Multiple-Omics analysis revealed the high fiber diets promote the gluconeogenesis and inhibit the glycolysis process in muscle

Jianghong Wu (wujianghonglong@126.com)
https://orcid.org/0000-0003-4027-2345

Ding Yang
Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences

Husile Gong
Inner Mongolia University for Nationalities

Yunxia Qi
Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences

Hailian Sun
Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences

Yongbin Liu
Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences

Yahong Liu
Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences

Xiao Qiu
Inner Mongolia Academy of Agricultural and Animal Husbandry Sciences

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Abstract

Background In a previous study, we found that the human essential fatty acid is significantly increased in the mutton fed with the Ceratoides pellets comparing with the alfalfa pellets, which is not consistent with common sense. Results In this study, to investigate the influence mechanisms of meat quality of twins sheep with two kinds of feed high fiber low protein (HFLP) forage (Ceratoides) and low fiber high protein (LFHP) forage (alfalfa), multi-omics techniques were utilized for integration analysis based on the feed nutrition, microbiome, transcriptome, metabolome and fatty acids profile. The results showed that the production performance and the muscle components of lambs were significantly affected by feeds. The lamb muscles fed HFLP have increased the content of essential fatty acid (linoleic acid and arachidonic acid). The diversity of rumen microbes in lamb with HFLP has increased than those of LFHP. The ratio of the Bacteroidetes and the Firmicutes in the rumen of the sheep fed the LFHP was 2.6 times higher than that of the HFLP. The transcriptome analysis of muscle revealed that those genes related with the glucose metabolic process and fat acids biosynthesis were significantly differentially expressed in the muscle of the lamb with two feeds. Conclusion The feed system could affect the epigenetic regulation of those genes that are involved in glucose metabolic pathway. HFLP feeds could induce the gluconeogenesis to maintain the glucose level in blood, which resulted in the fat content of muscle decreasing. The multiple-Omics analysis showed that the microbiota structure is significantly correlated with the metabolome and the gene expression in muscle. This study laid a theoretical foundation for controlling the nutrient intake of sheep, modifying its fatty acid spectrum, and removing the material which was detrimental to the quality of mutton, which could guide the directive sheep feeding for functional mutton.

Background

Sheep (Ovis aries) are important livestock providing meat, milk, and fiber. Genetic basis and diet compositions both can influence the lamb quality[1]. While differences between individual animals of the same breed in the composition of fatty acids have been observed[2], the basis for this variation remains to be delineated[3]. Like all ruminants, sheep have a specialized digestive organ, the rumen, which breaks down cellulose from plant material into simpler carbohydrates. The sheep rumen encompasses a complex microbiota, serves as the primary site for microbial fermentation of ingested feed[4]. Rumen microbes are intermediate links between the diet and the nutrition that are absorbed by sheep. The plant material was fermented by microbes in the rumen, and then converted to mycoprotein, carbohydrates, and lipids that are direct sources of nutrition for ruminants[5]. The rumen microbiota is a dynamic system in which the microbial diversity and community structure are shaped when exposed to different diet compositions [6, 7]. High roughage diet feed can improve microbial protein synthesis in rumen[8]. High-grain feeding also may be able to alter the bacterial microbiota composition and fermentation and affect the local inflammation[9]. In addition, the feeding pattern may also influence rumen microbial ecology system[10]. The quality of mutton would be changed after stall-feeding, so it is urgent to clarify the molecular mechanism of nutritive material affecting meat quality.

Although the rumen microbiota is considered to have an important role in sheep physiology, the mechanisms of how diets affect ruminants physiology remain poorly defined. With the Multiple-omics technology development, the system biology became much powerful to analyze the complex metabolism trait. Recently, research revealed that gut microbiota plays an essential role to host metabolism[11] and energy harvest[12]. The fat deposition process of the host will be changed, when the microbiota of obese mice transplanting into the lean mice. The molecular interaction has been reported between the metabolome of gut microbiota and the human physiology[13, 14]. These researches mostly focused on monogastric animals. Although a lot of research has focused on the relationship between the diet and the microbiota composition in cattle rumen, rumen microbial community composition varies with the host [15, 16].

