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Chemical and Fatty Acids Composition of Yak Milk from Qinghai-Tibetan Plateau in Different Seasons

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ABSTRACT

Background: Yak milk is the main source of dietary protein, fat and micronutrients for Qinghai-Tibetan Plateau residents. However, few studies have focused on the seasonal variation of nutritional components in natural grazing (without supplementary feeding) yak milk.

Methods: Yaks living at high altitudes (average altitude of more than 4200 metre) were selected (summer, n=18; winter, n=18; spring, n=15) to analyzed seasonal influence on chemical and fatty acids composition of yak milk.

Result: Results showed that the contents of fat, protein, lactose, non-fat milk solid, vitamin A and the main fatty acids ($C_{6:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{20:0}$, cis-9 $C_{16:1}$, cis-9 $C_{18:1}$, trans-9 $C_{18:1}$, LA, *etc*.) in winter yak milk were significantly different from those in summer and spring yak milk ($p<0.05$). This suggests that chemical and fatty acids composition of yak milk were influenced by seasons.

Key words: Fatty acid, Protein, Season, Yak milk.

INTRODUCTION

Yaks (*Bos grunniens*) live in the high reaches of the Himalayan region (Nepalese Himalayas,Indian Kashmir and Mongolia) and the Qinghai-Tibetan plateau (China). People living on the Tibetan Plateau rely on yaks for survival, as the region's native cattle give daily sustenance in the form of milk, butter, cheese, yoghurt and meat (Luming *et al*., 2008; Rhode *et al*., 2007).

Guoluo Tibetan Autonomous Prefecture is located in the southeastern part of Qinghai Province. Its geographic coordinates are 97°54′-121°50′E on the East longitude, 32°31′-35°40′N on the North latitude, with an average altitude of more than 4200 meters. The area of 4000-5000 meters above sea level accounts for about 80% of the whole area. Poor, cold and anoxic, atmospheric oxygen content is only 60% of sea level, annual average temperature is below -1.0°C, extreme minimum temperature is -48.1°C, with typical plateau continental climate characteristics (Shan and Ma, 2018).This area belong to pure pastoral area and the yaks are qinghai plateau yaks (Tao *et al*., 2002), natural grassland grazing with no feeding supplement, yaks in this area can only get enough pasture supplement in summer (June to September), but not in other seasons (Xue *et al*., 2005), which may bring about the differences in nutritional composition of yak milk.

In recent years, most reports on the nutritional components of yak milk focus on yaks living at altitude of 3000-4000 m and focus on the basic chemical indicators (such as protein, fat, lactose and ash) in different regions, breeds and parityies (Bin *et al*., 2011; Zuo-Lin *et al*., 2011; Zhanxing *et al*., 2017; He *et al*., 2011). A lack of reports on natural grazing of plateau yaks on natural grassland at high altitude (average altitude above 4200 m) is noted. In this paper, the effects of seasons on the chemical composition and fatty acids composition of plateau yak milk were analyzed.

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MATERIALS AND METHODS

Collection of yak milk

In this study, 51 yak milk samples (ranches from 6 different counties, traditional management, without fencing in open pastures) were used throughout the experiment and 18 from the yaks grazed naturally in middle later green grass period in the summer (August), 18 from the yaks grazed naturally in the early stages of grass withered period in the winter (November), 15 from the yaks naturally graze in the late grass withered period during spring (May). The yaks were milked once daily at around 0600 to 0800 h and at each milking, all the milk of the yak's breast was squeezed out and milk was sampled. Then, the milk was put into a sterilized container, stored in the freezer within 10 minutes of collection and kept at -20°C until analyzed.

Chemical analysis

Yak milk samples were kept cold during thawing and stirred before analysis. Concentrations of protein, fat, lactose and total solids (TS) were analyzed according to the method describe by He *et al*. (He *et al*., 2011). Fat soluble vitamins A content and calcium(Ca) was analyzed according to the method describe by Duan *et al*. (Duan *et al*., 2021). Fatty acids compositions were analyzed by the method of AOAC Official Method 996.06 (AOAC, 2001).

Statistical analysis

All data were catalogued in EXCEL software (Microsoft Office 2010). Data was analysed by SPSS 21 statistic software using one-way analysis of variance (ANOVA). The least significant difference at a 95% confidence level ($p \leq 0.05$) was set in statistical tests.

