

Article

Effects of Concentrate Supplementation Under Grazing Conditions on Milk Yield and Milk Nutritional Composition in Yili Mares

Zihao Xu [†], Mengfei Li [†], Long Sun, Zhiqiang Cheng, Yingying Yu, Yong Chen, Fengming Li and Changjiang Zang ^{*}

Xinjiang Herbivore Nutrition Laboratory for Meat & Milk, College of Animal Science, Xinjiang Agricultural University, Urumqi 830052, China; 18139298055@163.com (Z.X.); lmf0212@126.com (M.L.); muxiangmu163@163.com (L.S.); cheng07162022@163.com (Z.C.); yyychnxj@yeah.net (Y.Y.); cy@xjau.edu.cn (Y.C.); lifming@xjau.edu.cn (F.L.)

^{*} Correspondence: zcj780@xjau.edu.cn

[†] These authors contributed equally to this work.

Abstract

Under grazing conditions, it is difficult for lactating Yili mares to meet their nutritional requirements and those of their suckling foals solely through the consumption of natural pasture. Furthermore, seasonal variations and rainfall significantly influence the quality and nutrient content of forage, which severely constrains the healthy breeding of Yili horses and the industrial development of mare milk resources. Therefore, this study aimed to investigate the effects of concentrate supplementation on lactation performance and milk concentrations of amino acids, fatty acids, and mineral elements in Yili horses under grazing conditions. Twenty-two healthy Yili mares in early lactation, with similar ages (3–4 years), foaling dates, and body weights (391.5 ± 13.74 kg), were randomly assigned to either a grazing group (G, $n = 11$) or a grazing + supplementation group (GS, $n = 11$). Mares in group G grazed naturally on pasture, while those in group GS received 1 kg of concentrate supplement twice daily (totaling 2 kg/day) in addition to grazing. The experimental period lasted for 100 days, including a 10-day adaptation period and a 90-day formal experimental period. The results showed that: (1) In terms of lactation performance, the GS group exhibited highly significant increases in milk yield and lactose yield ($p < 0.01$), as well as significant increases in milk protein and milk fat yields ($p < 0.05$), with an extended duration of the peak lactation period. (2) Regarding the amino acid profile, the concentrations of threonine (Thr), serine (Ser), glycine (Gly), and alanine (Ala) in the milk of the GS group were significantly higher than those in the G group ($p < 0.05$), whereas the proline (Pro) content was significantly lower ($p < 0.01$); supplementation improved the uptake of certain functional amino acids by the mammary gland. (3) Concerning the fatty acid profile, the concentrations of polyunsaturated fatty acids (PUFA) and alpha-linolenic acid in the milk of the G group were significantly or highly significantly higher than those in the GS group ($p < 0.05$ or $p < 0.01$). (4) For mineral elements, concentrate supplementation highly significantly decreased the potassium (K) content and the K/Na ratio in horse milk ($p < 0.01$), highly significantly increased the levels of iron (Fe) and cobalt (Co) ($p < 0.01$), and significantly enhanced the chromium (Cr) content ($p < 0.05$). In conclusion, concentrate supplementation during grazing improved lactation performance in Yili mares, primarily by increasing milk yield and extending the peak lactation period. However, grazing alone was more favorable for maintaining higher PUFA and α -linolenic acid proportions in milk. Therefore, concentrate supplementation should be regarded as a nutritional strategy that increases milk output and modifies amino acid and mineral element composition, but may involve a trade-off with some beneficial fatty acids.



Academic Editor: Petru Alexandru Vlaicu

Received: 17 April 2026

Revised: 12 May 2026

Accepted: 12 May 2026

Published: 14 May 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\) license](https://creativecommons.org/licenses/by/4.0/).

Keywords: Yili horses; feeding system; lactation performance; amino acids; fatty acids; mineral elements

1. Introduction

The Yili horse, an elite native breed unique to China, is known for its distinctive biological traits, including high tolerance to roughage, strong cold resistance, and exceptional athletic ability. This breed has significant economic importance in endurance sports and meat production; additionally, its potential for milk production has gained increasing interest in recent years [1]. The nutritional profile of mare milk features low fat and protein levels and a high lactose content. Its overall composition closely resembles that of human breast milk and is rich in bioactive substances, including unsaturated fatty acids, lysozyme, and lactoferrin [2]. Previous research shows that horse milk is less allergenic than cow milk and may positively influence lipid metabolism, boost immune function, and support gut microbiota [3].

The Xinjiang Uyghur Autonomous Region has abundant natural grassland resources and is a key area for pastoral animal husbandry in China. Natural pasture is the main feed resource for Yili horses in grazing systems; however, its nutrient supply is not constant and can vary markedly with season, precipitation, plant growth stage, and pasture quality [4,5]. During periods of reduced forage availability or lower nutritive value, natural pasture may not consistently provide sufficient digestible energy, available protein, limiting amino acids, fatty acid precursors, and mineral elements to meet the high nutritional demands of lactating Yili mares. Therefore, concentrate supplementation may help compensate for temporal fluctuations in pasture nutrient supply and support milk production under grazing conditions. At the same time, as market demand for mare milk products continues to grow, competition for milk between humans and foals has become more intense. This results in insufficient milk intake for suckling foals, leaving young stock in a chronic energy and protein deficiency, which worsens the tension between production and resource allocation [6]. To effectively address the nutritional gaps of natural pasture and improve the lactation performance of Yili mares, scientific concentrate supplementation has become a vital recent strategy in feeding management. Mara et al. [7] reported that supplementing pasture-fed dairy cows with concentrate increased milk production. Pyle et al. [8] fed mares diets with different levels of non-structural carbohydrates (NSC) and found that mares in the high-NSC group produced substantially more milk and had higher lactose content than those in the low-NSC group. Zhang et al. [9] showed that protein supplementation during the warm season significantly boosted milk yield in lactating yaks.

For Yili mares, existing studies have mainly focused on the effects of corn or concentrate supplementation on milk yield, basic milk composition, blood biochemical indices, and intestinal microbiota [10,11]. Nevertheless, limited information is available on how concentrate supplementation under grazing conditions affects the broader nutritional composition of Yili mare milk, particularly amino acid profiles, fatty acid composition, and mineral element deposition. Moreover, the relationship between pasture mineral supply and milk mineral composition in grazing Yili mares remains poorly understood. Therefore, we hypothesized that concentrate supplementation under grazing conditions would improve milk yield in Yili mares and modify milk nutritional composition, although responses across nutrient classes, including amino acids, fatty acids, and mineral elements, may differ due to changes in dietary nutrient supply. To test this hypothesis, this study compared grazing alone with grazing supplemented with concentrate to evaluate their effects on milk yield, milk composition, amino acid profiles, fatty acid composition, and mineral

element concentrations in Yili mares. The findings are expected to provide breed- and system-specific evidence to improve the nutritional management of lactating Yili mares and to support the development of mare milk resources in Xinjiang.

2. Materials and Methods

2.1. Ethical Considerations

All animal care and handling procedures adhered to the Guidance of the Care and Use of Laboratory Animals in China and were approved by the Animal Care Committee of Xinjiang Agricultural University (Animal protocol number: 2023059).

2.2. Experimental Animals and Design

This experiment was conducted from May to September 2023 in the Kuder pastoral area of Zhaosu County, Ili Kazakh Autonomous Prefecture, Xinjiang Uygur Autonomous Region. Twenty-two healthy Yili mares of similar age (3–4 years) and body weights averaging 391.5 ± 13.7 kg were selected under natural grazing conditions. The mares were randomly divided into two groups ($n = 11$ per group): the grazing group (G) and the grazing + supplementation group (GS). Mares in group G grazed naturally on pasture. Mares in group GS received concentrate supplements at 11:00 and 19:00 daily, each at 1 kg (totaling 2 kg per day), under the same grazing conditions. The two groups had access to the same pasture resources, drinking water, shade, and general grazing environment. The only planned difference between the two groups was that mares in the GS group received concentrate supplementation. The entire experiment lasted 100 days, including a 10-day adaptation period and a 90-day formal experimental phase.

2.3. Feeding Management and Diet Composition

During the experimental period, all mares were kept under the same grazing and husbandry conditions. Each day at 10:30 a.m., foals were temporarily separated from the mares and housed in nearby pens to maintain contact with the mares. During the separation period, foals were provided with clean drinking water and forage. Mares were then herded back for milking at approximately 2 h intervals, with five milkings performed per day. After the final milking session, foals and mares were reunited and allowed to suckle and graze together.

The pasture vegetation was mainly made up of graminaceous species. The concentrate supplement was prepared according to the NRC nutrient requirements of horses [12], specifically designed to fulfill the nutritional needs of lactating mares weighing 400 kg. The ingredients and nutrient levels of the concentrate supplement are shown in Table 1. Throughout the experimental period, all mares were under veterinary supervision.

Table 1. Ingredient composition and analyzed nutrient composition of the concentrate supplement and pasture (DM basis, %).

Concentrate Ration		Nutrient Levels ²		
Ingredients	Contents	Item	Concentrate	Pasture
Corn	52.00	CP	16.95	10.57
Bran	12.00	EE	7.52	3.31
Barley	10.00	NDF	25.86	60.44
Soybean meal	20.00	ADF	3.15	29.93
CaHPO ₄	2.00	Ash	8.07	7.71

Table 1. Cont.

Concentrate Ration		Nutrient Levels ²		
Ingredients	Contents	Item	Concentrate	Pasture
Salt	2.00	Ca	1.32	1.16
Limestone powder	1.00	P	0.74	0.23
Premix ¹	1.00	GE, MJ/kg	18.86	17.65
Total	100.00			

¹ The premix provided the following amounts per kilogram of concentrate supplement: Co, 0.5 mg; Cu, 100 mg; I, 3.5 mg; Fe, 500 mg; Mn, 400 mg; Zn, 400 mg; Se, 1 mg; vitamin A, 24,000 IU; vitamin D, 2640 IU; vitamin E, 400 IU; vitamin B₁, 30 mg; vitamin B₂, 20 mg; vitamin B₃, 240 mg; choline chloride, 120 mg; and phytase, 50 mg.

² Ingredient proportions of the concentrate supplement are formulated values. Nutrient levels of the concentrate supplement and pasture are analyzed.