Here it was hypothesized that the grass species may have an effect on the rumen microbiota and meat quality of sheep due to differences in fiber, protein content and nutrient availability. In this study, to globally analyze the association between the feeds and lamb quality, we utilized the system biology to integrate the metagenomics data, such as the feed nutrition, microbiome, transcriptome, metabolome and fatty acids profile. This research will provide a fundament for the understanding of the mechanism as to how the feeds affect the lamb quality.

Methods

Pellets feed preparation

We prepared two kinds of pellet feed, one of which consisted of 25 % concentrate feed and 75 % Alfalfa hay, another one consisted of 25 % concentrate feed and 75 % *C. arborescens* hay. Because of the higher protein content of Alfalfa, it is the most prevalent forage grass to livestock in worldwide. *C. arborescens* is a forage grass with the higher fiber content and widely distributed in the north of China. The *C. arborescens* pellets have 57.2% fiber and 11.8% crude protein (HFLP). The Alfalfa pellets have 46.2% fiber and 16.1% crude protein (LFHP) (Table S1).

Animal experiment and sampling

To decrease the influence of genetic background as possible as we can, four pairs of Chinese Sunit sheep (*Ovis aries*) female twins weighing 24 ± 2.3 kg were used in the matched pairs experiment, which were randomly divided into two groups and were fed for 12 weeks with HFLP and LFHP. These Sunit sheep (Ovis aries) twins were selected from the sheep breeding farm of Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences that located at Siziwangqi, Jining, China (N41.798°E111.858°). Both two groups were kept in the small corral with free access to food and fresh water. After a fasting period, these lamb twins were slaughtered in a local abattoir according to the standards of the Animal Care and Use Committee in Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, China. After the slaughter, a piece of Longissimus dorsi muscle from the loin between the 12th and 13th ribs was sampled for the extraction of total RNA and the detection of fatty acid profile and metabolome, and was preserved in a nitrogen canister and in a refrigerator at -196°C and -80 °C, respectively. On the other

hands, the liquid and solid phases were separated by squeezing rumen and proximal duodenum contents through four layers gauze (1 mm mesh) (Figure S2). The fluid was then centrifuged at 500 g for 30 min at 4°C to isolate residual particles and was preserved in −80 °C until extraction of genomic DNA.

Microbiome DNA preparation and 16s gene sequencing and processing

In this study, we sequenced the 16S rRNA gene of the microbiome in sheep rumen to identify the bacterial community structure associated with animals under two diet conditions. Enterotypes were strongly associated with long-term diets, particularly protein and animal fat (Bacteroides) versus carbohydrates (Prevotella). Sunit sheep were fed for 12 weeks with (1) the high fiber and low protein diet (Ceratoides arborescens 75% and concentrate 25%) (HFLP), (2) the low fiber high protein diet (clover 75% and concentrate 25%) (LFHP). 15 mL of the ruminal fluids was sampled from four animals for each treatment. Thereafter, total DNA was isolated and 16S rRNA gene was amplified for subsequent metagenomic sequencing by using the Illumina MiSeq PE300. Microbiome DNA was extracted using the MoBio PowerSoil kit according to the operation manual. Bacterial 16S genes were amplified from microbiome DNA using V3-V4 region primers and sequenced using the Illumina MiSeq PE300. After filtering and merging, 868,901Tags were acquired for 8 samples (an average of 108,613 tags per sample). Then, we used the UCLUST[17] algorithm of QIIME (version 1.8.0)[18] to cluster the thousands of tags with 97% of similarity and obtain the operational taxonomic units (OTUs). The annotation of these OTUs was carried out using the Silva database[19]. The alpha and beta diversity was calculated using the Mothur software version v.1.30 (http://www.mothur.org/) [20] and UniFrac method[21].

The fat acid profile and meat quality ofLongissimus dorsi muscle

Crude fat was extracted using the hexane from muscle tissue. Using the sulfuric acid-methanol method, we prepared the ester derivatives of fatty acids for gas chromatography-mass spectrometry analysis (Agilent 6890-5973N, USA). Gas chromatography conditions were set to nitrogen carrier gas (ultra-high purity) 1.0 ml/min, DB-23 quartz capillary column (60 m×0.25 mm×0.25μm), 270°C injector temp, 280°C sample transfer line, Gradient: 130°C start, 6.5°C/min to 170°C, 1.5°C/min to 215°C, 40°C/min to 230°C, 3 minute hold. Mass spectrometer conditions were set to Electron Ionization, 150°C Quad temp, 230°C Source temp, 70 eV, and in full scan mode over the atomic mass range of 35-380amu.

