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Assessment of the Amino Acid Composition of Shubat from Western Kazakhstan

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Abstract

Background and Objective: Fermented foods of camel milk are important components of the diet for people living in arid lands. Shubat is a widely consumed fermented camel milk product in Kazakhstan. However, a comprehensive assessment of its protein quality, specifically amino acid composition, is lacking. This study aimed to assess biological value of the protein component of traditionally prepared shubat from Western Kazakhstan.

Material and Methods: Totally, 12 shubat samples were collected from the regions of Atyrau, Aktobe, Mangystau and West Kazakhstan. All samples were set to the national standard ST RK 117-2015. Amino acid composition was assessed using high-performance liquid chromatography with pre-column derivatization. The biological value was assessed by calculating the essential amino acid content; then, amino acid score was compared to the Food and Agriculture Organization/World Health Organization (2011) reference score and the amino acid composition index (U_A) based on Harrington's desirability scale. Data were present as mean ± standard deviation (n = 3).

Results and Conclusion: Significant regional variance was observed. Fat and protein contents varied from 5.47 to 6.69% and from 2.14 to 3.21%, respectively. The essential amino acid content constituted 43–46% of total amino acids. The presence of limiting amino acids was a key factor reducing the protein quality. The first limiting amino acid was histidine in samples from Atyrau and West Kazakhstan and leucine in samples from Aktobe and Mangystau. The identification of these region-specific limiting amino acids highlights potential nutritional considerations for populations relying on shubat as a primary protein source. The amino acid composition index (U_A) was highest for the sample from Mangystau (0.66) rated as "good," while samples from other regions were rated "satisfactory" (0.56–0.58).

Keywords: Amino acid composition index (U_A), Amino acid score, Biological value (BV), Camel milk, Chemical compositionm Essential amino acids (EAAs), Fermented foods, Protein content.

What is "already known" on this topic:

- Camel milk is a vital nutritional resource in arid regions, and its fermented product, Shubat, is a traditional staple in Kazakhstan.
- The general protein and fat composition of camel milk is documented, but detailed amino acid profiling of Shubat is limited.
- The protein quality of foods is commonly assessed by comparing their essential amino acid profile to a FAO/WHO reference standard.

What this article adds:	➢ This study provides the first comprehensive amino acid assessment of traditionally prepared Shubat from four regions of Western Kazakhstan. ➢ We identify region-specific limiting amino acids (Histidine and Leucine) that constrain the protein quality of Shubat, rated as "satisfactory" to "good." ➢ The findings establish a scientific basis for potential nutritional interventions and highlight the influence of regional production on final product quality.

1. Introduction

Camel milk is an essential component of the diet in arid and semi-arid regions where other livestock are less resilient to extreme climatic conditions, serving as a vital nutritional source for populations in these areas [1]. In recent years, an increasing global interest in camel milk as an alternative to other types of milk is reported due to its hypoallergenic, anti-carcinogenic and anti-diabetic characteristics [2,3,4]. Camel milk contains significant quantities of bioactive compounds such as lactoferrin, immunoglobulins, lysozyme and vitamin C, which contribute to immune support and prevention of non-communicable diseases [5,6]. The milk unique biochemical characteristics are largely attributed to its protein composition, particularly low content of β-casein and absence of β-lactoglobulin, making it appropriate for individuals with milk allergies [7,8]. Despite these advantages, there is a limited research on the detailed composition of camel milk and its fermented derivatives, especially regarding their nutritional potential and use in the food industry [9,10,11]. One of the most effective ways to enhance the nutritional and functional value of milk is through fermentation, a process that improves digestibility and enriches the product with bioactive compounds [12,13]. Fermented dairy products differ across the world for production methods, microbial cultures and names, including yogurt, kefir, matsoni, dahi, ghioddu, leben, tarag, unda, shubat (chal), suusak (susa) and garris [14,15,16].

