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Gangba sheep in the Tibetan plateau: Validating their unique meat quality and grazing factor analysis

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ARTICLE INFO

Article history:

Received 26 September 2019

Revised 17 June 2020

Accepted 18 June 2020

Available online 24 August 2020

Keywords:

Gangba sheep

Mutton quality

Amino acids

Fatty acids

Mineral elements

ABSTRACT

Gangba sheep are known for having typical sensory characteristics attributed to free range conditions and grazing on wild plants. The genuine Gangba mutton was selected as the experimental group, and the commercial Tibetan mutton was selected as the control group, the nutritive composition of basic chemical components, amino acids and fatty acids in mutton were investigated to correlate its unique meat quality and eating satisfaction. The results showed that fatty acids were significantly higher ($P < 0.05$) in Gangba mutton than in commercial mutton, and the higher content of flavoring amino acids (glutamic acid and aspartame) were primarily responsible for the taste attributes umami of meat juices. Moreover, the trace elements analysis in mutton and grazing factors (forage, water source and soil) were conducted, to explain the source of essential trace elements in mutton. The concentrations of essential trace elements show that the Gangba mutton was a valuable source for highly available Cu and Zn in human nutrition, and well managed with few detected of toxicity metal. The concentrations of essential trace elements in mutton are closely related to the trace elements in environmental grazing factors. In conclusion, the congenital grazing conditions (a highly mineralized water resource, natural forages and clean soils) were shown to contribute to the unique meat characteristics of Gangba sheep.

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Introduction

The global demand for safe and high-quality mutton has increased dramatically in recent years. Mutton is an excellent source of essential micronutrients, contributing to physiological and biochemical function in humans. Nutritionally, mutton is typically higher in saturated fatty acids, with sheep-like flavor, which overshadow its nutritional value and influences the temptation to consumers (Abdalla Filho et al., 2017; Arsenos et al., 2006).

However, in the Tibetan Plateau, mutton is one of the high consumption meat products, due to the meat flavor, high nutritional

value, low levels of contaminants. These unique sensory quality are closely related to the distinct environment and natural grazing system. Gangba sheep is considered as the typical sheep in Tibet. Their feeding process is still that of natural grazing. The geographical isolation, cool climate, unpolluted air condition, unfertilized grazing pastures and sufficient mineral water sources in the Tibetan Plateau make it ideal for sheep breeding with low levels of contaminants. Gangba mutton is extremely tender, and juicy and has a smooth texture; additionally, this mutton has an exceptionally delicious taste without a sheep-like flavor. Confidence in the unique sensory quality, in combination with the natural grazing of the sheep, leads to consumers being willing to purchase it. Currently, the Gangba sheep industry has no system that accurately measures quality characteristics that are indicative of eating satisfaction.

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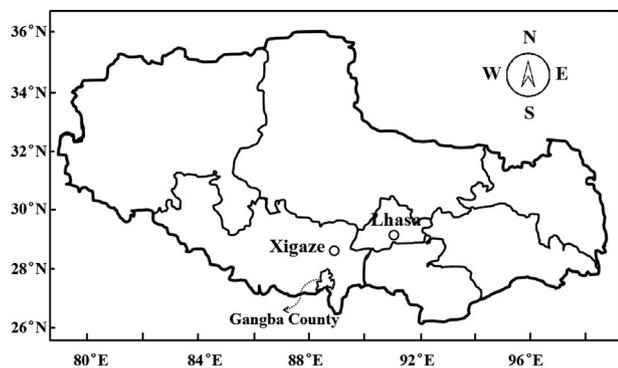


Fig. 1 – The location of Gangba County.