RESULTS AND DISCUSSION

Chemical composition of yak milk

Table 1 showed that there were significant seasonal effects on the chemical composition of yak milk. Especially, there are significant differences in the protein content of yak milk in different seasons. In addition, from Table 1, we could also find that fat was the most volatile index of all indicators. There were great differences between individuals in green grass period (summer) and grass withered period (winter and spring). The highest value in summer and spring was nearly three times the lowest value and the highest value in winter was twice the lowest value. It is well documented that the chemical composition of yak milk is affected by altitudes and parities (Bin *et al*., 2011; Zuo-Lin *et al*., 2011; Peng *et al*., 2008; Wu *et al*., 2009). However, there are few data on the chemical composition of yak milk in different seasons in the same area. In the present study, we observed that changes in the grass composition of pasture according to the seasons led to changes in the chemical composition of their milk, especially fats (higher in winter) and proteins (lower in winter and spring). Ding *et al*. (2013) found that fat content of yak milk in winter is higher than that in summer and spring, this result is consistent with ours. The reason that he proposed for this was that yaks increase their fibre intake in winter, resulting in high concentrations of acetate in the ruminal fluid, which is the main substrate for synthesising milk fat. And another possible reason is that during winter (early stages of grass withered period), yaks have a large reserve of fat that can be mobilized for milk secretionis. However, our study has opposite results with Ding *et al.* (2013) concerning the content of protein, our study showed that protein content in winter samples were lower than that in the summer samples. On the other hand, in our study the content of protein of Qinghai yak milk in the same season was higher than that in the Guo's report (5.63 vs 4.79 g/100 g, respectively) (Xian *et al*., 2015).

Concentrations of vitamin A and calcium

Fat soluble vitamin A (VA) and calcium content in different seasons were listed in Fig 1, respectively. ANOVA analysis showed that the content of vitamin A was significantly lower in winter than that in summer and spring (52.7 vs 72.7 µg/100 g, 52.7 vs 75.7 µg/100 g respectively), but the content of calcium was no significant difference in yak milk in different seasons. Moreover, for the vitamin A content, there was a great difference between yak individuals in summer and spring (with larger standard deviations). Studies on fat-soluble vitamins of yak milk are rare, but there are many research achievements on calcium content in yak milk and the reported values of yak milk was higher than holstein cows (about 106 mg/100 g) (McSweeney and Fox ,2009). The present study found a higher VA content compared to those in previous studies, it is about 1.63 times (summer and spring yak milk) and 1.18 times (winter yak milk) than that of white yak milk in Gansu (44.5 µg/100 g) (Hai-Jun *et al*., 2007) and it is about 2.10 times (summer and spring yak milk) and 1.52 times (winter yak milk) than holstein cows (34.7 µg/100 g, data came from brightdairy unpublished laboratory data). The present study also found that the VA content related to different seasons, this might be due to the low vitamin A precursor (β-carotene) in grass in winter (Lindqvist *et al*., 2012) , which affected the synthesis of vitamin A in yaks.

Fatty acid composition

The AOAC method allowed for sensitive analysis of 44 fatty acids by gas chromatography, the results were shown in Table 2. The main fatty acids in yak milk were $C_{6:0}$, $C_{10:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$, cis-9 $C_{16:1}$, cis-9 $C_{18:1}$ and trans-9 $C_{18:1}$. This result was similar to that in the literature (Peng *et al*., 2008; Ding *et al*., 2013; Xian *et al*., 2015; Liu *et al*., 2011; Feng, Hua and Peilei, 2006). For saturated fatty acids (SFA), more than 80% of the total SFA were composed of $C_{14:0}$ (myristic acid), $C_{16:0}$ (palmitic acid) and $C_{18:0}$ (stearic acid). For monounsaturated fatty acid (MUFA), cis-9 $C_{18:1}$ (oleic acid), accounted for 70.6%, 82.9% and 80.5% of total MUFA in summer, winter and spring yak milk, respectively. For

Table 1: Chemical analysis of yak milk obtained at different seasons (Mean values±standard deviations; minimum-maximum in parentheses).

