.05$ indicates significant difference

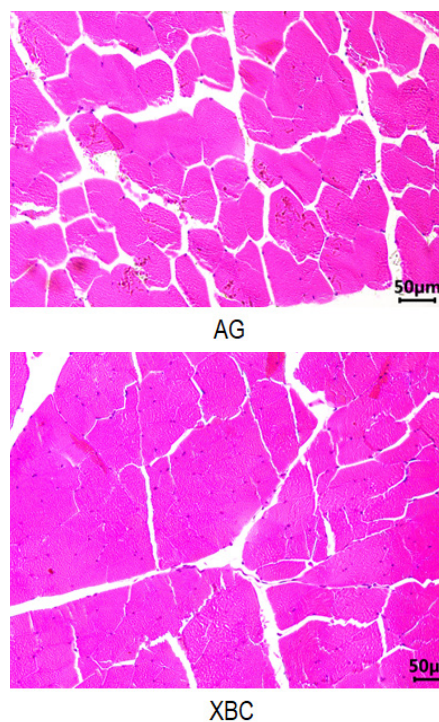


Figure 1. Hematoxylin-eosin staining of the longissimus dorsi muscle (200 \times)

higher than that of Japanese black cattle; however, the adipogenic capacity of Angus cattle was higher in comparison to Japanese black cattle. The average muscle fibre diameter of Japanese black cattle was larger, but the muscle mass was smaller. In our present study, the results showed that the muscle fibre area of *longissimus dorsi* in Xinjiang brown cattle was significantly higher compared to Angus cattle, which might be one of the factors affecting meat tenderness in this breed. Subcutaneous fat deposition is a necessary stage in the fattening process of cattle (Du et al., 2010), thus backfat thickness is another important biomarker of MQTs (Taniguchi et al., 2008). In our study, Xinjiang brown cattle and Angus cattle fed under identical conditions showed that backfat thickness of Xinjiang brown cattle was significantly smaller compared to Angus cattle, suggesting that the fat deposition capacity of Angus cattle was better than that of Xinjiang brown cattle.

Structure of gut microbiota

There were no significant differences in the Chao1, Shannon, Faith_pd and α -diversity indices, as well as observed operational taxonomic units (OTUs) of the gut microbiota between AG and XBC (Table 3). Principal co-ordinates analysis (PCoA), visualized using PERMANOVA test, showed that β -diversity of the gut microbiota between AG and XBC was not significantly different, but tended to differ ($q = 0.08$) (Figure 2).

Table 3. Comparison of α -diversity in the gut microbiota

	Chao1	Shannon	Observed OTUs	Faith_pd
AG	149.1 ± 12.28	6.54 ± 0.17	149.1 ± 12.28	12.64 ± 0.83
XBC	144.8 ± 7.80	6.54 ± 0.11	144.8 ± 7.80	12.49 ± 0.45
P-value	0.78	0.99	0.78	0.89

OTUs – operational taxonomic units, AG – Angus cattle, XBC – Xinjiang brown cattle; data are presented as mean ± SEM (standard error of the mean); $P > 0.05$

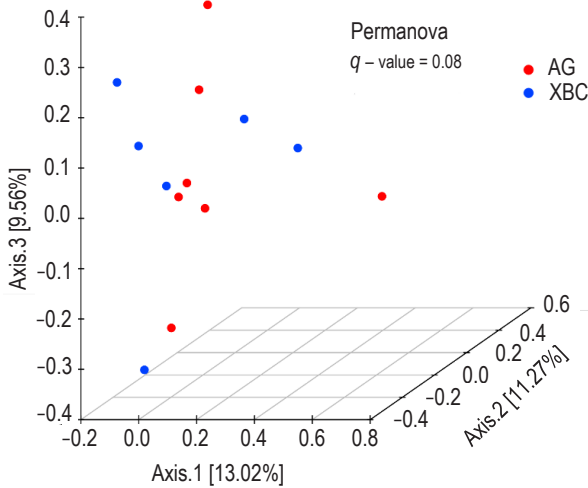


Figure 2. Principal co-ordinates analysis

Composition of gut microbiota

The top 20 phyla and genera in the gut microbiota of XBC and AG are presented in Figure 3, including 7 phyla and 20 genera (Table 5) with a relative abundance above 0.1% ($RA > 0.1\%$). The most abundant phyla with $RA > 1\%$ were Firmicutes, Bacteroidetes and Spirochaetes (Table 4), whereas the most abundant genera with $RA > 1\%$ included *CF231*, *Oscillospira*, *5_7N15*, *Treponema*, *Phascolarctobacterium* and *Clostridium*. The relative