In general, eating satisfaction is characterized by the combination of tenderness, flavor and juiciness. The sensory quality of meat is influenced by intrinsic (gender, genotype) and extrinsic (diet) factors, which are generally associated with geographic regions of origin. In regard specifically to flavor, the flavor intensity is altered depending on the types of both forage and grain consumed. It is reported that sheep fed primarily on grass-based forage has a pastoral flavor (Gkarane et al., 2019). The effect of diet on the sensory characteristics of mutton has been previously investigated (Almela et al., 2010; Gkarane et al., 2019; Watkins et al., 2013). As demonstrated, dietary differences between regions has a significant effect on the sensory profiles and fatty acid composition of mutton (Erasmus et al., 2016, 2017). Researchers have mainly focused on nutritional strategies via diet to improve meat quality (Brand et al., 2018; Teixeira et al., 2011; Turner et al., 2014). The purpose of this work was to investigate how the breeding environment and diet impacts mutton quality. To date, there is no clear conclusion about the unique qualities of Gangba mutton, and no complete research has revealed the scientific reason explaining the eating satisfaction of Gangba mutton, but there are some speculations: (1) the water drunk by Gangba sheep is highly mineralized (Can et al., 2017); (2) natural forages eaten by Gangba sheep are mineral-rich; and (3) the Gangba sheep species have been selectively bred and cultivated over several years.

Accordingly, the main objective of this research was to evaluate the nutritional index (including amino acids, fatty acids and trace elements) of Gangba sheep raised in grazing systems. The environmental breeding factors (water source, soil and forage) were selected to determine the mineral elements that may explain the source of essential trace elements in mutton. Our study was expected to provide a scientific, theoretical basis for the development and utilization of high-quality sheep resources in Tibet.

1. Materials and methods

1.1. Sample collection and grazing systems

The samples were collected from Chana Village (88°35'E; 28°19'N), Gangba County in December 2017. The location of Gangba County is shown in Fig. 1. Gangba sheep was selected as normal ram under natural grazing. It weighed approximately 25 kg and was healthy and disease-free. After slaughtering, the longissimus dorsi, forelegs and gluteus medius muscles were collected under sterile conditions and divided into small pieces of approximately 200 g into zippered polyethylene bags in boxes with ice. After collection, duplicate muscle samples were transported to the laboratory, freeze-dried, ground and stored at -20°C until analysis. For comparison, the gluteus medius muscles of commercial sheep were randomly selected and purchased in a local market (Lhasa, Tibet) and used as the control group.

The Gangba County is located in southern Tibet, which is an alpine pasture environment characterized by herbaceous pastures, with an average altitude of over 4700 m. The famous Qudennima glacier is located in Gangba County, which is a source of mineral water. The water drunk by Gangba sheep mainly comes from glacial snow melting. Natural forages eaten by Gangba sheep were meadow grasses like oats, tame grass mixture, kobresia humilis, ryegrass and lucerne, etc. To analyze the source of essential trace elements in Gangba mutton, soil, five meadow grasses and four environmental water samples were collected from the pasture area for element analysis.

1.2. Analysis of basic mutton composition

The homogenized muscle samples were collected and used to determine the chemical composition of the mutton (Brand et al., 2018). The moisture content was obtained by drying 0.3 g sample in an oven at 100°C for 24 hr. The ash content was measured after the dried sample calcined in an oven at 500°C for 6 hr. The fat content was determined from a 5 g sample using the 2:1 (V/V) chloroform/methanol rapid solvent extraction method. The crude protein content was assayed by Kjeldahl determination.

1.3. Analysis of amino acids in the mutton

In general, acid hydrolysis is the most common method used for hydrolyzing protein samples before amino acid analysis, according to the procedure described by Cai et al. (2010). Briefly, the homogenized muscle (0.1 g) was placed into a tube, followed by adding 40 mL of 0.6 mol/L HCl (Merck, Germany) for hydrolysis. Afterwards, the sample was frozen in a dry ice bath with acetone (Aladdin, China), flame-sealed, then hydrolyzed at 110°C for 24 hr under vacuum to obtain a completely hydrolyzed product. After hydrolysis, the resultant solutions were dried and dissolved in 0.02 mol/L HCl for amino acid analysis using ion-exchange chromatography coupled to an automatic amino acid analyzer (L-8900, Hitachi, Japan).

