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Review article
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FEEDING RATION OF YAKS OF THE KYRGYZ POPULATION AND ITS INFLUENCE ON THE BIOCHEMICAL COMPOSITION OF MILK

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KEY WORDS:
yak milk, protein fractions, casein, whey proteins, amino acid composition, season, ration

ABSTRACT

Yaks are the large herbivorous ruminants that live in high mountain pastures at altitudes from 2,000 to 4,000 m above sea level. This environment is quite harsh, and yaks calmly tolerate extreme cold — down to minus 40 °C. The main food of yaks are herbaceous plants, the vegetation season of which in a cold mountain climate is short, about 3–4 months, and therefore they feed mostly on wilted pasture, which is, in principle, sufficient for these animals. At the end of winter, when the amount of pasture feed decreases, the animals become malnourished and lose weight. Therefore, yak farmers often face the need to feed their animals in winter. A complete ration of an animal, along with other factors, has a significant impact on the composition of milk and its productive ability. The aim of the work is to study the chemical composition, in particular the protein fraction, of the milk of yaks living in the mountainous regions of Kyrgyzstan in comparison with the milk of yaks from different regions of the world. The object of the study was milk of yaks inhabiting the highland regions of Naryn province of Kyrgyzstan. Samples were collected and tested according to standard methods. Studies have shown that the milk of yaks from the Kyrgyz population contains more protein (5.66%) than milk from yaks in the mountains of China, Russia and Mongolia (4.95, 4.55 and 5.3%, respectively). Milk we studied is high in amino acids such as methionine, lysine, proline, phenylalanine, and alanine. Seasonal (spring, summer) changes in the protein composition (whey proteins, casein proteins), amino acid profile, total nitrogen, non-protein nitrogen of yak milk were also studied. The total content of amino acids in the milk of Kyrgyz female yaks is noticeably higher in spring (6189.96 mg/100 g) than in summer (5101.47 mg/100 g). The research results presented in this article showed that the milking season and the associated feeding ration of yaks affects the composition of milk, which is associated with the nutritional value of grass feed and its nutrient content. Spring milk contains more proteins and amino acids than summer milk, which is due to the high protein content of plants in the spring period before the flowering phase. Milk of Kyrgyz yak with proven high nutritional and biological value is an alternative to cow milk.

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РАЦИОН КОРМЛЕНИЯ ЯКОВ КЫРГЫЗСКОЙ ПОПУЛЯЦИИ И ЕГО ВЛИЯНИЕ НА БИОХИМИЧЕСКИЙ СОСТАВ МОЛОКА

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КЛЮЧЕВЫЕ СЛОВА:
молоко яка, фракции белков, казеин, сывороточные белки, аминокислотный состав, сезон, корм

АННОТАЦИЯ

Яки — крупные травоядные жвачные животные, обитающие на высокогорных пастбищах на высоте от 2000 до 4000 м над уровнем моря. Эта среда достаточно суровая, и яки спокойно переносят экстремальные холода — до минус 40 °С. Основной пищей яков являются травянистые растения, вегетационный период которых в холодном горном климате короткий, примерно 3–4 месяца, в связи с чем они питаются большей частью увядшим подножным кормом, что в принципе достаточно для этих животных. В конце зимы, когда количество подножного корма уменьшается, животные недоедают и теряют в весе. Поэтому перед яководами часто возникает необходимость зимней подкормки животных. Полноценный рацион животного, наряду с другими факторами, оказывает существенное влияние на его продуктивную способность и состав молока. Цель работы — исследование химического состава, в частности белковой фракции, молока якоматок, обитающих в горных регионах Кыргызстана, в сравнении с молоком ячих разных регионов мира. Объектом исследования являлось молоко ячих, обитающих в высокогорных регионах Нарынской области Кыргызстана. Сбор проб и исследования проводили в соответствии со стандартными методами. Исследования показали, что в молоке якоматок кыргызской популяции содержится больше белка (5,66%), по сравнению с молоком животных, обитающих в горах Китая, России и Монголии (4,95, 4,55 и 5,3% соответственно). В исследованном нами молоке высокое содержание аминокислот, таких как метионин, лизин, пролин, фенилаланин и аланин. Также изучены сезонные (весна, лето) изменения белкового состава (сывороточные белки, казеиновые белки), аминокислотного профиля, общего азота, небелкового азота молока якоматок. Суммарное содержание аминокислот в молоке яков кыргызской популяции заметно выше в весеннее время (6189,96 мг/100 г),

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чем летом (5101,47 мг/100 г). Результаты исследований, приведенные в данной статье, показали, что сезон удоя и связанный с ним рацион питания ячих влияет на состав молока, что связано с питательностью травяного корма и содержанием в нем нутриентов.