Shubat (chal), a traditional fermented camel milk beverage, is widely consumed in Turkey, Turkmenistan and Kazakhstan, where it has historically been produced through spontaneous fermentation. Traditionally, shubat is prepared by mixing fresh camel milk with warm water (1:1 ratio) in goatskin or ceramic containers and inoculating it with 1/3–1/5 of previously fermented milk as a starter culture. The fermentation typically lasts 3–4 h at 25–30 °C, followed by an additional maturation phase of nearly 8 h at a similar temperature, promoting mixed lactic and alcoholic fermentation [17,18]. In modern Kazakhstan, shubat is manufactured on an industrial scale using defined starter cultures such as *Lactobacillus casei* and *Streptococcus thermophiles* with yeasts [19]. Under industrial conditions, camel milk fermentation occurs at 25 °C for approximately 8 h, followed by incubation at 20 °C for nearly 16 h. The final product must meet the quality and safety criteria established by the National Standard of the Republic of Kazakhstan (ST RK 117-2015 “Shubat. General Technical Conditions”) and relevant international guidelines [20].

Studies of the amino acid (AA) composition of camel milk indicate the presence of 17 AAs, excluding tryptophan, with essential amino acids (EAAs) comprising approximately 43.5–43.9% of total amino acids (TAAs), values that exceed the Food and Agriculture Organization/World Health Organization (FAO/WHO) recommended standards. The ratios of EAAs/TAAs and EAAs/NEAAs (non-essential amino acids) are greater than 40 and 75%, respectively [21]. Furthermore, fermentation significantly affects the protein profile of camel milk, increasing the content of antioxidant peptides, likely due to the unique structure of β-casein, which is shorter and richer in proline residues. Hydrolysis of β-casein leads to the release of bioactive peptides and AAs such as phenylalanine and tryptophan with antioxidant activity [22]. Regarding limited data on the biochemical characteristics of fermented camel milk products, this study aimed to assess the biological value of the protein fraction of shubat from the western regions of the Republic of Kazakhstan, focusing on EAA profiles. The assessment was based on the AA score, AA composition index (UA) and Harrington’s desirability scale to provide a comparative assessment of nutritional quality in regional samples [23].

2. Materials and Methods

2.1. Samples collection

Totally, 12 samples of traditionally prepared fermented product, shubat, were selected from western regions of the Republic of Kazakhstan, where Bactrian camels were widely bred (Atyrau, Aktobe and Mangystau, West Kazakhstan). Samples were collected randomly from local markets and households in each region, representing the traditional supply chain. As shubat was a traditionally fermented product, specific data on camel breed, lactation stage and feed were not controlled, reflecting the typical consumption product. This initial survey of 12 samples (four per region) provided a foundational mapping of the AA profile across Western Kazakhstan. The samples (250 ml each) were collected in sterile screw-cap bottles and stored at low refrigeration temperature (4 ±2 °C) until use.

All samples were positively assessed for quality and safety indicators based on ST RK 117-2015 “Shubat. General Technical Conditions” and Technical Regulations of the Customs Union - TR CU 033/2013 “On the Safety of Milk and Dairy Products” and approved for further research [24,25].

2.2. Amino Acid Composition Analysis

Assessment of the AA composition of the samples was carried out using high-performance liquid chromatography (HPLC) and an Agilent 1200 liquid chromatograph device, USA, with diode-array detection at 254 nm [26]. Prior to analysis, proteins were hydrolyzed with 6M HCl for 24 h at 110 °C under a nitrogen atmosphere. Tryptophan was analyzed after alkaline hydrolysis. The hydrolysates were derived using AccQ-Tag reagent kit (Waters, USA) according to the manufacturer's instructions. A C18 column was used at the column thermostat temperature of 16 °C. Acetonitrile and acetate buffer at pH 6.0 were used as the mobile phase in a gradient elution mode with flow rate of 1.0 ml min^{-1} [27]. Qualitative and quantitative analyses were carried out based on retention time and the internal standard method (norvaline was used as an internal standard). Calibration was carried out using standard AA mixture (Sigma-Aldrich, USA). All analyses were carried out in triplicate (n = 3) [28].