1.4. Analysis of fatty acids in the mutton

The fatty acid composition was based on a previously published solvent extraction procedure with minor modifications (Flakemore et al., 2017). Briefly, the homogenized muscle (1 g) was progressively added to 10 mL chloroform/methanol 2:1 (V/V). Then, the mixture was vigorously shaken for 2 hr. Afterwards, the mixture was filtered, and the aqueous layer was removed. The extract was then dried using a rotary evaporator.

The preparation of fatty acid methyl esters for fatty acid analysis was carried out in accordance with a previously reported procedure (Kalbe et al., 2018). Briefly, the above lipid extracts were redissolved in 300 μ L toluene (containing 17:0 methyl ester as an internal standard), and a 25 mg aliquot was used for methyl ester preparation. Next, 2 mL of 0.5 mol/L NaOH/methanol solution was transferred to the mixture. Then, the methyl esterification was carried out at 60°C for approximately 1.5 hr. Subsequently, 1 mL of 14% boron trifluoride in methanol was added to the mixture, mechanically shaken for 10 min at 60°C, and combined with 2 mL saturated NaHCO_3 . The fatty acid methyl esters were extracted twice in 2 mL *n*-hexane to achieve complete extraction. The final *n*-hexane extracts were dried with Na_2SO_4 and K_2CO_3 (10:1, W/W). The fatty acid methyl esters were resuspended in 100 μ L *n*-hexane and stored at -18°C until gas chromatographic analysis. The above mentioned solid reagents and solvents were of analytical grade and purchased from Aladdin Corporation (Shanghai, China).

1.5. Analysis of trace elements

In the digestion procedure, the collected samples (soil, plant and mutton) were sequentially dried, ground, sieved and stored in sealed

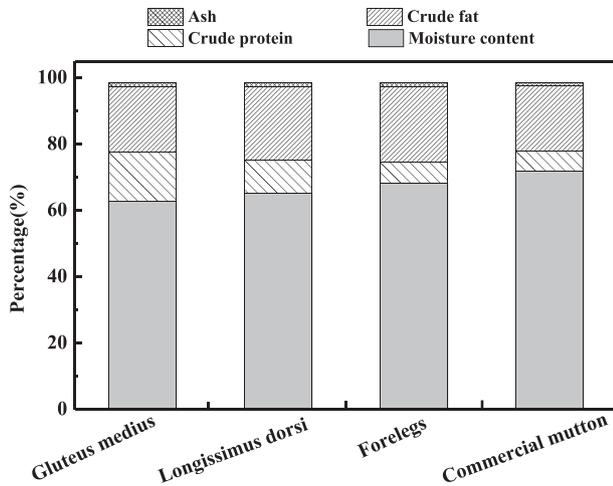


Fig. 2 – The percentage of basic chemical components in the mutton samples.

bags. A total of 0.2 g of each of the above mentioned samples were weighed and transferred into digestion vessels, followed by the adding of 5 mL concentrated analytical grade HNO_3 (Merck, Germany) and 1 mL H_2O_2 (Beijing Chemical Company, China). Additionally, soil samples were combined with 1 mL hydrofluoric acid. Afterwards, the vessels were maintained at 85°C for 30 min to predigest, followed by microwave digestion (Marsx, CEM, USA). The digestion procedures were set up as recommend by Shao et al. (2016). After digestion, the obtained solutions were diluted with deionized water (Millipore, Bedford, USA) and centrifuged. The supernatant was measured by ICP-MS (ELAN DRC-e, Perkin Elmer, USA) for trace elemental analysis.

1.6. Quality control

The accuracy of the proposed method for element analysis was validated only in this work. Specifically, both spinach and sediment certified reference materials (CRMs) were analyzed after digestion. All measurements were obtained after subtracting the blank values. For all CRMs, the determined values were in good agreement with the certified values (Appendix A Table S1). As seen, the recoveries for all elements in the spinach and sediment were in the range of 73.6%-107.6% and 74.5%-107%, respectively, indicating that the proposed procedures for analysis the elements were accurate.

