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Posted Date: 3 September 2024

doi: 10.20944/preprints202409.0164.v1

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Fatty Acids Composition of Grass, Yak Milk and Yak Ghee from the Four Altitudes of Qinghai-Tibet Plateau: A Predictive Modelling Approach to Evaluate the Correlation among Altitude, Grass, Yak Milk and Yak Ghee

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Simple Summary: This study investigates how the fatty acid composition of grass, yak milk, and yak ghee changes at different altitudes on the Qinghai-Tibet Plateau. The research aims to understand how environmental factors, such as altitude, influence the nutritional value of these products. The findings reveal that certain fatty acids, such as lauric and myristic acids, are significantly higher at specific altitudes, indicating that yaks adapt to their high-altitude environment. These adaptations affect the quality of yak milk and ghee, which are important food sources for local communities. By highlighting the relationship between altitude and fatty acid profiles, this research can help improve dairy practices, ultimately contributing to better nutrition and food security for residents in high-altitude regions. This knowledge is valuable for enhancing the quality of dairy products and ensuring that local populations have access to nutritious food options.

Abstract: This study investigates the effect of altitude on the fatty acid composition of grass, yak milk, and yak ghee on the Qinghai-Tibet Plateau, aiming to understand how environmental factors influence the nutritional quality of these products. Samples were collected from four different altitudes and analyzed for fatty acid profiles using gas chromatography. The findings reveal significant differences in fatty acid profiles, with notably higher concentrations of lauric acid (C12:0) and myristic acid (C14:0) at altitude A2 compared to others ($p < 0.001$). Furthermore, the fatty acid composition in yak milk and ghee is influenced by the grass consumed and the metabolism of rumen microorganisms. The results indicate that yaks adapt their lipid biosynthesis to high-altitude conditions, as evidenced by a significant increase in stearic acid (C18:0) levels at higher altitudes, which subsequently affects the nutritional value of their milk and ghee. These insights are crucial for improving yak breeding and dairy production practices, ultimately enhancing food security and nutritional health for local communities residing in high-altitude areas. The study underscores the necessity for further research on optimizing these natural resources for better health outcomes.

Keywords: Qinghai-Tibet plateau; fatty acids composition; correlation analysis; yak ; altitude

1. Introduction

Yaks are remarkable animals adapted to harsh environments, primarily found in the Qinghai-Tibet Plateau region. Having unique genetic potential and physiological traits, to thrive in high-altitude, low-oxygen environments [1] . Over 90% of the world's yaks are found in China, where they are adapted to extreme environments and provide livestock products for plateau inhabitants[2,3]. Yaks are vital for the livelihoods and food security of residents, providing high quality meat and fur for human consumption, as well as providing highly nutritious milk. Additionally. the yak milk is also processed to produce yak ghee [4]. Residents of these highlands have historically used yak ghee for various purposes, including as food, a medicinal agent for wound healing, and in religious rituals due to its purity. The reliance on yak ghee has increased because of the limited availability of plant-based oils, making it the primary oil used in their daily diet [5].

Yak milk, often referred to as natural concentrated milk [6], which is characterized by high solids content (16.3 %-19.0 %), the fat (5.6%-8.8%), and protein range from 4.68%-5.41% [7]. Milk composition influenced by various environmental factors including the breeding area, climate, breeding season, the growth stage of grass, and its quality [8,9]. The composition of yak milk, as the raw material for yak ghee, directly affects the composition and quality of the yak ghee[10]. Yak ghee, a traditional handmade dairy product common in the Qinghai-Tibet Plateau, is a key ingredient in Tibetan cuisine and an essential energy source for plateau residents to combat cold temperatures [11,12]. It is bright yellow in color, high in fat content derived from yak milk, by naturally fermentation process. Yak ghee is characterized by its bright yellow color and high fat content, reaching up to 87.7% due to the natural fermentation of yak milk, with approximately two-thirds being SFA such as myristic, palmitic, and stearic acids [13]. Consuming yak ghee can reduce the need for additional saturated fats among pastoralists and offers health benefits, including anti-cancer, anti-atherosclerosis, and osteoporosis inhibition, primarily due to its high conjugated linoleic acid (CLA) content [15,16]. Compared to regular ghee, yak ghee contains higher levels of UFA, making it a significant source of CLA [4].

Yak milk fatty acids differ with grass composition across altitudes, causing variability in yak ghee. Studies have documented the fatty acid profiles of yak ghee from high-altitude regions in Nepal [17], as well as seasonal and altitude-based variations in yak milk on the Qinghai-Tibet Plateau [18,19]. However, there is currently a lack of research on the composition of yak ghee from different altitude areas on the Qinghai-Tibet Plateau, and there is also a deficiency in studies on how the composition of grass at different altitudes affects the nutritional content of yak milk and ghee.

This study examines how fatty acid composition varies with altitude in the grass-milk-ghee system and within a single altitude. It aims to create predictive models, explore unique fatty acid biosynthesis in high-altitude yaks, and assess its impact on milk and ghee quality. The results will reveal how environmental factors influence the health benefits of yak products for plateau residents.

2. Materials and Methods

2.1. Sample Collection

The experiment was carried out from August to September on the Qinghai-Tibet Plateau, with an average temperature of 10°C and humidity at 45%. The 22 counties across six regions in Northern Tibetan Plateau (Yushu, Guoluo, Hainan, Huangnan, Haixi, and Haibei) were selected. The sampling points are divided into four groups A1-A4 according to the elevation gradient of every 500m. Grass samples were collected from each pastoralist's own pasture using several quadrats (1 m×1 m). Collected grass samples were processed and unnecessary materials like stones, woods, and inedible objects were removed, after that grass samples were placed in dry ice and immediately transported stored at -20°C for further analysis.