2.4. Sample Collection

2.4.1. Forage Samples

To collect forage samples, five grazing cages were uniformly placed along the grazing routes using the grazing cage method. Forage species selectively consumed by the mares within the cages were harvested at ground level. A 500 g composite sample was obtained after mixing, which was then shade-dried in a cool, well-ventilated area before storage. For the concentrate supplement, a total of 1 kg was randomly collected from various bags and thoroughly mixed to obtain a 200 g representative subsample for storage. Upon arrival at the laboratory, the forage and feed samples were oven-dried and pulverized. The resulting powder was passed through a 40-mesh sieve and subsequently stored in self-sealing plastic bags for further analysis.

2.4.2. Milk Samples

Milk samples were collected on days 0, 30, 60, and 90 of the formal experimental period. Day 0 marked the start of the formal experiment following the 10-day adaptation period. On each sampling day, mares were milked five times at approximately 2 h intervals during the daytime milking period. Milk obtained from the five milkings of each mare on the same day was pooled to obtain one composite daily milk sample per mare. The composite sample was thoroughly mixed and then aliquoted into 10 mL tubes for subsequent analyses of milk composition, amino acid composition, fatty acid composition, and mineral elements. All milk samples were stored at -20°C until laboratory analysis.

2.5. Sample Determination and Analysis

2.5.1. Nutrients in Feed

Representative pasture and concentrate supplement samples were analyzed for nutrient composition. Samples were oven-dried at 65°C for 48 h, ground, and passed through a 1 mm sieve before analysis. Dry matter (DM; method no. 930.15), ether extract (EE; method no. 989.05), and crude protein (CP; method no. 976.05) were determined according to the procedures of the Association of Official Analytical Chemists [13]. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were determined according to the Van Soest method [14] using fiber bags and an ANKOM 220 Fiber Analyzer (ANKOM Technology Company, Macedonia, NY, USA). All analyses were performed using representative samples of pasture and concentrate supplement.

2.5.2. Milk Yield

During the experimental period, the total daily milk yield was recorded every other day. Data were weighed and recorded using an electronic scale, and the daily milk yield

was calculated by summing all individual milkings collected that day. The 24 h milk yield was then converted using the following formula:

$$24 \text{ h milk yield (kg)} = \frac{\text{Total daily milk collected (kg)} \times 24}{\text{Total daily milking duration (h)}}$$

2.5.3. Milk Composition

Milk samples were thawed at room temperature and preheated in a 37 °C water bath before analysis. After gentle mixing, milk fat, milk protein, lactose, total solids, and non-fat milk solids were determined using a calibrated milk composition analyzer (FOSS FT3; FOSS, Hillerød, Denmark). The instrument was calibrated before analysis according to the manufacturer's instructions. Milk component yields were calculated by multiplying daily milk yield by the corresponding milk component percentage.

2.5.4. Amino Acid Composition

Milk samples collected on day 90 of the formal experimental period were used for amino acid composition analysis. Sixteen amino acids, excluding tryptophan, were measured by acid hydrolysis in accordance with the national standard GB 5009.124-2016 [15].

Sample Preparation: Briefly, 1 g of the milk sample was accurately weighed into a hydrolysis tube. Then, 1 mL of concentrated hydrochloric acid (HCl), 10 mL of 6 mol/L HCl solution, and 3–4 drops of n-octanol were added sequentially. The tube was flushed with high-purity nitrogen (N₂) and placed in a constant-temperature drying oven at 110 ± 1 °C for 22 h for hydrolysis. After cooling, the hydrolyzed solution was filtered into a 50 mL volumetric flask and diluted to volume. A 1 mL aliquot of the resulting solution was evaporated at 60 °C using a concentrator. The residue was dissolved in HCl (pH 2.2) and passed through a 0.22 µm filter. Each sample was processed in duplicate for subsequent analysis.

Chromatographic Conditions: An automatic amino acid analyzer (L-8900; Hitachi High-Tech Corporation, Tokyo, Japan) equipped with a hydrolysis amino acid analysis column (LCAK06/Na, 4.6 mm × 150 mm; LCA K06/Na, 4.6 × 150 mm; Sykam GmbH, Eresing, Germany) was used. The column temperature was set with a gradient from 58 to 74 °C, while the reaction temperature was kept at 130 °C. The flow rates of the mobile phase and ninhydrin were 0.45 mL/min and 0.25 mL/min, respectively. Detection was performed with a dual-wavelength detector at 44 nm and 570 nm, with an injection volume of 50 µL.

2.5.5. Fatty Acid Composition

Milk samples collected on day 90 of the formal experimental period were used for fatty acid composition analysis. Fatty acid composition was determined according to GB 5009.168-2016 [16].

Milk fat was extracted and converted to fatty acid methyl esters before gas chromatographic analysis. Fatty acid methyl esters were analyzed using a gas chromatograph (GC-2010, Shimadzu Corporation, Kyoto, Japan) equipped with an SH-2560 capillary column (100 m × 0.25 mm × 0.20 µm). The injector temperature was 250 °C, the injection volume was 1 µL, and the split ratio was 10:1. The oven temperature was held at 100 °C for 5 min, increased to 150 °C at 6 °C/min, then increased to 240 °C at 2 °C/min and held for 8 min. The flame ionization detector temperature was 260 °C. Fatty acids were expressed as relative percentages using the area normalization method.

2.5.6. Mineral Elements

Mineral elements in milk were determined according to GB 5009.268-2016 [17] using inductively coupled plasma mass spectrometry (ICP-MS). Briefly, 0.5 g of the milk sample was accurately weighed and digested with nitric acid using a microwave digestion system. After digestion, the solution was cooled, diluted to a constant volume with ultrapure water, and analyzed by ICP-MS. The major elements measured included Ca, K, Mg, Na, and P, while trace elements included Cu, Fe, Mn, Zn, Cr, Se, Mo, and Co. The Ca/P and K/Na ratios were calculated based on the measured concentrations. Mineral elements in pasture and concentrate supplement samples were determined according to GB/T 13885-2017 [18]. Results are shown in Table 2.

Table 2. Mineral element content in natural pasture and concentrate supplements (DM-basis, mg/kg).

Items	0 d	30 d	60 d	90 d	Concentrate
Ca	4817	7095	3981	10,703	11,130
K	24,527	23,532	11,443	11,939	8924
Na	124	227	230	335	9274
Mg	1252	1425	815	1776	2846
Fe	187	319	282	819	634
Mn	45.2	158	30.2	67.8	255
Zn	24.6	24	19.7	26.3	239
Cu	7.29	6.88	3.78	8.92	51.6
Co	0.06	0.11	0.09	0.24	0.90
Se	0.08	0.05	0.06	0.09	0.58

Note: The measured values of mineral elements in forage on different experimental days were obtained from mixed samples of forage collected on the corresponding day.

2.6. Statistical Analysis

Data were initially organized using Excel 2019 and analyzed using SPSS 26.0 software (IBM Corp., Armonk, NY, USA). Before statistical analysis, normality was assessed using the Shapiro–Wilk test, and homogeneity of variance was evaluated using Levene’s test. Lactation performance and mineral element data were analyzed using a general linear model (GLM), with Group, Time, and the Group \times Time interaction included as fixed effects. For variables that did not meet model assumptions, data transformation or appropriate robust/non-parametric methods were applied when necessary. Amino acid and fatty acid data were analyzed as follows: Variables satisfying normality and homogeneity of variance assumptions were analyzed using Student’s independent-samples *t*-test. Variables with unequal variances were analyzed using Welch’s *t*-test, whereas variables not satisfying normality were analyzed using the Mann–Whitney U test. Results are presented as means (Mean), with variability expressed as the standard error of the mean (SEM). Statistical significance was set at $p < 0.05$, and $p < 0.01$ was considered highly significant.

3. Results

3.1. Effects of Concentrate Supplementation Under Grazing Conditions on Lactation Performance of Yili Mares

As shown in Figure 1, the lactation curve of Yili mares showed an initial rise followed by a decline throughout the experimental period, with peak lactation occurring on Day 40. Milk yield in the GS group was higher than in the G group throughout the entire experimental period, with highly significant differences observed at 50–60 days after feeding began ($p < 0.01$) and significant differences at 40–50 days and 60–90 days ($p < 0.05$).

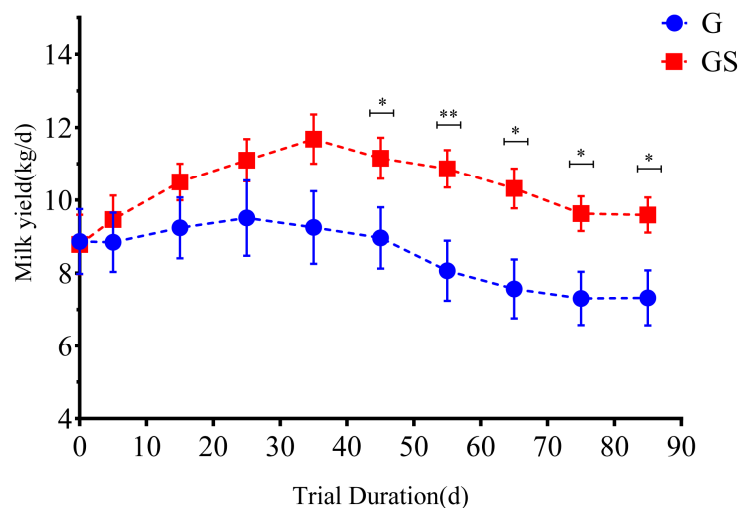


Figure 1. Effects of concentrate supplementation under grazing conditions on lactation trends of Yili mares. Milk yield is expressed as kg/d. Day 0 represents the beginning of the formal experimental period after the 10-day adaptation period. Values represent the average milk yield of Yili mares in each group at 10-day intervals. G indicates the grazing group, and GS indicates the grazing with concentrate supplementation group. * $p < 0.05$; ** $p < 0.01$.

As shown in Table 3, compared to the G group, the GS group exhibited highly significant increases in milk yield and lactose yield during the experimental period ($p < 0.01$), and significant increases in milk fat yield and milk protein yield ($p < 0.05$). No significant differences were observed between the two groups for other indicators ($p > 0.05$).

Table 3. Effects of Concentrate Supplementation Under Grazing Conditions on Lactation Performance of Yili Mares.