1.7. Statistical analysis

The obtained data were performed by multiple statistical analyses using SPSS 19.0 software. Spearman correlation was applied to find the correlation between the concentrations of minerals in the mutton. In all analyses, statistical significance was assumed at $P < 0.05$.

2. Results and discussion

2.1. Evaluation of nutritional composition

2.1.1. Basic composition

The contents of basic chemical components present in the tested samples are depicted in Fig. 2. The muscles of Gangba sheep had the unique advantage of higher ($P < 0.05$) protein and lower ($P < 0.05$) moisture contents than those of the commercial mutton. Moreover, it is worth mentioning that the moisture content in Gangba mutton was nearly 10% lower than that of goat meat (Ivanovic et al., 2016). However, the average crude fat content in Gangba mutton was higher ($P < 0.05$) than that of commercial mutton. It is generally accepted that the juiciness of meat is positively related to the intramuscular fat

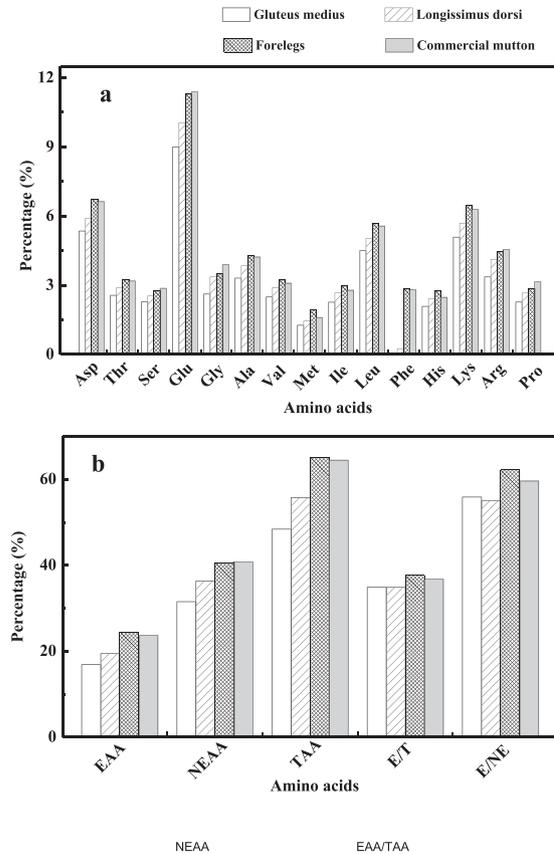


Fig. 3 – The contents of single amino acid (a) and calculated amino acids (b) in the mutton samples. EAAs: essential amino acids; NEAAs: nonessential amino acids; TAAs: total amino acids; E/T: the ratio of EAAs/TAAs; E/NE: the ratio of EAAs/NEAAs.

content, with very low levels of intramuscular fat resulting in dry and less flavorful meat (Hocquette et al., 2010). The accumulation of fat between the muscle fibers of Gangba sheep was sufficient, which is one of the reasons for its superior juiciness.

2.1.2. Amino acids

The amino acid profile and composition in mutton is an important index for evaluating meat flavor, tenderness and taste, and directly affects the nutritional value of meat protein (Watkins et al., 2013). As presented in Fig. 3, six essential amino acids (EAAs) and nine nonessential amino acids (NEAAs) were detected. Nutritionally, EAAs are essential for function, not synthesized in the human body, and must be obtained from the diet (Wu, 2010). According to the FAO/WHO model (FAO/WHO/UNU, 1985), for better quality proteins, the ratio of EAAs/total amino acids (TAAs) should be approximately 40%, and that of EAAs/NEAAs should be over 60%. The EAAs/TAAs and EAAs/NEAAs ratios of the commercial mutton were 36.8% and 59.6%, respectively. However, the EAAs/TAAs and EAAs/NEAAs ratios of the foreleg muscles of Gangba mutton were 37.6% and 62.2%, respectively, there is no significant difference between the foreleg muscles of Gangba mutton and commercial mutton ($P > 0.4$). And the both Tibetan mutton were in accordance with the ideal model of the FAO/WHO, indicating that the protein composition of the mutton was reasonable and indicative of a high-quality protein source.