In this study, standard samples of proteinogenic AAs were used, including aspartic acid (Asp), glutamic acid (Glu), serine (Ser), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), proline (Pro), tyrosine (Tyr), valine (Val), methionine (Met), isoleucine (Ile), leucine (Leu), tryptophan (Trp), phenylalanine (Phe) and lysine (Lys). These AAs are major structural elements for protein biosynthesis and characterized by unique physicochemical parameters that set their functional characteristics in biological systems. Ratio of EAA to NEAA was calculated mathematically.

2.3. Assessment of Amino Acid Composition

The EAA content was calculated to g per 100 g of protein using Eq. 1.

$$\text{Content of EAA} = A * 100 / B \quad \text{Eq. 1}$$

Where, A was the mass fraction of an EAA in a sample, g per 100 g of sample; and B was the mass fraction of protein in a sample, g. To calculate the AA score, the content of each EAA of a sample was compared with its content in the reference protein (FAO/WHO, 2011) using Eq. 2.

$$\text{Amino acid score} = Ai / Ari * 100, \% \quad \text{Eq. 2}$$

Figure 1. Regions of the Republic of Kazakhstan. Source: Agency for Strategic planning and reforms of the Republic of Kazakhstan. Available from: https://stat.gov.kz/

Where, A_i was the mass fraction of an EAA of a sample, g per 100 g of protein; and A_{ri} was the mass fraction of an EAA in the reference protein, g per 100g of protein. An AA score rate of less than 100% was addressed as "limiting". In a presence of several limiting AAs in a sample, an AA with the lowest AA score was addressed as "first limiting". Assessment of the biological value of the protein component was carried out using an AA composition index (U_A) based on Liebig's law Eq. 3.

$$U_A = \sqrt[m]{\prod_{i=1}^{m} d_{Ai}} \quad \text{Eq. 3}$$

As

$$d_{(A_i)} = (A_i/A_{ri}),\ \text{if } A_i \le A_{ri}$$

$$d_{(A_i)} = (A_{ri}/A_i),\ \text{if } A_i \ge A_{ri}$$

Where, m was the quantity of EAAs based on FAO/WHO, 2011 (nine EAAs). The inversion in the formula for $d_{(A_i)}$, when $A_i \ge A_{ri}$ ensured that any deviation from the ideal reference protein, whether a deficit or an excess, negatively affected the index. This was because an excess of one EAA could not compensate for a deficit in another (the "law of the minimum"). To analyze data, Harrington's function known as the desirability scale was used. The desirability scale was divided in several ranges from 0 to 1 by five subranges of [0–0.2] "very bad", [0.2–0.37] "bad", [0.37–0.63] "satisfactory", [0.63–0.8] "good" and [0.8–1] "very good".

2.4. Statistical Analysis

All measurements were carried out in triplicate and results were expressed as mean ±SD (standard deviation). Differences in chemical composition and AA content between the regions were assessed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons and SPSS software v.26 (IBM, USA). A p-value less than 0.05 was considered statistically significant.

3. Results and Discussion

Chemical composition of the samples was assessed based on the requirements of ST RK 117-2015 "Shubat. General Technical Conditions", where protein content is not standardized. Results of this assessment are present in Table 1. Literary, data report on chemical composition of camel milk in the Republic of Kazakhstan highlights its fat content in a range from 4.47 to 5.17, as well as total protein content from 3.5 to 4.45 [29]. Increase in fat content and decrease in total protein in the samples were recorded, previously described by the global research community [30]. The present study showed that all samples exceeded the established indicators of ST RK 117-2015, in particular, the minimum requirements for fat content by 1.5–2 times (Table 1).