Items	G	GS	SEM	p-Value		
				Group	Time	Group × Time
Milk yield, kg/d	8.55 ^{Bb}	10.05 ^{Aa}	0.375	0.006	0.182	0.272
Milk fat, %	1.27	1.26	0.032	0.980	0.001	0.352
Milk fat yield, kg/d	0.11 ^b	0.13 ^a	0.006	0.023	0.008	0.490
Milk protein, %	2.03	2.01	0.026	0.666	<0.001	0.628
Milk protein yield, kg/d	0.17 ^b	0.20 ^a	0.008	0.024	0.234	0.248
Milk lactose, %	6.81	6.85	0.027	0.250	<0.001	0.739
Milk lactose yield, kg/d	0.58 ^{Bb}	0.69 ^{Aa}	0.026	0.004	0.081	0.263
Total solid, %	10.50	10.45	0.060	0.567	<0.001	0.812
Non-fat milk solid, %	9.23	9.23	0.036	0.965	<0.001	0.117

Note: SEM = standard error of the mean. Within the same row, values with different uppercase letters indicate highly significant differences ($p < 0.01$), whereas values with different lowercase letters indicate significant differences ($p < 0.05$). Values without letter superscripts indicate no significant difference ($p > 0.05$); the same applies to subsequent tables. G indicates the grazing group ($n = 11$), and GS indicates the grazing with concentrate supplementation group ($n = 11$).

3.2. Partial Cost–Benefit Analysis of Concentrate Supplementation

Based on the local market conditions in Zhaosu County, a partial cost–benefit analysis was conducted to evaluate the economic return of concentrate supplementation. As shown in Table 4, compared with the G group, the GS group showed an average increase in milk yield of approximately 1.50 kg/d per mare. The additional milk income was estimated to be 2700.00 CNY per mare, corresponding to 29,700.00 CNY for the 11 supplemented mares. The total cost, including concentrate and labor costs, was estimated to be 1509.09 CNY per mare and 16,600.00 CNY for the 11 supplemented mares. After subtracting

these costs, the estimated net benefit was 1190.91 CNY per mare and 13,100.00 CNY for the 11 supplemented mares, with a benefit–cost ratio of 1.79.

Table 4. Partial cost–benefit analysis of concentrate supplementation in grazing Yili mares over a 100-day period.

Items	Calculation	Per Mare	11 Mares
Additional milk yield (kg)	1.50 kg/d × 90 d	135.00	1485.00
Additional milk income (CNY)	Additional milk yield × 20 CNY/kg	2700.00	29,700.00
Concentrate cost (CNY)	2 kg/d × 3 CNY/kg × 100 d	600.00	6600.00
Labor cost (CNY)	3000 CNY/month ÷ 30 d × 100 d	909.09	10,000.00
Total cost (CNY)	Concentrate cost + labor cost	1509.09	16,600.00
Net benefit (CNY)	Additional milk income – total cost	1190.91	13,100.00
Benefit–cost ratio	Additional milk income/total cost	1.79	1.79

Note: Additional milk income was calculated based on the milk yield difference observed during the 90-day formal experimental period. Concentrate and labor costs were calculated over the entire 100-day supplementation period, including the 10-day adaptation period, to provide a conservative estimate. The average mare milk purchase price was set at 20 CNY/kg, and the concentrate supplement cost was estimated at 3 CNY/kg based on local market prices in Zhaosu County. Labor cost was calculated based on a hired worker wage of 3000 CNY/month and was allocated to the 11 supplemented mares. Net benefit = additional milk income – total cost. Benefit–cost ratio = additional milk income/total cost.

3.3. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Amino Acid Composition in Yili Mares

3.3.1. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Amino Acid Composition in Yili Mares

As shown in Table 5, the proportions of Thr, Ser, and Gly in mare milk were significantly higher in the GS group than in the G group ($p < 0.05$). In contrast, the proportions of Leu, Phe, and Pro were significantly lower in the GS group than in the G group ($p < 0.05$). Ala showed an increasing trend in the GS group, but the difference was not statistically significant ($p > 0.05$). No significant differences were observed between the two groups for the other amino acids ($p > 0.05$).

Table 5. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Amino Acid Composition in Yili Mares (%).

Items	G	GS	SEM	<i>p</i> -Value
Asp	8.65	8.61	0.261	0.904
Thr	2.87 ^b	3.50 ^a	0.275	0.041
Ser	3.89 ^b	5.23 ^a	0.580	0.040
Glu	19.20	20.82	0.875	0.080
Gly	1.50 ^b	1.61 ^a	0.049	0.045
Ala	2.75 ^b	3.12 ^a	0.171	0.041
Val	4.08	4.24	0.128	0.239
Met	3.52	3.48	0.083	0.610
Ile	3.57	3.62	0.086	0.552
Leu	9.83	9.29	0.249	0.051
Tyr	4.50	4.34	0.107	0.157
Phe	5.26	4.78	0.260	0.079
His	5.16	5.14	0.204	0.930
Lys	8.49	7.48	0.574	0.093
Arg	5.91	5.40	0.270	0.077
Pro	10.82 ^a	9.34 ^b	0.530	0.011

Note: Asp = aspartic acid; Thr = threonine; Ser = serine; Glu = glutamic acid; Gly = glycine; Ala = alanine; Val = valine; Met = methionine; Ile = isoleucine; Leu = leucine; Tyr = tyrosine; Phe = phenylalanine; His = histidine; Lys = lysine; Arg = arginine; Pro = proline. Different lowercase letters within the same row indicate significant differences ($p < 0.05$). G indicates the grazing group ($n = 11$), and GS indicates the grazing with concentrate supplementation group ($n = 11$).

3.3.2. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Amino Acid Profiles in Yili Mares

Principal component analysis (PCA) was conducted on the milk amino acid contents of both the G group and the GS group to assess the separation trend and variation in amino acids. As shown in Figure 2, the first two principal components under the two feeding systems explained 75.04% of the total variance, with PC1 accounting for 60.05% and PC2 for 14.99%. No clear separation in amino acid composition was observed between the G group and the GS group. Figure 3 shows the contents of EAA, NEAA, DAA, BCAA, and FAA in mare milk from the two groups. The contents of NEAA, DAA, BCAA, and FAA appeared to increase in the GS group, but these differences were not statistically significant ($p > 0.05$).

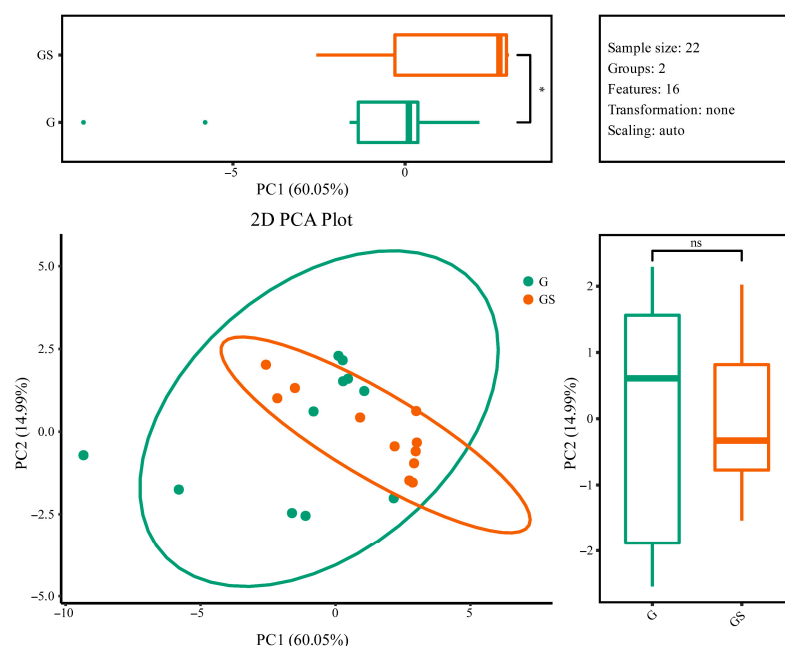


Figure 2. Principal Component Analysis of Milk Amino Acids in Response to Concentrate Supplementation Under Grazing Conditions. * $p < 0.05$; ns, not significant. G indicates the grazing group ($n = 11$), and GS indicates the grazing with concentrate supplementation group ($n = 11$).

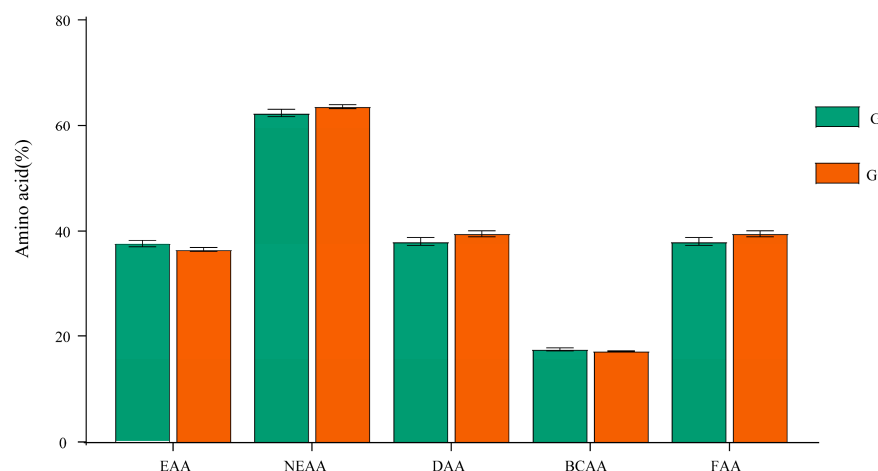


Figure 3. Milk amino acid profiles in response to concentrate supplementation under grazing conditions. EAA = Val + Met + Ile + Leu + Phe + Lys + His + Arg; NEAA = Asp + Thr + Ser + Glu + Gly + Ala + Tyr + Pro; BCAA = Val + Ile + Leu; FAA = Glu + Leu + Arg; LAA = Met + Lys; DAA = Asp + Glu + Gly + Ala + Met + Arg; TAA = EAA + NEAA. G indicates the grazing group ($n = 11$), and GS indicates the grazing with concentrate supplementation group ($n = 11$).