The metabolome of contents of the Longissimus dorsi muscle

In this study, metabolites were first isolated from the content of the *Longissimus dorsi* muscle, and subsequently analyzed by liquid chromatography followed by mass spectrometry (LC/MS). We performed un-targeted metabolomics analysis of Longissimus dorsi muscle obtained from the loin between the 12th and 13th ribs of four pair twins lamb. The identifications of components were based on the untargeted analysis workflow reviewed by (Gary J. Patti, Oscar Yanes and Gary Siuzdak). After data preprocessing, the differences between two groups were revealed by principal components analysis (PCA) and partial least squares discriminant analysis (PLS-DA). To reach the demand of sample number for metabolome, we created two new samples through the equal mixing A and B to E, then C and D to F. In

total we detected 4013 ions of which 2293 could be putatively assigned to metabolites according to five databases including Human Metabolome Database (HMDB) (http://www.hmdb.ca), Metlin (http://metlin.scripps.edu), massbank (http://www.massbank.jp/), LipidMaps (http://www.lipidmaps.org), and mzclound (https://www.mzcloud.org). To explain differences in the muscle tissue, we normalized the peak areas using autoscaling (Centering, scaling, and transformations: improving the biological information content of metabolomics data).

RNA library construction and sequencing

Total RNA was extracted with Trizol reagent according to the manufacturer's protocol. We detected the concentration, integrity and purity of RNA using Nanodrop, Qubit 2.0 and Aglient 2100. The ratio of (OD) 260/280 between 1.8 to 2 and RIN >7 is generally accepted as decent RNA for following procedure. The mRNAs were enriched with oligo (dT) magnetic beads. Then we used fragmentation buffer to implement randomly fragmenting, which can be used as a template to synthesize the cDNA with random hexamers, dNTP, RNase H and DNA polymerase I. After purifying the cDNA, we performed end repaired and added the adaptor to purified cDNA, and then used AMPure XP beads to carry out fragment size selection. Then, we performed the following quality control analysis on cDNA librarian. Sequencing was conducted on the Illumina HiSeq 2500.

Gene expression Analysis of RNA-seq data of Longissimus dorsi muscle

After filtering the raw data of *RNA-seq,* 5.43 GB to 6.92 GB clean bases were acquired for eight muscle tissues. We used the Trinity software to perform the *de novo* transcriptomes assembly [22]. The command line used for assembly was perl Trinity.pl --seqType fq --JM 200G --left left-reads1.fq --right right-reads1.fq --min_kmer_cov 2 --inchworm_cpu 22 --min_contig_length 300 --CPU 22. Subsequently, assembly statistics were performed using TrinityStats.pl scripts. We calculated the gene expression value of reads per kilobase million (RPKM) for each muscle sample using RSEM software [23]. The significantly differential expression genes were determined by edgeR[24]. We performed the enrichment analysis to these differential genes with ClueGO [25]that is one of the plugins of Cytoscape[26].

Multiple-omics integrative analysis

To integrate and explore the different kinds of Omics dataset, the method of DIABLO of mixOmics R package was used[27]. The differential expressed genes, the metabolome of muscle and the microbiome of rumen were analyzed to find a highly correlated multi-omics signature that could explain the different characters of mutton under two diets.

Results

Growth performance and carcass traits

The results showed that the group feeding with the LFHP pellets has significantly higher weight compared with the HFLP group. The average daily gain is 185.9 grams per day for the LFHP group, which

is significantly higher than the HFLP group (89.8 grams/day, P<0.001). Those carcass traits suggested that the LFHP group has better growth performance. The fat content of muscle is also higher in the LFHP group.