Initial data on the AA composition of samples, focusing on EAAs, were present in g per 100 g of sample (Figure 2 and Table 2). A variety was revealed in the AA composition of samples, which might be due to the differences in their chemical composition. It should be stated that the proportion of EAAs was 43–46%, similar to that reported in the global data [31,32,33].

Figure 3 presents a comparative analysis of the biological value of protein component in the samples using AA score method based on the reference protein data (FAO/WHO, 2011) [35].

To assess biological value of the protein component of samples, AA composition data were recalculated based on the units of the reference protein (FAO/WHO) in g per 100 g of protein (Table 3) [34].

In addition, biological value of the protein component of samples was assessed using AA composition index (U_A) and summarized data based on Harrington's desirability function [36,37]. The calculation results are present in Table 4.

Table 1. Chemical composition of the samples from Western Regions of the RK

Mass of nutrients	Requirements to Shubat*	Chemical composition of samples from regions of the RK, g/ 100 g of sample			
		Atyrau	Aktobe	Mangystau	West Kazakhstan
Fat	no less than 3.2	6.69±0.03	6.61±0.02	5.70±0.04	5.47±0.05
Protein	-	2.14±0.02	2.32±0.03	3.04±0.03	3.21±0.04
Lactose	3.22	3.91±0.05	3.87±0.04	4.12±0.01	4.05±0.05
Ash	-	0.81±0.02	0.79±0.01	0.85±0.02	0.83±0.02

*data of ST RK 117-2015"Shubat. General Specifications"
Different superscript letters within a row indicate significant differences ($p < 0.05$)