3.4. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Fatty Acid Composition in Yili Mares

As shown in Table 6, the proportion of alpha-linolenic acid (ALA, C18:3n3) was significantly higher in the G group than in the GS group ($p < 0.001$). Consistently, n-3 PUFA was also significantly higher in the G group ($p < 0.001$), whereas the n-6/n-3 ratio was significantly lower in the G group than in the GS group ($p < 0.05$). No significant difference was observed in n-6 PUFA between the two groups ($p > 0.05$).

Table 6. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Fatty Acid Composition in Yili Mares (%).

Items	G	GS	SEM	p-Value
C6:0	0.18	0.19	0.017	0.509
C8:0	1.77	2.10	0.198	0.104
C10:0	3.64	4.39	0.469	0.126
C12:0	4.41	5.03	0.528	0.470
C14:0	5.49	5.88	0.420	0.369
C14:1	0.49	0.50	0.049	0.599
C15:0	0.34	0.30	0.018	0.066
C16:0	21.29	21.33	0.690	0.962
C16:1	6.14	6.03	0.450	0.814
C17:0	0.23	0.22	0.014	0.744
C18:0	1.08	1.15	0.087	0.428
C18:1n9t	0.04	0.05	0.002	0.114
C18:1n9c	19.89	22.42	1.290	0.064
LA, C18:2n6c	10.68	11.27	0.914	0.529
C20:0	0.05	0.05	0.010	0.599
C18:3n6	0.03	0.03	0.004	0.431
C20:1n9	0.22	0.24	0.017	0.175
ALA, C18:3n3	23.04 ^{Aa}	17.89 ^{Bb}	1.246	<0.001
C20:2	0.20	0.23	0.016	0.070
C20:3n6	0.04	0.04	0.003	0.115
C22:1n9	0.04	0.04	0.004	0.634
C20:3n3	0.47	0.42	0.031	0.088
C23:0	0.05	0.05	0.004	0.246
AA, C20:4n6	0.05	0.04	0.012	0.694
C24:0	0.04	0.03	0.005	0.293
EPA, C20:5	0.04	0.03	0.004	0.325
C24:1	0.02	0.02	0.002	0.691
DHA, C22:6n3	0.03	0.04	0.013	1.000
SFA	38.56	40.72	2.077	0.311
UFA	61.44	59.28	2.077	0.311
MUFA	26.85	29.30	1.536	0.126
PUFA	34.58 ^a	29.97 ^b	1.521	0.013
n-3 PUFA	23.58 ^a	18.38 ^b	1.265	<0.001
n-6 PUFA	10.80	11.37	0.911	0.543
n-6/n-3	0.46 ^b	0.63 ^a	0.059	0.030

Note: In this table, SFA = C4:0 + C6:0 + C8:0 + C10:0 + C12:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C22:0 + C24:0; MUFA = C14:1 + C16:1 + C18:1n9t + C18:1n9c + C20:1n9 + C22:1n9 + C24:1; PUFA = LA(C18:2n6c) + C18:3n6 + ALA(C18:3n3) + C20:2 + C20:3n6 + C20:3n3 + AA(C20:4n6) + EPA(C20:5) + DHA(C22:6n3); UFA = MUFA + PUFA; n-3 PUFA = ALA (C18:3n3) + C20:3n3 + EPA (C20:5) + DHA (C22:6n3); n-6 PUFA = LA (C18:2n6c) + C18:3n6 + C20:3n6 + AA (C20:4n6). The n-6/n-3 ratio was calculated for each individual milk sample before group means were calculated. Different uppercase letters within the same row indicate highly significant differences ($p < 0.01$), whereas different lowercase letters indicate significant differences ($p < 0.05$). G indicates the grazing group ($n = 11$), and GS indicates the grazing with concentrate supplementation group ($n = 11$).

3.5. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Mineral Elements in Yili Mares

3.5.1. Effects of Concentrate Supplementation Under Grazing Conditions on Major Elements in Milk of Yili Mares

As shown in Table 7, compared to the G group, the GS group exhibited highly significant decreases in the potassium (K) content and the K/Na ratio in Yili mare milk ($p < 0.01$). No significant differences were found between the two groups for other macroelements ($p > 0.05$). Additionally, no Group \times Time interaction was observed for macroelement contents in milk ($p > 0.05$).

Table 7. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Major Element Composition in Yili Mares.

Items	G	GS	SEM	p-Value		
				Group	Time	Group \times Time
Ca, mg/kg	977.19	980.88	27.580	0.925	<0.001	0.976
K, mg/kg	643.28 ^{Aa}	578.66 ^{Bb}	13.963	0.002	<0.001	0.979
Mg, mg/kg	84.07	78.98	2.179	0.104	<0.001	0.892
Na, mg/kg	171.06	171.56	2.464	0.662	0.055	0.264
P, mg/kg	475.94	463.41	10.058	0.382	<0.001	0.356
Ca/P	2.05	2.11	0.033	0.218	0.052	0.589
K/Na	3.76 ^{Aa}	3.39 ^{Bb}	0.079	0.002	<0.001	0.757

Note: Ca/P and K/Na were calculated based on the measured concentrations of the corresponding elements. Different uppercase letters within the same row indicate highly significant differences ($p < 0.01$), whereas different lowercase letters indicate significant differences ($p < 0.05$). G indicates the grazing group ($n = 8$), and GS indicates the grazing with concentrate supplementation group ($n = 8$).

3.5.2. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Trace Element Composition in Yili Mares

As shown in Table 8, Fe and Co contents were higher in GS than in G ($p < 0.01$), and Cr content was higher in GS ($p < 0.05$). No significant differences in other trace elements were observed between the two groups ($p > 0.05$).

Table 8. Effects of concentrate supplementation under grazing conditions on milk trace element composition in Yili mares.

Items	G	GS	SEM	p-Value		
				Group	Time	Group \times Time
Cu, mg/kg	0.30	0.27	0.014	0.111	<0.001	0.033
Fe, mg/kg	12.90 ^{Bb}	15.05 ^{Aa}	0.403	<0.001	<0.001	0.004
Mn, mg/kg	0.24	0.22	0.013	0.105	<0.001	0.062
Zn, mg/kg	3.27	3.46	0.085	0.131	<0.001	0.040
Cr, mg/kg	0.41 ^b	0.44 ^a	0.011	0.047	<0.001	0.413
Mo, μ g/kg	14.98	15.47	0.552	0.437	0.788	0.695
Se, μ g/kg	19.22	20.85	0.794	0.150	0.002	0.364
Co, μ g/kg	4.16 ^{Bb}	4.87 ^{Aa}	0.139	<0.001	<0.001	0.283

Note: Different uppercase letters within the same row indicate highly significant differences ($p < 0.01$), whereas different lowercase letters indicate significant differences ($p < 0.05$). G indicates the grazing group ($n = 8$), and GS indicates the grazing with concentrate supplementation group ($n = 8$).

A highly significant Group \times Time interaction was detected for Fe (Figure 4A) ($p < 0.01$). Significant interactions were observed for Cu (Figure 4B) and Zn (Figure 4C) ($p < 0.05$). No interaction effects were found for the remaining trace elements ($p > 0.05$).

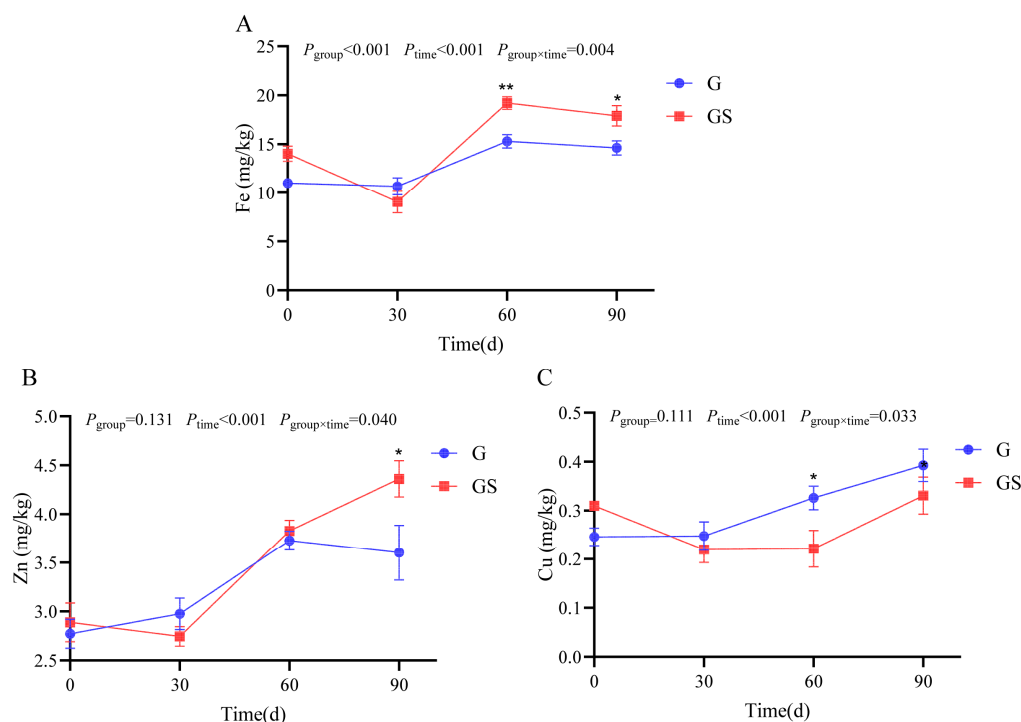


Figure 4. Group \times Time interaction effects on Fe, Cu, and Zn contents in milk. (A) trend plot of Fe content; (B) trend plot of Zn content; (C) trend plot of Cu content. * $p < 0.05$; ** $p < 0.01$. G indicates the grazing group ($n = 8$), and GS indicates the grazing with concentrate supplementation group ($n = 8$).

3.5.3. Correlation Between Mineral Elements in Pasture and Milk

To explore trends in the association between mineral elements in forage and those in milk, linear mixed models (LMMs) were constructed. As shown in Table 9, the concentrations of K, P, Co, Fe, Cr, and Mn in milk were highly significantly influenced ($p < 0.01$) by the corresponding elements in the forage, while Ca, Se, and Cu were significantly affected ($p < 0.05$). Based on the fixed-effect estimates, K, P, Co, Fe, and Cu in the forage exhibited significant positive regression effects on their respective concentrations in milk; conversely, Ca, Se, and Mn showed significant negative regression effects.