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1. Introduction

Vast high-mountain territories with a complex combination of natural, climatic and feeding conditions are the favorite habitat of yaks. They are adapted to the specific conditions of the highlands, which are characterized by sharp changes in temperature and atmospheric pressure. The feeding ration is quite meager and mostly consists of low-growing vegetation 0.7 cm high. Thanks to the specific structure of the tongue and upper lip, such short grass is accessible to the yaks. Their tongue is spade-shaped, the tip is flat and thin, which gives it special mobility; the upper lip is also particularly mobile, like that of sheep [1]. The most suitable grass height for yaks is considered to be 15–18 cm. Daily feed consumption in winter is 4–6 kg, in summer — 12–15 kg.

The habitat of wild yaks currently spans to the highlands of Tibet and a small part of Nepal. Semi-wild yaks are bred in many mountainous regions of Asia: Western China, Tibet, Nepal, Bhutan, Mongolia, Altai, Tajikistan and Kyrgyzstan. They are kept only in the open air, drink running water and eat pasture. Despite their high adaptive capacity, temperature changes force yaks to change pastures depending on the season [2]. In summer, yaks try to go up higher, to where it is not so hot, but green parts of herbaceous plants have already appeared on the pastures. June and August are the best times in terms of nutritional value, phytomass and easy digestibility of herbs. The nutritional value of green food is higher due to its high carbohydrate content. At the end of summer, the grass becomes coarser and the diet is replenished with withered stems of forbs, leaves of shrubs and various high-mountain berries. With the onset of cold weather, the diet of yaks becomes more meager, which significantly affects their feeding regime, and their main food is pasture grass. A shallow layer of snow is not a hindrance for the yak; it easily breaks and digs the snow with its muzzle and extracts food from under the snow. Breeding yaks contributes to the effective use of high-mountain pastures at different times of the year, and ability of yaks to quickly gain muscle mass and accumulate fat with a meager diet indicates the high ecological plasticity of the species.

It has been established that yaks consume 49 species of forage plants, of which 16 species are cereals, 4 legumes, 6 sedges, and more than 20 species of forbs and shrubs. Their favorite delicacies are bluegrass, fescue, Koeléria, sedge, cold wormwood and Gmelin's wormwood, kochia, and teresken subshrub (*Krascheninnikovia*). There are also plants that are not eaten by them (Gentian grandiflora, Desurainia filamentosa, Dracocephalum, Cinquefoil anseri, Quinoa spear-shaped, Velcro bristly, etc.), and even poisonous for the animals: poisonous wekh, long-rooted sorrel, black cohosh, toadflax, etc. [3]. The food composition of yaks depends on their habitat, since each region has its own specific floristic set, phenology of plant vegetation, weather conditions and other meteorological factors. The growing flora of pastures has a special effect on the population and geographic feeding characteristics of these animals.

The grassland flora of different regions is influenced by several factors, including climate and topography. On the Qinghai-Tibetan Plateau (altitude is more than 3000 m above sea level), where the largest yak population lives, desert steppes predominate (precipitation is about 50 mm per year), there are less alpine steppes (about 200 mm precipitation per year), and alpine meadows are located below (up to 600 mm of precipitation per year) with a variety of vegetation: sedge, shrubs and herbs [4].

Altai alpine meadows are used as pastures mainly in the summer. The main food of Altai yaks is cereal grass (wort grass, bluegrass, Koeléria) and forbs, which are good food for accumulating the animals fat. Sparse vegetation is replaced by the fauna of shrubby tundra, the soil surface of which is often covered with mosses and bushy lichens (cladonia and cetraria), over which thickets of gray willow and round-leaved birch, only 20–40 cm high, form. Claytonia, fescue, and cobresia are occasionally found [5].