Surprisingly, the content of glutamic acid (Glu) in Gangba mutton and in the commercial mutton was more than 10%, which was significantly higher ($P < 0.01$) than that of other mutton reported in the literature (less than 2.7%) (Xie et al., 2018). As an amino acid flavor component, Glu- and aspartame (Asp) are the most important

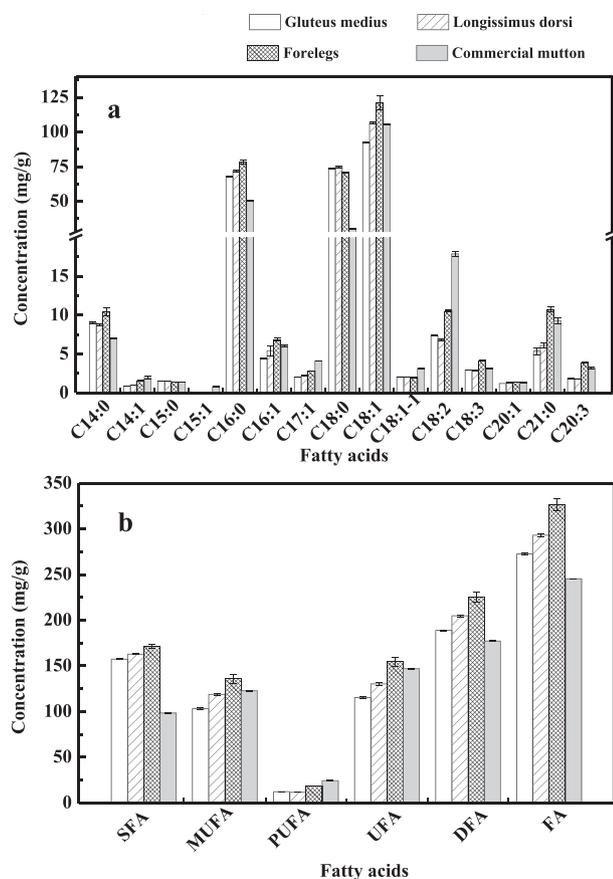


Fig. 4 – The contents of single fatty acid (a) and calculated fatty acids (b) in the mutton samples. SFAs: saturated fatty acids; MUFAs: monounsaturated fatty acids; PUFAs: polyunsaturated fatty acids; UFAs: unsaturated fatty acids; DFAs: desirable fatty acids; FAs: fatty acids.

source of food flavor and play an important role in improving the taste of mutton (Watkins et al., 2013). The higher the flavor component content, the more delicious the mutton will taste. In addition, Glu- has a good regulating effect on nerve degradation, is used in the treatment of gastric diseases and protects liver function (Wong et al., 2011). The high content of flavoring amino acids in Gangba mutton is an important reason for its unique delicious taste. The reasons are related to grazing intensity and forages quality. Gangba sheep walk long distances to obtain their daily dietary requirements, thus increasing the amount of ATP in muscles, so the ability of synthesizing flavor substances can be improved. On the other hand, Gangba sheep are fed on high quality natural forages in the whole feeding process, the muscles are rich in antioxidants, the integrity of tissue and cell membrane is well after slaughter, and the loss of flavor substances is reduced (Luo et al., 2019).

2.1.3. Fatty acids

It is worth emphasizing that pasture-fed sheep have higher levels of polyunsaturated fatty acids (PUFAs) than those of sheep raised indoors and are more conducive to consumer health (Aurousseau et al., 2007). The composition of fatty acids is an important indicator for evaluating meat flavor and nutritional quality. Unquestionably, research has shown that 4-ethylcatanoic fatty acid is mainly responsible for the sheep-like flavor (Ivanovic et al., 2016). The contents of individual fatty acids in the muscles were identified and are displayed in Fig. 4a. The main constituents of the fatty acid profile were oleic (C18:1), palmitic (16:0), and stearic (18:0) acids, constituting approxi-

mately 70% of the total fatty acids, which is consistent with the fatty acid profile in Australian lamb (Flakemore et al., 2017).