Table 4. The relative abundance of differential phyla in gut microbiota

Taxonomy	AG	XBC	P-value
<i>Firmicutes</i>	52.99 ± 3.46	58.19 ± 2.41	0.258
<i>Bacteroidetes</i>	39.98 ± 2.93	35.98 ± 2.21	0.313
<i>Spirochaetes</i>	2.39 ± 0.88	3.91 ± 0.76	0.226
<i>Verrucomicrobia</i>	1.65 ± 1.13	0.45 ± 0.19	0.357
<i>Tenericutes</i>	1.06 ± 0.46	0.41 ± 0.14	0.229
<i>Proteobacteria</i>	0.92 ± 0.22	0.37 ± 0.11	0.068
TM7	0.31 ± 0.11	0.39 ± 0.23	0.742

AG – Angus cattle, XBC – Xinjiang brown cattle; data are presented as mean ± SEM (standard error of the mean); $P > 0.05$

Table 5. The relative abundance of differential genera in gut microbiota ($RA > 0.1\%*$)

Taxonomy	AG	XBC	P-value
<i>CF231</i>	10.59 ± 3.33	5.86 ± 1.51	0.248
<i>Oscillospira</i>	4.25 ± 0.68	3.44 ± 0.45	0.359
<i>5_7N15</i>	4.33 ± 0.82	2.56 ± 0.57	0.116
<i>Treponema</i>	2.32 ± 0.87	3.91 ± 0.76	0.203
<i>Phascolarctobacterium</i>	2.11 ± 0.47	1.23 ± 0.45	0.208
<i>Clostridium</i>	1.66 ± 0.35	1.49 ± 0.51	0.789
<i>Anaerovibrio</i>	0.77 ± 0.16	2.75 ± 1.13	0.087
<i>Roseburia</i>	0.45 ± 0.31	1.39 ± 0.32	0.059
<i>Ruminococcus</i>	0.72 ± 0.22	1.21 ± 0.36	0.271
<i>Prevotella</i>	0.81 ± 0.17	0.87 ± 0.36	0.881
<i>Epulopiscium</i>	0.15 ± 0.11	0.73 ± 0.63	0.348
YRC22	0.84 ± 0.39	0.68 ± 0.14	0.719
<i>Dorea</i>	0.45 ± 0.29	0.61 ± 0.35	0.736
<i>Coprococcus</i>	0.81 ± 0.51	0.55 ± 0.14	0.661
<i>Paludibacter</i>	0.73 ± 0.16	0.52 ± 0.19	0.412
<i>Turicibacter</i>	0.34 ± 0.07	0.51 ± 0.14	0.282
<i>Akkermansia</i>	0.41 ± 0.11	0.37 ± 0.14	0.845
<i>rc4_4</i>	0.61 ± 0.09	0.35 ± 0.07	0.049
<i>Succinivibrio</i>	0.37 ± 0.11	0.18 ± 0.11	0.257
<i>Bacteroides</i>	0.36 ± 0.18	0.09 ± 0.03	0.219

* $RA > 0.1\%$ – relative abundance $> 0.1\%$, AG – Angus cattle, XBC – Xinjiang brown cattle; data are presented as mean ± SEM (standard error of the mean), $P < 0.05$ indicates significant difference