Item	Season		
	Summer (n=18)	Winter (n=18)	Spring (n=15)
Fat (g/100 g)	6.37±1.55 (3.40-9.23)[b]	8.49±1.17 (6.77-11.8)[a]	6.18±1.77 (3.16-10.70)[b]
Protein (g/100 g)	5.63±0.60 (4.56-6.79)[a]	4.91±0.13 (4.62-5.14)[b]	4.33±0.45 (3.56-5.20)[c]
Lactose (%,w/w)	4.86±0.52b (4.20-5.70)[a]	3.79±0.14 (3.60-4.10)[b]	4.88±0.57(3.80-5.50)[a]
Total solids content (%,w/w)	17.57±2.04 (13.60-21.10)[ab]	18.41±1.31 (15.90-21.30)[a]	16.49±1.77 (14.00-21.30)[b]
Non-fat milk solid (%,w/w)	11.20±0.93 (10.00-13.30)[a]	10.31±0.63 (8.89-11.50)[b]	10.31±0.50 (9.47-11.30)[b]
Ash (%,w/w)	0.86±0.09 (0.69-1.00)[a]	0.75±0.05 (0.65-0.82)[b]	0.73±0.06 (0.57-0.82)[b]

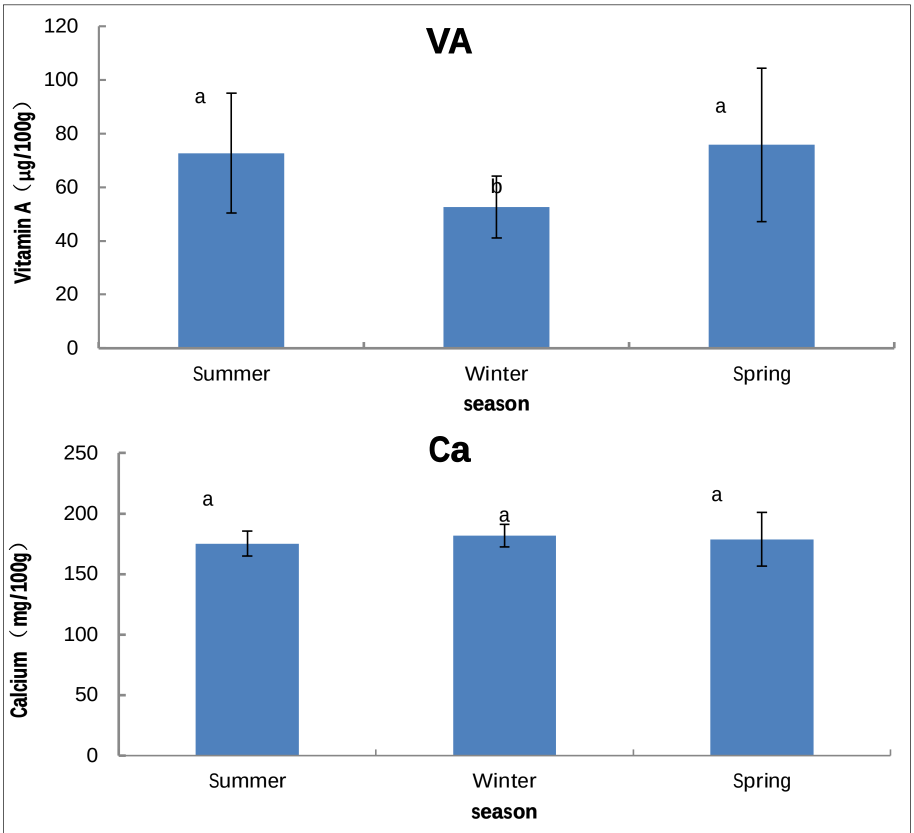
n: Number of samples.

* Means in the same row is significantly different (P<0.05).

polyunsaturated fatty acid (PUFA), cis-9,12 $C_{18:2\ n\text{-}6}$ (linoleic acid, LA), cis-9,12,15 $C_{18:3\ n\text{-}3}$ (linolenic acid, ALA), trans-9,12 $C_{18:2\ n\text{-}6}$ (trans linoleic acid, TLA) were the highest content PUFA, the content of LA and ALA accounts for more than 75% of the PUFA. This value was similar to that found in the yak milk (Li *et al*., 2017) and Tibet yak butter (Feng andPeilei, 2006) from Sichuan and it was higher than that yak milk and holstein milk from Gansu (Yu-jiao *et al*., 2013). For main fatty acids of yak milk, the contents of $C_{6:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{17:0}$, $C_{18:0}$, cis-9 $C_{16:1}$, cis-9 $C_{18:1}$ and LA in yak milk were significant higher in winter than in summer and spring and the main trans fatty acids (trans-9 $C_{18:1}$ and trans-9, 12 $C_{18:2\ n\text{-}6}$) of yak milk were significantly lower in winter than in summer. As for trans fatty acids, the types reported in this paper are slightly different from those reported by Ding (Ding *et al*., 2013) and Liu (Liu *et al*., 2011), but the content change of trans fatty acids in different seasons are the same.