In the area where grass was collected, six yaks from the herds of local herdsmen were also selected for the collection of yak milk, a total of 15 samples (50 ml) of mixed yak milk from each location. before morning grazing. The milking process was involved allowing the calves to stimulate milk letdown their mothers followed by manually milk samples were collected by hand milking.

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After milking, samples were placed into centrifuge tubes. Additionally, yak ghee made by pastoralists themselves was collected using double-headed stainless-steel spoons and stored in falcon tubes of 15 mL. All collected yak milk and yak ghee samples were cooled and transported to the laboratory, then frozen at -20°C for storage. The distribution of sampling points is shown in Figure 1.

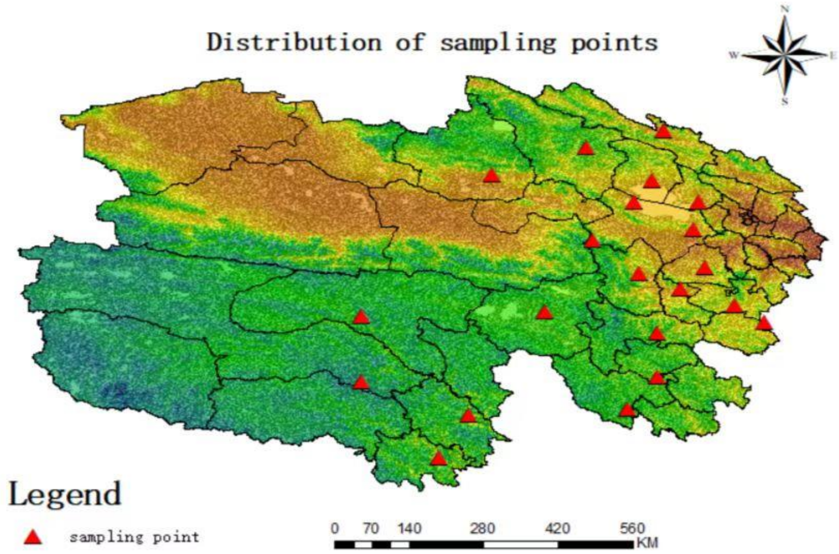

Figure 1. Distribution of sampling points for grass, milk and ghee sample collection.

2.2. Sample Analysis

The fat content in grass was determined using an XT15i automatic fat analyzer ANKOM XT15i，ANKOM，USA). The fat content in yak ghee and yak milk was determined according to standards (AOAC,1999) [20] , the 10 mL of ghee or milk liquid was mixed with 1.5 mL concentrated ammonia solution, 10 mL ethanol, 10 mL petroleum ether, and 10 mL ether successively in a separation funnel. After that the upper layer was collected, and the solvents (ether and petroleum ether) were removed by heating, then weighed. The fatty acid composition of yak milk and yak ghee were evaluated according to Luo, et al. [21] , the processed involved the frozen samples were thawed at room temperature and weighed 0.5 g (analytical balance, METTLER TOLEDO, Switzerland) and transferred to a 25ml of dry glass tube. After that 5.0 mL of toluene and 6 mL 10% acetyl chloride-methanol solution were added successively. The tubes were tightly capped, mixed by shaking, and heated in a water bath at (80±1 °C) for 2 hours, the samples were shaked manually for 20 minutes. After cooling to room temperature, the reaction solution was transferred to a 50 mL centrifuge tube. The tubes were washed three times with 3 mL sodium carbonate solution, and the washings were combined in the centrifuge tube and thoroughly mixed. After centrifugation at 5000 rpm for 5 minutes, the supernatant was transferred to a gas chromatography column (AT-FFAP capillary column, 30.0m*0.32μm*0.32μm) and analyzed using a gas chromatography-mass spectrometer (GC2014C, Shimadzu, Company Origin).

2.3. Data Analysis

Experimental data were organized and edited using Excel 2019 software. Subsequently, multivariate analysis of variance (MANOVA) was performed using RStudio statistical analysis software （version 4.4.1）to validate whether fatty acids in grass-milk-ghee systems at different altitudes showed significant changes ($p<0.05$). Linear discriminant analys. The overall approach of this experiment is shown in Figure 2.

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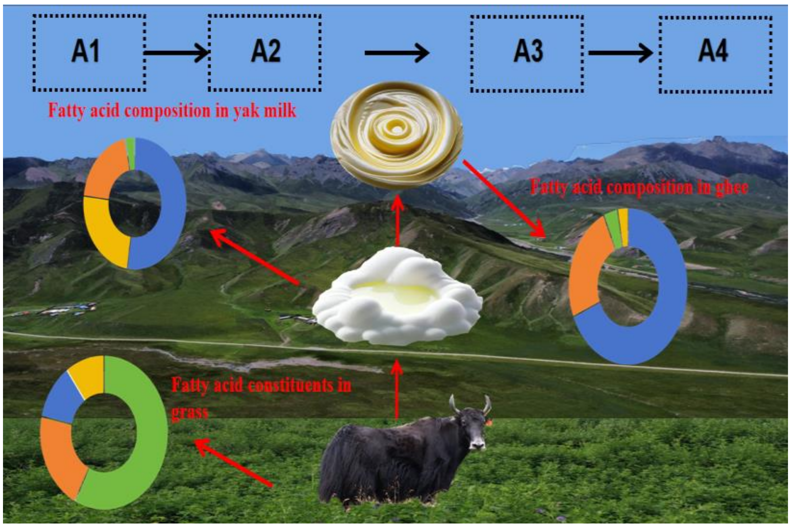

Figure 2. Diagram of the overall idea of the experiment(The blue area represents the proportion of saturated fatty acids, the orange area represents the proportion of unsaturated fatty acids, the yellow area represents the proportion of polyunsaturated fatty acids, and the green area represents the proportion of polyunsaturated fatty acids to saturated fatty acids).