Attractively, as illustrated in Fig. 4b, the profile of total fatty acids in Gangba mutton is significantly higher ($P < 0.01$) than that in commercial mutton. However, excessive intake of saturated fatty acids (SFAs) can increase the level of cholesterol in the body, which may cause cardiovascular disease. While C18:0 is generally considered neutral, it is also beneficial for lowering cholesterol (De Abreu et al., 2019). The SFAs content in Gangba mutton was higher than that in commercial mutton, and the neutral component C18:0 was significantly higher ($P < 0.01$) than in commercial mutton. In contrast, unsaturated fatty acids (UFAs = MUFAs + PUFAs) are considered hypocholesterolemic and beneficial to human health. The UFAs content in the gluteus medius muscle of Gangba mutton was the highest. Combining the quantity of monounsaturated fatty acids (MUFAs) and PUFAs, the average UFAs content in the Gangba mutton was comparable to that of the control group. Markedly, the PUFAs content in the mutton was significantly lower ($P < 0.05$) than in the commercial mutton. It should be further emphasized that low levels of PUFAs have a positive effect on juiciness because high PUFAs levels are often associated with lipid oxidation and a rancid taste in meat (Saudo et al., 2013). Additionally, the total amount of desirable fatty acids (DFAs = MUFAs + PUFAs + C18:0) was calculated according to Landim et al., (2011). The DFAs content in Gangba mutton was significantly higher ($P < 0.01$) than in the control group. Ruminants that finish on pasture can lead to a more reasonable and desirable fatty acid profile (Raes et al., 2004). The Gangba sheep finishing permanently on pasture leads to a reasonable fatty acid profile and high nutritional value.

2.2. Evaluation of minerals and trace elements

2.2.1. Minerals and trace elements in mutton

The mineral content in the mutton can provide an indication of the nutritional status of the animals. In general, minerals are essential for maintaining the metabolic activity of several biochemical pathways, and individuals obtain required minerals through a balanced diet. The Gangba sheep were adequately supplied with mineral-rich diets from the foraged grass. As depicted in Table 1, the concentrations of Mg, Ca and Cr in the Gangba mutton are significantly higher ($P < 0.01$) than that in control group. Similarly, the observed results show that the concentrations of the minerals were close to values reported for lambs in the literature (Islam et al., 2015; Xie et al., 2018; MacLachlan et al., 2016; Sezgin et al., 2011). It was found that Gangba mutton is rich in minerals nutrition. Moreover, determination of the toxic metal concentrations in mutton is important for assessing the nutritional status of the animals and the contaminant intake by humans (MacLachlan et al., 2016). For trace elements, heavy metals such as Pb, Cd, Ni, As, Co and Sb, which are not needed by the human body, were not detected, while the trace elements such as Zn, Mn, Cu and Cr, which are needed by the human body, were detected in the mutton. Additionally, Gangba mutton is a valuable source of highly available Cu and Zn in human nutrition. The contents of major and trace elements in muscles exhibited marked differences between different regions, which are closely related to sheep grazing factors such as forage grasses, soil and water resources (Bellof et al., 2006, 2007; Erasmus et al., 2017).

2.2.2. The source of minerals and trace elements in mutton

Water and forage grasses are the main ways for sheep to uptake minerals. Diet based on mineral-rich forage can lead to mineral accumulation in sheep tissues, ultimately, affect the quality of animal meat (Cazarotto et al., 2019). Thus, determining the level of conventional mineral elements in forage grasses, soil and water is of great significance for explaining the source of essential trace elements in mutton.