Firmicutes/Bacteroidetes ratio in rumen and Microbiota of HFLP Compared with LFHP

To characterize the microbial community structure of rumen fed with two pellet feeds, we sequenced the 16s rRNA gene of rumen bacteria. 1,230 OTUs were identified among eight rumen samples. Analysis of the relative abundance of bacterial OTUs revealed that the two kinds of diets have a different impact on the rumen microbiota. The biodiversity of rumen microbiota is higher in the *HFLP* than in LFHP, which was based on the alpha diversity by Mothur software (Table 2). LFHP showed a significant enrichment in Bacteroidetes and depletion in Firmicutes (P<0.001), with a unique abundance of bacteria from the genus Prevotella and Succiniclasticum, known to contain a set of bacterial genes for cellulose and xylan hydrolysis, lacking in the HFLP.

We hierarchically clustered phylum-level bacterial phylotypes by the similarity of their dynamics across feeds and samples. The results showed that the structure of rumen microbiota was significantly changed according to the feeds rather than the genetics of individuals. Notably, the phylum Bacteroidetes and Firmicutes, two of the leading sources of rumen microbiota, were hypothesized to be sensitive to feeds intake. The Bacteroidetes/Firmicutes ratio in rumen microbiota of the sheep feeding the clover pellet was 2.6 times higher than that of the ceratoides. However, the structure of proximal duodenum microbiota was the reversal, which the predominant phylum was the Firmicutes rather than the Bacteroidetes (data no show).

Enterotypes were strongly associated with feeds, clover pellet (Bacteroidetes) versus ceratoides pellets (Firmicutes). The clover pellets showed a significant enrichment in Bacteroidetes and depletion in Firmicutes (P<0.001), with a unique abundance of bacteria from the genus *prevotella*, known to contain a set of bacterial genes for cellulose and xylem hydrolysis, reducing in the Ceratoides pellets.

The fat acid profile of Longissimus dorsi muscle

To determine the difference of fatty acids composition in *Longissimus dorsi* muscle of twins under different feeding systems, gas chromatography-mass spectrometry analysis was utilized to detect fat acid profile. Thirty kinds of fatty acids were detected in eight muscle tissues. Of these fatty acids, eighteen kinds of fatty acids were a significant difference between twins under different feeding systems. Seven kinds of fatty acids decreased concentration in the muscle of sheep fed with HFLP diet compare with the LFHP diet and eleven kinds of fatty acids increased concentration in the muscle of sheep fed with the HFLP diet (Table3).

Differential gene expression of twins affected by feeds

To compare the transcriptomes from twin's sheep that fed with two different feeds, we utilized the illumina Hi-Seq 2500 to sequence eight *Longissimus dorsi* muscle samples. 22.5 to 28.5 million 125-bp

clean paired-end reads were generated by sequencing. We used the Trinity software to assemble *Longissimus dorsi* muscle de novo transcriptome with RNA-seq reads from eight samples. This reference transcriptome contained 347,335 transcripts; average length is 2166.29 bp. The expression value of transcripts of eight samples was calculated by RSEM. We used the edgeR R package to analyze the differential expression genes (DEGs) between the twin lambs under different feeds. 487 DEGs were obtained while set the *P* value to 0.0001 and False discovery rate (FDR) to 0.05. Of these genes, the PDK4 gene is highest expressed level in *Longissimus dorsi* muscle samples, which plays a key role in regulation of glucose and fatty acid metabolism.

To investigate the function of the DEGs, we performed a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis using the ClueGO. 105 enriched pathways were identified from DEGs. These terms divided 22 clusters and most of these involved epigenetic modification, muscle cell development and muscle fiber assembly, the regulation of glucose metabolic process, RNA transport, steroid hormone mediated signaling pathway and so on. In function of epigenetic modification, KALRN, NTM1B and TET1 genes were high expressed in muscle feeding with clover pellets (LFHP). While DSRAD, DOT1L, KAT2A, KMT2B, KMT2E, HSF4, PHF1, PICK1 genes were high expressed in muscle feeding with ceratoides pellets (HFLP). These results showed that HFLP could upregulated more genes related with the epigenetic modification comparing with LFHP and inactivated those genes of steroid hormone mediated signaling pathway such as NR1D1, NR4A1 and NR4A2.

