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Lipid Metabolomics Reveals Metabolic Responses of Subcutaneous Fat in Yaks under Different Feeding Conditions during the Cold Season

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ABSTRACT

Background: The aim of this experiment was to investigate the differences in subcutaneous fat metabolism expression in yaks under natural grazing and housed feeding conditions in the cold season. Eighteen 18-month-old yaks with similar body weights and non-significant differences in dorsal subcutaneous fat were randomly divided into three groups.

Methods: One group (G18_SF) of yaks was selected for slaughter at the beginning of the experiment (October), one group continued to graze for 6 months and then slaughtered (G24_SF) and one group was housed for 6 months and then slaughtered (F24_SF) and subcutaneous dorsal fat of the three groups was collected for metabolomic analysis, screening of differential metabolites and enrichment of KEGG pathway.

Result: In this experiment, 110 differential metabolites were detected in G24_SF vs F24_SF and 83 metabolites were screened for down-regulation and 27 metabolites were up-regulated and the differential metabolites were mainly enriched in Alanine, aspartate and glutamate metabolism, Arachidonic acid metabolism and D-Glutamine and D-glutamate metabolism pathways. Ninety-seven differential metabolites were detected in G18_SF vs G24_SF and 41 metabolites were screened for down-regulation and 56 metabolites were screened for up-regulation, with the two groups of differential metabolites mainly enriched in Alanine, aspartate and glutamate metabolism, Caffeine metabolism and Starch and sucrose metabolism pathways. The results of this experiment revealed the information of differential metabolites and enrichment pathways of yak fat in different feeding methods and different months of age and laid the foundation for further in-depth study of subcutaneous fat deposition in yaks.

Key words: Housed feeding, Lipid metabolomics, Natural grazing, Subcutaneous fat, Yak.

INTRODUCTION

Yaks live on the Qinghai-Tibetan Plateau and its surrounding areas and are the main domestic animals kept by plateau herders (Luo *et al*. 2018) and their milk, meat and their by-products not only greatly contribute to the development of the pastoral animal husbandry economy, but also increase the herders' income. Yaks graze naturally and the growth of pasture and the climatic conditions of the plateau directly affect the growth performance of yaks. Their habitat has a cold season of 6-7 months in a year, during which the pasture grass is the most scarce and has the lowest nutrient value, which directly leads to insufficient nutrient intake of yaks and the energy stored in the body is used to maintain the basic activities of the life of yaks and the fat content of yaks is the lowest in this stage. As the pastureland becomes green and the quantity and nutritional value of the pasture gradually increase, the yaks consume enough nutrients and the body fat begins to be slowly deposited to make energy reserves for the long cold season and after the cold season the yaks begin to decompose the fat to provide energy and the cycle continues. In short, yak body fat undergoes the process of "decomposition~deposition~maximum content~decom position". Fat deposition in animals is a complex regulatory process that is influenced by a combination of neuromodulation, humoral regulation, a range of hormones and fat metabolizing enzymes (Newsholme *et al*., 2003; Du *et al*., 2018; Khan *et al*., 2020; Roy Kumar Ashwani *et al*., 2020; Singh Jeet Ravinder, 2018). The different nutrients absorbed by animals under housed and grazing conditions directly lead to changes in the corresponding physiological functions and metabolic mechanisms of animals. Generally, yaks can lose 25% of their body weight during the cold season (Long *et al*., 2005; Jayanthi *et al*., 2020). It was found that the capacity of fat deposition was greater in housed yaks than in grazing

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yaks and that triglycerides in yak subcutaneous fat were mainly regulated by AGPAT2 and DGAT2 genes (Xiong *et al*. 2023). The fatty acid composition of housed feeding yak meat is healthier for consumers in terms of nutritional value (Xiong *et al*. 2022). In summary, housed feeding yaks during the cold season can significantly improve their productive performance.

Metabolomics analysis can visualize the state of the organism and its overall effects, making the study of complex genes and proteins at the level of complex genes and proteins to become simple (Kokova *et al*., 2020) and roughly elaborating the mechanism of the organism's response to stimuli (Middelkoop *et al*., 1993; Monaghan *et al*., 2009; Brand and Anderson, 2011). Previous studies on the metabolic mechanisms of subcutaneous fat deposition in yaks by housed feeding and grazing are scarce. The aim of this study was to compare the pattern of subcutaneous fat deposition in yaks under different feeding methods (grazing and housed feeding) and to detect and analyze the metabolites of yaks under different feeding conditions, with a view to revealing the mechanism of the effect of different feeding methods on subcutaneous fat deposition in yaks. After slaughtering the yaks at the end of the experiment, the subcutaneous fat on the back was collected, put into a liquid nitrogen tank and then transported back to the laboratory and stored at -80 m for reserve.

