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Fatty acid profiles in Chinese Maiwa yak milk across the lactation cycle

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An experiment was conducted to determine fatty acid (FA) profiles in Chinese Maiwa yak milk across the lactation cycle. Sixty-one healthy, similar weight, 4-7 years old, 3-6 parity Maiwa yaks with unrelated background were selected randomly from Hongyuan County, Sichuan province, in southwest of China (with the altitude over 3500 m). The yaks graze on natural pasture all year around without irrigation, fertilizer, or other changes to the pasture. The samples for each animal were collected separately at 1, 2, 3, 4, 5, 6, 7, 15, 30, 60, 120 and 180 days postpartum for fatty acid composition analysis. The most abundant FA species in yak milk were C16:0, C18:1c9, C18:0 and C14:0, all of which varied significantly with the lactation periods. C10:0, C12:0, C14:0 and C16:0 was positively correlated with each other, and negatively correlated with C18:0. C18:2t9c11 was negatively correlated with saturated FA (SFA). C18:1t11, iso-C15:0, C22:0 and C16:1c7 were located in the central positions of the correlation network. C18:1t11 showed close correlation with C18 unsaturated fatty acid (UFA) (C18:1t13 and C18:3n3), C16:0, C19:0, and anteiso-C17:0. Besides with each other, iso-C15:0 and C22:0 were associated with C15:0, anteiso-C15:0, C19:0, C20:0, and C20:0, anteiso-C15:0, iso-C14:0, 9,10-hexyl-C17:0, respectively. C16:1c7 was closely associated with C13:0, C17 FA (C17:0 and 9, 10-hexyl-C17:0), C16:1c9, C20:5n3 and C20:4n6. In conclusion, these data will be informative for the study on the regulatory mechanism of milk FA formation in yaks.

Key words: Correlations, fatty acid profile, lactation periods, Qinghai-Tibetan plateau, yak.

INTRODUCTION

Yak is a species that lives in mountainous areas of Central Asia, mainly found in the highlands of the Nepalese Himalayas, Indian Kashmir, Tibet, Mongolia, and Bhutan (Neupaney et al., 2003; Dhanapati et al., 2003), at an altitude of 2,500 000 to 5,500 000 m (Shi et al., 2019; Zi et al., 2008). The total world population of yaks is estimated at around 14.214.7 million of which China has the largest number of yaks in the world with approximately 13 14 million that comprises > 93.795% of all planet yaks (Sheng-Hua et al., 2013; Dong et al., 2007). Yak milk is a component of the diet in those areas contains $16.5 \pm 2.8116.9$ -17.7% dry matter (DM), 4.90-5.39% protein, 5.53-7.28.8% fat, 4.9 \pm 0.61 4.5-5.0% lactose, and 5.4 \pm 2.43%0.8-0.9% minerals (Shi et al., 2019; Or-Rashid et al., 2008), thus is considered to be a naturally concentrated milk (Ren et al., 2014), accounting for 15 to 32% of a herder's daily fat intake (Liu et al., 2011). Considering the importance of fatty acids (FA) in affecting cheese quality, which is the main product of yak milk in Qinghai-Tibetan plateau

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons</u> <u>Attribution License 4.0 International License</u> (Liu et al., 2011; Gottardo et al., 2017), it will be of great value to determinate the FA composition of yak milk.

Milk fat of yak at very high altitudes is richer in polyunsaturated FA (PUFA) (Gottardo et al., 2017; Nikkhah, 2011). The amounts of conjugated linoleic acid (CLA) cis-9, trans-11 in yak cheese has been found to be 4.2 times greater than that in cow cheese (Jiang et al., 2007; Or-Rashid et al., 2008). Eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C22:6n3, DHA)(Gottardo et al., 2017; Liu et al., 2011) are recognized as playing an essential role in human health, and are particularly important for the proper function of the brain, heart, and retina (Simopoulos, 1991; Kris-Etherton et al., 2003; Din et al., 2004; 1991). The greater content of low molecular weight volatile acids (Li et al., 2010) might prevent some human metabolic illness (Williams, 2000; Haenlein, 2004). Although the milk yield of yak has been reported (147 -487 200-300 kg) and the lactation duration is just 150-200 days daily milk yield is commonly 1.5-2 kg (Dong et al., 2007; Zhou et al., 2014), considering the milk FA composition in each month varies a lot with the change of diet in dairy cow (Palmquist et al., 2006), determining the specific FA profile alteration across the lactation cycle may be beneficial for the utilization of yak milk.

The synthesis of FA are regulated by complex networks (Bionaz and Loor, 2008), results in the close association between different FA compositions (Samková and Kalač, 2021) (Maroteau et al., 2014). Many genes have reported to be responsible for the synthesis of different parts of FA (Stergiadis et al., 2020), such as short and medium chain FA (SMFA) (Zhu et al., 2014), monounsaturated FA (MUFA) (Shi et al., 2013), PUFA (Park et al., 2009) and very long chain FA (VLFA) (Matsuzaka et al., 2007). The milk fatty acids based on genetic and herd parameters was characterized in cow. Although the pathway leading to the formation of FA will not differ between yaks and cows, concentrations in milk fat are probably different. Investigate the exact association among yak milk FA compositions may enhance our understanding about the formation in yaks. The objective of present study was to investigate the FA profile of yak milk across the lactation cycle, and to identify the correlation among yak milk FA compositions. These data may be better for understanding the lactation physiology of yak, and facilitating the utilization of yak milk.