3. Results and Discussion

3.1. Fatty Acid Spectrum in Grass-Yak Milk-Ghee

Tables 1 and 2 present the fatty acid compositions of grass and yak milk. Lauric acid (C12:0) levels were significantly elevated (P < 0.001) at A2 altitude compared to A1, A3, and A4. Similarly, myristic acid (C14:0) was significantly higher at A2 than at A1, A3, and A4. Conversely, stearic acid (C18:0) was significantly more prevalent (P < 0.001) at A1, A2, and A3 compared to A4. Additionally, C18:2n6c was significantly higher (P < 0.001) at A4 and A1 compared to A2 and A3. Significant altitude-related changes were observed for linolenic acid and eicosadienoic acid, whereas stearic acid, arachidic acid, and specific polyunsaturated fatty acids did not show significant variation, consistent with previous findings [19]. Altitude also affects the content of long-chain fatty acids in grass, such as Eicosadienoic acid（C20:2） and linoleic acid（C18:2）, corroborated by earlier studies. Factors influencing grass fatty acid content include type, growth stage, fertilization, and preservation methods[22] , and yak milk’s fatty acid profile is influenced by grass[19] . Therefore, these changes may relate to the plant’s adaptation mechanisms to high-altitude conditions [23,24] .

Table 1. Fatty acid content in grass（g/100 g） samples collected form four altitude in Qinghai Province.

	Altitude gradient						
Fatty acids	A1	A2	A3	A4	average	SEM	p-value
C4:0	0.20	0.21	0.21	ND	0.207	0.016	0.975
C6:0	0.44	0.49	0.55	0.50	0.495	0.038	0.878
C8:0	0.26	0.30	0.31	0.30	0.293	0.021	0.095
C10:0	0.19	0.09	ND [1]	ND	0.140	0.050	0.051
C11:0	0.90	0.72	0.67	0.79	0.770	0.077	0.798
C12:0	0.77^b	1.44^a	0.59^b	0.39^b	0.798	0.091	<0.001
C13:0	0.50	0.46	0.84	0.57	0.593	0.067	0.448
C14:0	1.15^b	1.86^a	1.02^b	1.11^b	1.285	0.075	<0.001
C15:0	0.90	1.06	0.96	0.97	0.973	0.120	0.975
C16:0	20.60	21.31	19.93	20.69	20.63	0.443	0.731
C16:1	0.43	0.40	0.38	0.43	0.410	0.018	0.746
C17:0	0.37	0.59	0.36	0.30	0.405	0.052	0.231

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C17:1	0.27	1.23	0.22	0.32	0.510	0.169	0.108
C18:0	4.83^{a}	5.40^{a}	5.13^{a}	3.36^{b}	4.680	0.166	<0.001
C18:1n9t	0.22	0.30	0.27	0.37	0.290	0.022	0.096
C18:1n9c	4.03	4.03	2.94	2.84	3.460	0.296	0.301
C18:2n6c	13.34^{a}	10.26^{b}	11.02^{b}	13.86^{a}	12.12	0.333	<0.001
C20:0	1.52^{ab}	1.25^{b}	1.90^{a}	2.01^{a}	1.670	0.098	0.016
C18:3n6 [2]	5.25^{bc}	5.92^{ab}	6.28^{a}	4.56^{c}	5.503	0.157	<0.001
C18:3n3	33.89	35.99	40.00	37.66	36.88	1.140	0.280
C21:0	0.22^{b}	0.37^{a}	0.29^{ab}	0.25^{ab}	0.283	0.024	0.050
C20:2	2.39^{ab}	2.64^{ab}	2.28^{b}	3.40^{a}	2.678	0.118	<0.001
C22:0	1.75^{ab}	1.36^{b}	1.99^{ab}	2.24^{a}	1.835	0.022	0.024
C24:0	4.69	4.82	3.58	4.01	4.275	0.362	0.071
C20:5n3	1.37^{ab}	1.11^{b}	1.11^{b}	1.50^{a}	1.273	0.602	0.032
C22:6n3	0.64^{b}	0.79^{b}	2.04^{a}	0.25^{b}	0.930	0.196	0.002

A1 was grazed on rangeland at an elevation of about 3100 m; A2 was grazed on rangeland at an elevation of about 3600 m; A3 was grazed on rangeland at an elevation of about 4100 m; A4 was grazed on rangeland at an elevation of about 4600 m, the means with different superscript within the same row bearing different superscript are significantly different (P<0.05).

Table 2. Fatty acid content in yak milk（g/100 g）samples collected form four altitude in Qinghai Province. A1 was grazed on rangeland at an elevation of about 3100 m; A2 was grazed on rangeland at an elevation of about 3600 m; A3 was grazed on rangeland at an elevation of about 4100 m; A4 was grazed on rangeland at an elevation of about 4600 m , the means with different superscript within the same row bearing different superscript are significantly different (P<0.05).