MATERIALS AND METHODS

Experimental design

The experimental yaks in this study were obtained from the Meilong Palm Livestock Management Specialized Cooperative (Qilian, Qinghai), which is located at an average altitude of 3.169 m above sea level and has a plateau continental climate. Eighteen newborn male calves (142.45±2.65 kg) at 18 months of age were selected to carry out the experiment and all the experiment cows were healthy and in good growth condition, with non-significant differences in body weight. At the beginning of the trial in October, 6 yaks were randomly slaughtered (G18_SF) and the remaining 12 yaks were randomly divided into 2 groups, one group was naturally grazed in the original group for 6 months to 24 months of age (G24_SF) and the other group was fully housed and fattened for 6 months to 24 months of age (F24_SF).

Feeding and management

Yaks in the grazing group grazed in the same pasture for 11 hours per day and the yaks had free access to food and water throughout the grazing period. The grazed pasture was dominated by dominant forages such as needlegrass, morning-glory, dwarf tarragon and small tarragon. Table 1 shows the nutrient levels of the forages in the free-grazing group. The TMR total mixed ration pattern was used to feed housed yaks once at 07:00 and 17:00, respectively. The pellet concentrates used in this experiment were obtained from a feed mill in Minle County, Gansu Province and the composition and nutrient levels of the basal rations are shown in Table 2.

Test method

Sample handling

At the end of the experiment the yaks were slaughtered, samples were collected and stored at -80°C. Accurately weigh 50 mg of each sample into a 2 ml centrifuge tube, add a 6 mm diameter grinding bead; add 400 μL of extraction solution (methanol: water = 4:1 (v:v)) with 0.02 mg/mL of the internal standard (L-2-chlorophenylalanine);

Table 1: Nutrient levels of natural herbage (DM basis).

Items	Grass period	Hay period
Moisture (%)	26.01	11.16
Crude protein (%)	9.96	7.06
Ether extract (%)	2.55	1.33
Ash (%)	15.79	21.77
Crude fiber (%)	29.75	38.25
Acid detergent fiber (%)	34.81	53.31
Neutral detergent fibre (%)	46.35	66.47
Calcium (%)	2.82	1.10
Total phosphorus (%)	0.14	0.04

Note: All the nutrients were the actual test values.

Table 2: Diet composition and nutrient levels (DM basis).

Items	Proportion (%)	Nutrient levels	Content
Corn	43.71	Combined net energy (MJ/kg)	4.28
Concentrates	10.93	Crude protein (%)	16.63
Rapeseed meal	3.0	Ether extract (%)	5.49
Soybean meal	3.0	Crude fiber (%)	21.17
Oaten hay	10.93	Acid detergent fiber (%)	28.61
Silage	27.33	Neutral detergent fibre (%)	46.09
NaCl	0.55	Calcium (%)	0.30
$NaHCO_3$	0.55	Total phosphorus (%)	0.16
Total	100		

Note: (1) Concentrate feed provided the following per kg of diet vitamin A 3000 IU, vitamin D 800IU, vitamin E 40IU, copper 15 mg, iron 50 mg, cobalt 0.4 mg, zinc 30 mg, manganese 40 mg, selenium 0.3 mg, iodine 0.4mg.(2) All the nutrients were the actual test values.

grind the samples for 6 min in a freezing tissue grinder (-10°C, 50 Hz); extract the samples by cryo-sonication for 30 min (5°C, 40 KHz); leave the samples at -20°C for 30 min; centrifuge for 15 min (13000 g, 4°C); transfer the supernatant into an injection vial with an insert tube for analysis; in addition, each sample is analyzed separately in an injection vial with an insert tube; and each sample is analyzed separately. The samples were allowed to stand at -20°C for 30 min, centrifuged for 15 min (13000 g, 4°C) and the supernatant was pipetted into an injection vial with an internal cannula for analysis. 20 μL of the supernatant was pipetted from each sample and mixed as the quality control sample.

LC-MS detection

The instrument platform for this LC-MS analysis was a Thermo Fisher Ultra High-Performance Liquid Chromatography Tandem Fourier Transform Mass Spectrometer (UHPLC-Q Exactive HF-X) system.

Chromatographic conditions: The column was ACQUITY UPLC HSS T3 (100 mm×2.1 mm i.d., 1.8 ìm; Waters, Milford, USA); mobile phase A was 95% water+5% acetonitrile (containing 0.1% formic acid) and mobile phase B was 47.5% acetonitrile+47.5% isopropanol+5% water (containing 0.1% formic acid), with an injection volume of 2 μL and the temperature of the column was 40°C (Table 3).