MATERIALS AND METHODS

Animals and sampling

The Animal Care and Use Committee of the Southwest University for Nationalities approved all procedures and experiments (Ministry of Science and Technology, China, revised in June 2004). Sixty-one healthy, similar weight, 4-7 years old, 3-6 parity *Maiwa* yaks with unrelated background were selected randomly from Hongyuan County, Sichuan province, in southwest of China (with the altitude over 3500 m) (Figure 1). The yaks graze on natural pasture all year around without irrigation, fertilizer, or other changes to the pasture. The experiment was conducted from May to November 2013. The samples for each animal were collected separately at 1, 2, 3, 4, 5, 6, 7, 15, 30, 60, 120 and 180 days postpartum. The yaks were hand milked once daily between 7 am to 8 am. About 100 ml milk samples (divided into 2 tubes after mixture) for each animal were then transferred using ice boxes from the farm to the laboratory in 6 h, and stored at -80° C until analysis.

Fatty acid extraction and analyses

The milk FA were extracted and methyl-esterified protocol was that 2 ml milk was completely mixed with 50 ml chloroform/methanol (2:1) solution (Bligh and Dyer, 1959; Folch et al., 1957). After being centrifuged at 3000 rpm for 5 min, the solution was transferred into a new tube and mixed with 10 ml 20% NaCl following by violent shock. Folch solution (chloroform:methanol:water/3:47:48) was then used for washing the tube wall. After standing for 5 min, the supernatant was removed. A total of 5 ml 0.5 M NaOH-CH₃OH was supplemented into the sample for methylation at 60°C for 30 min. Nitrogen gas was then used for evaporating the solution. 5 ml 14% BF₃-CH₃OH solution was added to the sample following standing at 80°C for 5 min. After the sample was cooled down to room temperature, 8 ml hexane was supplemented and shocked. The saturated NaCl solution was added until hierarchy. Following standing, the supernatant was transferred to a new tube. CuSO₄ was used for removing the water, and the supernatant was then used for gas chromatography-mass spectrometry (GC-MS. Agilent 7890A/5970C) analysis equipment with a HP88 column (60 m x 250 µm x 0.2 µm). Approximately 1 µl methylated lipid samples was injected into the GC system with 1:30 split ratio. The temperature of injector was 280°C. The initial temperature of the column was 120°C, which was temperature-programmed at 8°C/min to 145°C, continued to increase to 220°C at 3°C/min and maintained at 220°C for 3 min. The speed of carrier gas was 1 ml/min. The ionization mode was EI, with the ionization energy of 70 eV with 35 to 500 amu mass number. The temperature of interface, ion source and quadrupole rod were 280, 200 and 100°C respectively. The solvent delay was 3 min.

Statistical analyses

The analysis was carried out in duplicates and the data generated was expressed as mean ± standard deviation. Data obtained were statistically analysed by one-way ANOVA using the Statistical Package for the Social Sciences, version 22.0 (SPSS, Chicago, IL, USA), for significant F-statistics. If the overall F-test was significant (P < 0.05), a Fischer's T-test was performed to significant differences. The correlation analysis among different FA was calculated by Pearson method (2-tailed) using SPSS 22.0. The |r| ≥ 0.65 with P < 0.05 was defined as correlated significantly.

RESULTS

Differences in milk saturated fatty acids across lactation cycle

A total of 38 FA and groups were detected. The FA individuals shorter than C10:0 could not be quantified due to solvent delay. Table 1 shows the fat content and the major FA profile of milk from primiparous yaks across the lactation cycle. The most abundant FA in yak milk were C16:0, C18:1c9, C18:0 and C14:0 across the lactation cycle, consistent with goat, sheep and cow (Markiewicz-Kęszycka et al., 2013). In detail, the relative content of C16:0 in yak milk had the highest content level at 2 days, and was decreased in a fluctuation model until 120 days postpartum, however

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Figure 1. Map showing the sample collection site of Longri Seed Storage Chang, Hongyuan County, Sichuan Province. Source: AMAP software

with a sharp increase at 180 days to the similar level as 1 day postpartum. An inverse trend was observed in the relative content of C18:0, which increased in a fluctuation model, peaked at 7 and 120 days postpartum, with a sharp decrease at 180 days. The relative content of C14:0 was continually decreased across the whole lactation periods except a slight increase at 180 days. Considering that the relative content of FA compositions was seriously affected with the decrease of milk yield in the late lactation period, however, different results were observed at 180 days compared with the previous data (except C16:0) (Liu et al., 2011). Although the discrepancy of breeds, grazing model and pasture may result in different FA profile, the exact reason underlying the divergence remains unknown.

In ruminants, about one-half of milk FA are synthesized de novo (Frutos et al., 2020; Bauman and Davis, 1974), mainly including short- and medium- chain FA (C4-C14) and a portion of 16-carbon FA. Consistent with the data in Gannan yak (Liu et al., 2011), the relative content of C10:0 and C12:0 increased continually until 6 days, and turned down till the end of the lactation cycle. Following a stable period, the relative content of C17:0 was increased significantly at 120 days, however, with no significant change in 9,10-hexyl-C17:0, which was proved to increase cyclooxygenase activity in human. For the LCFA, no significant change was observed in C20:0

except for the significant increase after 30 days postpartum.

In milk fat odd branched-chain fatty acids (OBCFA) are important bioactive component due to their essential role in the gut and potential activity against human breast cancer cells (Gomez-Cortes et al., 2017). In the present study, similar as in dairy cow (Gomez-Cortes et al., 2017), C15:0, C17:0, anteiso-C15:0, iso-C15:0 and anteiso-C17:0 are most abundant OBCFA in yak milk fat. Differently, while a continue increase trend was observed in the content of C15:0 across the lactation cycle, the content of C17:0 decreased continually during colostrum period and increased continually till the end of lactation. However, we did not find iso-C17:0 in the present study. Besides, we also found 9. 10-hexyl-C17:0, the main cyclopropane FA in human adipose tissue and serum, in yak milk, with a continually decrease during colostrum period and turned up till the end of lactation. This may indicate the variation of cyclooxygenase activity during lactation in yaks (Sledzinski et al., 2013).