Table 9. Linear mixed model analysis of the effects of pasture mineral elements on mineral element concentrations in milk.

Items	Regression Coefficient (β)	SEM	p -Value	Direction of Association
K	0.0063	0.001	<0.001	Positive
Ca	-0.0175	0.004	0.026	Negative
P	0.0960	0.0082	<0.001	Positive
Se	-0.0949	0.0394	0.019	Negative
Na	0.0053	0.0256	0.816	Positive
Co	0.0171	0.0016	<0.001	Positive
Mo	0.0001	0.0002	0.963	Positive
Fe	0.0094	0.0018	<0.001	Positive
Cu	0.0142	0.0066	0.037	Positive
Mg	-0.0060	0.0038	0.123	Negative
Cr	0.0679	0.0079	<0.001	Positive
Zn	-0.2961	0.0286	0.308	Negative
Mn	-0.0012	0.0002	<0.001	Negative

3.5.4. Establishment of Mineral Element Fingerprints in Milk Under Different Feeding Systems

To achieve rapid and accurate traceability of mare milk and its products, mineral element fingerprint profiles were developed for both the grazing and supplementation groups at four time points (0, 30, 60, and 90 days). These profiles were based on 13 mineral elements, arranged from macroelements to trace elements. To display all elements together in a single figure, elements with lower concentrations were multiplied by the same factor to make their magnitudes comparable. Specifically, Fe was multiplied by 10; Zn, Mn, Se, Cu, Cr, and Mo by 100; and Co by 1000. The resulting fingerprint profiles are shown in Figure 5. As shown in Figure 5, the fingerprint profiles of the different elements in mare milk were generally similar, and the levels of K, Ca, P, Zn, and Fe throughout the experiment were strongly influenced by the corresponding element levels in forage.

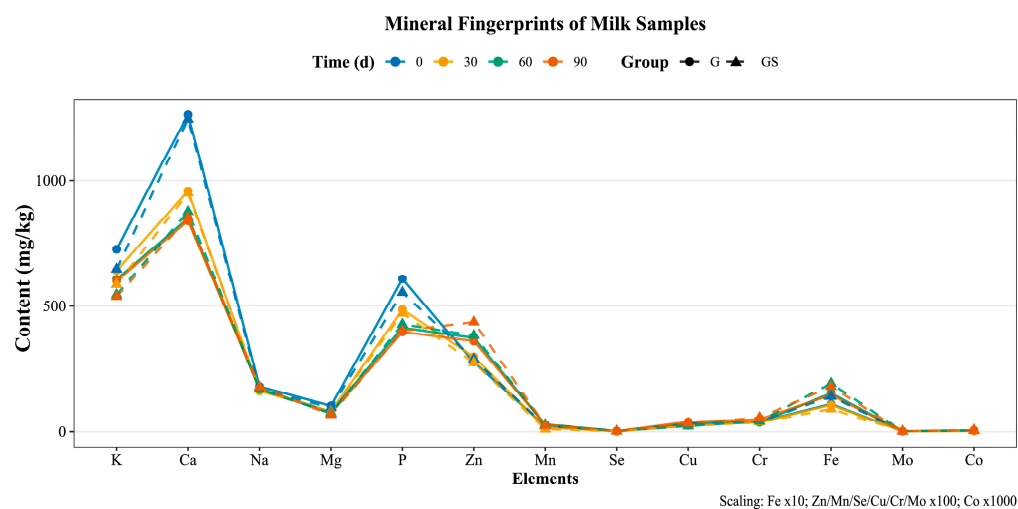


Figure 5. Fingerprint profiles of 13 mineral elements in milk under different feeding systems. The solid line represents the G group, and the dashed line represents the GS group. G indicates the grazing group, and GS indicates the grazing with concentrate supplementation group.

4. Discussion

4.1. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Yield and Milk Composition in Yili Mares

Grassland resources in Xinjiang show strong seasonal and regional variations. During the growing season, natural pastures supply crude fiber and some minerals [19]. However, when grasslands degrade, sward height decreases, or during dry periods, nutrient availability becomes limited. Lactating mares have high nutritional needs, with energy and amino acids required for milk production increasing rapidly [20]. Under grazing conditions, energy intake may not fully meet the demands of lactation and pregnancy, leading to a negative energy balance and insufficient protein, which can reduce milk yield [21].

Milk production and quality serve as key indicators of the economic worth of horse milk, reflecting how effectively nutrients are digested and utilized. These qualities are influenced by genetics, feeding methods, environment, and diet makeup. A previous study has indicated that dietary and physiological factors are associated with lactation performance in mares [22]. Consequently, concentrate supplementation can help balance pasture supply with the demands of milk production in grazing systems. Caprioli et al. [23] reported that concentrate supplementation during summer reduced the decline in milk yield in grazing sheep and boosted overall lactation output. Similar positive effects of supplementation on lactation performance have also been observed in cows [24]. In this study, concentrate supplementation increased milk yield in Yili mares, and the lactation

curve shown in Figure 1 suggests that the GS group maintained higher milk yield for a longer period around peak lactation. Although the lactose percentage did not significantly differ between groups, an increasing trend was observed in GS. Lactose, the primary carbohydrate in milk, is closely linked to milk production; generally, higher lactose content correlates with greater milk output [25]. It is noteworthy that concentrate supplementation significantly increased milk fat yield, milk protein yield, and lactose yield, whereas the percentages of milk fat, milk protein, and lactose were not significantly changed. This indicates that the improvement in milk component yields was mainly driven by higher total milk yield rather than by higher component concentrations. Therefore, the primary effect of concentrate supplementation under grazing conditions may be to improve the overall lactation output of Yili mares by increasing nutrient and energy supply, rather than directly altering the proportional composition of milk. This finding suggests that supplementation improved milk secretion capacity while maintaining relatively stable milk composition. Based on the local average mare milk purchase price in Zhaosu County, which was estimated at 20 CNY/kg, the additional 1.50 kg/d milk yield in the GS group could generate approximately 30.00 CNY/d of extra milk income per mare. After deducting concentrate and labor costs, concentrate supplementation still produced an estimated net benefit of 1490.91 CNY per mare over the 100-day supplementation period, indicating its potential economic feasibility under local grazing conditions.

The small intestine of horses has a limited capacity for starch digestion. When starch supply exceeds the small-intestinal digestive capacity to digest it, undigested starch enters the hindgut and is rapidly fermented by microorganisms, producing volatile fatty acids (VFA) such as propionate and butyrate [26]. High-concentrate diets increase fermentable substrates in the hindgut and may shift fermentation from an acetate-dominant pattern to a propionate-dominant one [27]. However, the link between hindgut propionate production and milk lactose synthesis is indirect. In mares, dietary carbohydrates may support lactose synthesis mainly by increasing the availability of digestible energy and glucose precursors. Glucose absorbed from small-intestinal starch digestion can be directly used by the mammary gland for lactose synthesis, whereas propionate produced in the hindgut may contribute indirectly through hepatic gluconeogenesis and overall energy metabolism. Hoffman et al. [28] reported that using corn instead of fiber as the main energy source in concentrates for grazing mares increased milk lactose content. Lu et al. [11] found that supplementation with 1 kg of concentrate or fat did not significantly change milk composition but did increase milk fat, milk protein, and lactose yields. Similarly, Morrison [29] observed that concentrate supplementation boosted dry matter intake in grazing dairy cows and improved milk yield and lactose content. In the present study, concentrate supplementation increased milk yield and lactose yield, while lactose percentage remained relatively stable. This suggests that supplementation mainly enhanced total milk secretion rather than markedly changing lactose concentration. Because fecal pH, volatile fatty acids, lactic acid concentration, and hindgut microbial composition were not measured, the contribution of hindgut fermentation to the observed lactation response should be interpreted cautiously.

4.2. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Amino Acid Composition in Yili Mares

Amino acids are the essential building blocks of proteins, and their concentration and composition are not only vital for assessing the nutritional value of mare milk but also closely linked to its flavor characteristics [30]. The findings of this study showed that, compared to the grazing group, concentrate supplementation significantly increased the levels of threonine (Thr), serine (Ser), glycine (Gly), and alanine (Ala) in mare milk, while significantly reducing the proportion of proline (Pro). During lactation, the mammary gland fulfills the needs for milk protein synthesis by regulating blood flow and removing amino

acids from the plasma. At the same time, the mTOR signaling pathway controls milk protein synthesis by integrating signals from nutrients and growth factors [31,32]. Serine and Ala can be produced from the glycolytic intermediates 3-phosphoglycerate and pyruvate, respectively, via enzymes such as PHGDH, PSAT1, and PSPH. Moreover, Gly can be formed from Ser via a reversible reaction mediated by SHMT and then participate in carbon metabolism [33]. In this study, concentrate supplementation increased energy intake and available protein intake in lactating mares. It is believed that such supplementation helps direct intermediates produced during glycolysis more effectively toward the biosynthetic pathways of Ser and Ala. Leu is a typical branched-chain amino acid and an important nutritional signal that regulates the mTOR pathway. Gao et al. [34] reported that Leu supplementation in bovine mammary epithelial cells promoted milk protein synthesis by activating the mTOR signaling pathway. Phe, as an aromatic essential amino acid, is also involved in the regulation of milk protein synthesis and amino acid transport. Guo et al. [35] showed that Phe can affect casein synthesis and amino acid utilization via the LAT1–mTOR signaling pathway in dairy cows, suggesting a close association with milk protein synthesis. Similarly, Xing et al. [36] reported that Phe concentration significantly influenced casein synthesis in bovine mammary epithelial cells. In the present study, the proportions of Leu and Phe were significantly lower in the GS group than in the G group. Given that both milk yield and milk protein yield increased following concentrate supplementation in Yili mares, the decreased proportions of Leu and Phe may not necessarily indicate reduced absolute secretion of these amino acids, but rather reflect a redistribution of the milk amino acid profile. This may be associated with changes in amino acid supply and mammary amino acid utilization induced by concentrate supplementation. Lin et al. [37] discovered that supplementing lysine and threonine under grazing conditions increased their respective concentrations in mare milk and improved milk quality. This indicates that the dietary supply of amino acids, especially limiting amino acids, can influence the amino acid profile of milk by modulating amino acid metabolism and mammary biosynthetic processes. Additionally, Meng [38] found that branched-chain amino acid (BCAA) supplementation could similarly alter the levels of multiple amino acids in mare milk. This suggests that various nutritional interventions can modify the milk amino acid profile through multiple pathways, including regulation of systemic amino acid metabolism and modulation of gut microbiota.