	Altitude gradient						
Fatty acids	A1	A2	A3	A4	Average	SEM	p-value
C4:0	1.12	1.11	1.03	1.14	1.100	0.179	0.131
C6:0	1.84	1.82	1.71	1.86	1.808	0.026	0.167
C8:0	1.16	1.93	1.10	1.21	1.350	0.023	0.303
C10:0	2.36	2.43	2.18	2.39	2.340	0.048	0.258
C12:0	1.80^{ab}	1.96^{a}	1.74^{b}	1.89^{ab}	1.848	0.035	0.050
C13:0	0.08^{b}	0.08^{b}	0.08^{b}	0.09^{a}	0.083	0.001	0.004
C14:0	9.02^{ab}	9.60^{a}	8.78^{b}	9.28^{ab}	9.170	0.111	0.038
C14:1	0.36^{b}	0.42^{a}	0.37^{b}	0.39^{ab}	0.385	0.008	0.020
C15:1	1.57^{a}	1.57^{a}	1.52^{ab}	1.45^{b}	1.528	0.019	0.050
C16:0	34.51^{ab}	34.78^{a}	32.71^{b}	33.67^{ab}	33.918	0.330	0.978
C16:1	2.24^{b}	2.47^{a}	2.23^{b}	2.22^{b}	2.290	0.024	0.009
C17:0	1.02	1.08	0.99	1.10	1.048	0.027	<0.001
C17:1	0.37^{ab}	0.46^{a}	0.36^{b}	0.38^{ab}	0.393	0.016	0.037
C18:0	17.65^{ab}	16.40^{b}	18.71^{a}	17.48^{ab}	17.560	0.296	0.035
C18:1n9t	0.28^{ab}	0.25^{b}	0.30^{a}	0.27^{ab}	0.275	0.007	0.049
C18:1n9c	21.00	21.2	22.17	21.34	21.428	0.250	0.480
C18:2n6t	0.29^{c}	0.24^{b}	0.29^{b}	0.35^{a}	0.293	0.008	0.010
C18:2n6c	1.41^{a}	1.19^{b}	1.58^{a}	1.48^{ab}	1.415	0.033	0.011
C20:0	0.37	0.40	0.39	0.36	0.380	0.007	0.272
C20:1	0.19^{ab}	0.23^{a}	0.23^{a}	0.14^{b}	0.198	0.026	0.030
C18:3n3	1.31^{a}	0.98^{b}	1.52^{a}	1.38^{a}	1.298	0.097	<0.001
C21:0	0.16	0.11	0.13	0.17	0.143	0.010	0.101
C22:0	0.15	0.18	0.15	0.16	0.160	0.006	0.325
C20:4n6	0.17	0.17	0.16	0.13	0.158	0.009	0.669
C24:0	ND	0.12	0.10	ND	0.110	0.010	0.192

In yak milk, palmitic acid (C16:0) is the most abundant fatty acid, followed by oleic acid (C18:1n9c) and stearic acid (C18:0). Notable differences in medium- to long-chain fatty acids were observed with altitude. Group A2 had higher levels of caprylic acid (C8:0), capric acid (C10:0), and lauric acid (C12:0) compared to group A3 ($P < 0.001$). Group A4 had the highest concentrations of margaric acid (C17:0), caproic acid (C6:0), and tridecanoic acid (C13:0). Alpha-linolenic acid (C18:3n3) was highest in group A3 ($P < 0.001$), while group A4 had the highest levels of tricosanoic acid (C21:0). These results are consistent with previous studies on yak milk fatty acids[25]. The increase in long-chain fatty acids at high altitudes may be attributed to yaks mobilizing fat in response to lower oxygen levels, a phenomenon also linked to higher long-chain fatty acids in high-altitude grass [26] .

In yak ghee,(Table 3), palmitic acid (C16:0) and oleic acid (C18:1n9c) are the most prevalent. Butyric acid (C4:0) was significantly higher at A4 compared to A1, A2, and A3 ($P < 0.001$), showing a trend of increasing with altitude. The rise in butyric acid (C4:0) along the altitude gradient enhances the energy value of Tibetan yak ghee, making it advantageous for high-altitude adaptation [27]. Short and medium-chain fatty acids are major components in Tibetan yak ghee across altitudes, aligning with previous research from Qinghai Guoluo [14], This may be due to smaller fat globules in yak milk compared to cow milk, offering a larger interfacial area for gastric lipase, which preferentially releases these fatty acids [28]. The composition of yak milk will directly affect the composition of yak ghee

Conjugated linoleic acid (CLA) C9T11 was detected in Tibetan yak ghee at proportions of 21.57%, 23.88%, 23.18%, and 22.54% in samples A1, A2, A3, and A4, respectively,

Table 3. Fatty acid content in ghee（g/100 g）samples collected form four altitude in Qinghai Province. A1 was grazed on rangeland at an elevation of about 3100 m; A2 was grazed on rangeland at an elevation of about 3600 m; A3 was grazed on rangeland at an elevation of about 4100 m; A4 was grazed on rangeland at an elevation of about 4600 m. The means bearing different superscript within the same row are significantly different ($P<0.05$).