Differences in the Longissimus dorsi muscle metabolome of twins affected by feeds

To find feeds related differences in metabolism, we performed PLS-DA to analyze the differences between two groups. 16 compounds with VIP >1.5, P value<0.05 and FDR <0.05 were identified as key lineages for separating the muscle compounds in the two groups. Most of them were involved in the glucose metabolism, such as phosphatidylethanolamine, methylhistamine, phosphoenolpyruvate, alpha-D-glucose 6-phosphate and, taurine. Phosphoenolpyruvate and alpha-D-glucose 6-phosphate are involved in the gluconeogenesis, which is activated in the muscle of HFLP group. Furthermore, we observed a change in the concentration of methylhistamine that is an inactive metabolite of histamine and plays a role in the activation of histamine H2/H4 receptor. Methylbutyric acid is related to the odor of bioproduct. Adenine hydrochloride and Taurine have been considered as essential factors for the differentiation and growth of skeletal muscles.

Integrated analysis of microbiota, transcriptome and metabolome

We utilized DIABLO to compare 8 twins lamb feeding two different feeds, profiled for rumen microbiome, RNA expression, and metabolome of muscles. The correlation between components from each data set has been maximized as specified in the design matrix. The results showed that these four kinds of data on the different level have a higher correlation. Certainly, 5 OTUs, 10 genes, 2 fatty acids and 4 metabolites assigned to this correlation demonstrated notable divergences between two diets.

Discussion

As the rumen microbiota changes, the fatty acids profile and the metabolome of *Longissimus dorsi muscle* of twin sheep have been affected by two kinds of feeds. The integrated analysis showed that the Arachidic acid and Linolenic acid were significant correlation with the microbiota of rumen, the differential expression genes and the metabolism of *Longissimus dorsi muscle.* The Arachidic acid and Linolenic acid both are essential fatty acids for human, which implied that we could produce the functional mutton for consumers.

The microbiota of the rumen is an intermediate link between the diet and the nutrition that are absorbed by sheep. In this study, the feedstuffs have affected the microbiota structure of sheep rumen. The ceratoides pellets have increased the diversity of rumen's microbiota comparing to the clover pellets, which was consistent with the previous study in human that the high-fiber diet could increase the potentially beneficial microbiome [28]. In this paper, the major organisms in sheep rumen microbiota are members of the Bacteroidetes and the Firmicutes phyla. While the predominant phylum was the Firmicutes rather than the Bacteroidetes in proximal duodenum microbiota. The Bacteroidetes / Firmicutes ratio of rumen significantly changed with feedstuffs of ceratoides pellets and clover pellets (3.63 and 9.61 respectively). The ratio of Bacteroidetes / Firmicutes considered as an index for gut homeostatsis in human[11], which also changes with the age of persons[29]. The different kinds of nutrition from diet intake have different effects on the ratio of Bacteroidetes/Firmicutes. However, the high-energy diet could increase the Firmicutes and decrease Bacteroidetes in human [28] and mice, while the change trend was opposite in sheep rumen. The reason may be is that sheep, human and mice have different digestion types[30]. The rumen microbiota has some special microbial species to help ruminants to degrade feeds. In this paper, the genus Prevotella showed a decrease in the high fiber diet, which is not consistent with the previous study in human and pigs [31]. Prevotella is largely responsible for much of the proteolytic activity within the rumen and is important for breaking down plant protein into usable nitrogen, which becomes accessible for other microbes within the rumen[32].

In this study, the low-quality feeds (HFLP) could not provide enough nutrition for sheep. After the microbial fermentation in the rumen, those metabolites were absorbed by a host and then these nutrition molecules could regulate the status of host physiology. Therefore, FOXO1 and PDK4 gene are highly expressed in *Longissimus dorsi* muscle of HFLP. The FOXO1 could upregulate the expression level of the PDK4 gene through direct binding the promoter region. PDK4 plays an important role in maintaining normal blood glucose levels and increases fat metabolism in response to prolonged fasting[33] and starvation[34]. The gene expression analysis also showed that the Methylation process was downregulated in muscle feeding with the HFLP pellets. For instance, the heat shock factor protein 4 (HSF4) of these genes is higher expressed in HFLP muscle, which could be the response of gene regulation to the low-quality feed under the stress of starvation. The interaction between HSF4 and FKBP5 plays a role in the intracellular trafficking of heterooligomeric forms of steroid hormone receptors maintaining the complex into the cytoplasm when unliganded. Moreover, the steroid hormone mediated signaling pathway was inactivated, which decreased the food uptake and activity of a number of