Mass spectrometry conditions: The samples were ionized by electrospray ionization and the mass spectrometry signals were collected in positive and negative ion scanning modes, respectively. The specific parameters are shown in Table 4.

Data processing

After the raw data were imported into the metabolomics processing software ProgenesisQI (WatersCorporation, Milford,USA), a series of pre-processing was carried out and a combination of multidimensional and unidimensional analyses was used to screen for differential metabolites in yak subcutaneous fat under different feeding conditions for volcano diagram analysis, using a combination of multidimensional and unidimensional analyses, with the VIP value of the first principal component of the OPLS-DA model>1 and the P value returned by the t-test (Wilcox-test)<0.05 were used as criteria to screen the differential metabolites of yak subcutaneous fat under different feeding conditions for volcano plot analysis. Online cloud platform (https://report.majorbio.com/wholerna/geneset_overview/task_id/3u9i_gd0m9blvcljkgt4jsbc2a9.html) was used to compare the differential metabolites with KEGG and HMDB databases and KEGG pathway enrichment analysis was performed after obtaining the annotation information of metabolites in the databases.

RESULTS AND DICUSSION

Principal component analysis

From the principal component analysis in Fig 1, it can be seen that there is a certain grouping trend of subcutaneous

Table 3: Mobile phase elution gradients.

Time (min)	Flow rate (ml/min)	A (%)	B (%)
0	0.4	100	0
3.5	0.4	75.5	24.5
5	0.4	35	65
5.5	0.4	0	100
7.4	0.6	0	100
7.6	0.6	48.5	51.5
7.8	0.5	100	0
9	0.4	100	0
10	0.4	100	0

Table 4: Mass spectral parameters.

Description	Parameter
Scan type (m/z)	70-1050
Sheath gas flow rate (arb)	50
Aux gas flow rate (arb)	13
Heater temp (°C)	425
Capillary temp (°C)	325
Spray voltage (+) (V)	3500
Spray voltage (-) (V)	-3500
S-Lens RF Level	50
Normalized collision energy (eV)	20,40,60
Resolution (Full MS)	60000
Resolution (MS2)	7500

Table 5: Details of differences between F24_SF and G24_SF groups.

Metabolite	Regulate	Mode	P_value	FDR
Ophthalmic acid	down	neg	2.71E-05	0.00536
Dimethylglycine	down	pos	3.52E-05	0.007981
1-Linoleoylglycerophosphocholine	up	pos	4.91E-05	0.007981
2-Pentyl-3-phenyl-2-propenal	down	pos	0.000221	0.01995
Xanthine	down	neg	0.0002243	0.01587
N-Acetylneuraminic acid	down	neg	0.0003618	0.02002
(S)-10,16-Dihydroxyhexadecanoic acid	down	neg	0.0009419	0.03193
5-Hydroxyindoleacetic acid	down	pos	0.001221	0.04279
Dihyroxy-1H-indole glucuronide I	down	neg	0.001251	0.03724

fat under different feeding conditions and the difference between the G24_SF and F24_SF groups is large and the difference with the G18_SF group is small, in which the QC samples are clustered together, which indicates that the samples have good reproducibility, the quality of the QC is high and the data are reliable and can be subjected to further analysis of variance.

Screening for differential metabolites

Venn diagrams show the metabolites that are shared or unique between metabolic sets. To obtain the differential metabolites between metabolic sets, differential metabolite analysis was performed. As shown in Fig 3, there were 97 differential metabolites between the G24_SF and G18_SF sets and 110 differential metabolites between the F24_SF and G24_SF sets (Fig 2).

Bioinformatics analysis

Classification of KEGG compounds

The analyzed metabolites were compared to the KEGG Compound database to obtain an overview of metabolite classification, as shown in Fig 3a and it was found that the metabolites differing between the F24_SF and G24_SF groups were classified into a total of seven major types, including vitamins and cofactors, peptides, nucleic acids; the different metabolites produced were mainly peptides and lipid compounds. It was found that the differential metabolites of the G18_SF and G24_SF groups were categorized into five major types, including peptides, nucleic acids, lipids, hormones and transmitters and carbohydrates; the differential metabolites produced were mainly peptides and carbohydrates (Fig 3b).