Differences in milk unsaturated fatty acids across lactation cycle

The C18:2t9t12 was the most abundant FA among the 3 kinds of linoleic acids observed in the present study.

Traits	0d	1d	2d	3d	4d	5d	6d	7d	15d	30d	120d	180d
C10:0	1.54±0.47ª	1.97±0.60°	2.24±0.58 ^d	2.31±0.63 ^{de}	2.32±0.57 ^{de}	2.46±0.70 ^e	2.27±0.64 ^{de}	2.01±0.55°	1.82±0.35 ^{bc}	1.66±0.36ª	1.53±0.29 ^{ab}	1.60±0.26ª
C10:1t4	0.03±0.02ª	0.04±0.02 ^b	0.05±0.03 ^{bc}	0.06±0.03 ^{cd}	0.06±0.02 ^{cd}	0.07 ± 0.03^{de}	0.06±0.03 ^d	0.07±0.03 ^{de}	0.08±0.04 ^{ef}	0.09±0.04 ^{fg}	0.09±0.04 ^{fg}	0.10±0.05 ⁹
C12:0	1.68±0.34ª	1.97±0.48 ^b	2.16±0.48°	2.17±0.49°	2.20±0.50°	2.30±0.61°	2.13±0.57°	1.87±0.49 ^b	1.65±0.27ª	1.62±0.39ª	1.57±0.46ª	1.65±0.29ª
C13:0	0.03±0.02ª	0.04±0.01 ^{ab}	0.04±0.02 ^{ab}	0.04±0.01 ^{abc}	0.04±0.01 ^{abc}	0.04±0.01 ^{bcd}	0.04±0.01 ^{abcd}	0.04±0.01 ^{abc}	0.04±0.02 ^{abc}	0.04±0.02 ^{cd}	0.04±0.02 ^{cd}	0.05±0.03d
iso-C14:0	0.10±0.05ª	0.11±0.03ª	0.12±0.04 ^{ab}	0.13±0.04 ^b	0.13±0.03 ^b	0.14±0.05 ^b	0.13±0.04 ^b	0.13±0.04 ^b	0.14±0.05 ^b	0.19±0.10⁰	0.21±0.07d	0.21±0.07 ^{cd}
C14:0	10.32±1.89d	9.67±1.32 ^{bc}	10.02±1.44 ^{cd}	9.81±1.26 ^{bc}	9.75±1.34 ^{bc}	9.73±1.35 ^{bc}	9.36±1.17⁵	8.87±1.06ª	8.51±0.69ª	8.55±1.07ª	8.41±1.03ª	8.81±0.69ª
lso-C15:0	0.28±0.11ª	0.29±0.08 ^{ab}	0.29±0.10ª	0.32±0.10 ^{bcd}	0.33±0.08 ^{bcd}	0.33±0.09 ^{cd}	0.32±0.09bcd	0.32±0.08bcd	0.30±0.07 ^{abc}	0.35±0.07d	0.46±0.06°	0.46±0.12°
Anteiso-C15:0	0.66±0.21ª	0.69±0.19ª	0.72±0.24ª	0.80±0.23b	0.82±0.19 ^b	0.82±0.20b	0.82±0.18 ^b	0.91±0.17°	0.96±0.17°	1.06±0.11₫	1.18±0.12⁰	1.18±0.19⁰
C15:0	1.03±0.21ª	1.13±0.17 ^{bc}	1.08±0.21ab	1.12±0.17 ^{bc}	1.13±0.15b⁰	1.12±0.17 ^{bc}	1.13±0.16 ^{bc}	1.15±0.13 ^{bc}	1.15±0.15⁰	1.24±0.11₫	1.35±0.19⁰	1.34±0.21⁰
C16:0	36.01±5.51de	35.07±3.95 ^{cd}	36.81±4.14⁰	35.77±4.30 ^{de}	35.18±3.74 ^{cd}	36.01±3.99 ^{de}	35.58±3.94 ^{cde}	34.03±3.54 ^{bc}	34.41±3.06 ^{bcd}	33.31±4.14 ^{ab}	32.28±2.60ª	34.87±3.90 ^{cd}
C16:1c9	0.06±0.06 ^{bc}	0.04±0.04 ^{ab}	0.02±0.03ª	0.03±0.04ª	0.03±0.04ª	0.04±0.05 ^{ab}	0.04±0.05 ^{ab}	0.07±0.08 ^{cd}	0.10±0.08°	0.09±0.06 ^{de}	0.07±0.05 ^{cd}	0.03±0.04ª
C16:1c7	0.16±0.12₫	0.15±0.10 ^{cd}	0.10±0.09ª	0.11±0.08ª	0.11±0.08 ^{abc}	0.12±0.08 ^{abc}	0.12±0.09 ^{abc}	0.11±0.10 ^{abc}	0.13±0.09 ^{abcd}	0.14±0.09 ^{bcd}	0.13±0.10 ^{abcd}	0.11±0.09 ^{ab}
C16:1t2	0.76±0.33 ^{cd}	0.74±0.29 ^{bcd}	0.60±0.30ª	0.62±0.29ª	0.63±0.27 ^{ab}	0.61±0.28ª	0.59±0.24ª	0.67±0.22 ^{abc}	0.83±0.33de	0.86±0.35de	0.94±0.34°	1.11±0.48 ^f
Anteiso-C17:0	0.32±0.16 ^{bcd}	0.32±0.14 ^{abcd}	0.26±0.14ª	0.29±0.14 ^{abc}	0.30±0.12 ^{abcd}	0.27±0.14 ^{ab}	0.27±0.13 ^{ab}	0.26±0.12ª	0.27±0.12ª	0.27±0.11ab	0.35±0.17₫	0.35±0.16 ^{cd}
C17:0	0.97±0.34°	0.95±0.29 ^{bc}	0.78±0.28ª	0.85±0.27 ^{ab}	0.85±0.20 ^{ab}	0.84±0.25 ^{ab}	0.85±0.23ab	0.79±0.25ª	0.80±0.25ª	0.85±0.23ab	1.08±0.24₫	1.11±0.41d
9,10-hexyl-C17:0	0.13±0.10 ^{bc}	0.11±0.08 ^b	0.07±0.06ª	0.08±0.05ª	0.08±0.05ª	0.08±0.06ª	0.08±0.05ª	0.07±0.05ª	0.09±0.05ª	0.13±0.10 ^{bc}	0.15±0.08°	0.18±0.09 ^d
C18:0	16.48±3.51ª	17.62±2.78 ^{bc}	17.83±2.94 ^{bcd}	18.49±2.79 ^{cde}	18.78±2.79 ^{de}	18.39±2.78 ^{cde}	19.04±2.21°	19.24±2.04e	18.71±1.87 ^{de}	18.68±2.37 ^{de}	19.12±1.94e	16.96±2.03ab