The short- and medium-chain fatty acids (C4-C16) can be synthesised de novo *e.g*. from carbohydrates in the rumen and the metabolism of the animals, but the long-chain fatty acids (C_{18}-C_{24}), such as cis-9 $C_{18:1}$, trans-9 $C_{18:1}$, cis-9, 12 $C_{18:2\ n\text{-}6}$ (LA) and trans-9,12 $C_{18:2\ n\text{-}6}$ cannot be synthesized in vivo, mainly derived from the diet (Or-Rashid *et al*., 2008). The present study showed that the main long-chain fatty acids cis-9 $C_{18:1}$ and LA were higher in winter than in summer and spring and the main long-chain fatty acids trans-9 $C_{18:1}$ and trans-9,12 $C_{18:2\ n\text{-}6}$ were lower in winter and spring than in summer. The result of cis-9 $C_{18:1}$ is consistent with that of Ding (Ding *et al*., 2013) and contrary to that of Liu (Liu *et al*., 2011) and for LA in this paper is contrary to that of Ding and Liu. And, conflicting results may be due to other factors, breed, feeding, natural grass and altitude gradient. Our data also showed the content of LA was higher in the winter yak milk than that in the summer and spring yak milk. The total content of n-6 series PUFAs in yak milk significantly decreased ($p<0.05$) with seasonal changes (summer-winter-spring), while the content of n-3 series PUFAs in summer yak milk and winter yak milk showed no significant difference, but both were significantly higher than in spring. When we further analyzed the n-6 series PUFAs, we found that the reason for the great difference of n-6 series fatty acids was that the trans-9,12 C18:2 n-6 content in summer milk samples was significantly higher than that in winter and spring milk samples and its average value was more than twice that in winter more than triple that in spring.

In the total SFA of yak milk in different seasons, the SFA of winter samples was significantly higher than that of summer and spring samples ($p<0.05$). The MUFA and PUFA levels in yak milk showed no significant difference between summer and winter samples, but both were significantly higher than those in spring samples. In addition, the n-6 PUFA in PUFA showed significant differences between



*Means significantly different, p<0.05 (ANOVA).

Fig 1: Vitamin A and calcium of yak milk from different seasons.

Table 2: Fatty acids composition of yak milk from different seasons (g/100 g, Mean values±standard deviations; minimum-maximum in parentheses).