Kyrgyzstan is a mountainous country predominantly covered by vast high-mountain pastures, where grazing of the main agricultural livestock is impossible or difficult. These pastures have long been effectively used for keeping yaks, which do not require the construction of the winter stalls and the preparation of feed, since they feed only on pasture and are not afraid of cold and icy winds. Therefore, the cost of yak production is low. In the warm season, milk is obtained from the yak cows, the composition of milk depends on various factors, including the animal's diet and the type of plant food.

The type and composition of vegetation of mountain pastures in Kyrgyzstan is also determined by the height of the mountain ranges. In the northern part, where the climate is more humid, steppes and meadows predominate; in the southern regions, deserts and semi-deserts are found more often. High-mountain meadows are characterized by low grass height, which does not exceed 25–35 cm. The most common grasses that grow are kobrezei, awnless brome, creeping wheatgrass, bluegrass, pinnate short-legged grass, vetch, aconite, cinquefoil, locoweeds, etc. [6]. At an altitude of more than 3000 m, the grass stand is formed from the most cold-resistant plants: cobresia, ptilagrostis, white bluegrass, fescue, reed grass, mosses and lichens. Significant areas are wastelands, which in the summer are the pastures for yaks, where they feed on high-mountain cushions of dryad flowers, Saussurea alpine, which begin to grow in a cushion-shaped form to preserve heat and moisture [7].

The main population of the Kyrgyz yaks lives on the pastures of the Aksai-Chatyrkul valley of the Inner Tien Shan, which is located on the border of Kyrgyzstan and the People's Republic of China. The total area of pastures is 9,250 km^2 at absolute altitudes of 3,100–3,600 m above sea level [8]. High-mountain pastures are used, most often, as distant lands for grazing animals.

The Aksai-Chatyrkul valley is one of the largest summer pastures of the Kyrgyz Republic. The geological structure of the valley and the surrounding ridges is quite complex. The vegetation cover is dominated by cryophyte steppes and cryophyte meadows in combination with cobresia (Kobresia cappiliformis), fescue (Festuca kryloviana), white grass (Festuca olgae), sheep (Helictotrichon desertorum), barley (Hordeum turkestanicum), fescue (Festuca valesiaca) and wormwood-cereals (Stipa orientalis, Artemisia rhodantha) wealds [9]. High-mountain soddy-semi-peaty and meadow-steppe alpine soils, chestnut takyr-like desert soils, and meadow-steppe carbonate subalpine soils are developed. Surface waters of the region are represented by the Western and Eastern Aksai rivers and their numerous tributaries.

It has been established [10] that depending on the altitude of the pasture, the content of vitamins and minerals in yak milk changes, the content of fat-soluble vitamins A and E increases, and, conversely, the content of vitamins B1 and B2 decreases. The content of magnesium and iron in the milk of yaks grazing on higher pastures is also higher, but the content of heavy metals is lower. Thus, the height of the pasture and, accordingly, the flora growing in the highlands play a large role in the formation of the chemical composition of yak milk.

The purpose of the work is to study the influence of the feeding ration of the Kyrgyz yak on the formation of the chemical composition of milk, to study the amino acid (AA) composition of milk proteins, and also to analyze changes in the composition of the protein fraction of milk depending on the milking season.

2. Materials and methods

The object of research conducted between 2019 and 2021 was yak milk obtained from the Naryn region of the Kyrgyz Republic at an altitude of 2,500–3,000 m above sea level. Naryn region is the largest region in Kyrgyzstan. More than 85% of the region's territory is occupied by the Tien Shan mountain ranges, which are covered with large tracts of natural pastures, the area of which is 2,875.5 thousand hectares.

Filtered yak milk immediately after milking was placed into special sterile milk storage bags. Milk samples were stored at minus 20 °C in a freezer until analyzed in the laboratory.

The analyzes were carried out on the basis of the Laboratory of Technochemical Control of the All-Russian Scientific Research Institute of the Dairy Industry (Moscow).