enzymes involved in hepatic TAG synthesis[35]. It could be the reason for the lower fat content of muscle in the HFLP sheep group. The compounds of gluconeogenesis were found to be activated in the HFLP group based on the metabolome analysis, which could maintain the content of glucose in blood during the starvation. Meanwhile, the content of methylhistamine in HFLP muscle was higher than LFHP muscle from the metabolomic analysis. The Methylhistamine is the agonist for histamine H2/H4 receptor which could stimulate the gastric acid secretion[36] and induce the increase of the cAMP[37]. With the increase of cAMP concentration, the pyruvate kinase would be phosphorylated by protein kinase A, the activity of phosphorylated pyruvate kinase was decreased[38] and the glycolysis process was inhibited in HFLP muscle. While, the Taurine and the Adenine hydrochloride were both upregulated in LFHP group and could promote the differentiation and growth of muscles [39, 40]. We utilized the *Multiple-omics integrative analysis*, which resulted in glucose metabolism as the most significant pathway. Certainly, 5 OTUs, 10 genes, 2 fatty acids and 4 metabolites assigned to this correlation demonstrated notable divergences between two diets. However, it needs more advanced genome editing methods in the future to identify which is more critical for altering the content and quality of the mutton.

Conclusions

The feed system could affect the microbiota structure of rumen, and change the epigenetic regulation of those genes that are involved in glucose metabolic pathway. Low quality feeds induce the gluconeogenesis to maintain the glucose level in blood, which resulted in the fat content of muscle decreasing. The multiple Omics analysis showed that the microbiota structure is significantly correlated with the metabolome and the gene expression in muscle. This study laid a theoretical foundation for controlling the nutrient intake of sheep, modifying its fatty acid spectrum, and removing the material which was detrimental to the quality of mutton, which could guide the directive sheep feeding for functional mutton.

Abbreviations

HFLP: high fiber low protein

LFHP: low fiber high protein

OTUs: operational taxonomic units

PCA: principal components analysis

PLS-DA: partial least squares discriminant analysis

RPKM: reads per kilobase million

DEGs : differential expression genes

FDR: False discovery rate

KEGG: Kyoto encyclopedia of genes and genomes

Declarations

Ethical Approval and Consent to participate

My manuscript report data collected from sheep. After a fasting period, these lamb twins were slaughtered in a local abattoir according to the standards of the Animal Care and Use Committee in Inner Mongolia University for Nationalities, China.

Consent for publication

All authors provided consent for publication.

Availability of supporting data

The multiple Omics data used, analysis pipeline used in this study will be made available from the corresponding author upon request.

Competing interests

The authors declare that there are no competing interests.

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Authors' contributions

Conceived and designed the experiments: JW. Performed the experiments: JW DY YQ HS YL1 XQ YL2. Analysed the data: HG JW. Contributed reagents/materials/ analysis tools: DY YQ. Wrote the paper: JW HG.

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Authors' information

Jianghong Wu (wujianghonglong@126.com, 1.College of Animal Science and Technology, Inner Mongolia University for Nationalities, Tongliao, 028000, China

2. Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, 010031, China)

Ding Yang (yangding81@126.com, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, 010031, China)

Husile Gong (huslee@163.com, College of Life Science, Inner Mongolia University for Nationalities, Tongliao, 028000, China)

Yunxia Qi (qi_yunxia@163.com, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, 010031, China)

Hailian Sun (sunlian1053@qq.com, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, 010031, China)

Yongbin liu (ybliu117@126.com, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, 010031, China)

Yahong Liu (liuyahong2008@163.com, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, 010031, China)

Xiao Qiu (284454352@qq.com, Inner Mongolia Academy of Agricultural & Animal Husbandry Sciences, Hohhot, 010031, China)

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Tables

Table 1 Carcass traits of twins sheep fed the two diets.