C18:1t8	3.30±1.40 ^b	3.83±1.49°	3.88±1.33°	4.33±1.37 ^{cde}	4.44±1.34 ^{de}	4.50±1.34°	4.87±1.35 ^{ef}	6.00±1.68 ^g	6.11±1.49 ^g	5.13±1.63 ^f	3.92±1.25 ^{∞d}	2.71±0.78ª
C18:1c9	22.08±3.65 ^{de}	21.32±4.01d	19.59±3.28 ^{bc}	19.03±2.91 ^{abc}	18.76±3.18 ^{ab}	18.37±3.12ª	18.44±3.15ª	19.36±2.56 ^{abc}	19.90±1.70°	21.56±2.29 ^d	22.35±1.03 ^{de}	23.10±1.75°
C18:1t11	0.47±0.22 ^{abc}	0.48±0.17°	0.40±0.20ª	0.43±0.17 ^{abc}	0.45±0.16 ^{abc}	0.40±0.16ª	0.42±0.14 ^{abc}	0.45±0.16 ^{abc}	0.44±0.15 ^{abc}	0.44±0.16 ^{abc}	0.47±0.15 ^{bc}	0.41±0.17 ^{ab}
C18:1t13	0.11±0.09ª	0.11±0.08 ^{ab}	0.10±0.09ª	0.12±0.09 ^{ab}	0.15±0.09 ^{bcd}	0.13±0.08 ^{ab}	0.13±0.08 ^{abc}	0.16±0.09 ^{cde}	0.18±0.09 ^{def}	0.20±0.11 ^f	0.20±0.10 ^{ef}	0.17±0.10 ^{cde}
C18:2c9c12	0.21±0.12ª	0.22±0.11 ^{ab}	0.19±0.12ª	0.24±0.12 ^{abc}	0.27±0.11 ^{bcd}	0.26±0.12 ^{bcd}	0.27±0.13 ^{bcd}	0.28±0.14 ^{cd}	0.28±0.12 ^{cde}	0.31±0.12 ^{de}	0.39±0.16 ^f	0.33±0.15°
C18:2t9t12	1.25±0.45 ^f	1.11±0.35 ^e	0.90±0.33 ^{cd}	0.91±0.29 ^{cd}	0.96±0.24 ^d	0.88±0.28 ^{cd}	0.82±0.29bc	0.73±0.26 ^{ab}	0.67±0.20ª	0.67±0.28ª	0.83±0.26 ^{bc}	0.62±0.16ª
C18:2t9c11	0.69±0.37ª	0.78±0.32 ^{abc}	0.74±0.38 ^{ab}	0.84±0.37 ^{abc}	0.87±0.36 ^{bcd}	0.87±0.34 ^{bcd}	0.91±0.33 ^{cd}	1.30±0.56 ^{fg}	1.46±0.53 ^g	1.34±0.519	1.16±0.47 ^{ef}	1.01±0.39de
C18:2c9c15	0.01±0.02 ^{ab}	0.01±0.01ab	0.01±0.01ª	0.01±0.02ª	0.01 ± 0.02^{ab}	0.02 ± 0.02^{ab}	0.01±0.02 ^{ab}	0.02±0.02 ^{ab}	0.02±0.03 ^{abc}	0.03±0.04 ^{bcd}	0.03±0.03 ^{cd}	0.04±0.10 ^d
C18:3n3	0.61±0.24 ^b	0.61±0.19 ^b	0.53±0.24 ^{ab}	0.58±0.21⁵	0.60±0.21⁵	0.57 ± 0.19^{ab}	0.56±0.20 ^{ab}	0.54±0.20ab	0.49±0.15ª	0.60±0.21 ^b	0.80±0.24°	0.60±0.19 ^b
C19:0	0.13±0.08ª	0.15±0.06 ^{abc}	0.13±0.07 ^{ab}	0.15±0.07 ^{abc}	0.17±0.06 ^{cd}	0.15±0.06 ^{abc}	0.16±0.06 ^{bc}	0.15±0.06 ^{abc}	0.15±0.06 ^{abc}	0.15±0.05 ^{abc}	0.18±0.07d	0.17±0.08 ^{cd}
C20:0	0.20±0.11bc	0.21±0.08 ^{bc}	0.19±0.11 ^{abc}	0.22±0.09bc	0.22±0.08°	0.20±0.08 ^{abc}	0.21±0.08 ^{bc}	0.18±0.06 ^{ab}	0.16±0.05ª	0.22±0.08 ^{bc}	0.40±0.18d	0.44±0.23⁰
C22:0	0.04±0.03ª	0.04±0.03ª	0.04±0.04ª	0.05±0.03ª	0.05±0.03ª	0.05±0.03ª	0.05±0.03ª	0.05±0.03ª	0.05±0.03ª	0.07±0.04 ^b	0.13±0.06°	0.14±0.07°
C20:4n6	0.03±0.03°	0.03±0.03 ^{de}	0.02±0.03 ^{abcd}	0.03±0.02 ^{cde}	0.03±0.02 ^{cde}	0.03±0.02 ^{cde}	0.02±0.02 ^{bcde}	0.01±0.02 ^{ab}	0.01±0.01ª	0.02±0.03 ^{abc}	0.02±0.02 ^{abc}	0.03±0.05 ^{cde}
C20:5n3	0.05±0.04d	0.05±0.05d	0.03±0.03ab	0.03±0.03 ^{abc}	$0.04 \pm 0.03^{\text{abcd}}$	0.04±0.03 ^{abcd}	0.03±0.03 ^{abc}	0.02±0.03ª	0.03±0.03 ^{ab}	0.04±0.04 ^{cd}	0.05±0.04d	0.04±0.04 ^{bcd}
MCFA	15.67±1.90 ^{bc}	15.91±2.10 ^{bcd}	16.73±2.08d	16.77±1.87d	16.78±2.06d	17.02±2.38d	16.30±2.17⁰	15.35±1.91ab	14.68±1.17ª	14.68±1.55ª	14.88±1.57ª	15.35±1.12 ^{ab}
LCFA	83.87±2.08 ^{bcdef}	83.72±2.04ª	82.98±2.02 ^{abcd}	82.90±1.81 ^{abcd}	82.87±2.01 ^{abc}	82.66±2.34 ^{ab}	83.35±2.11 ^{abcde}	84.28±2.02 ^{cdef}	85.06±1.17 ^f	84.56±2.20ef	84.39±1.48 ^{def}	83.87±1.54 ^{bcdef}
VLCFA	0.32±0.18 ^{abc}	0.33±0.16 ^{bc}	0.28±0.19 ^{abc}	0.33±0.16 ^{bc}	0.34±0.14°	0.32±0.14 ^{abc}	0.32±0.14 ^{abc}	0.26±0.13ab	0.25±0.12ª	0.35±0.16⁰	0.62±0.27d	0.65±0.29d
SFA	70.15±3.97°	70.39±3.91°	72.83±3.88 ^d	72.62±3.92d	72.36±3.53d	73.18±3.93 ^d	72.60±3.97d	70.15±3.73°	69.26±2.86 ^{abc}	68.15±3.35ª	68.53±2.83 ^{ab}	69.56±2.46 ^{bc}