To determine the protein composition, the following analytical methods were used:

- mass fraction of protein and total nitrogen — by the Kjeldahl method according to GOST 23327–98[1];
- whey protein content — according to GOST 34536–2019[2];
- non-protein nitrogen content — according to GOST R55246–2012[3];
- content of casein proteins — according to STB ISO 17997–1–2012[4];
- lactoferrin content — according to GOST 33600–2015[5];
- mass fraction of protein — according to GOST R52054–2003[6];
- fat content — according to GOST 5867–90[7];
- lactose content — according to GOST 34304–2017[8];
- content of dry matter — according to GOST 3626–73[9];
- determination of density — according to GOST 3625–84[10].

The amino acid composition of milk was determined according to the method M 04–38–2009[11] using the capillary electrophoresis system "Kapel-205" (Lumex LLC, Russia).

The Kjeldahl method is based on the mineralization of the milk sample under the influence of sulfuric acid, potassium sulfate and copper sulfate, followed by distillation of ammonia. The resulting ammonia is titrated with sulfuric or nitric acid, and the titration results are used to calculate the content of protein and total content of nitrogen. Samples were prepared for analysis. The studied yak milk was preliminarily defatted by centrifugation at 10,000 rpm for 10 minutes. Mineralization was carried out in the presence of sulfuric acid, an oxidizing agent and a catalyst at a temperature of 420 °C in a HYP-320 digester (Hanon, China). Ammonia was distilled off after complete mineralization using an automated distiller K9840 (Hanon, China).

To study the peptide composition of milk samples, a high-performance liquid chromatography (HPLC) technique was used, which is based on the separation of protein components in the mobile phase due to differences in interactions with the sorbent. In this work, the separation was carried out using a ReproSil-Pur 300 ODS-3.5 µm 250×4.6 mm chromatographic column with a chemically grafted octadecylsilanol phase, which is capable of retaining proteins due to hydrophobic bonds, and a pore size of 300 Å, allowing the peptides to fully bind to the stationary phase. The separation was carried out using a chromatographic system from Maestro (Russia), equipped with two pumps and a dynamic mixer, which allow gradient elution of analytes in a programmable composition of the mobile phase. Double-distilled water with the addition of trifluoroacetic acid as an ion-pair reagent in an amount of 0.1% by volume and acetonitrile as an organic solvent with the addition of 0.1% trifluoroacetic acid were used as the components of the mobile phase. The samples were analyzed at room temperature with a mobile phase flow rate of 1 ml/min. The volume of the injected sample was 20 µL. The proportion of acetonitrile during the analysis was increased from 5 to 60% within 30 minutes. Detection was performed at 214 nm using a spectrophotometric detector. The measurements were made in 3 parallel versions.

The amino acid composition was determined using the capillary electrophoresis system "Kapel-205" (Lumex LLC, Russia). The measurements were carried out in 2 replicates. Methodology M 04–38–2009[11] was taken as a basis. When preparing samples with a view to obtaining derivatives, acid or alkaline hydrolysis was used to convert amino acids into free form.

To determine tryptophan, alkaline hydrolysis using a hot 50% barium hydroxide solution and direct determination without obtaining derivatives were used. Hydrolysis was carried out while maintaining a high temperature of 110 °C for 14–16 hours. Next, the hot hydrolyzate was diluted with demineralized water, a few drops of methyl red and a solution of 2.0 mol/dm^3 sulfuric acid were added until neutralization (pink color). The finished solution was cooled to room temperature and brought to a volume of 50 cm^3 with demineralized water. 1.5 cm^3 of the resulting solution were taken and placed in an Eppendorf tube, which was centrifuged for 5 minutes at 5,000 rpm.

To determine the content of amino acids *Arg, Lys, Tyr, Phe, His, Leu+Ile, Met, Val, Hyp, Pro, Thr, Ser, Ala, Gly*, samples were taken and subjected to acid hydrolysis (5 cm^3 of concentrated hydrochloric acid and 2.5 cm^3 of demineralized water).

The measurement technique makes it possible to determine the total content of amino acids in the samples (total free and bound forms). Since asparagine and glutamine are quantitatively hydrolyzed to aspartic and glutamic acids respectively, during sample decomposition, data on the content of aspartic and glutamic acids represent the total content of these acids and the corresponding amides. Similarly, data for cystine content represent the total content of cystine and cysteine, after their preliminary oxidation to cysteic acid. Under measurement conditions, leucine and isoleucine are not separated, so their total determination is provided.