Trait	A1	B1	C1	D1	A2	B2	C2	D2
Start weight(kg)	21.90	23.60	25.15	24.65	22.40	21.50	25.00	25.20
Slaughter weight(kg)	31.35	31.35	32.5	39.6	37.65	35.7	49.7	52.85
Carcass weight(kg)	13.65	13.65	13.5	16.9	17.5	16.09	22.99	21.75
Dressing %	43.54	43.54	41.54	42.68	46.48	45.07	46.25	41.15
Loin eye area (cm2)	17.80	17.59	17.58	18.02	22.14	22.76	29.63	36.32
Water holding capacity	15.98	18.73	18.63	16.98	23.23	20.11	18.51	22.23
protein	18.79	20.35	20.14	20.70	18.34	19.97	20.32	18.38
Lipid	5.58	1.45	3.54	4.10	10.97	8.23	4.99	9.30
Moisture	73.08	75.66	75.12	73.43	68.60	69.93	73.21	70.85
Fiber	0.66	0.64	1.15	1.21	0.73	0.70	1.76	0.83

Table 2 the statistic results of alpha diversity in rumen microbiota

Sample_ ID	Sobs	Ace	Chao1	Shannon	Simpson
A1	1015.00	1085.01	1094.10	5.12	0.02
A2	932.00	997.04	1031.62	4.02	0.13
B1	1073.00	1117.13	1129.29	5.06	0.02
B2	928.00	1000.85	1031.89	4.21	0.05
C1	968.00	1034.45	1041.22	5.21	0.01
C2	952.00	1017.24	1032.67	4.24	0.06
D1	912.00	970.03	973.26	4.84	0.02
D2	908.00	954.23	973.27	4.46	0.03

Table 3 the fatty acid profile of *Longissimus dorsi* muscle

	HFLP	STDEVP	LFHP	STDEVP	p_value
Palmitic acid	23.5827	2.4614	25.9489	3.2206	0.0170
Oleic acid	43.2356	2.2468	45.1598	2.3060	0.0149
Linolenic acid	0.2154	0.0157	0.3904	0.0391	0.0000
Margaric acid	0.7006	0.0602	0.8209	0.0603	0.0000
Margaroleic acid	0.2320	0.0421	0.3068	0.0349	0.0002
Stearic acid b2	0.3347	0.0404	0.3739	0.0369	0.0002
Jecoleic acid	0.0530	0.0163	0.0698	0.0194	0.0286
Tridecanoic acid	0.0080	0.0032	0.0054	0.0023	0.0016
Myristic acid b	0.0298	0.0066	0.0218	0.0085	0.0002
Nonadecanoic acid	0.1013	0.0200	0.0893	0.0126	0.0472
Arachidonic acid	0.0949	0.0324	0.0794	0.0214	0.0225
Pentadecenoic acid b2	0.1116	0.0276	0.0926	0.0240	0.0014
Palmitic acid b	0.1156	0.0220	0.0965	0.0229	0.0008
Palmitelaidic acid t	0.2393	0.0330	0.2146	0.0262	0.0021
Arachidic acid	0.1654	0.0265	0.1129	0.0235	0.0003
Elaidic acid	0.4570	0.1368	0.2768	0.1089	0.0019
cis Linolelaidic acid	2.5112	0.3750	2.1234	0.2158	0.0002
Stearic acid	23.6041	3.8339	19.6163	2.9588	0.0101

Table 4 the compounds of *metabolome in Longissimus dorsi* muscle

Compounds	VIP	pvalue	FDR	log2FC (LFHP / HFLP)
Adenine hydrochloride hydrate	1.81	0.02	0.03	-3.28
Dihydrouracil	1.63	0.04	0.04	-1.30
Phosphoenolpyruvate	2.08	0.00	0.03	-1.24
Methylbutyric acid	1.74	0.04	0.04	-0.66
Methylhistamine	1.79	0.04	0.04	-0.48
Hydroxybenzotriazole	1.97	0.02	0.03	-0.46
Vanillylmandelic acid	1.98	0.02	0.03	-0.36
Proline	1.78	0.04	0.04	0.48
Phosphatidylethanolamine lyso 16:0	1.71	0.03	0.04	0.60
Creatinine	2.11	0.01	0.03	0.71
Alpha-d-glucose 6-phosphate	1.99	0.01	0.03	0.71
Trimethylenediamine	2.09	0.01	0.03	0.96
Glutaric acid	1.79	0.02	0.04	1.06
Citric acid	1.64	0.04	0.04	1.18
Taurine	2.00	0.01	0.03	1.81
Acetamidobenzoic acid	2.31	0.00	0.01	3.09

Note: FDR: Significances were considered at q<0.05, VIP, variable importance in the projection.

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