	Altitude gradient					
Fatty acids	**A1**	**A2**	**A3**	**A4**	**Average**	**SEM**
C4:0	3.67^{ab}	3.46^{b}	4.04^{ab}	4.63^{a}	3.950	0.183
C6:0	4.99	3.36	3.64	4.41	4.100	0.335
C8:0	1.68	1.55	1.65	1.95	1.708	0.073
C10:0	3.01	2.71	2.87	3.36	2.988	0.118
C11:0	0.025^{a}	0.020^{b}	0.022^{ab}	0.025^{a}	0.023	0.001
C12:0	2.22	1.95	2.11	2.24	2.130	0.069
C14:1	0.932	0.942	1.106	0.900	0.970	0.054
C14:0	9.46	9.05	9.10	9.32	9.233	0.138
C15:0	1.37^{ab}	1.43^{a}	1.41^{ab}	1.32^{b}	1.383	0.017
C16:1	1.38	1.35	1.35	1.41	1.373	0.021
C16:0	30.44	30.37	29.42	29.59	29.95	0.388
C18:0	14.06	14.40	14.03	12.83	13.83	0.265
T9C18:1	0.39	0.41	0.40	0.37	0.393	0.016
C9C18:1	18.77^{ab}	19.07^{a}	18.79^{ab}	17.67^{b}	18.57	0.229
T6C18:2	0.30^{ab}	0.33^{a}	0.27^{b}	0.29^{ab}	0.298	0.009
C6C18:2	1.02^{b}	0.88^{b}	1.20^{a}	1.00^{b}	1.025	0.034
C20:0	0.30	0.36	0.33	0.29	0.320	0.011
N3C18:3	1.28^{b}	1.20^{b}	1.55^{a}	1.27^{b}	1.325	0.039
C21:0	1.11^{a}	0.10^{ab}	0.09^{ab}	0.08^{b}	0.345	0.004
C20:2	0.07^{ab}	0.08^{a}	0.07^{ab}	0.06^{b}	0.070	0.018
C8C20:3	0.17	0.10	0.10	0.08	0.113	0.005
C22:0	0.005^{b}	0.016^{b}	0.047^{a}	0.003^{b}	0.018	0.001
C22:1	0.006^{b}	0.016^{a}	0.017^{a}	0.011^{ab}	0.013	0.021

C20:4	0.10	0.16	0.09	0.07	0.105	0.003
C23:0	0.08	0.10	0.09	0.08	0.088	0.002
C20:5	0.070^{b}	0.067^{a}	0.068^{ab}	0.059^{ab}	0.066	0.025
C24:0	0.095^{ab}	0.189^{a}	0.106^{ab}	0.103^{ab}	0.123	0.002
C24:1	0.018^{a}	0.020^{a}	0.013^{ab}	0.008^{ab}	0.015	0.002
C22:6	0.030^{a}	0.025^{a}	0.025^{a}	0.015^{b}	0.024	0.002
anteisoC13:0	0.063	0.061	0.069	0.069	0.066	0.001
isoC13:0	0.022	0.026	0.023	0.023	0.024	0.021
C13:0	0.080	0.077	0.168	0.080	0.101	0.021
isoC14:0	0.22	0.25	0.26	0.25	0.245	0.006
anteisoC15:0	0.49^{ab}	0.52^{a}	0.49^{ab}	0.45^{b}	0.488	0.011
isoC15:0	0.72^{b}	0.80^{ab}	0.81^{a}	0.77^{ab}	0.775	0.014
isoC16:0	0.37	0.39	0.39	0.36	0.378	0.008
anteisoC17:0	0.35^{ab}	0.37^{a}	0.32^{b}	0.27^{c}	0.328	0.010
isoC17:0	0.50^{ab}	0.53^{a}	0.50^{ab}	0.45^{b}	0.495	0.012
C17:0	0.74	0.81	0.74	0.80	0.773	0.027
C9C17:1	0.34	0.34	0.34	0.37	0.348	0.012
C9T11CLA	1.57	1.68	1.62	1.59	1.615	0.052

surpassing the CLA content in cow milk ghee [29]. This elevated CLA level underscores the superior nutritional value of Tibetan yak ghee compared to standard ghee [17]. The high concentrations of palmitic acid (C16:0) and oleic acid (C18:1n9c) in Tibetan yak ghee are linked to the elevated linoleic acid (C18:2n6c) content in yak pasture. Linoleic acid undergoes biohydrogenation in yaks' rumen, yielding palmitic and stearic acids (C18:0) [30]. These fatty acids enhance the antioxidant properties and storage stability of yak ghee[31,32].

3.2. Correlation Analysis of Fatty Acids in Grass-Yak Milk-Ghee Across Different Altitudes

According to the results from correlation heatmaps（Figure.3), the proportion of saturated fatty acids（SFA）in grass from different altitudinal regions shows correlations with both the saturated and unsaturated fatty acids in yak milk and yak ghee. There is a strong positive correlation between the proportion of unsaturated fatty acids（UFA）in grass and the ratio of polyunsaturated to saturated fatty acids in yak milk. Additionally, the proportion of polyunsaturated fatty acids (PUFA) in grass shows a strong positive correlation with the PUFA proportion in both yak milk and yak ghee. This is because the grass, as the sole feed source for yaks, provides a sustainable source of PUFA in the milk fat [33], confirming the superiority of grass-fed yak milk fat.