To determine the content of amino acids *Asp+Asn, Glu+Gln* and *Cys-Cys*, a freshly prepared mixture of hydrogen peroxide and formic acid in a ratio of 1:9 was used as an oxidizing agent. Next, the mixture was evaporated in a stream of hot air until a dry residue was obtained, which was then dissolved in 10 cm^3 of hydrochloric acid diluted with distilled water in a 1:1 ratio.

1.5 cm^3 were taken from the prepared hydrolyzed solutions, placed in an Eppendorf tube and centrifuged for 5 minutes at 5000 rpm. Detection was carried out in the UV region of the spectrum at a wavelength of 254 nm with a data acquisition rate of 2.5 Hz.

Statistical processing and generalization of experimental data were carried out using application service programs Microsoft Office Excel 2010. Tabular results are presented as the arithmetic mean ± confidence interval. The reliability of differences between data samples was determined by the method of confidence intervals. Differences between means were compared at a significance level of $p < 0.05$.

3. Results and discussion

The content of the basic nutrients in yak milk depends on the time of year, seasonal food of the animals, namely the flora of the yaks' habitat. The fat content in yak milk is higher in the cold season than in the warm season, and the total protein and lactose content is higher in the warm season. This dependence on seasonal grass vegetation and climate change was revealed by Chinese scientists Li et al. (2011) [11]. Yaks' food is plentiful in warm weather, when there is plenty of green grass, but with cold weather, short and coarse grass, as well as shrubby plants, begin to predominate in the diet. The high content of nutrients in milk, including fat, makes it possible to provide calves with everything they need to survive in the harsh conditions of the highlands. The content of main nutrients in yaks' milk depending on their habitat is given in the Table 1.

Table 1. **Chemical composition of yak milk from different regions**

Таблица 1. **Химический состав молока яков разных регионов обитания**

Biochemical composition	Yak milk, from				
	Kyrgyzstan (own data) (n=2)	Tibet [11]	Tuva [12]	Altai [13]	Mongolia [13]
Fat, %	5.40±0.22	6.12	6.96	7.7	7.29
Protein, %	5.66±0.06	4.95	4.55	5.3	5.31
Lactose, %	5.60±0.3	5.50	—	5.1	5.21
Dry matter, %	17.40±0.25	17.66	17.07	19.4	18.71

As can be seen from the Table 1, the content of nutrients in the milk of yaks from different habitat regions does not differ significantly, except for the mass fraction of fat. The milk of the animals from Altai contains the most fat. The milk of the yaks living in Kyrgyzstan is the least fatty. However, Kyrgyz yak milk contains more protein. The ratio of the mass fraction of fat and protein in milk can also be used to judge the condition of the animal and its diet [14]. The optimal ratio of these two indicators is considered to be 1:1.1–1.5. In the milk of Kyrgyz yaks, signs of acidosis are observed, because the ratio of the mass fraction of fat and protein was 1:0.96. This occurs when the animal has oxygen deficiency, a lack of fiber and an excess of easily digestible carbohydrates in the feed [15].

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[11] M 04–38–2009 Feeds, compound feeds and raw materials for their production. Methodology for measuring the mass fraction of amino acids by capillary electrophoresis using the Kapel capillary electrophoresis system" Retrieved from https://www.lumex.ru/metodics/22ARU03.15.01–1_amino_acids-feed.pdf Accessed November 22, 2023.

The density of the compared types of milk is almost the same (1.033–1.034 g/cm^3) with some overestimation for the milk of Altai yaks (1.038 g/cm^3), associated with an increased content of dry substances.

As is known, the quality of a protein is determined by its amino acid composition. Therefore, we analyzed the amino acid profile of milk of the animals from different regions of habitat (Table 2).