Table 1. The fat content (g/100 g) and the major FA profile (% in total fatty acid methyl esters, g/100 g total FAs) of milk from yaks across the lactation cycle.

Table 1. Contd.

USFA	29.85±3.97 ^b	29.58±3.90 ^b	27.17±3.88ª	27.38±3.92ª	27.64±3.53ª	26.82±3.93ª	27.36±3.98ª	29.85±3.73 ^b	30.74±2.86 ^{bc}	31.73±3.27°	31.35±2.73°	30.45±2.45 ^{bc}
MUFA	26.98±3.56 ^{bc}	26.74±3.55 ^b	24.73±3.31ª	24.72±3.38ª	24.85±3.10ª	24.17±3.52ª	24.69±3.51ª	26.94±3.09 ^b	27.77±2.34 ^{bcd}	28.67±2.85d	28.16±1.78 ^{cd}	27.91±1.79 ^{bcd}
PUFA	2.87±1.01 ^{bcd}	2.84±0.79 ^{bcd}	2.44±0.97ª	2.65±0.77 ^{ab}	2.76±0.72 ^{abcd}	2.65±0.70 ^{ab}	2.70±0.61 ^{abc}	2.91±0.90 ^{bcd}	3.02±0.74 ^{cde}	3.07±0.74de	3.30±0.95⁰	2.64±0.75 ^{ab}

^{a-g}Different superscript letters indicate significant differences (P <0.05) within the same row. MCFA, medium-chain FA (sum of C10-C15); LCFA, long-chain FA (sum of C16-C22); VLCFA (sum of C20-C22); SFA, saturated FA (sum of C10:0, C12:0-C16:0, anteiso-C17:0 -C18:0, C19:0-C22:0); USFA, unsaturated FA (sum of C10:1n4, C16:1, C18:1, C18:2, C18:3, C20:4, C20:5); MUFA (sum of C10:1n4, C16:1, C18:1); PUFA (sum of C18:2, C18:3, C20:4, C20:5). Source: Authors

Although there was no significant change for the total content of linoleic acids, C18:2t9t12 continually decreased during lactation periods. Different from our results that cis-9, trans-11 CLA and trans-10, cis-12 CLA were the main CLA in dairy cow (Harvatine et al., 2009; and Wang et al., 2012), dairy goat (Maroteau et al., 2014) and even in Gannan vak (Liu et al., 2011). A possible reason was that the GC system was not sufficient for distinguishing different CLA types only using individual cis-9, trans-11 CLA and trans-10, cis-12 CLA references in yak milk (Liu et al., 2011). In the present study, a GC-MS system was used for more reliable results by comparing the different types of CLA with the NIST mass spectra library (2009). Obviously, more research by GC-MSMS in yak milk is essential for confirming the results in the future.