	Season		
	Summer (n=18)	Winter (n=18)	Spring (n=15)
$C_{4:0}$	ND	ND	ND
$C_{6:0}$	0.12±0.03 (0.07-0.17)^{b}	014±0.02 (0.09-0.21)^{a}	010±0.03 (0.05-0.17)^{c}
$C_{8:0}$	0.06±0.02 (0.03-0.10)^{a}	0.07±0.01 (0.04-0.10)^{a}	0.05±0.02 (0.02-0.11)^{a}
$C_{10:0}$	0.10±0.04 (0.05-0.18)^{a}	0.12±0.02 (0.08-0.18)^{a}	0.10±0.05 (0.04-0.24)^{a}
$C_{11:0}$	ND	ND	ND
$C_{12:0}$	0.08±0.03 (0.04-0.14)^{b}	0.10±0.02 (0.06-0.13)^{a}	0.07±0.04 (0.03-0.20)^{b}
$C_{13:0}$	ND	ND	ND
$C_{14:0}$	0.39±0.10 (0.22-0.56)^{b}	0.53±0.08 (0.40-0.74)^{a}	0.34±0.13 (0.16-0.69)^{b}
$C_{15:0}$	0.08±0.02 (0.05-0.12)^{b}	0.13±0.03 (0.08-0.20)^{a}	0.07±0.03 (0.03-0.12)^{b}
$C_{16:0}$	1.40±0.29 (0.82-1.96)^{b}	2.22±0.34 (1.76-3.06)^{a}	1.29±0.47 (0.64-2.09)^{b}
$C_{17:0}$	0.09±0.02 (0.05-0.11)^{b}	0.14±0.03 (0.10-0.19)^{a}	0.06±0.02 (0.03-0.09)^{c}
$C_{18:0}$	0.95±0.21 (0.59-1.26)^{a}	1.08±0.21 (0.79-1.68)^{a}	0.67±0.26 (0.33-1.22)^{b}
$C_{20:0}$	0.03±0.01 (0.02-0.05)^{b}	0.06±0.01 (0.04-0.08)^{a}	0.03±0.01 (0.01-0.06)^{b}
$C_{21:0}$	ND	ND	ND
$C_{22:0}$	0.01±0.01 (0.00-0.02)$^{b'\$}$	0.03±0.01 (0.02-0.04)^{a}	0.01±0.00 (0.00-0.02)$^{bd\$}$
$C_{23:0}$	ND	ND	ND
$C_{24:0}$	0.00±0.00 (0.00-0.01)$^{ba\$}$	0.01±0.00 (0.01-0.02)^{a}	0.01±0.00 (0.00-0.01)$^{ae\$}$
Total SFA	3.30±0.65 (1.95-4.34)^{b}	4.62±0.69 (3.53-6.60)^{a}	2.81±1.01 (1.43-4.66)^{b}
Total MCT	0.16±0.05 (0.08-0.28)^{a}	0.19±0.04 (0.12-0.28)^{a}	0.15±0.07 (0.05-0.35)^{a}
cis-9 $C_{14:1}$	0.02±0.01 (0.00-0.05)$^{ab'\$}$	0.02±0.00 (0.02-0.03)^{a}	0.01±0.00 (0.00-0.02)$^{bf\$}$
trans-9 $C_{14:1}$	ND	ND	ND
cis-10 $C_{15:1}$	0.02±0.01 (0.01-0.03)	ND	ND
cis-9 $C_{16:1}$	0.09±0.03 (0.05-0.19)^{b}	0.14±0.03 (0.08-0.17)^{a}	0.07±0.03 (0.04-0.11)^{b}
trans-9 $C_{16:1}$	ND	ND	ND
cis-10 $C_{17:1}$	ND	ND	ND
cis-9 $C_{18:1}$	1.25±0.29 (0.67-1.93)^{b}	1.65±0.19 (1.26-2.10)^{a}	0.95±0.34 (0.51-1.45)^{c}
trans-9 $C_{18:1}$	0.39±0.10 (0.23-0.59)^{a}	0.19±0.04 (0.12-0.29)^{b}	0.17±0.10 (0.06-0.37)^{b}
cis-11 $C_{20:1}$	ND	ND	ND
trans-11 $C_{20:1}$	ND	ND	ND
cis-13 $C_{22:1}$	ND	ND	ND
cis-15 $C_{24:1}$	ND	ND	ND
Total MUFA	1.77±0.40 (1.06-2.72)^{a}	1.99±0.23 (1.53-2.58)^{a}	1.18±0.44 (0.60-1.83)^{b}
cis-9,12 $C_{18:2\ n\text{-}6}$ (LA)	0.07±0.02 (0.05-0.11)^{b}	0.10±0.02 (0.07-0.16)^{a}	0.08±0.03 (0.04-0.14)^{b}
trans-9,12 $C_{18:2\ n\text{-}6}$	0.14±0.03 (0.08-0.21)^{a}	0.06±0.01 (0.04-0.08)^{b}	0.04±0.02 (0.02-0.07)^{c}
cis-6,9,12 $C_{18:3\ n\text{-}6}$	ND	ND	ND
cis-9,12,15 $C_{18:3\ n\text{-}3}$ (ALA)	0.07±0.02 (0.04-0.11)^{a}	0.07±0.01 (0.05-0.10)^{a}	0.04±0.02 (0.02-0.08)^{b}
cis-11,14 $C_{20:2\ n\text{-}6}$	ND	ND	ND
cis-8,11,14 $C_{20:3\ n\text{-}6}$	ND	ND	ND
cis-11,14,17 $C_{20:3\ n\text{-}3}$	ND	ND	ND
cis-5,8,11,14 $C_{20:4\ n\text{-}6}$(ARA)	0.00±0.00 (0.00-0.01)$^{b\$}$	0.00±0.00 (0.00-0.01)$^{b\$}$	0.00±0.00 (0.00-0.01)$^{a\$}$
cis-5,8,11,14,17 $C_{20:5\ n\text{-}3}$ (EPA)	ND	ND	ND
cis-13,16 $C_{22:2\ n\text{-}6}$	ND	ND	ND
cis-13,16,19 $C_{22:3\ n\text{-}3}$	ND	ND	ND
cis-7,10,13,16 $C_{22:4\ n\text{-}6}$	ND	ND	ND
cis-4,7,10,13,16 $C_{22:5\ n\text{-}6}$	ND	ND	ND
cis-7,10,13,16,19 $C_{22:5}$ n-3 (DPA)	0.01±0.00 (0.00-0.02)$^{cc\$}$	0.02±0.01 (0.01-0.02)^{a}	0.01±0.00 (0.00-0.02)$^{bf\$}$
cis-4,7,10,13,16,19 $C_{22:6\ n\text{-}3}$ (DHA)	ND	ND	ND
Total PUFA	0.28±0.07 (0.17-0.45)^{a}	0.25±0.04 (0.19-0.36)^{a}	0.17±0.08 (0.07-0.30)^{b}
Total n-3 PUFA	0.07±0.03 (0.04-0.13)^{a}	0.08±0.01 (0.06-0.12)^{a}	0.05±0.02 (0.02-0.10)^{b}