Functional analysis of the KEGG database

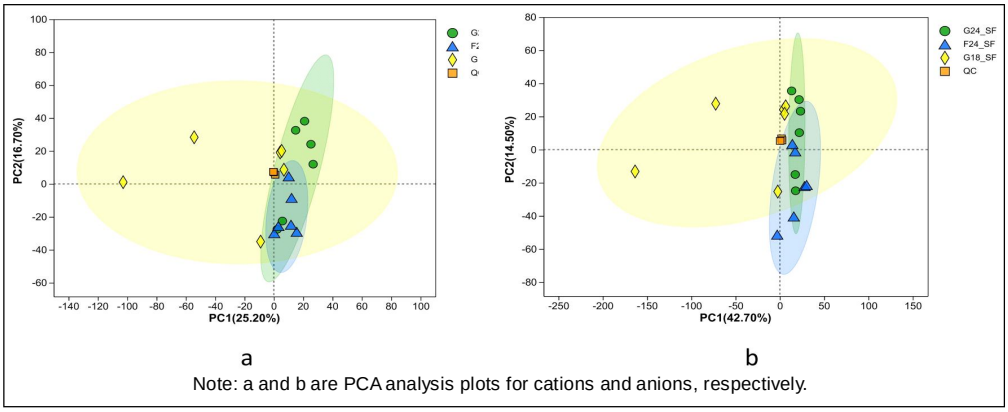
The experiment searched the KEGG database for the differential metabolites of each sample group and obtained 31 and 18 signaling pathways belonging to 6 and 5 classes, respectively and the results showed (Fig 4) that the differential metabolites of each sample group were significantly annotated to the metabolism of amino acid metabolism, carbohydrate metabolism and lipid metabolism, among others; Digestive system and Nervous system in the system of organismal systems, *etc*.; Cancer: overview in the human disease, *etc*.; Membrane transport in the environmental information processing, *etc.*

Hierarchical cluster analysis of differential metabolites

Cluster analysis can provide a better understanding of the relationship between the differential metabolites screened above. Fig 5 Horizontal coordinate is the sample name, vertical coordinate is the differential metabolite, red color indicates high expression of the substance content and blue color indicates low expression of the substance content. As shown in Fig 5a, the G24_SF group showed high expression of phosphatidyl ethanolamine (PE) and phosphatidylcholine (PC) related metabolites and N-acetylneuraminic acid and low expression of LysoPE, LysoPC, Beta-D-Glucosamine and Methycholine. Low expression of docosapentaenoic acid, ophthalmic acid, N-acetylneuraminic acid and PE and PC related metabolites and high expression of LysoPE, LysoPC, Beta-D-Glucosamine and Methycholine in the F24_SF group.As can be seen in Fig 5b, in the G24_SF group, the expression of PE and PC related metabolites and LysoPE-related metabolites was low and the expression of Palmitoyl-L-carnitine and 2-Pentyl-3-phenyl-2-propenal was high. The expression of PE and PC related metabolites, LysoPE-related metabolism and Beta-D-Glucosamine was high in the G18_SF group;low expression of Palmitoyl-L-carnitine and 2-pentyl-3-phenyl-2-propene.

Volcanic composition

Each dot in Fig 6 represents a specific metabolite. The dots on the left are metabolites that are differentially down-regulated in expression and the dots on the right are metabolites that are differentially up-regulated in expression. In Fig 6a, 83 metabolites were down-regulated and 27 metabolites were up-regulated in the G24_SF relative to the F24_SF group and in Fig 6b, 41 metabolites were down-regulated and 56 metabolites were up-regulated in the G24_SF relative to the G18_SF group. Table 5 and Table 6



Note: a and b are PCA analysis plots for cations and anions, respectively.

Fig 1: PCA analysis of the population sample.

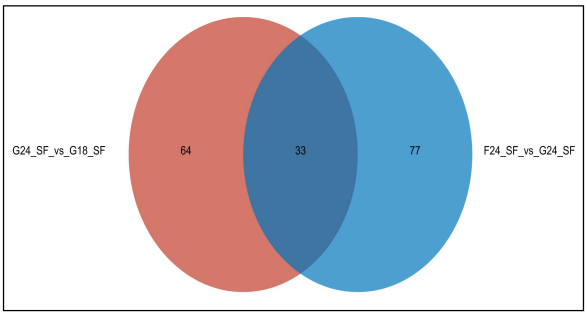
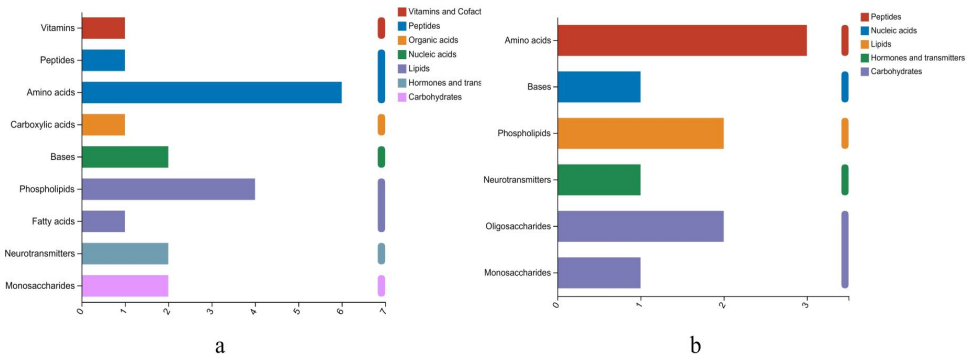


Fig 2: Differential metabolite venn plot.

show that products with annotations were detected in the G24_SFvsF24_SF and G24_SFvsG18_SF groups, respectively.