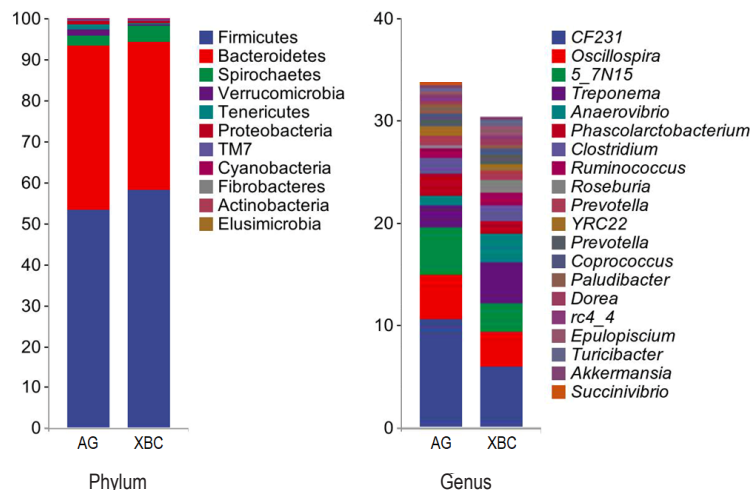


Figure 3. Composition of microbiota at phylum and genus levels (Top 20)

abundance of Proteobacteria in AG tended to be higher than in XBC ($P = 0.068$). The relative abundance of the genus *rc4_4* was significantly higher in AG compared to XBC ($P < 0.05$), and the relative abundance of *Anaerovibrio* ($P = 0.087$) and *Roseburia* ($P = 0.059$) tended to be lower in AG compared to XBC. Proteobacteria have been shown to be associated with several chronic diseases, such as obesity and metabolic syndrome in both human and animal studies (Crovesy et al., 2020; Saiyasit et al., 2020). Ziętak et al. (2016) found that low temperature environment could improve diet-induced obesity, reduce the abundance of *rc4_4* in the gut microbiota, and proved that *rc4_4* was associated with obesity. *Anaerovibrio* are typical fat decomposers that can hydrolyse triglycerides to glycerol and fatty acids, and play a key role in lipolysis, a fundamental requirement in the subsequent steps of lipid metabolism in rumen liquor, bacterial membrane structure formation and cell replication (Mannelli et al., 2018). *Roseburia* were reported to produce butyric acid (Kim et al., 2018) and the abundance of *Roseburia* in the gut microbiota in obese individuals was lower than in lean subjects (Tamanai-Shacoori et al., 2017). In the present experiment, there were no significant differences in the structure of the gut microbiota between AG and BC detected, but the relative abundance in the gut microbiota of some phyla and genera involved in fat deposition/lipid metabolism differed between these two cattle breeds, suggesting that the gut microbiota could be associated with different MQT values in AG and XBC related to the aforementioned parameters.

The results of the linear discriminant analysis effect size (LefSe) showed that species of the family *Peptostreptococcaceae* were the characteristic microorganisms of the XBC microbiota (Figure 4). *Peptostreptococcaceae* belonging to the order *Clostridiales* are a group of bacteria characterized by short chain fatty acid production in the process

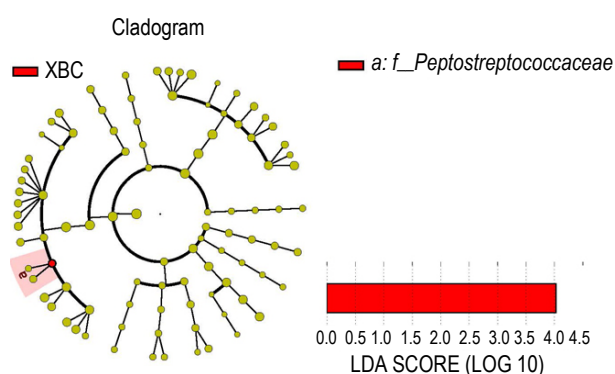


Figure 4. Linear discriminant effect size analysis

of degradation of plant-derived cellulose and hemicellulose components (Bernad-Roche et al., 2021). Previous studies demonstrated that the abundance of the family *Peptostreptococcaceae* in the gut microbiota of castrated bulls was higher than in uncastrated bulls, and they exhibited distinct serum and muscle amino acid profiles, with increased muscular fat storage (Whon et al., 2021).