Furthermore, concentrate supplementation in this study did not significantly alter the overall total amino acid (TAA) levels in the milk; however, the EAA/TAA ratio ranged from 36% to 37%, and the EAA/NEAA ratio ranged from 57% to 61%. Research by Liang et al. [39] on donkey milk suggested that for high-quality protein, the EAA/TAA ratio should be about 40% and the EAA/NEAA ratio should be around 60%. These results indicate that the amino acid quality of the mare milk in the present experiment was high, with a well-balanced composition. Overall, providing concentrate supplements to lactating mares on a grazing basis can increase the levels of certain functional and flavor-related amino acids (such as Thr, Ser, Gly, and Ala) in milk, potentially enhancing the nutritional quality of mare milk.

4.3. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Fatty Acid Composition in Yili Mares

The fatty acid (FA) composition of milk influences the appearance, physical properties, and nutritional value of milk and dairy products. Fatty acids in mare milk are mainly unsaturated fatty acids (UFAs), similar to the profile of human breast milk. Differences in FA composition between mare milk and bovine milk depend on their respective feeding systems and the unique structure of the gastrointestinal tract; specifically, the biohydrogenation process typical of ruminants rarely occurs in the equine gastrointestinal tract. As

a result, among the UFAs, the most common fatty acid in mare milk is α -linolenic acid (ALA) [40,41]. As an n-3 polyunsaturated fatty acid (PUFA), ALA not only increases total PUFAs but also plays various physiological roles, such as anti-inflammatory, lipid-lowering, and cardiovascular-protective effects [42].

A study by Doreau et al. [43] demonstrated that mares fed a forage-rich diet (95% hay, 5% concentrate) produced milk with higher levels of milk fat, milk protein, and ALA, but lower levels of linoleic acid, compared to those fed a concentrate-rich diet (50% hay, 50% concentrate). The present study showed a decreasing trend in ALA content following concentrate supplementation, which is consistent with the aforementioned research. Under grazing conditions, the intake of fresh green pasture usually provides abundant ALA, the majority of which can be directly transported via the circulatory system to the mammary gland after digestion and absorption, and subsequently secreted into the milk [30]. In contrast, the proportion of ALA in the milk of the GS group was significantly lower than that of the G group, leading to a concurrent decrease in the total PUFA proportion. This suggests that concentrate supplementation altered the milk's fatty acid composition. Saturated fatty acids (SFAs) can produce significant amounts of ATP through β -oxidation to supply energy for physiological functions; they also play an essential role in aiding the absorption of fat-soluble nutrients and preserving the integrity and function of cell membranes [44]. However, excessively high levels of SFAs can raise low-density lipoprotein (LDL) concentrations, which can negatively impact lipid metabolism [45]. Research has shown that monounsaturated fatty acids (MUFAs) help lower blood LDL-C levels, thereby preventing the development of hypercholesterolemia and atherosclerosis [46]. In this study, concentrate supplementation based on grazing did not significantly affect the levels of SFAs and MUFAs in mare milk. Further analysis of omega fatty acids confirmed the influence of feeding system on the fatty acid composition of mare milk. In the present study, the G group had a significantly higher proportion of n-3 PUFA and a significantly lower n-6/n-3 ratio than the GS group, whereas no significant difference in n-6 PUFA was observed between the two groups. Benbrook et al. [47] reported that grass-fed or pasture-based diets generally increase the n-3 PUFA content of milk and reduce the n-6/n-3 ratio, findings consistent with those of the present study. This difference was mainly attributable to the higher content of alpha-linolenic acid (ALA) in milk from grazing-only mares. Therefore, the higher n-3 PUFA proportion and lower n-6/n-3 ratio in the G group likely reflect a greater contribution of fresh pasture-derived fatty acids to milk fat synthesis. From a nutritional perspective, a higher n-3 PUFA proportion and a lower n-6/n-3 ratio are generally considered favorable indicators of milk lipid quality. Thus, although concentrate supplementation improved lactation performance in Yili mares, grazing alone appeared to be more beneficial for promoting n-3 fatty acid deposition in mare milk.

4.4. Effects of Concentrate Supplementation Under Grazing Conditions on Milk Mineral Element Composition in Yili Mares

Minerals are indispensable nutritional components of mare milk, playing a critical role in the skeletal development, nerve conduction, and immune system maturation of suckling foals [48]. In the present study, the concentrations of Ca, P, K, and Na in the milk of both the G and GS groups were generally consistent with the findings of Gao [49] in Mongolian horses. However, the Mg content in both groups was higher than that reported by Gao [49], which may be attributed to regional differences or breed variation. In this experiment, the concentrate supplement consisted of grains (e.g., barley and maize), soybean meal, calcium hydrogen phosphate, and premix. Supplementation meets the animal's energy requirements while concurrently reducing forage intake, leading to significant increases in milk yield and routine milk components. There are often inherent differences in mineral element profiles—particularly the K/Na ratio and the supply of

soluble electrolytes—between concentrate supplements and natural forage. Consequently, the effects of concentrate supplementation are primarily reflected in electrolytic minerals such as potassium (K). K is involved in the synthesis of muscle and nerve tissues, participates in protein and carbohydrate metabolism, and contributes to the regulation of acid–base balance and osmotic pressure in body fluids [50]. In the current study, the K concentration in the milk of the GS group was significantly lower than that of the G group. Furthermore, K and P exhibited a significant positive correlation in the linear mixed model, suggesting that fluctuations in forage supply are more readily reflected in synchronized changes in their milk concentrations. Research has shown that mineral elements such as K and Na are frequently selected as key variables for the authentication and traceability of the geographical origin of animal milk and dairy products, as they are closely linked to feed and water sources [51]. The forage in this study contained extremely high levels of potassium; therefore, concentrate supplementation reduced the proportion of forage in the diet, thereby decreasing the K concentration in the mare milk.

In mare milk, the trace element Fe is a key component of hemoglobin and myoglobin, directly involved in oxygen transport and energy metabolism; its deficiency can cause anemia and insufficient cellular energy supply. Cu serves as an essential cofactor for important enzymes, such as superoxide dismutase (SOD) and cytochrome c oxidase, and plays a vital role in antioxidation, nerve conduction, and skeletal health. Co, as a major part of vitamin B₁₂, aids in the biosynthesis of vitamin B₁₂ in the body [52,53]. In this study, the levels of Fe and Zn in the milk of naturally grazing Yili mares were significantly higher than those reported by Gao [49] and Salimei [54]. Additionally, concentrate supplementation notably increased Fe, Co, and Cr levels. Alipour et al. [55] reported that the concentrations of Fe and Co in raw mare's milk were 180.69–230.21 µg/L and 1.12–9.30 µg/L, respectively; conversely, Bilandžić et al. [56] reported that the Co content in the milk of Croatian Coldblood mares was below the detection limit, while the average Cr content was 26.7 µg/kg. These findings indicate that current data regarding the levels of iron, cobalt, and chromium in mare's milk remain limited, and that their concentrations vary depending on breed, geographical region, lactation stage, feeding regimen, the mineral composition of forage, and the analytical methods employed. In the present study, the higher levels of iron, cobalt, and chromium observed in the milk of mares in the GS group may be attributed to increased dietary mineral intake resulting from the supplementation of concentrate feeds and the addition of premixes. Although the cobalt concentration increased significantly, the absolute difference between the G and GS groups remained marginal. Consequently, this variation likely reflects a change primarily in the transfer of dietary trace elements into milk. Given that this study did not evaluate foal growth performance or mineral intake, the physiological significance of the observed increases in Fe, Co, and Cr levels warrants further investigation. Grazing horses often experience seasonal deficiencies of trace elements in soil or forage. The higher levels of Fe, Co, and Cr observed in this study may be due to concentrate supplementation enhancing the dietary intake of these minerals (via the premix) and improving the digestibility of the diet in Yili horses, which in turn increases the efficiency of Fe and Co absorption and transport, boosting their transfer into milk [57].

In this study, a mineral-element fingerprint profile of mare milk was developed for 13 mineral elements. The profile indicated that the levels of K, Ca, P, Zn, and Fe during the experiment were heavily influenced by their respective contents in forage. In traceability research of milk and dairy products, combining multi-element and isotopic analyses can achieve high accuracy in differentiating samples from various geographic regions. Elements such as K, Na, Ca, and Zn are often identified as key variables [58–60]. Therefore, for distinguishing mare milk from different feeding systems, K and the K/Na

ratio may serve as potential discriminative indicators, while Fe, Co, and Cr may reflect forage-related differences. However, since the number of animals in this study was limited, future research should include more production regions and a larger sample size to enhance the traceability of mare milk and dairy products through fingerprint profiles and to improve the representativeness of the results.

4.5. Limitations and Future Perspectives

This study has several limitations. First, the 10-day adaptation period may have been relatively short for mares to fully adapt to concentrate supplementation, especially when starch-containing feeds were introduced. Second, forage biomass, individual pasture intake, shade availability, and soil moisture were not quantitatively evaluated, which limited a more precise assessment of pasture nutrient supply. Third, although significant differences were observed in several milk amino acids, the post hoc power analysis indicated that the statistical power for some amino acid variables was below the commonly accepted threshold of 0.80, likely due to individual variation among mares. Therefore, these amino acid results should be further validated in larger-sample studies. Fourth, foal growth performance, milk intake, behavioral responses, and detailed health indicators were not evaluated. Finally, because this study was conducted in a single grazing area and compared only two feeding systems, the application of mineral element profiles for mare milk traceability and the economic feasibility of concentrate supplementation should be further validated in future studies involving multiple regions, seasons, feeding systems, and larger sample populations.