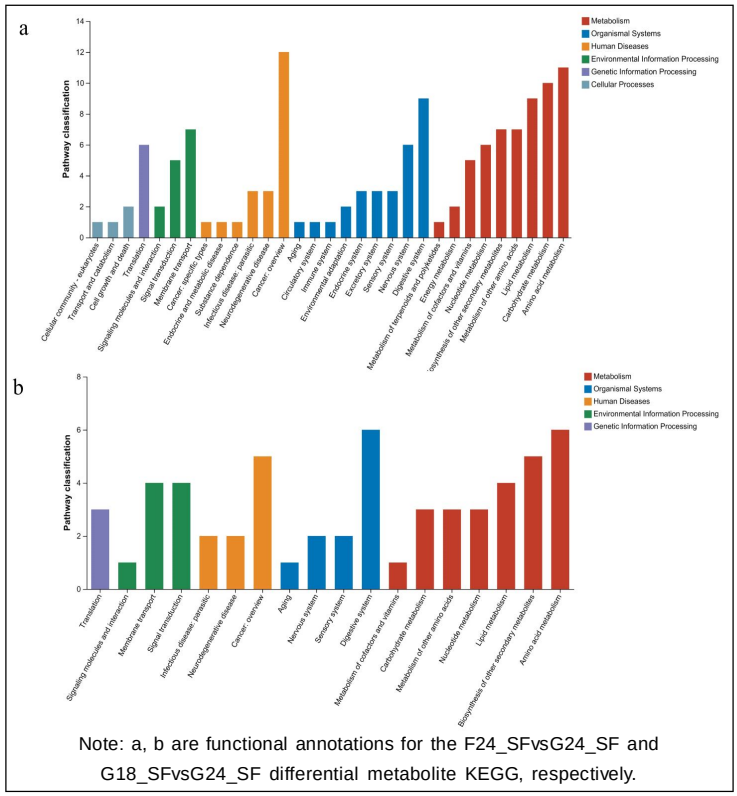
KEGG pathway enrichment

By comparing the F24_SF with the G24_SF group (Fig 7a and Table 7), KEGG pathway enrichment was found to involve metabolic pathways such as Alanine, aspartate and glutamate metabolism, Arachidonic acid metabolism, D-Glutamine and D-glutamate metabolism and others;By comparing the G18_SF with the G24_SF group (Fig 7b and Table 8), KEGG pathway enrichment was found to involve



Note: a, b are compound classifications of the F24_SFvsG24_SF and G18_SFvsG24_SF differential metabolite KEGG, respectively.

Fig 3: Compound classification of the differential metabolite KEGG.



Note: a, b are functional annotations for the F24_SFvsG24_SF and G18_SFvsG24_SF differential metabolite KEGG, respectively.

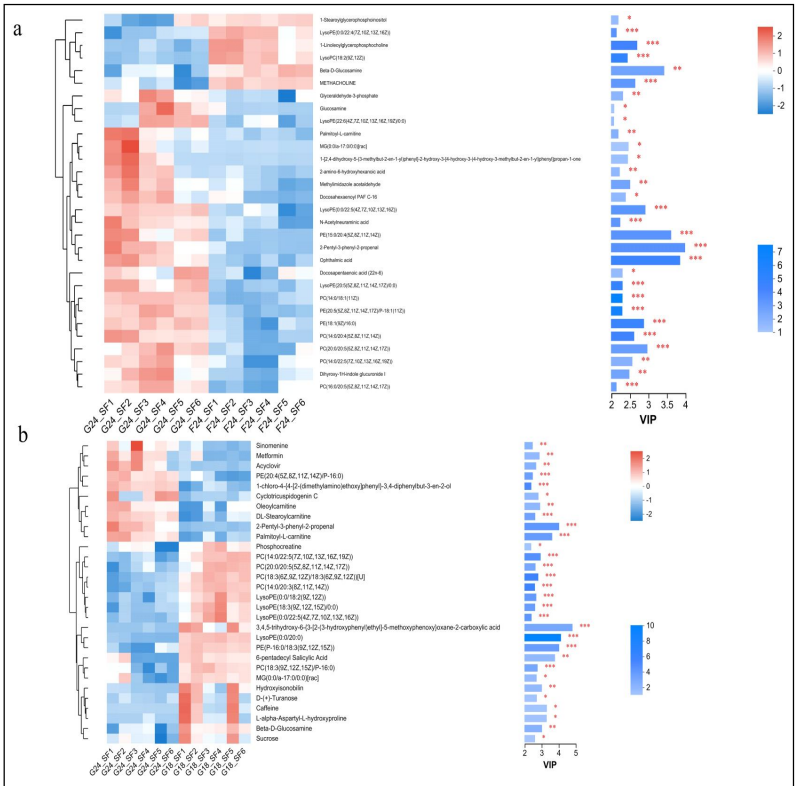
Fig 4: Functional annotation of the differential metabolite KEGG.