Correlation analysis

Spearman's correlation analysis was performed between MQTs and 22 genera with RA > 0.1% in the gut microbiota. The results showed that 4 genera in the gut microbiota of AG were significantly correlated with MQTs, i.e. eye muscle area was negatively correlated with *Roseburia*; IMF content was positively correlated with *YRC22*; moisture content was positively correlated with *Phascolarctobacterium*; ash content was positively correlated with *Akkermansia* and negatively correlated with *Roseburia* (Figure 5A). Eight genera in the gut microbiota of XBC were significantly correlated with MQTs, i.e. backfat thickness was positively correlated with *Clostridium*, *Roseburia* and *Turicibacter*; eye muscle area was positively correlated with *CF231*, *5_7N15*, *Phascolarctobacterium*, and negatively correlated with *Turicibacter*; moisture content was positively correlated with *Prevotella*; ash content was positively correlated with *Clostridium* and negatively correlated with *Oscillospira* (Figure 5B). Furthermore, when the data between AG and XBC were pooled to conduct the correlation analysis to reveal the general rule of gut microbiota association with MQTs, the results showed that 13 genera were significantly correlated with MQTs, where backfat thickness positively correlated with *Succinivibrio*. Eye muscle area was positively correlated with *CF231*, *Phascolarctobacterium* and *Akkermansia*, while negatively correlated with *Roseburia*. Eye muscle area was negatively correlated with *Oscillospira* and *Bacteroides*, while IMF content was positively correlated with *YRC22*; moisture content was positively correlated with *Ruminococcus* and negatively with *Prevotella* and *Succinivibrio*; ash content was negatively correlated with *Oscillospira* (Figure 5C). Khan et al. (2018) found that *Oscillospira*, *Ruminococcus* and *YRC22* were all associated with changes in cholesterol levels in rats, and cholesterol was present in blood lipoproteins. In our present experiment, it was also found that *YRC22* was positively correlated with IMF, indicating that *YRC22* could increase intramuscular fat deposition by regulating cholesterol