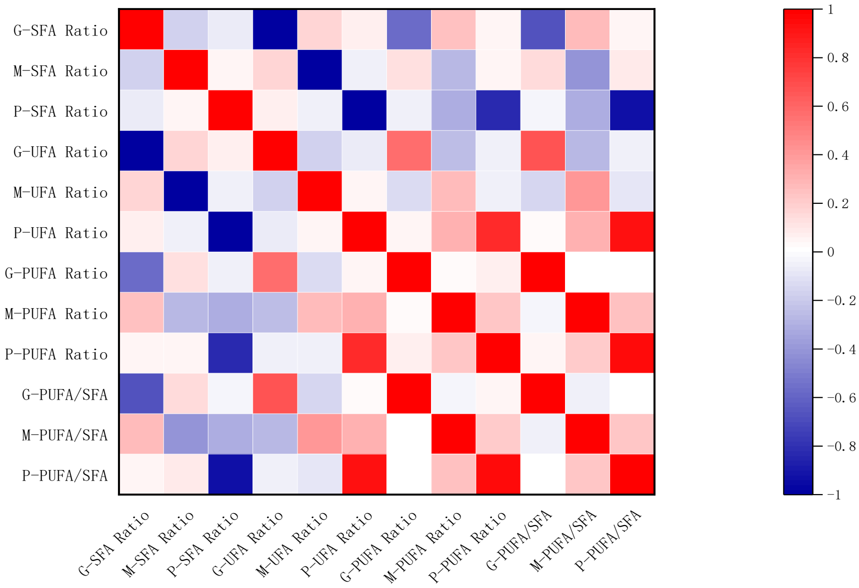

Figure 3. Correlation heat map of four fatty acids in grass-yak milk-yak ghee P grassPasture, M, yak milk, G, yak ghee.

Fatty acid composition varied significantly across different altitudinal samples ($p<0.05$). Linear Discriminant Analysis (LDA) based on altitude (Figure 4A) indicates that the first and second discriminant functions account for 75.76% and 17.72% of the variance, Together, they cover 93.48% of the total variance, indicating adequate separation of fatty acid data across four altitude levels (A1 to A4). Specifically, the first discriminant function distinguishes the groups with elevation gradients A3 and A4, and the second discriminant function distinguishes the groups with elevation gradients A1 and A2. The divisions based on altitude gradients are primarily attributed to environmental factors influencing the transfer between grass, milk, and ghee. The fatty acids in the grass are affected by temperature [34] and sunlight [35] , and with increasing altitude, temperatures decrease while sunlight intensity increases. As shown in Figure 5, unsaturated fatty acids, which are predominant in grass, significantly increase in proportion with altitude within a 1000 m gradient. This is because UFA maintain cell membrane fluidity and function, which is crucial for the survival of grass in extreme environments [36] . The proportion of UFA in yak milk from summer pastures in Qinghai-Tibet Plateau is similar to previous findings, and higher than those from winter pastures, due to the increased proportion of fresh grass in the diet leading to an increase in unsaturated fatty acids in the milk [37].

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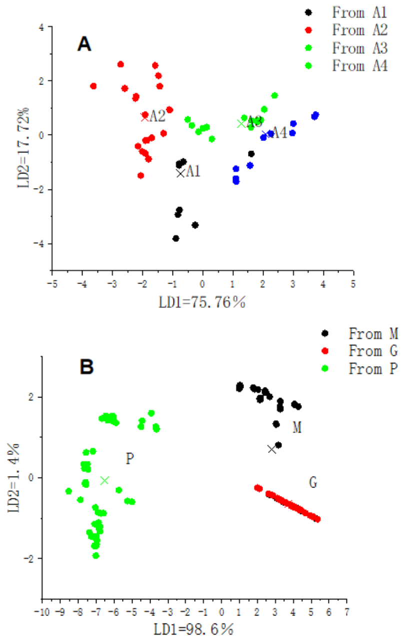

Figure 4. A is an LDA plot based on the altitudinal gradient, B is an LDA plot based on the type of sample, P, pasture, M, yak milk, G, yak ghee A1 was grazed on rangeland at an elevation of about 3100 m; A2 was grazed on rangeland at an elevation of about 3600 m; A3 was grazed on rangeland at an elevation of about 4100 m; A4 was grazed on rangeland at an elevation of about 4600 m.

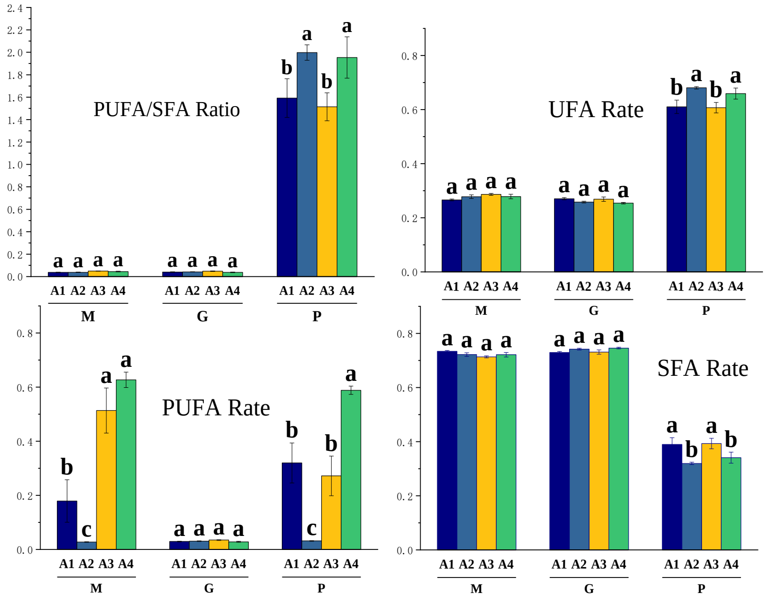

Figure 5. Multifactorial bar chart of multivariate analysis for pasture-yak milk-yak ghee fat, P, M, yak milk, G, yak ghee fat, A1 was grazed on rangeland at an elevation of about 3100 m; A2 was grazed on rangeland at an elevation of about 3600 m; A3 was grazed on rangeland at an elevation of about 4100 m; A4 was grazed on rangeland at an elevation of about 4600 m. Different letters on bar indicate significant differences P<0.05.