5. Conclusions

This study showed that concentrate supplementation under grazing conditions improved lactation performance in Yili mares, mainly by increasing milk yield and the daily yields of milk fat, milk protein, and lactose, rather than by changing their percentages in milk. Supplementation also altered the nutritional composition of mare milk, as reflected by changes in several amino acids and mineral elements. However, grazing alone was more favorable for maintaining higher proportions of alpha-linolenic acid, n-3 PUFA, and total PUFA in milk, indicating a trade-off between increased milk yield and the preservation of beneficial fatty acids. Mineral analysis showed that supplementation reduced milk K concentration and the K/Na ratio while increasing Fe, Co, and Cr concentrations, suggesting that the feeding system can influence the mineral profile of mare milk. Overall, moderate concentrate supplementation may be a useful strategy to improve milk production in grazing Yili mares, but its effects on fatty acid nutritional quality, foal growth and digestive health require further evaluation.

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

Funding: This research was funded by the National Key R&D Program Special Project (2022YFD1600103).

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Animal Experiment Ethics Committee of Xinjiang Agricultural University (2023059) on 1 February 2023.

Data Availability Statement: The data presented in this study are available on request from the corresponding author due to ongoing research and further data analysis.

Acknowledgments: We acknowledge the support of the College of Animal Science, Xinjiang Agricultural University.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Wang, C.; Zeng, Y.; Wang, J.; Wang, T.; Li, X.; Shen, Z.; Meng, J.; Yao, X. Estimation of Genetic Parameters of Body Conformation and Racing Performance Traits in Yili Horses. *J. Equine Vet. Sci.* **2025**, *146*, 105378. [CrossRef]
2. Chen, B.; Zhu, H.; Zhang, Y.; Wang, X.; Zhang, W.; Wang, Y.; Pang, X.; Zhang, S.; Lv, J. Comparison of Species and Lactation of Different Mammalian Milk: The Unique Composition and Stereospecificity of Fatty Acids of Mare Milk. *Int. Dairy J.* **2024**, *150*, 105822. (In Chinese) [CrossRef]
3. Duan, C.; Ma, L.; Cai, L.; Li, X.; Ma, F.; Chen, J.; Huo, G.; Li, D. Comparison of Allergenicity among Cow, Goat, and Horse Milks Using a Murine Model of Atopy. *Food Funct.* **2021**, *12*, 5417–5428. [CrossRef]
4. Wang, G.; Li, Y.; Fan, L.; Ma, X.; Liang, Y.; Hui, T.; Zhang, W.; Li, W.; Mao, J. Assessment of Grassland Carrying Capacity Drivers and Evaluation of Pasture-Livestock Balance: A Case Study of Xinjiang, China. *Glob. Ecol. Conserv.* **2024**, *55*, e03203. [CrossRef]
5. Ma, L.; Zheng, J.; Pen, J.; Xiao, X.; Liu, Y.; Liu, L.; Han, W.; Li, G.; Zhang, J. Monitoring and Influencing Factors of Grassland Livestock Overload in Xinjiang from 1982 to 2020. *Front. Plant Sci.* **2024**, *15*, 1340566. [CrossRef]
6. Yu, X.; Fang, C.; Liu, L.; Zhao, X.; Liu, W.; Cao, H.; Lv, S. Transcriptome Study Underling Difference of Milk Yield during Peak Lactation of Kazakh Horse. *J. Equine Vet. Sci.* **2021**, *102*, 103424. [CrossRef] [PubMed]
7. O'Mara, F.P.; Murphy, J.J.; Rath, M. The Effect of Concentrate Supplements Differing in Ruminal Protein Degradability on Milk Production and Blood Metabolite Concentrations of Dairy Cows Grazing Perennial Ryegrass Pasture. *Livest. Prod. Sci.* **2000**, *64*, 183–191. [CrossRef]
8. Pyles, M.B.; Fowler, A.L.; Crum, A.D.; Hayes, S.H.; Schendel, R.R.; Lawrence, L.M. Dietary Nonstructural Carbohydrates Influence Synthesis of Mare Milk Carbohydrates and Yield. *J. Anim. Sci.* **2025**, *103*, skaf213. [CrossRef]
9. Wei, M.; Ci, R.; Wang, S.; Yang, Z.; Zhang, Y.; Nan, J.; Xie, Z.; Cuose, Y.; Yang, Y.; Zhang, G.; et al. Effects of warm season supplementary feeding on the milk performance of lactating yaks grazing on natural grassland in an alpine pastoral area. *Pratacultural Sci.* **2024**, *41*, 195–206. (In Chinese) [CrossRef]
10. Li, Q.; Dou, J.; Li, H.; Huang, X.; Li, X.; Guo, Y.; Liao, C.; Ma, Y.; Li, X. Effects of Supplementing Different Levels of Corn on Milk Yield and Milk Quality of Yili Mares. *Chin. J. Anim. Sci.* **2021**, *57*, 168–171. (In Chinese) [CrossRef]
11. Lu, H.; Zhang, W.; Sun, S.; Mei, Y.; Zhao, G.; Yang, K. Effect of Supplementary Feeding on Milk Volume, Milk Composition, Blood Biochemical Index, and Fecal Microflora Diversity in Grazing Yili Mares. *Animals* **2023**, *13*, 2415. [CrossRef]
12. NRC. *Nutrient Requirements of Horses*, 6th ed.; National Research Council of the National Academies: Washington, DC, USA, 2007; pp. 296–297.
13. AOAC. *Official Methods of Analysis*, 18th ed.; Association of Official Analytical Chemists International: Washington, DC, USA, 2010.
14. Van Soest, P.J.; Robertson, J.B.; Lewis, B.A. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci.* **1991**, *74*, 3583–3597. [CrossRef] [PubMed]
15. GB 5009.124-2016; National Food Safety Standard—Determination of Amino Acids in Foods. National Health and Family Planning Commission of the People's Republic of China: Beijing, China; China Food and Drug Administration: Beijing, China, 2016.
16. GB 5009.168-2016; National Food Safety Standard—Determination of Fatty Acids in Foods. National Health and Family Planning Commission of the People's Republic of China: Beijing, China; China Food and Drug Administration: Beijing, China, 2016.
17. GB 5009.268-2016; National Food Safety Standard—Determination of Multi-Elements in Foods. National Health and Family Planning Commission of the People's Republic of China: Beijing, China; China Food and Drug Administration: Beijing, China, 2016.
18. GB/T 13885-2017; Animal Feeding Stuffs—Determination of the Contents of Calcium, Copper, Iron, Magnesium, Manganese, Potassium, Sodium and Zinc—Method Using Atomic Absorption Spectrometry. State Administration for Quality Supervision, Inspection and Quarantine of the People's Republic of China: Beijing, China; Standardization Administration of the People's Republic of China: Beijing, China, 2017.
19. Li, J.; Liu, J.; Fan, T.; Wang, N.; Li, J.; Yang, G.; Nai, B. Evaluation of nutritional value of natural herbage of different grassland types in Xinjiang. *China Feed* **2021**, *13*, 92–97. (In Chinese) [CrossRef]
20. Douhard, F.; Lemaître, J.-F.; Rauw, W.M.; Friggens, N.C. Allometric Scaling of the Elevation of Maternal Energy Intake during Lactation. *Front. Zool.* **2016**, *13*, 32. [CrossRef]