Consistent with previous study in goat (Haile et al., 2016), dairy cow (Chilliard and Ferlay, 2004) and *Gannan yak* (Liu et al., 2011), the C18:1c9 was the most abundant USFA. Different with the fluctuation model of C16:0 and C18:0, the relative content of C18:1c9 was continually decreased during colostrum period and turned over after 5 days postpartum. Similar change model was observed in C16:1c9 and C16:1t2 which was the most abundant derivative of palmitic acid. In ruminants, stearoyl-coenzyme A desaturase (*SCD*) is a primary candidate gene for altering the proportion of SFA versus MUFA in milk with the palmitic acid and stearic acid as substrates

(Shi et al., 2013). The relative content of MUFA C18:1c9, compared to the sum of C16:0 and C18:0, was significantly lower than that in dairy goat (Haile et al., 2016) and dairy cow (Cortes et al., 2010), may indicate a lower activity of SCD in yak milk. However, in *Gannan yak* the value of MUFA/SFA was much higher than that in dairy cow (Wang et al., 2012). Although different breeds of yak may be different in terms of milk fatty acid profile, obviously more controlled study is still needed for elucidating the discrepancy. We also observed small portion of ARA and EPA which presented the activity of fatty acid desaturase 1 (FADS1) (Park et al., 2009), bottomed at 3 and 9 /8 days postpartum, respectively.

Correlations between milk FA

Table 2 showed the FA correlations in Maiwa yak milk, while the Figures 1 and 2 showed the significant correlations. In general, C16:1c7, C18:1t11, C22:0 and iso-C15:0 correlated with most of the FA individuals. As expected, the FA de novo synthesized in mammary gland, including C10:0, C12:0, C14:0 and part of C16:0, significantly correlated with each other, and negatively correlated with C18:0 (r=-0.64). Close correlation was observed among C18 FAs, including C18:1t11, C18:1t13, C18:2t9t12, ALA, and also anteiso-C17:0 (Ntambi and Miyazaki, 2004). While the

C16:1c7 was then converted to C18:1c7, C18:1c9 was mainly desaturated from C18:0 (Green et al., 2010). Although C18:2t9c11 was not correlated with the individuals, there was a significantly negative correlation with the total content of SFA, supporting the results of MFD effect on de novo fatty acid synthesis (Piperova et al., 2000).

Close correlations were observed among C15 FAs, C20:0 and C22:0, indicating that anteiso-C15:0, iso-C15:0 and C15:0 may have the same source (Gomez-Cortes et al., 2017). The close correlation between iso-C15:0, as well as 9, 10-hexyl-C17:0 and C17:0, and the total content of VLCFA may indicate their involvement in FA elongation. The C22:0 may be the elongation product of C20:0 in yak milk. Iso-C14:0 was correlated with C13:0 instead of C14:0, indicating that they may share similar source in yak milk (Gomez-Cortes et al., 2017).

DISCUSSION

Although the fatty acid profile of yak milk from the Qinghai-Tibetan plateau in different seasons has been identified (Liu et al., 2011), the present study is novel in that we investigated the variation of milk FA profile across the whole lactation cycle, and analyzed the correlation between FA individuals in grazed yak without the transfer from summer pasture to winter

Traits	C10:0	C10:1t4	C12:0	C13:0	iso-C14:0	C14:0	iso-C15:0	anteiso-C15:0	C15:0	C16:0	C16:1c9	C16:1c7	C16:1t2	anteiso-C17:0	C17:0	9,10-h exyl-C 17:0
C10:0	1															
C10:1t4	0.20	1														
C12:0	0.90	0.16	1													
C13:0	0.06	0.64	0.05 ^{NS}	1												
iso-C14:0	-0.23	0.60	-0.28	0.74	1											
C14:0	0.62	-0.06 ^{NS}	0.73	-0.12	-0.38	1										
Iso-C15:0	-0.31	0.44	-0.23	0.48	0.61	-0.45	1									
Anteiso-C15:0	-0.40	0.54	-0.38	0.41	0.61	-0.56	0.84	1								
C15:0	-0.39	0.21	-0.39	0.34	0.53	-0.60	0.78	0.82	1							
C16:0	0.43	-0.04 ^{NS}	0.35	-0.18	-0.23	0.63	-0.57	-0.55	-0.58	1						
C16:1c9	-0.13	0.38	-0.19	0.61	0.43	-0.23	0.20	0.27	0.17	-0.21	11					
C16:1c7	-0.19	0.35	-0.18	0.71	0.47	-0.21	0.32	0.25	0.22	-0.28	0.77	1				
C16:1t2	-0.44	0.18	-0.37	-0.05 ^{NS}	0.14	-0.38	0.47	0.58	0.51	-0.33	-0.06 ^{NS}	0.03 ^{NS}	1			
Anteiso-C17:0	-0.40	-0.10	-0.30	-0.01 ^{NS}	0.13	-0.42	0.53	0.49	0.64	-0.58	-0.06 ^{NS}	0.11	0.67	1		
C17:0	-0.39	0.27	-0.32	0.52	0.54	-0.39	0.64	0.48	0.56	-0.48	0.39	0.67	0.26	0.45	1	
9,10-Hexyl-C17:0	-0.42	0.33	-0.37	0.48	0.54	-0.35	0.56	0.52	0.48	-0.36	0.44	0.67	0.48	0.47	0.73	1
C18:0	-0.10	-0.19	-0.11	0.01 ^{NS}	0.03 ^{NS}	-0.45	0.21	0.12	0.24	-0.64	0.01 ^{NS}	-0.01 ^{NS}	-0.25	0.11	0.09	-0.12
C18:1t8	-0.02 ^{NS}	-0.06 ^{NS}	-0.10	0.07 ^{NS}	-0.06 ^{NS}	-0.31	0.01 ^{NS}	0.21	0.21	-0.36	0.35	0.08	-0.05 ^{NS}	0.09	-0.20	-0.11
C18:1c9	-0.71	-0.05 ^{NS}	-0.65	-0.10	0.20	-0.53	0.27	0.31	0.27	-0.48	-0.05 ^{NS}	0.11	0.46	0.36	0.41	0.38
C18:1t11	-0.33	-0.02 ^{NS}	-0.18	0.10	0.02 ^{NS}	-0.37	0.48	0.41	0.52	-0.67	0.14	0.30	0.57	0.79	0.45	0.47
C18:1t13	-0.23	0.32	-0.10	0.30	0.22	-0.39	0.55	0.57	0.50	-0.64	0.32	0.32	0.41	0.54	0.32	0.39
C18:2c9c12	-0.29	0.31	-0.25	0.32	0.37	-0.41	0.56	0.58	0.48	-0.46	0.26	0.27	0.45	0.44	0.34	0.44
C18:2t9t12	-0.20	-0.29	-0.04 ^{NS}	-0.01 ^{NS}	-0.15	0.02 ^{NS}	0.12	-0.08	0.12	-0.29	-0.05 ^{NS}	0.29	0.22	0.49	0.42	0.33
C18:2t9c11	-0.35	0.22	-0.39	0.14	0.20	-0.55	0.35	0.62	0.52	-0.50	0.42	0.19	0.52	0.41	0.12	0.26
C18:2c9c15	-0.11	0.48	-0.11	0.71	0.67	-0.12	0.35	0.24	0.17	-0.03 ^{NS}	0.70	0.63	-0.02 ^{NS}	0.02 ^{NS}	0.38	0.43
C18:3n3	-0.29	0.13	-0.13	0.24	0.24	-0.28	0.60	0.46	0.52	-0.56	0.13	0.29	0.43	0.60	0.54	0.46
C19:0	-0.20	0.26	-0.13	0.47	0.39	-0.38	0.70	0.58	0.64	-0.63	0.31	0.47	0.37	0.60	0.64	0.62
C20:0	-0.35	0.18	-0.33	0.22	0.49	-0.41	0.68	0.57	0.65	-0.41	0.04 ^{NS}	0.20	0.54	0.61	0.60	0.64
C22:0	-0.33	0.45	-0.33	0.47	0.71	-0.42	0.73	0.65	0.62	-0.36	0.24	0.34	0.43	0.41	0.63	0.71