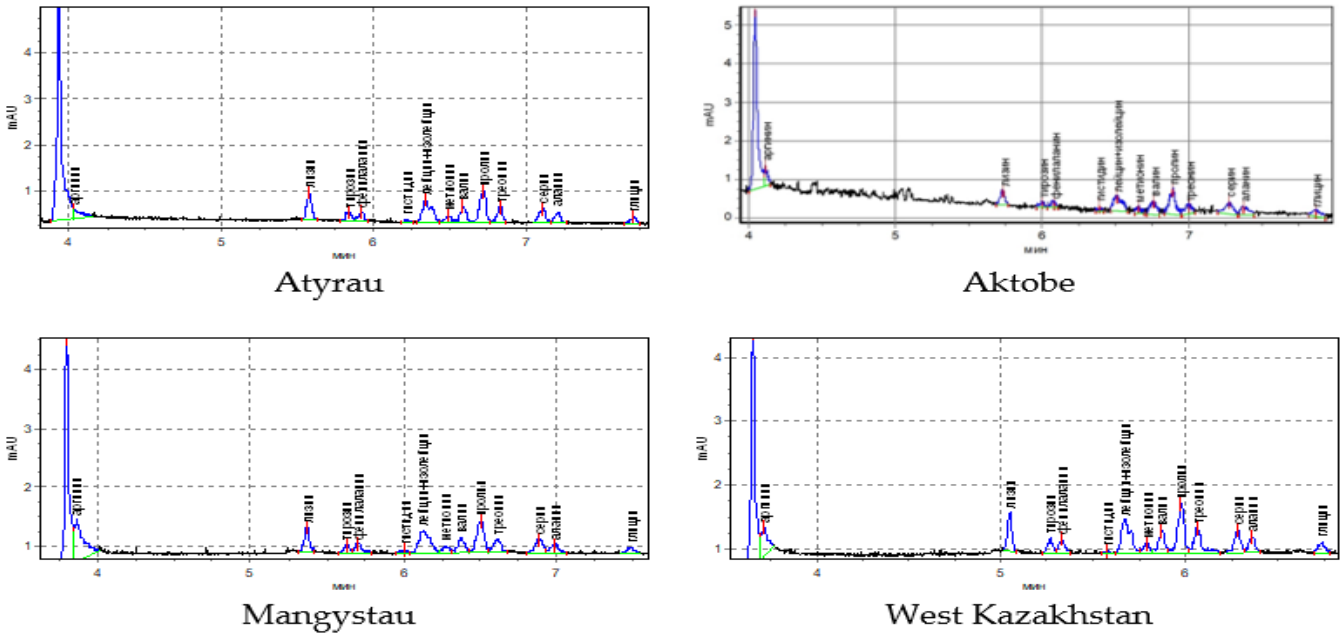

Figure 2. Chromatogram of the shubat samples

Table 2. Mass fraction of the essential amino acids of samples from western regions of the Republic of Kazakhstan

Essential amino acids (EAAs)	Mass fraction of EAAs of samples from regions of the RK, g/ 100 g of sample			
	Atyrau	Aktobe	Mangystau	West Kazakhstan
Histidine	0.02±0.01	0.05±0.03	0.05±0.03	0.03±0.02
Leucine	0.13±0.05	0.12±0.05	0.14±0.06	0.20±0.08
Isoleucine	0.07±0.05	0.06±0.05	0.08±0.06	0.11±0.08
Lysine	0.16±0.05	0.13±0.04	0.15±0.05	0.22±0.08
Methionine + Cysteine*	0.11±0.03	0.13±0.04	0.14±0.04	0.17±0.05
Phenylalanine + Tyrosine*	0.30±0.05	0.25±0.04	0.32±0.05	0.37±0.06
Threonine	0.17±0.07	0.17±0.07	0.16±0.06	0.30±0.12
Tryptophan	0.06±0.01	0.06±0.02	0.06±0.03	0.07±0.03
Valine	0.17±0.07	0.22±0.09	0.17±0.07	0.26±0.10

* a need for one EAA can be covered by the presence of another one, then the pairs are summed up