21. Miraglia, N.; Burger, D.; Kapron, M.; Flanagan, J.; Langlois, B.; Martin-Rosset, W. Local Animal Resources and Products in Sustainable Development: Role and Potential of Equids. In *Livestock Farming Systems*; Rubino, R., Sepe, L., Dimitriadou, A., Gibon, A., Eds.; Brill | Wageningen Academic: Wageningen, The Netherlands, 2006; pp. 215–233, ISBN 978-90-8686-565-9.
22. Santos, A.S.; Silvestre, A.M. A Study of Lusitano Mare Lactation Curve with Wood's Model. *J. Dairy Sci.* **2008**, *91*, 760–766. [[CrossRef](#)]
23. Caprioli, G.; Kamgang Nzekoue, F.; Fiorini, D.; Scocco, P.; Trabalza-Marinucci, M.; Acuti, G.; Tardella, F.M.; Sagratini, G.; Catorci, A. The Effects of Feeding Supplementation on the Nutritional Quality of Milk and Cheese from Sheep Grazing on Dry Pasture. *Int. J. Food Sci. Nutr.* **2020**, *71*, 50–62. [[CrossRef](#)]
24. Della Rosa, M.M.; Boshier, T.J.; Khan, M.A.; Sandoval, E.; Dobson-Hill, B.; Duranovich, F.N.; Jonker, A. Effect of Supplementing High-Fiber or High-Starch Concentrates or a 50:50 Mix of Both to Late-Lactation Dairy Cows Fed Cut Herbage on Methane Production, Milk Yield, and Ruminant Fermentation. *J. Dairy Sci.* **2025**, *108*, 7036–7050. [[CrossRef](#)] [[PubMed](#)]
25. Costa, A.; Lopez-Villalobos, N.; Sneddon, N.W.; Shalloo, L.; Franzoi, M.; De Marchi, M.; Penasa, M. Invited Review: Milk Lactose—Current Status and Future Challenges in Dairy Cattle. *J. Dairy Sci.* **2019**, *102*, 5883–5898. [[CrossRef](#)]
26. Julliand, V.; De Fombelle, A.; Varloud, M. Starch Digestion in Horses: The Impact of Feed Processing. *Livest. Sci.* **2006**, *100*, 44–52. [[CrossRef](#)]
27. Medina, B.; Girard, I.D.; Jacotot, E.; Julliand, V. Effect of a Preparation of *Saccharomyces Cerevisiae* on Microbial Profiles and Fermentation Patterns in the Large Intestine of Horses Fed a High Fiber or a High Starch Diet¹. *J. Anim. Sci.* **2002**, *80*, 2600–2609. [[CrossRef](#)]
28. Hoffman, R.M.; Kronfeld, D.S.; Herbein, J.H.; Swecker, W.S.; Cooper, W.L.; Harris, P.A. Dietary Carbohydrates and Fat Influence Milk Composition and Fatty Acid Profile of Mare's Milk. *J. Nutr.* **1998**, *128*, S2708–S2711. [[CrossRef](#)]
29. Morrison, S.J.; Patterson, D.C. The Effects of Offering a Range of Forage and Concentrate Supplements on Milk Production and Dry Matter Intake of Grazing Dairy Cows. *Grass Forage Sci.* **2007**, *62*, 332–345. [[CrossRef](#)]
30. Boranbayeva, T.; Dossimova, Z.; Zhalelov, D.; Zhunisbek, A.; Bolat, A.; Toishimanov, M. Influence of Lactation, Age and Foaling Factors on the Quality Composition, Fatty and Amino Acid Profile of Mare's Milk under Pasture Conditions. *Foods* **2025**, *14*, 2880. [[CrossRef](#)]
31. Cant, J.P.; Kim, J.J.M.; Cieslar, S.R.L.; Doelman, J. Symposium Review: Amino Acid Uptake by the Mammary Glands: Where Does the Control Lie? *J. Dairy Sci.* **2018**, *101*, 5655–5666. [[CrossRef](#)] [[PubMed](#)]
32. Manjarin, R.; Bequette, B.J.; Wu, G.; Trottier, N.L. Linking Our Understanding of Mammary Gland Metabolism to Amino Acid Nutrition. *Amino Acids* **2014**, *46*, 2447–2462. [[CrossRef](#)] [[PubMed](#)]
33. Chandel, N.S. Amino Acid Metabolism. *Cold Spring Harb. Perspect. Biol.* **2021**, *13*, a040584. [[CrossRef](#)]
34. Gao, H.; Hu, H.; Zheng, N.; Wang, J. Leucine and Histidine Independently Regulate Milk Protein Synthesis in Bovine Mammary Epithelial Cells via mTOR Signaling Pathway. *J. Zhejiang Univ. Sci. B* **2015**, *16*, 560–572. [[CrossRef](#)]
35. Guo, L.; Zheng, C.; Chen, J.; Du, R.; Li, F. Phenylalanine Regulates Milk Protein Synthesis via LAT1–mTOR Signaling Pathways in Bovine Mammary Epithelial Cells. *Int. J. Mol. Sci.* **2024**, *25*, 13135. [[CrossRef](#)]
36. Xing, Y.; Luo, Y.; Sun, M.; Yang, J.; Lin, S.; Mu, X.; Niu, X.; Li, D.; Liu, Y. Phenylalanine Modulates Casein Synthesis in Bovine Mammary Epithelial Cells by Influencing Amino Acid Transport and Protein Synthesis Pathways. *Front. Nutr.* **2025**, *12*, 1598191. [[CrossRef](#)]
37. Lin, J.; Jing, H.; Wang, J.; Lucien-Cabaraux, J.-F.; Yang, K.; Liu, W.; Li, X. Effects of Lysine and Threonine on Milk Yield, Amino Acid Metabolism, and Fecal Microbiota of Yili Lactating Mares. *Front. Vet. Sci.* **2024**, *11*, 1396053. [[CrossRef](#)] [[PubMed](#)]
38. Meng, C.; Zeng, Y.; Wang, J.; Yao, X.; Meng, J. BCAA Supplementation Enhances Milk Fat Synthesis in Yili Mares and Promotes Foal Growth through Remodeling of Intestinal Amino Acid Metabolism. *Front. Microbiol.* **2025**, *16*, 1699614. [[CrossRef](#)]
39. Liang, X.S.; Yue, Y.X.; Zhao, Y.L.; Guo, Y.M.; Guo, X.Y.; Shi, B.L.; Yan, S.M. Effects of Dietary Concentrate to Forage Ratio on Milk Performance, Milk Amino Acid Composition and Milk Protein Synthesis of Lactating Donkeys. *Anim. Feed Sci. Technol.* **2022**, *292*, 115444. [[CrossRef](#)]
40. Millet, S.; Van Oeckel, M.J.; Aluwé, M.; Delezie, E.; De Brabander, D.L. Prediction of in Vivo Short-Chain Fatty Acid Production in Hindgut Fermenting Mammals: Problems and Pitfalls. *Crit. Rev. Food Sci. Nutr.* **2010**, *50*, 605–619. [[CrossRef](#)]
41. Pieszka, M.; Łuszczynski, J.; Zamachowska, M.; Augustyn, R.; Długosz, B.; Hędrzak, M. Is Mare Milk an Appropriate Food for People?—A Review. *Ann. Anim. Sci.* **2016**, *16*, 33–51. [[CrossRef](#)]
42. Yuan, Q.; Xie, F.; Huang, W.; Hu, M.; Yan, Q.; Chen, Z.; Zheng, Y.; Liu, L. The Review of Alpha-Linolenic Acid: Sources, Metabolism, and Pharmacology. *Phytother. Res.* **2022**, *36*, 164–188. [[CrossRef](#)]
43. Doreau, M.; Boulot, S.; Bauchart, D.; Barlet, J.-P.; Martin-Rosset, W. Voluntary Intake, Milk Production and Plasma Metabolites in Nursing Mares Fed Two Different Diets. *J. Nutr.* **1992**, *122*, 992–999. [[CrossRef](#)]
44. Saber, S.H.; Yak, N.; Yong, X.L.H.; Bong, Y.T.; Leeson, H.; Dai, C.-Y.; Binder, T.; Lu, S.; Purushothaman, R.; Lenaerts, A.-S.; et al. DDHD2 Provides a Flux of Saturated Fatty Acids for Neuronal Energy and Function. *Nat. Metab.* **2025**, *7*, 2117–2141. [[CrossRef](#)]

45. Haug, A.; Høstmark, A.T.; Harstad, O.M. Bovine Milk in Human Nutrition—A Review. *Lipids Health Dis.* **2007**, *6*, 25. [[CrossRef](#)] [[PubMed](#)]
46. Capurso, C. Whole-Grain Intake in the Mediterranean Diet and a Low Protein to Carbohydrates Ratio Can Help to Reduce Mortality from Cardiovascular Disease, Slow down the Progression of Aging, and to Improve Lifespan: A Review. *Nutrients* **2021**, *13*, 2540. [[CrossRef](#)] [[PubMed](#)]
47. Benbrook, C.M.; Davis, D.R.; Heins, B.J.; Latif, M.A.; Leifert, C.; Peterman, L.; Butler, G.; Faergeman, O.; Abel-Caines, S.; Baranski, M. Enhancing the Fatty Acid Profile of Milk through Forage-based Rations, with Nutrition Modeling of Diet Outcomes. *Food Sci. Nutr.* **2018**, *6*, 681–700. [[CrossRef](#)] [[PubMed](#)]
48. Schryver, H.F.; Oftedal, O.T.; Williams, J.; Soderholm, L.V.; Hintz, H.F. Lactation in the Horse: The Mineral Composition of Mare Milk. *J. Nutr.* **1986**, *116*, 2142–2147. [[CrossRef](#)]
49. Gao, D. Determination on Macro and Trace Minerals of Four Livestock Milk of Inner Mongolia and Analysis on Minerals Profile. Master's Thesis, Inner Mongolia Agricultural University, Hohhot, China, 2017. (In Chinese)
50. Affonfere, M.; Chadare, F.J.; Fassinou, F.T.K.; Linnemann, A.R.; Duodu, K.G. In-Vitro Digestibility Methods and Factors Affecting Minerals Bioavailability: A Review. *Food Rev. Int.* **2023**, *39*, 1014–1042. [[CrossRef](#)]
51. Zhu, Y.; Guo, H.; Zheng, H.; Wen, W.; Schmidt, O.; Ma, L.; Bu, D. Application of Isotope and Multi-Element Fingerprinting in Subregional Milk Authentication—The Case of Ningxia, China. *Food Chem.* **2025**, *467*, 142261. [[CrossRef](#)]
52. Weyh, C.; Krüger, K.; Peeling, P.; Castell, L. The Role of Minerals in the Optimal Functioning of the Immune System. *Nutrients* **2022**, *14*, 644. [[CrossRef](#)] [[PubMed](#)]
53. Xue, Y. The Effect of Copper, Manganese and Iodine on Yak's Rumen Fermentation, Blood Indexes and Growth Performance. Master's Thesis, Qinghai University, Xining, China, 2016. (In Chinese)
54. Salimej, E.; Park, Y.W. Mare Milk. In *Handbook of Milk of Non-Bovine Mammals*; John Wiley & Sons, Ltd.: Hoboken, NJ, USA, 2017; pp. 369–408, ISBN 978-1-119-11031-6.
55. Alipour, A.; Akrami Mohajeri, F.; Javdan, G.; Pourramezani, F.; Fallahzadeh, H.; Khalili Sadrabad, E. Concentration of Mineral and Heavy Metals in Raw Mare (Horse) Milk Consumed in Yazd, Iran: A Risk Assessment Study. *Vet. Med. Sci.* **2023**, *9*, 1592–1598. [[CrossRef](#)]
56. Bilandžić, N.; Sedak, M.; Đokić, M.; Solomun Kolanović, B.; Varenina, I.; Božić, Đ.; Končurat, A. Concentrations of Microelements al, Co, Cr, Li, Mo, Ni, Sb and Sr in the Milk of Croatian Coldblood Mares. *Mljekarstvo Časopis Za Unaprjeđenje Proizv. I Prerade Mlijeka* **2013**, *63*, 150–157.
57. Zhou, Y.; Hao, L.; Liu, S. Effects of Supplementary Concentrate in Cold Season on Milk Yield and Milk Mineral Element Contents in Lactating Yaks in Alpine Pastures in Sanjiangyuan Region. *Chin. J. Anim. Nutr.* **2020**, *32*, 4194–4204. (In Chinese)
58. Nečemer, M.; Potočnik, D.; Ogrinc, N. Discrimination between Slovenian Cow, Goat and Sheep Milk and Cheese According to Geographical Origin Using a Combination of Elemental Content and Stable Isotope Data. *J. Food Compos. Anal.* **2016**, *52*, 16–23. [[CrossRef](#)]
59. Osorio, M.T.; Koidis, A.; Papademas, P. Major and Trace Elements in Milk and Halloumi Cheese as Markers for Authentication of Goat Feeding Regimes and Geographical Origin. *Int. J. Dairy Technol.* **2015**, *68*, 573–581. [[CrossRef](#)]
60. Camin, F.; Wehrens, R.; Bertoldi, D.; Bontempo, L.; Ziller, L.; Perini, M.; Nicolini, G.; Nocetti, M.; Larcher, R. H, C, N and S Stable Isotopes and Mineral Profiles to Objectively Guarantee the Authenticity of Grated Hard Cheeses. *Anal. Chim. Acta* **2012**, *711*, 54–59. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.