metabolic pathways such as Alanine, aspartate and glutamate metabolism, Caffeine metabolism, Starch and sucrose metabolism and others.

In this study, yaks were grazed and housed during the dry grass period and yak subcutaneous fat was used as the object of study to investigate the effects of different feeding methods and different ages on yak fat metabolism by analyzing the metabolomic changes of yak subcutaneous fat at the ages of 18 months and 24 months.

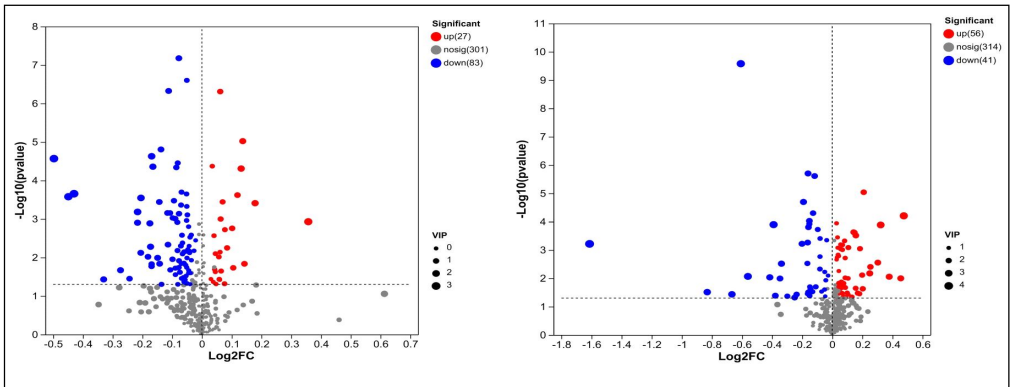
Differential metabolite analysis

2-Pentyl-3-phenyl-2-propenal, N-Acetylneuraminic acid and Xanthine were the most abundant in F24_SF, followed by G24_SF and the least abundant in G18_SF.N-



Note: a, b are F24_SFvsG24_SF and G18_SFvsG24_SF metabolite expression profiles and VIP values, respectively.

Fig 5: Metabolite expression profiles and VIP values.

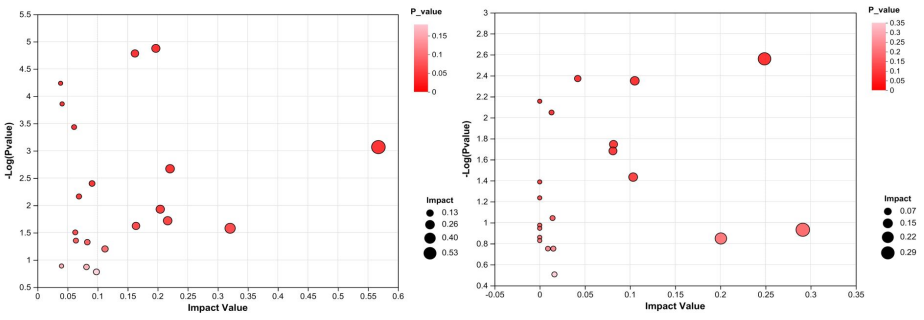


a b

Note: a, b are F24_SFvsG24_SF and G18_SFvsG24_SF differential metabolite volcano plots, respectively.