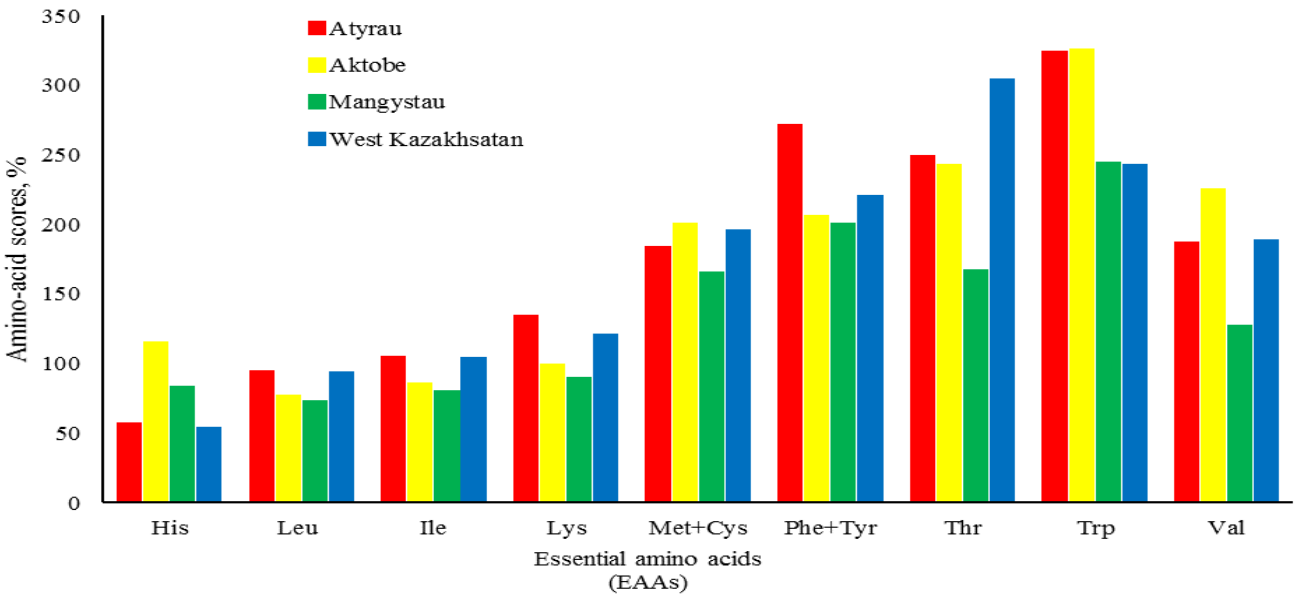

Figure 3. Amino acid scores of samples from western regions of the Republic of Kazakhstan (FAO/WHO, 2011)

Table 3. Amino acid compositions of samples from western regions of Republic of Kazakhstan

Essential amino acids (EAA)	Reference protein, g /100 g of protein	Amino acid composition of samples from regions of the RK, g /100 g of protein			
		Atyrau	Aktobe	Mangystau	West Kazakhstan
Histidine	2.00	1.12	2.28	1.65	1.06
Leucine	6.60	6.17	5.04	4.74	6.11
Isoleucine	3.20	3.32	2.72	2.53	3.30
Lysine	5.70	7.62	5.60	5.07	6.85
Methionine + Cysteine	2.70	4.95	5.39	4.44	5.26
Phenylalanine + Tyrosine	5.20	14.07	10.69	10.39	11.43
Threonine	3.10	7.71	7.50	5.16	9.41
Tryptophan	0.85	2.75	2.76	2.07	2.06
Valine	4.30	7.99	9.66	5.43	8.07

Table 4. Assessment of the amino acid composition index (U_A) of samples from regions of the Republic of Kazakhstan

Essential Amino Acids (EAAs)	d_{A_i}			
	Atyrau	Aktobe	Mangystau	West Kazakhstan
Histidine	0.56	0.88	0.83	0.53
Leucine	0.93	0.76	0.72	0.93
Isoleucine	0.96	0.85	0.79	0.97
Lysine	0.75	0.98	0.89	0.83
Methionine + Cysteine*	0.55	0.50	0.61	0.51
Phenylalanine + Tyrosine*	0.37	0.49	0.50	0.46
Threonine	0.40	0.41	0.60	0.33
Tryptophan	0.31	0.31	0.41	0.41
Valine	0.54	0.45	0.79	0.53
U_A	0.56	0.58	0.66	0.57

Based on this study, it could be concluded that all samples of shubat from the Western Kazakhstan regions met the requirements of regulatory framework of the Republic of Kazakhstan in accordance with ST RK 117-2015 "Shubat. General Technical Conditions" and significantly exceeded the minimum requirements for fat content. Specifically, the highest value of this value was observed in the sample from Atyrau (6.69%) and the lowest in a sample from West Kazakhstan (5.47%). Moreover, the highest value for protein content was reported in a sample from West Kazakhstan (3.21%), slightly a lower protein content in a sample from Mangystau (3.04%), significantly a lower protein content in a sample from Aktobe (2.32%) and the lowest value in a sample from Atyrau (2.14%). For lactose and ash in the samples, a relatively identical content of dry matter of nearly 13–14% was recorded, which corresponded to the literary data by other authors [38,39].

Assessment of the biological value of protein component in the samples using AA score method and AA composition index (U_A) showed that all the samples included satisfactory values. However, statistically significant variability in AA composition of samples was seen, which might be due to various chemical composition of the camel milk from the western regions of the country, affected by factors such as biodiversity of the forage base, regional environmental conditions and traditional husbandry practices.