As shown in Table 1, clearly, the forage grasses are rich in K (10,083–31,595 mg/kg), Mg (1261–2784 mg/kg), Ca (4730–37,083 mg/kg), Mn (23–215 mg/kg), Cu (2.9–4.3 mg/kg), and Zn (13–23 mg/kg). As expected, the same phenomenon was seen in the Gangba mutton. The concentrations of mineral elements in the forage grasses and “the

Table 1 – The average concentrations of minerals and trace element (mg/kg) in the mutton and grazing factors.

	Sample	K	Mg	Ca	Ba	Na	Cr	Cu	Mn	Ti	Zn
Mutton	Gangba mutton	3858	358	879	3.1	N.D.	0.37	0.92	0.24	0.56	24.4
	Commercial mutton	2923	170.8	124	0.08	N.D.	0.07	0.82	0.14	0.19	48.8
Water	River water-1	2.5	14.8	25.0	0.005	46.5	0.025	N.D.	N.D.	N.D.	N.D.
	River water-2	2.0	11.0	21.4	0.005	41.5	0.022	N.D.	N.D.	N.D.	N.D.
	Mineral water	5.1	1.1	23.8	0.004	2.2	0.016	N.D.	N.D.	N.D.	N.D.
	Hot spring water	20.3	1.0	6.3	0.075	400.7	0.32	0.006	N.D.	0.01	N.D.
Soil Forages	Surface soil	13266	1802	94497	198	6489	6.60	1.38	213	338	8.21
	Oats	12597	1261	4730	6.78	N.D.	7.74	3.28	23.4	6.36	12.84
	Tame grass mixture	18781	2468	7867	10.7	N.D.	17.94	4.25	215.4	6.72	22.86
	Kobresia humilis	16774	1634	18875	9.60	N.D.	10.86	3.26	52.9	7.74	12.60
	Ryegrass	31596	1848	37083	23.0	N.D.	43.14	4.27	70.8	30.12	14.22
	Lucerne	10084	2784	21416	13.2	N.D.	34.32	2.85	52.6	30.18	22.26
	Enrichment factor	From water to Gangba mutton	516.1	51.3	46.0	139.3	–	3.9	613.3	–	224.0
From forages to Gangba mutton		0.2	0.2	0.05	0.2	–	0.02	0.3	0.03	0.04	1.4

N.D.: not detected. -: result cannot be calculated.

Table 2 – Correlation matrix of the concentrations of minerals in the mutton.

	K	Mg	Ca	Ba	Cr	Cu	Mn	Ti	Zn
K	1								
Mg	0.421	1							
Ca	0.150	0.960*	1						
Ba	0.155	0.961*	0.972**	1					
Cr	0.241	0.982*	0.994**	0.995**	1				
Cu	0.665	0.041	-0.183	-0.176	-0.077	1			
Mn	0.482	0.839	0.748	0.753	0.810	0.503	1		
Ti	0.472	0.997**	0.940	0.941	0.969*	0.111	0.867	1	
Zn	-0.592	-0.557	-0.449	-0.447	-0.453	0.207	-0.140*	-0.549	1

* Correlation is significant at the 0.05 level (2-tailed);

** Correlation is significant at the 0.01 level (2-tailed).

specific commercial concentrate (pelleted) for lambs” were compared (Cazarotto et al., 2019). Except for Cu, Co and Zn, the mineral content in some forage grasses was higher than that in “the specific commercial concentrate for lambs”. Therefore, the forage grasses were considered mineral-rich. Compared with the maximum contamination levels of heavy metals in soil stipulated by the US Environmental Protection Agency (Duruibe et al., 2007), the level of heavy metals in the grassland soil was almost no pollution. A clean soil environment provides a prerequisite for high-quality water sources and pasture grasslands. As one of the grazing factors, water plays an indispensable role in the survival of plants, animals. Therefore, the representative water utilized by Gangba sheep were analyzed for elements analysis. As displayed in Table 1. Surprisingly, both river and hot spring water samples contained high concentrations of sodium that were, much higher ($P < 0.05$) than those of other water bodies in Tibet that have been reported in the literature (Xiang et al., 2009). Gangba sheep obtain saltwater through free-grazing processes. For hot spring water, the concentrations of K and Na were significantly higher ($P < 0.01$) than those in the river water. In Tibet, hot spring water usually using as gastric lavage water for sheep breeding. During the breeding process of Gangba sheep, herdsman wash their stomachs 2–3 times per year using hot spring. Therefore, sheep can supply adequate minerals and trace elements by drinking saltwater. Also, this process prevents diseases, improves the feed conversion rate and enhances the ketone body mass for Gangba sheep. A sufficient saltwater supply and suitable water for gastric lavage are the reasons for the delicious taste of mutton without a sheep-like flavor.