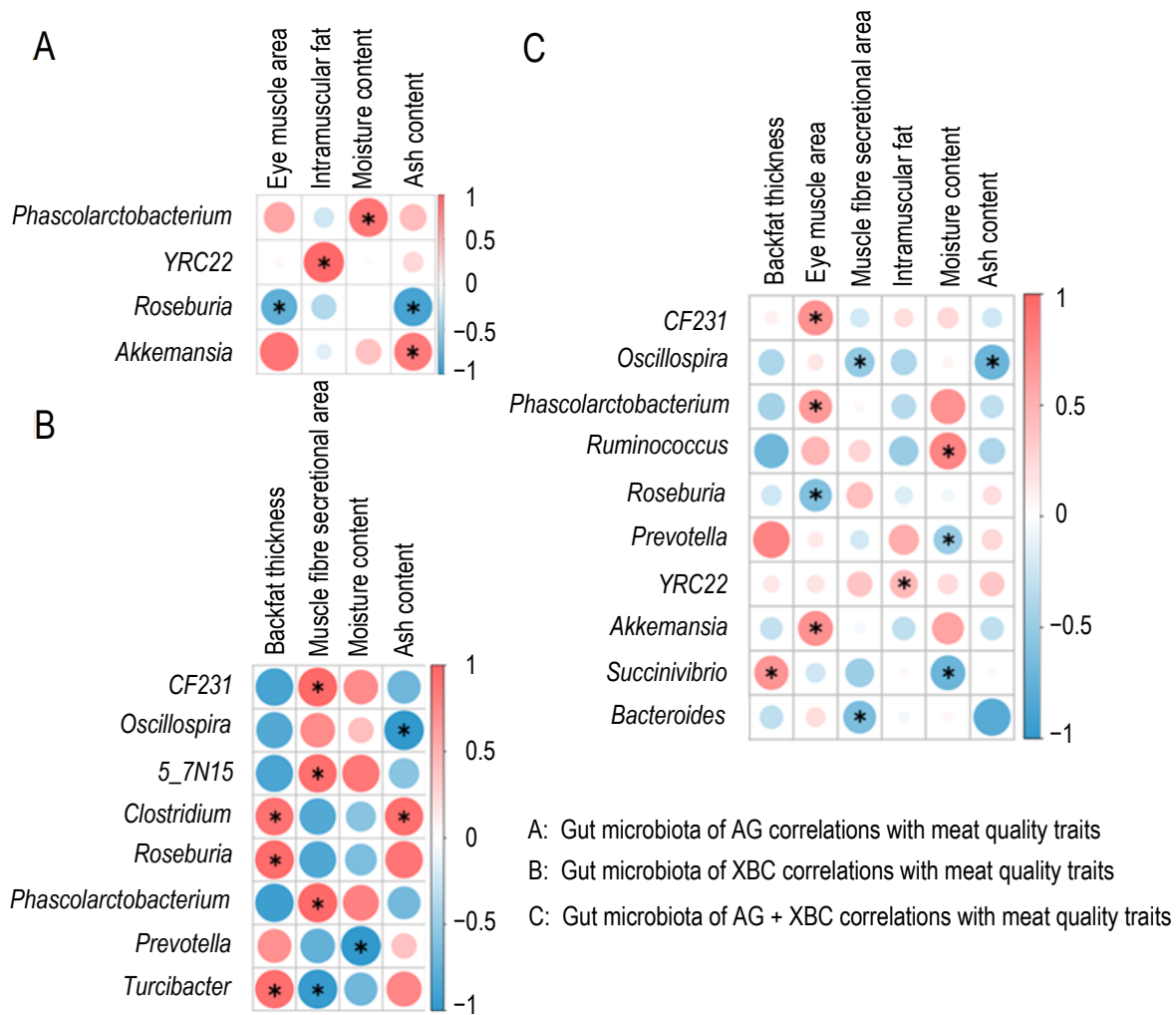


Figure 5. Correlation analysis between gut microbiota and meat quality. Data are presented as mean values \pm SEM; * indicates significant difference at $P < 0.05$

metabolism in cattle. Studies by Tun et al. (2017) found that *Oscillospira* was associated with a reduced risk of obesity. *Oscillospira*, *Ruminococcus*, *Phascolarctobacterium*, *Clostridium* and *Roseburia* all belong to the order *Clostridiales*. Magnusson et al. (2015) found that the count of *Clostridiales* in the gut microbiota of mice fed a high-fat, high-sugar and high-calorie diet increased significantly, which could lead to metabolic disorders and obesity. YRC22, *Bacteroides*, CF231 and *Prevotella* belong to the order *Bacteroidales*. *Prevotella* are one of the most abundant bacteria in the rumen, they decompose cellulose and use cellulose degradation products as energy sources in cattle (Delgado et al., 2019). Zheng et al. (2022) compared the gut microbiota and intramuscular differentially expressed genes between Angus cattle and Chinese Simmental cattle, and the results showed that the relative abundance of *Prevotella* in the gut microbiota of Simmental cattle was significantly lower. Hakkak et al.

(2017) showed that the count of *Bacteroides* in the gut microbiota of obese rats was higher, and the abundance of *Akkemansia* was significantly higher compared to lean mice. Studies proved that *Akkemansia* was associated with lipid metabolism and regulation of the brown fat to white fat ratio (Deng et al., 2020). Bergamaschi et al. (2020) analysed the correlation between the gut microbiota and carcass traits of pigs, and found that there was a significant correlation between *Succinivibrio* and backfat thickness, which was consistent with the results of this experiment.

Conclusions

There are differences in MQTs between Angus cattle and Xinjiang brown cattle related to fat metabolism/deposition that are associated with a relative abundance of certain gut microbial genera. They may serve as potential gut microbial

biomarkers, assisting in the improvement of meat quality in Xinjiang brown cattle. Further confirmation of these associations and the mechanism behind them is expected.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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