A negative factor in assessing biological values of the protein component of the samples was the presence of limiting AAs such as leucine, an AA score of which varied from 71.8 to 93.5% and histidine, an AA score of which varied from 53 to 82.5% (with the exception of a sample from Aktobe with a score of 114%), as well as isoleucine and lysine for samples from Aktobe and Mangystau (AA scores of isoleucine were 85 and 79.1% and those of lysine were 98.2 and 88.9%, respectively). This imbalance was demonstrated by the U_A index, which penalized deficits and excesses. However, increased values of EAAs were reported in the samples. In particular, phenylalanine and tyrosine exceeded the FAO/WHO 2011 values by an average of two times and tryptophan by 2–3 times, which negatively affected comprehensive assessment of the highlighted parameters.

It should be stated that in samples from Atyrau and West Kazakhstan, the first limiting AA was histidine, with AA scores of 56 and 53%, respectively. Histidine is critical for protein synthesis, tissue repair and production of histamine.

Deficiency of this AA can impair growth in children and negatively affect metabolic functions [40,41]. This factor may lead to disruptions in numerous physiological processes such as impaired protein synthesis and decreased muscle mass, slower metabolism and decreased energy exchange in the human body [42,43]. In samples from Aktobe and Mangystau, deficiency of leucine was observed, with AA scores of 76.4 and 71.8%, respectively. Leucine is a key regulator of muscle protein synthesis and metabolic signaling pathways [44,45]. This might negatively affect immunity and overall metabolism and lead to the following adverse consequences of impaired functioning of the nervous and hematopoietic systems, decreased antioxidant protection and weakened immune function of the body [46,47].

Compared to other fermented camel milks such as chal from Turkmenistan [48], EAA profile of the shubat samples showed high phenylalanine and tyrosine values as well as its specific limiting AAs, which might be attributed to the differences in camel breed, fermentation microbiota and/or regional diets. Based on the data (Table 4), it could be concluded that samples from western regions of the Republic of Kazakhstan such as Atyrau, West Kazakhstan and Aktobe, included similar AA composition index (U_A) values ranging from 0.56 to 0.58, assessed as “satisfactory” based on Harrington’s desirability function. A sample from Mangystau demonstrated the highest AA composition index (UA) value (0.66), which was characterized as “good” based on Harrington’s desirability scale.

4. Conclusion

This study provided the first comprehensive assessment of the AA composition and protein quality of traditionally prepared shubat from Western Kazakhstan. Results showed that although shubat was a rich source of fat and contained a substantial proportion of EAAs (43–46%); its protein quality was limited by region-specific deficiencies in histidine and leucine, leading to an overall "satisfactory" to "good" rating on the Harrington scale. It is necessary to expand a number of studied samples from other regions of the Republic of Kazakhstan as well as focus on studying chemical composition of the raw materials for shubat production (camel milk from similar regions) to identify correlations between the low EAA values in samples due to deficiencies in individual AAs in the raw materials. Furthermore, when studying qualitative composition of the raw material protein component, attention should be paid to seasonality of the camel milk production. Effect of the biodiversity of forage base and conditions, under which camels are bred in various regions of the Republic of Kazakhstan, should be addressed. Further studies should specifically aim to (1) link shubat composition with raw camel milk from similar regions and seasons; (2) investigate effects of specific fermentation strains on the release of bioactive peptides and the AA profile; and (3) carry out longitudinal monitoring of temporal variations in milk composition. Identification of the specific limiting AAs provides a scientific basis for the potential fortification strategies to enhance the nutritional completeness of shubat in public health initiatives.

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6. Declaration of competing interest

The authors report no conflict of interest.

7. Authors’ Contributions

Conceptualization, L.N. and A.O.; methodology, L.N. and R.M.; formal analysis, B.R. and R.M.; investigation, B.R. and A.O.; data curation, R.M.; writing-original draft preparation, B.R. and L.N.; writing-review and editing, L.N. and A.O.; visualization, A.O.; supervision, L.N.; project administration, A.O.; funding acquisition, A.O. All authors have read and agreed to the published version of the manuscript.