In order to further explore the source and the uptake pathway of trace metal elements in Gangba mutton, the enrichment factor from

forages/water to mutton were calculated and displayed in Table 1. For the enrichment factor from water source to mutton, the enrichment difference among the elements changed greatly from 3.9 (Cr) to 613.3 (Cu). However, the enrichment factor from forages to mutton, the enrichment factor for Zn was the highest (1.4), followed by K, Mg, Ba and Cu (0.2–0.3), and Ca, Cr, Mn and Ti were the lowest (0.02–0.05). In conclusion, Zn and Mn in mutton were mainly from forages, and Ca, Cr, and Ti were mainly from drinking water, while K, Mg, Ba and Cu were from drinking water and forages. Mineral content is closely related to meat quality (Cazarotto et al., 2019). Specifically, increasing dietary Ca can accelerate and improve muscle tenderness (Clare et al., 1997). Cazarotto et al. (2019) demonstrated that mineral supplementation with Zn, Cu, and Mn was capable of improving meat quality through the prevention of oxidative processes. Moreover, dietary Mn could improve the carcass quality of animals and reduce the visceral fat content (Holen et al., 2018). Consequently, Gangba sheep fed mineral-rich diets based on forage grass and water are the premise for the production of high-quality meat.

2.2.3. The correlation analysis in mutton

Moreover, the significant correlations of the minerals in mutton was conducted and summarized in Table 2. Significant correlations among the minerals suggest the common sources of the related elements. There are also some correlation characteristics between the minerals, significantly positive correlations were found among the concentrations of trace metals such as Ba, Mg, Ca, Cr and Ti, ($P < 0.05$), indicating that they have certain homology. As the result of element analysis in the forage grasses and water, these elements were detected only in the forage grasses, indication that these elements in

the mutton are mainly for forage grasses. Zn, Cu and K have a significant negative correlation with Ba, Mg, Ca, Cr and Ti, suggestion that the mutual inhibition between these heavy metals.

3. Conclusions

Gangba sheep are born, reared and slaughtered in a specified region of origin, fed a pasture-specific diet, have distinctive quality characteristic that are generally linked to the sheep breed, grazing system and diet. Gangba mutton is a valuable source of not only essential amino acids and fatty acids but also of Zn and Cu in human nutrition. In addition, the concentrations of trace elements showed that Gangba sheep were well managed with few cases of deficiency or toxicity detected. Moreover, the low content of PUFAs contributes to the mutton juiciness, and the high content of flavor-contributing amino acids leads to its exquisitely delicious taste. Further research is required to establish the influence of geographical environment on meat quality.

Acknowledgments

This work was supported by the High Level Training Programs for Postgraduates of Tibet University (No. 2017-GSP-018), the Reformation and Development Funds for Local Region Universities from China Government, the Project of the Open Fund of the State Key Laboratory of Environmental Chemistry and Ecotoxicology (No. KF2017-07), the Tibet University 2018, 2019 Central Financial Support Special Funds for Local Colleges and Universities (Nos. [2018]54, [2019]19), Tibet Economic and Social Development and Plateau Scientific Research Co-construction Innovation Fund (Nos. lzt2020002, lzt2020002).

Appendix A. Supplementary data

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2020.06.024.

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