Fig 6: Volcanic map of differential metabolites.

acetylneuraminic acid (Neu5Ac), also known as sialic acid, is an acetylated derivative of the acidic sugar neuraminic acid, Neu5Ac enhances intestinal absorption of minerals and vitamins and promotes bone development; and Neu5Ac has also been associated with neurotransmission, promotion of brain development, cosmetic whitening, promotion of the growth of probiotics, anti-tumor, anti-viral or bacterial infections and anti-Alzheimer's disease, among other functions (Bavaresco *et al*., 2008).Studies have shown that Neu5Ac enhances immunity in mice. Neu5Ac prevents HFD-induced hyperlipidemia in rats by regulating lipid-associated and coagulation-associated genes, which in turn induce changes in metabolites and proteins (Yida *et al*., 2015). Hypoxanthine, also known as 6-hydroxypurine, which can be obtained biologically by the removal of one amino group from adenine, is an important alkaloidal purine that is widely distributed in the body and is involved in the regulation of the body's physiological functions. Hypoxanthine may be used to assess health status (Mahanty and Xi, 2020). Arginine is an essential amino acid for young animals, which can regulate protein synthesis and metabolism, promote growth and



Note: a, b are KEGG pathway enrichment bubble plots for F24_SFvsG24_SF and G18_SFvsG24_SF, respectively.

Fig 7: KEGG pathway enrichment bubble map.

Table 6: Details of differences between G18_SF and G24_SF groups.

Metabolite	Regulate	Mode	P_value	FDR
2-Pentyl-3-phenyl-2-propenal	up	pos	6.22E-05	0.01123
N-Acetylneuraminic acid	up	neg	0.0002424	0.01246
Palmitoleamide	up	pos	0.0003595	0.02995
Citreoviridin C	up	neg	0.0006624	0.02131
5-Isopropyl-2-(2-methylpropyl)-2-cyclohexen-1-one	up	pos	0.001637	0.06409
Heptadecanoyl carnitine	up	neg	0.001924	0.04082
Nerolidol	up	pos	0.002193	0.07493
Sambutoxin	down	neg	0.002983	0.05389
Sinomenine	up	pos	0.00396	0.1041
Oleoylcarnitine	up	pos	0.006702	0.1278

Table 7: G24_SFvsF24_SF (top 10) KEGG pathway enrichment table.

Pathway_ID	Match_status	Pathway desciption	P_value	P_adjust
map00250	3|28	Alanine, aspartate and glutamate metabolism	0.00085694	0.005248758
map00590	2|37	Arachidonic acid metabolism	0.026319124	0.067875636
map00471	2|10	D-Glutamine and D-glutamate metabolism	0.002136784	0.0116336
map00010	2|31	Glycolysis/Gluconeogenesis	0.019143212	0.055177493
map00330	3|72	Arginine and proline metabolism	0.011779809	0.04122933
map00220	4|23	Arginine biosynthesis	1.33E-05	0.000326156
map00030	2|35	Pentose phosphate pathway	0.023836276	0.064887641
map00970	5|52	Aminoacyl-tRNA biosynthesis	1.65E-05	0.000269315
map00240	2|62	Pyrimidine metabolism	0.062980709	0.128585615
map00620	1|27	Pyruvate metabolism	0.165903637	0.246341764

Table 8: G18_SFvsG24_SF (top 10) KEGG pathway enrichment table.

Pathway_ID	Match_status	Pathway desciption	P_value	P_adjust
map00250	1|28	Alanine, aspartate and glutamate metabolism	0.116981185	0.194968642
map00232	2|18	Caffeine metabolism	0.002753068	0.034413348
map00500	1|35	Starch and sucrose metabolism	0.141655233	0.196743379
map00220	2|23	Arginine biosynthesis	0.004460699	0.027879367
map00330	2|72	Arginine and proline metabolism	0.036867796	0.102410544
map00564	2|48	Glycerophospholipid metabolism	0.017956646	0.064130879
map00970	2|52	Aminoacyl-tRNA biosynthesis	0.020765272	0.064891475
map00230	3|81	Purine metabolism	0.004244558	0.035371318
map00520	1|107	Amino sugar and nucleotide sugar metabolism	0.31144922	0.299470403
map00052	1|46	Galactose metabolism	0.177093532	0.215967722

development, improve intestinal development and the number of intestinal villi and increase the immunity of animals (Nieves and Langkamp-Henken, 2002; Peranzoni *et al*., 2007). It was shown that feeding 1.15% L-arginine to ZDF rats significantly reduced abdominal and perirenal fat in rats (Clapés and Rosa Infante, 2009; Fu *et al*., 2005). Therefore, it is hypothesized that the nutritional value of yaks housed at 24 months of age may be somewhat higher; and the above differential metabolites may affect fat deposition in yaks, which can be promoted later by regulating them.