8. Using Artificial Intelligent Chatbots

During preparation of this manuscript, the authors used ChatGPT (OpenAI) to improve language clarity and readability. After using this tool, the authors reviewed and edited the content as needed and accepted full responsibility for the content of the publication.

9. Ethical Consideration

This study did not involve human participants or animals. All research procedures complied with institutional guidelines on laboratory safety and good scientific practice.

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ارزیابی ترکیب اسیدهای آمینه شوبات از منطقه غربی قزاقستان

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چکیده

سابقه و هدف: غذاهای تخمیری شیر شتر از اجزای مهم رژیم غذایی ساکنان سرزمین‌های خشک محسوب می‌شوند. شوبات[۱] محصول تخمیری شیر شتر است که به طور گسترده در قزاقستان مصرف می‌شود. با این حال، ارزیابی جامعی از کیفیت پروتئین آن، به‌ویژه ترکیب اسیدهای آمینه، تاکنون انجام نشده است. هدف از این پژوهش، ارزیابی ارزش بیولوژیکی جزء پروتئینی شوبات تهیه‌شده به روش سنتی از قزاقستان غربی بود.

مواد و روش ها: در مجموع، ۱۲ نمونه شوبات از مناطق آتیرائو، آکتوبه، مانقیستاو و قزاقستان غربی جمع‌آوری شدند. تمامی نمونه‌ها با استاندارد ملی ST RK 117-2015 مطابقت داده شدند. ترکیب اسیدهای آمینه با استفاده از کروماتوگرافی مایع با کارایی بالا[۲] همراه با پیش‌ستونی مشتق‌سازی[۳] ارزیابی شد. ارزش بیولوژیکی با محاسبه میزان اسیدهای آمینه ضروری سنجیده شد و سپس، امتیاز اسید آمینه با امتیاز مرجع سازمان خواربار و کشاورزی/سازمان جهانی بهداشت (۲۰۱۱) و شاخص ترکیب اسید آمینه (UA) بر اساس مقیاس مطلوبیت هارینگتون مقایسه گردید. داده‌ها به‌صورت میانگین ± انحراف معیار (تعداد=۳) ارائه شدند.

یافته‌ها و نتیجه‌گیری: تغییرات منطقه‌ای معنی داری مشاهده شد. محتوای چربی و پروتئین به ترتیب از ۵,۴۷ تا ۶,۶۹ درصد و از ۲,۱۴ تا ۳,۲۱ درصد متغیر بود. محتوای اسیدهای آمینه ضروری ۴۳ تا ۴۶ درصد از کل اسیدهای آمینه را تشکیل می‌داد. وجود اسیدهای آمینه محدودکننده عامل کلیدی در کاهش کیفیت پروتئین بود. اولین اسید آمینه محدودکننده، هیستیدین در نمونه‌های مناطق آتیرائو و قزاقستان غربی و لوسین در نمونه‌های مناطق آکتوبه و مانقیستاو بود. شناسایی این اسیدهای آمینه محدودکننده خاص منطقه، ملاحظات تغذیه‌ای بالقوه‌ای را برای جمعیت‌هایی که به شوبات به عنوان منبع اصلی پروتئین متکی هستند، برجسته می‌سازد. شاخص ترکیب اسید آمینه (UA) برای نمونه منطقه مانقیستاو با مقدار ۰,۶۶ که در رده "خوب" طبقه‌بندی شد، بالاترین میزان را داشت، در حالی که نمونه‌های مناطق دیگر در رده "رضایت‌بخش" (۰,۵۶–۰,۵۸) قرار گرفتند.

واژگان کلیدی: شاخص ترکیب اسید آمینه(UA)، امتیاز اسید آمینه، ارزش بیولوژیکی(BV)، شیر شتر، ترکیب شیمیایی، اسیدهای آمینه ضروری(EAAs)، غذاهای تخمیری، محتوای پروتئین

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۲ HPLC
۳ pre-column derivatization