MANOVA revealed significant differences in fatty acid composition among grass, yak milk, and yak ghee ($p < 0.05$). The LDA plot (Figure 4B) shows that the first discriminant function explains

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98.6% of the variance, separating yak milk from yak ghee, while the second function (1.4% variance) distinguishes grass. This separation is due to microbial lipid synthesis in the rumen [38], which alters fatty acid profiles in milk and ghee [39]. Figure 4 illustrates distinct fatty acid profiles for grass compared to yak milk and ghee. The fatty acid compositions of yak milk and ghee are similar, with minimal changes during ghee production [19,40]. However, yak ghee has reduced polyunsaturated fatty acids compared to yak milk, likely due to processing-induced oxidative degradation [41].

3.3. Correlation Analysis of Fatty Acid Indicators in Grass-Milk-Ghee at the Same Altitude

Regression analysis was conducted on grass, milk, and ghee from the same altitude, as shown in Table 4. The results for the A4 group and A3 group show good linear fits for saturated, unsaturated, and especially polyunsaturated fatty acids, which may be due to the harsh high-altitude environment prompting grass and yaks to adjust their biosynthesis of lipids to adapt. The ruminal and metabolic mechanisms of Tibetan yaks on the Qinghai-Tibet Plateau significantly influence the dominant fatty acids in milk[42] . The R^2 values for linear fits in the A4 group were 0.662, 0.587, and 0.784, respectively. The regression equations indicate a negative correlation between grass fatty acid indicators and yak milk fatty acids, except for polyunsaturated fatty acids in group A3. Conversely, yak ghee shows a positive correlation with yak milk indicators. Linear fitting was less effective for groups A1 and A2, potentially due to a reduced impact of environmental factors on physiological processes in these regions or the influence of factors like soil type, water availability, and temperature fluctuations on fatty acid composition[43,44].

Table 4. Regression Analysis of Grass-Milk-Ghee at the Same Altitude. M, Yak Milk, P, Pasture Grass, G, Yak ghee, A1 was grazed on rangeland at an elevation of about 3100 m; A2 was grazed on rangeland at an elevation of about 3600 m; A3 was grazed on rangeland at an elevation of about 4100 m; A4 was grazed on rangeland at an elevation of about 4600 m. SFA Rate, Saturated fatty acids as a percentage of all fatty acids. UFA Rate, unsaturated fatty acids as a percentage of all fatty acids. PUFA Rate, polyunsaturated fatty acids as a percentage of all fatty acids. R2, Representing the goodness of fit of a regression equation, the higher the R^2, the better the model fits the data. P-Value, A measure of the statistical significance of the effect of an independent variable on a dependent variable.

Altitude	Content	R^2	P-Value	Regression equation
A4	SFA Rate	0.662	0.008	M-SFA Rate=-0.92+2.235G-SFA Rate-0.075P-SFA Rate
	UFA Rate	0.587	0.008	M-UFA Rate=-0.239+2.235G-UFA Rate-0.075P-UFA Rate
	PUFA Rate	0.784	0.000	M-PUFA Rate=0.615-13.856G-PUFA Rate-0.684P-PUFA Rate
A3	SFA Rate	0.647	0.004	M-SFA Rate=0.54+0.154G-SFA Rate-0.155P-SFA Rate
	UFA Rate	0.647	0.004	M-UFA Rate=0.151+0.154G-UFA Rate-0.155P-UFA Rate

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	PUFA Rate	0.739	0.001	M-PUFA Rate=1.536-37.874G-PUFA Rate+1.076P-PUFA Rate
A2	SFA Rate	0.225	0.112	-
	UFA Rate	0.225	0.112	-
	PUFA Rate	0.095	0.259	-
A1	SFA Rate	0.129	0.161	-
	UFA Rate	0.129	0.161	-
	PUFA Rate	0.315	0.034	-

3.4. Functional Fatty Acids in Grass-Milk-Ghee

Functional fatty acids, particularly ω-3 fatty acids, are present in varying concentrations in grass, yak milk, and yak ghee. Notably, α-linolenic acid (C18:3n3) is found in high levels in grass (Figure 6) and shows a significant increase ($P < 0.001$) along altitude gradients from A1 to A2 and from A3 to A4. Conversely, α-linolenic acid levels are markedly lower in yak milk and yak ghee ($P < 0.001$). The concentrations of eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA) in yak ghee are significantly higher than those in grass ($P < 0.001$), likely due to the in vivo conversion of α-linolenic acid into EPA and DHA [45]. The levels of EPA and DHA in grass are notably lower at the A2 altitude compared to A1, A3, and A4 ($P < 0.001$). In contrast, EPA content in yak ghee is significantly higher at A2 compared to A1, A3, and A4 ($P < 0.001$). DHA content peaks in grass at the A2 altitude and declines at higher altitudes, whereas in yak ghee, DHA content peaks at A3 altitude. γ-Linolenic acid (C18:3n6) was not detected in yak milk and ghee but is abundant in grass, particularly at A2 altitude. Research suggests that γ-linolenic acid (C18:3n6) synthesis pathways are upregulated in yak serum at high altitudes[46]. Asγ-linolenic acid (C18:3n6) contributes to cell membrane phospholipids and aids in protection against inflammation and oxidative stress, its increased availability at high altitudes may support yaks' adaptation to harsh conditions[46]. Consequently, the reduced levels of γ-linolenic acid (C18:3n6) in yak milk and ghee may reflect its greater utilization in cell membrane construction. Linoleic acid (C18:2n6c) was present in lower concentrations in yak milk and ghee (Figure 6). The correlation observed in Figure 4 indicates that as the linoleic acid (C18:2n6c) content in forage increases, so does the content of PUFA in yak milk. Linoleic acid (C18:2n6c) likely serves as a precursor for CLA and ω-3 . In yaks, linoleic acid (C18:2n6c) is preferentially used for PUFA synthesis, thus enhancing the PUFA content in milk fat[47].Oleic acid (C18:1n9c) is less prevalent in grass but reaches its peak concentration in yak milk and ghee at A3 and A1 altitudes, respectively. The higher C18:1n9c levels in yak milk and ghee may result from its conversion from palmitic acid rather than direct dietary intake from grass [48]. C18:1n9c is also associated with calf growth and development, leading to its incorporation into milk fat during yak milk synthesis[49].