Dimethylglycine, niacinamide and glutamine were lower than in the housed group. Dimethylglycine (DMG) is a chemical complex that appears in the pathway of choline to glycine metabolism and has the emulsifying effect of a surfactant, which can effectively emulsify oil-water mixtures, break down fat particles in the mixtures, promote the chemical action of digestive enzymes in the intestinal tract and increase the contact area between the villi of the small intestine and the nutrients to promote the digestive absorption of the nutrients. Nicotinamide (NAM) is the amide form of vitamin B3.NAM can regulate fat metabolism, enhance body immunity and promote fat deposition in lambs; it was found that peri rumen nicotinamide can promote the growth and development of fattening lake sheep and increase the average daily weight gain; and Li suggested that NAM, by inhibiting the activity of SIRT1 dehydrogenase to promote lipid synthesis in animals (Li *et al*., 2015). Glutamine (Gln) is a non-essential amino acid, on the one hand, it can promote the proliferation of intestinal cells and intestinal development, enhance the activity of small intestinal enzymes and promote the absorption of nutrients; on the other hand, it can promote the proliferation of immune cells and improve the immunity of the body.It was found that the addition of 0.8% Gln significantly increased the mean daily feed intake and mean daily weight gain of 1- and 4-week-old chicks; the addition of Gln to the diet increased the mean daily weight gain and apparent digestibility of weaned piglets (Hwang *et al*. 2015). The above DMG, hypoxanthine, NAM, Gln and GlcN all promoted fat deposition, which was lower in the G24_SF group, presumably because yaks are in a cold environment and the body must provide sufficient energy to resist the cold, resulting in excessively low fat deposition compared to housed yaks.DMG, NAM, arginine, Gln, GlcN (Shmagel *et al*., 2019; Bowman and Wolfgang, 2019), PRO, CAR (Salem *et al*., 2015; Brown *et al*., 2014; Bao *et al*., 2015), AA (Demetz *et al*., 2014; Zheng and Hasegawa, 2016) and Neu5Ac all improved the immunity of yaks and were lower in the G24_SF group. Lower temperatures in the cold season on the Tibetan Plateau, a decrease in drinkable water resources and an uneven distribution of pasture grasses resulted in a lower nutrient intake by yaks during the cold season, which reduced the immunity of yaks. Therefore, grazing may affect the expression levels of the above differential metabolites, which in turn affects fat deposition and immunity in yaks; secondly, the nutritional value of housed yak meat is a little bit better compared to grazed yak meat.

KEGG enrichment pathway analysis

The Alanine, aspartate and glutamate metabolism pathways are thanked as significantly different metabolic pathways common to G24_SF, F24_SF and G24_SF. The metabolites of alanine (Ala) can provide the body with carbon shelf, nitrogen source and energy. Aspartate (Asp) not only regulates the immune system, but also plays an important role in the proliferation of immune cells. Glutamate (Glu) regulates normal physiological activities such as cell proliferation, differentiation, secretion and contraction through glutamate receptors (Skerry and Genever 2001; Newsholme *et al*. 2003). Glutamine is synthesized from glutamate, NH3 and ATP in skeletal muscle by the action of glutamine synthetase. It has been found that glutamine promotes the cytokine TNF to enhance immunity (Calder and Yaqoob 1999). Differential metabolites enriched to this pathway may be related to fat deposition, immunocompetence and growth and development in yaks and are important for resolving the mechanisms of yak adipocyte proliferation and differentiation as well as immunoregulation.

In summary, these metabolites regulate fat metabolism and may promote fat deposition by inhibiting

adipocyte proliferation, promoting intestinal development and increasing nutrient absorption. Therefore, different feeding practices as well as different months of age will affect fat deposition in yaks and these significantly enriched pathways and differential metabolites will be the main metabolites and metabolic pathways that are highly affected during yak growth and development.

CONCLUSION

Using yak dorsal subcutaneous fat as test material, non-targeted metabolomics was used to investigate the effects of different feeding methods on fat deposition in yaks during the cold season and to further determine the changes of differential metabolites during production in different feeding methods and at different months of age, with the main conclusions as follows:

Feeding practices as well as growth and development may directly affect the type and amount of subcutaneous fat metabolites in yaks. Metabolic pathways of subcutaneous fat deposition in yaks are influenced by different feeding practices and different months of age. Differential metabolites such as 2-pentyl-3-phenyl-2-propene and N-acetylneuraminic acid may affect fat deposition in yaks, which could be promoted later by modulating them. Yak cold-season fattening can effectively improve the problem of yak cold-season fat loss and can promote the efficient production and fat deposition of yaks and improve the quality of yak meat.

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The animal welfare statement

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received.

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Institutional review board statement

The animal handling procedures in this study were approved by the Ethics Committee for the Use of Laboratory Animals, College of Animal Husbandry and Veterinary Science, Qinghai University (Permit No.2023-QHMKY-001).

Informed consent statement

Not applicable.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of interest

The authors declared that they had no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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