Table 2: Continue....

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Total n-6 PUFA	0.21±0.05 (0.13-0.32)[a]	0.16±0.03 (0.11-0.24)[b]	0.12±0.05 (0.05-0.21)[c]
n-6 PUFA/n-3 PUFA	3.02±0.59 (2.22-4.75)[a]	1.95±0.29 (1.38-2.38)[b]	2.75±0.53 (2.00-3.81)[a]
LA/ALA	1.07±0.17 (0.83-1.50)[c]	1.51±0.15 (1.17-1.71)[b]	2.16±0.27 (1.65-2.53)[a]
SFA/PUFA	12.05±1.70 (8.96-14.76)[b]	19.00±2.53 (15.45-24.38)[a]	17.84±3.18 (12.12-23.35)[a]
MUFA/PUFA	6.39±0.74 (5.05-7.88)[c]	8.19±0.89 (6.89-10.00)[a]	7.48±1.21 (5.40-9.66)[b]
Total TFA	0.53±0.13 (0.32-0.80)[a]	0.24±0.05 (0.16-0.37)[b]	0.20±0.11 (0.07-0.44)[b]

*Means in the same row is significantly different, p<0.05(ANOVA). ND, Not Detected; SFA, saturated fatty acids; MCT, medium chain triglycerides (C8:0-C10:0); MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; trans, trans double bond; cis, cis double bond; 1), of the group, only 16 samples were detected; 2), of the group, only 4 samples were detected; 3), of the group, only 1 sample was detected; 4), of the group, only 10 samples were detected; 5), of the group, only 12 samples were detected. 6), of the group, only 3 samples were detected; 7), of the group, only 9 samples were detected.

different seasons. The main groups of SFA, MUFA and PUFA clearly varied with seasons(p<0.05) (Table 2). For this seasonal variation in SFA, MUFA and PUFA, it maybe due to different diets in different seasons (summer diet contained fresh grass, while winter and spring diet did not contain fresh grass) (Liu *et al*., 2011); another possible reason is the mobilization of the yak's body reserved fat at this time in winter (Ding *et al*., 2013).

CONCLUSION

Yak milk is the main source of dietary protein, fat and micronutrients for Qinghai-Tibetan Plateau residents, who live at a high altitude and oxygen-deficient environment. Living at high altitudes (average altitude of more than 4000 metre) yaks were selected to analyzed the influence of season on the chemical and fatty acids composition of yak milk. Results showed that the chemical composition and fatty acid composition of winter yak milk were significantly different from those of summer and spring yak milk. For example, the contents of fat, protein, lactose, non-fat milk solid, vitamin A and the main fatty acids ($C_{6:0}$, $C_{12:0}$, $C_{14:0}$, $C_{15:0}$, $C_{16:0}$, $C_{17:0}$, $C_{20:0}$, cis-9 $C_{16:1}$, cis-9 $C_{18:1}$, trans-9 $C_{18:1}$, LA, *etc*.) in winter yak milk were significantly different from those in summer and spring yak milk (p<0.05). This suggests that chemical and fatty acids composition of yak milk were influenced by seasons.

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