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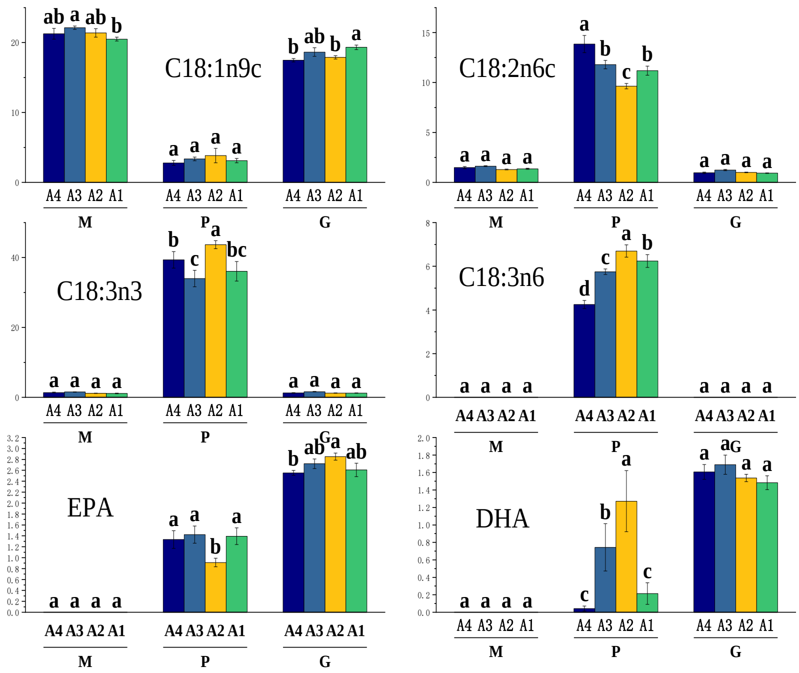

Figure 6. Multifactor bar chart of functional fatty acids in straw-milky-ghee, M, Yak Milk, P, Pasture Grass, G, Yak ghee, A1 was grazed on rangeland at an elevation of about 3100 m; A2 was grazed on rangeland at an elevation of about 3600 m; A3 was grazed on rangeland at an elevation of about 4100 m; A4 was grazed on rangeland at an elevation of about 4600 m. Different letters on bar indicate significant differences P<0.05).

4. Conclusion

This study analyzed fatty acid profiles in grass, yak milk, and yak ghee across different altitudes on the Qinghai-Tibet Plateau, highlighting the effects of environmental factors on fatty acid composition and its transmission. At high altitudes, adaptations in lipid biosynthesis are observed in both grass and yaks. The fatty acid composition of yak milk and ghee is influenced by grass and modified by rumen microbial metabolism. Omega-3 fatty acids, abundant in grass, are present in lower amounts in yak milk and ghee due to metabolic changes. Higher altitudes correlate with increased energy value and concentrations of fatty acids that help counter high-altitude sickness, benefiting local residents. This research supports improvements in yak breeding and dairy quality and suggests further study on fatty acid changes in the grass-yak milk-ghee system. Future research should focus on how breeding management practices can improve the nutritional value of yak milk and ghee, and how these natural resources can contribute to food security and nutritional health for residents of high-altitude areas.

Author Contributions: Conceptualization, L.H., R.W.and.J.Y; methodology, R.W.,M.M., Y.H.,Y.Y.,S.L., X.H., B.B., J.Y.; validation, M.M.and L.H.; formal analysis, R.W.,J.Y.; data curation, R.W.,J.Y., L.H.; writing—original draft preparation,R.W.; writing—review and editing, M.M.,Y.H., L.H.,Y.Y.,R.Z.,MM., B.B., S.L.X.H.; visualization R.W.; supervision, L.H.,B.B and M.M.; project administration, L.H.; funding acquisition L.H., All authors have read and agreed to the published version of the manuscript.

Funding: The authors thank the National Natural Science Foundation of China (32060766, U20A2002); the Qinghai Provincial Natural Science Fund for Distinguished Young Scholars (2024-ZJ-905); Provincial Key Laboratory Incentive Funds-Key Laboratory of Plateau Grazing Animal Nutrition and Feed Science of Qinghai Province (2024-1-8) for financial support.

Data Availability Statement: Data openly available in a public repository

Conflicts of Interest: The authors declare no potential conflict of interests.

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