Table 2. Pearson correlation of fatty acids in yak milk.

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Table 2. cont'd

C20:4n6	0.	05 ^{NS}	0.30	0.06 ^{NS}	0.62	0.46	(0.04 ^{NS}	0.18	0.02 ^{NS}	0.0	2 ^{NS}	-0.01 ^{NS}	0.40	0.67	-0	.07 ^{NS}	0.04 ^{NS}	0.50	0.5	52
C20:5n3	-0	.07 ^{NS}	0.38	-0.01 ^{NS}	0.66	0.50	-(0.06 ^{NS}	0.30	0.17	0.1	15	-0.18	0.57	0.72	0	.02 ^{NS}	0.10	0.56	0.5	59
MCFA	().84	0.21	0.92	0.11	-0.18		0.88	-0.19	-0.32	-0.3	37	0.48	-0.16	-0.14	-	0.33	-0.33	-0.29	-0.3	33
LCFA	-(0.80	-0.26	-0.87	-0.16	0.11		-0.82	0.11	0.25	0.2	29	-0.45	0.11	0.08	(0.29	0.28	0.20	0.1	6
VLCFA	-(0.31	0.35	-0.28	0.47	0.64		-0.37	0.69	0.56	0.5	59	-0.38	0.25	0.44	(0.43	0.49	0.70	0.7	77
SFA	().74	-0.02 ^{NS}	0.69	-0.05 ^{NS}	-0.22		0.72	-0.44	-0.56	-0.	53	0.75	-0.25	-0.28	-	0.59	-0.57	-0.44	-0.5	52
USFA	-0.74		0.02 ^{NS}	-0.69	0.05 ^{NS}	0.22		-0.71	0.43	0.56	0.5	53	-0.75	0.25	0.29	(0.60	0.58	0.44	0.5	52
MUFA	FA -0.75		-0.02 ^{NS}	-0.71	-0.01 ^{NS}	0.19		-0.71	0.37	0.51	0.4	46	-0.71	0.20	0.22	(0.54	0.50	0.40	0.4	1 6
PUFA	-(0.41	0.15	-0.32	0.27	0.24		-0.45	0.53	0.56	0.8	57	-0.62	0.38	0.45	(0.55	0.65	0.50	0.5	58
Traits	C18:0	C18:1t8	C18:1c9	C18:1c11	C18:1c13	C18:2c9c12	C18:2t9t,12	C18:2t9c11	C18:2c9c15	C18:3n3	C19:0	C20:0	C22:0	C20:4n6	C20:5n3	MCFA	LCFA	VLCFA	SFA	USFA	MUFA
C10:0																					
C10:1t4																					
C12:0																					
C13:0																					
ISO-C14:0																					
C14:0																					
ISO-C15:0																					
antelso-C15:0																					
C15.0																					
C10.0																					
C16:1c7																					
C16:1t2																					
anteiso-C17:0																					
C17.0																					
9.10-hexvl-C17:	0																				
C18:0	- 1																				
C18:1t8	0.40	1																			
C18:1c9	-0.02 ^{NS}	-0.30) 1																		
C18:1t11	0.22	0.28	0.26	1																	
C18:1t13	0.33	0.34	0.08	0.72	1																

Table 2. cont'd

C18:2c9c12	0.11	0.29	0.11	0.51	0.58	1															
C18:2t9t12	-0.00 ^{NS}	-0.21	0.20	0.58	0.20	0.15	1														
C18:2t9c11	0.08	0.70	0.14	0.50	0.54	0.56	-0.07	1													
C18:2c9c15	-0.14	0.03 ^{NS}	-0.06 ^{NS}	0.02 ^{NS}	0.18	0.34	-0.03 ^{NS}	0.20	1												
C18:3n3	0.21	-0.01 ^{NS}	0.18	0.71	0.63	0.56	0.66	0.27	0.15	1											
C19:0	0.33	0.20	0.11	0.68	0.60	0.55	0.38	0.38	0.23	0.67	1										
C20:0	0.11	-0.19	0.30	0.42	0.37	0.52	0.25	0.21	0.26	0.59	0.61	1									
C22:0	0.08	-0.19	0.28	0.29	0.41	0.50	0.08	0.22	0.47	0.52	0.59	0.87	1								
C20:4n6	-0.14	-0.23	-0.01 ^{NS}	0.15	0.13	0.13	0.33	-0.13	0.55	0.24	0.35	0.20	0.37	1							
C20:5n3	-0.06 ^{NS}	-0.12	0.06 ^{NS}	0.23	0.31	0.25	0.31	0.03 ^{NS}	0.58	0.38	0.45	0.29	0.48	0.83	1						
MCFA	-0.32	-0.21	-0.65	-0.27	-0.20	-0.26	-0.05 ^{NS}	-0.42	-0.10	-0.16	-0.16	-0.28	-0.25	0.11	0.03 ^{NS}	1					
LCFA	0.34	0.23	0.64	0.23	0.14	0.19	-0.03 ^{NS}	0.41	-0.08	0.09	0.11	0.16	0.15	-0.16	-0.10	-0.97	1				
VLCFA	0.06 ^{NS}	-0.22	0.27	0.39	0.40	0.51	0.25	0.17	0.47	0.59	0.66	0.92	0.94	0.50	0.58	-0.21	-0.01 ^{NS}	1			
SFA	-0.15	-0.26	-0.78	-0.62	-0.47	-0.46	-0.25	-0.65	-0.07 ^{NS}	-0.41	-0.42	-0.36	-0.32	0.03 ^{NS}	-0.12	0.74	-0.54	-0.32	1		
USFA	0.15	0.27	0.78	0.63	0.47	0.46	0.25	0.65	0.07 ^{NS}	0.41	0.43	0.36	0.32	-0.02 ^{NS}	0.12	-0.73	0.55	0.32	-0.99	1	
MUFA	0.14	0.21	0.84	0.52	0.37	0.36	0.14	0.57	0.11	0.27	0.31	0.30	0.26	-0.08	0.05 ^{NS}	-0.76	0.62	0.24	-0.98	0.98	1
PUFA	0.11	0.37	0.21	0.81	0.67	0.72	0.56	0.70	0.40	0.80	0.69	0.49	0.43	0.24	0.38	-0.35	0.04 ^{NS}	0.50	-0.66	0.67	0.52

NS, Not significantly different. MCFA, medium-chain FA (sum of C10-C15); LCFA, long-chain FA (sum of C16-C22); VLCFA (sum of C20-C22); SFA, saturated FA (sum of C10:0, C12:0-C16:0, anteiso-C17:0 -C18:0, C19:0-C22:0); USFA, unsaturated FA (sum of C10:1n4, C16:1, C18:1, C18:2, C18:3, C20:4, C20:5); MUFA (sum of C10:1n4, C16:1, C18:1); PUFA (sum of C18:2, C18:3, C20:4, C20:5). Source: Authors

pasture. This was predicted to be the main reason for the discrepancy between our results and Gannan yaks (Liu et al., 2011). In addition, the innovative use of GC-MS, instead of the GC equipment (Or-Rashid et al., 2008; Liu et al., 2011), guaranteed the believable qualitative results of FA composition in yak milk. Although more confirming studies remain needed, these data will be informative for the study of milk FA profile in the future.

We also recognized the limitation of relative quantitative of FA composition. However, this method was used for milk FA profile study in goat (Haile et al., 2016). We also had investigated the variation trend of yak milk FA composition across the whole lactation cycle, and built the correlation among different FA individuals based on a relative quantitative method, which were the main objectives in the present study. Although an absolute quantitative method may be beneficial for detecting the exact content of FA individuals in yak milk, our data also will be informative for understanding the yak milk FA formation regulation.

Another limitation was that we gave a possible that SCD and EVOVLs may be involved in the correlation of FA desaturation and elongation, especially for MUFA and VLCFA, we did not detect the expression of these genes. Obviously, if we could detect the expression of the genes associated with milk fat formation in the mammary gland (Zhu et al., 2014, 2015, 2016), and build their correlation with the FA individuals will enhance our understanding about the regulatory mechanism of FA profile formation in yak milk in the future.

Conclusion

This work studied the variation of milk FA profile across the lactation cycle in yak milk grazed at 3,500 m above sea level on the Qinghai-Tibetan plateau. The most abundant FA in yak milk are C16:0, C18:1c9, C18:0 and C14:0, and are varied significantly along with the lactation periods. Close correlations existed among different FA individuals, in which C18:1t11, iso-C15:0, C22:0 and C16:1c7 are at the central positions, indicating that they may paly important role



Figure 2. Significant correlation of fatty acids in yak milk. The correlation network was performed using the software of Cytoscape 3.1. Bigger node size was associated with more of FA individuals. The color of the node was mapped according to eccentricity. The $|\mathbf{r}| \ge 0.65$ with P < 0.05 was defined as correlated significantly. Source: Authors

in FA profile formation in yak milk. These data will be informative for the study about the regulatory mechanism underlying the milk FA formation in yak.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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