Table 2. **Amino acid content of yak milk (g of AA/100 g of milk)**

Таблица 2. **Аминокислотный профиль ячьего молока (г на 100 г молока)**

Amino acid	Yak milk, Kyrgyzstan (own data) (n=2)	Yak milk, Tibet [12]	Yak milk, Tuva [13]
Threonine	0.33 ± 19.57	0.19	0.22
Valine	0.37 ± 22.26	0.26	0.28
Methionine	0.20 ± 12.21	0.11	0.09
Lysine	0.59 ± 35.59	0.38	0.35
Phenylalanine	0.35 ± 20.93	0.22	0.21
Serine	0.36 ± 21.65	0.23	0.23
Alanine	0.25 ± 14.87	0.14	0.17
Proline	0.85±50.85	0.46	0.36

From the data presented (Table 2) it is clear that the milk of yak living on the pastures of Kyrgyzstan contains more amino acids, which is associated with a higher content of total protein. Amino acids such as methionine and lysine, which are especially important for the proper functioning of the human body, are higher in Kyrgyz yak milk by 2 and 1.5 times, respectively, compared to the yaks' milk from other regions (China, Russia). Lysine is useful for people suffering from cardiovascular diseases, has a positive effect on cardiac tone, and reduces cholesterol levels in the blood. Both of these amino acids are involved in calcium absorption, which is beneficial for bone formation.

The milk of yak living on the pastures of Kyrgyzstan also contains more proline (0.85 g per 100 g of milk) compared to the milk of yaks in other regions (0.46 g and 0.36 g). Proline is important in the prevention of diseases of the cardiovascular system, as it is responsible for strengthening the muscles of the heart. To improve the functioning of the human nervous system, amino acids such as alanine and phenylalanine are needed. Alanine in Kyrgyz yak milk is almost 2 times higher (0.25 g per 100 g of milk) compared to Tibetan yaks' milk (0.14 g) and Tuvan yaks' milk (0.17 g). The difference in phenylalanine content in yak milk from different regions is insignificant; however, yak milk from Kyrgyzstan contains more of this amino acid.

It is known that the macro- and micronutrient composition of milk depends on a number of factors, including the season of the year and, consequently, the feeding diet, as well as the stage of lactation. In this aspect, we studied changes in the protein composition of the milk of Kyrgyz yaks during different periods of lactation (spring, summer). The results obtained are shown in the Table 3.

The data from the Table 3 shows that in the spring, cow milk accumulates more proteins, including whey proteins, than in the summer, which is consistent with literature data [11]. Apparently this is due to the fact that in the pastures of the Naryn region at this time there is intensive grass growth and the yaks feed on abundant forbs, especially rich in vitamins, mineral elements, organic acids and other nutrients. For example, the content of propionic acid in herbs can affect the synthesis of lactose and protein, although the fiber content in spring is relatively low [16].

In Kyrgyzstan, the mountain steppes are used as pastures mainly in spring and autumn. The feature of the steppe grass stand is its high nutritional value throughout the growing season. The nutrient content decreases as plants progress through developmental phases, but remains high even in autumn. The protein concentration in plants decreases from 13.2 to 9.1% depending on the growth period. In years with favorable weather conditions, when precipitation falls and warm weather prevails, plants exhibit autumn tillering, which leads to a slight increase in their feed value. Due to the preservation of a high protein concentration (up to 10%), steppe grass is an excellent fattening food for the animals until late autumn. It is also known that the cinquefoil, which grows on the mountain ranges of Kyrgyzstan, is well eaten by sheep and cattle. In terms of its chemical composition, it is characterized by a high protein concentration, which decreases to 18.8% in the flowering phase and to 11.5% at the end of fruiting [17,18].

The chemical composition of the same plant may vary depending on the region of growth. Thus, the fescue plant in the phase of fruit shedding in the geological conditions of the Eastern Pamirs contains 7.2% protein, in the Alai Valley of Kyrgyzstan — 6.0%, while in the pastures of the fescue-wormwood steppe of the Kyrgyz Tien Shan range the protein concentration in the same period is already 9.5% and 11.8% in the cereal-forb meadow-steppe [17]. The high protein content in yak feed, especially in the spring before the flowering phase, accordingly affects the amount of protein in milk, which is proven by the results of our research (see Table 3).

Of all milk proteins, 20% are whey proteins, which are of particular value, close to the ideal protein and have a number of physiologically functional properties. Using capillary electrophoresis, we determined the fractional composition of whey proteins (WP) in yak milk depending on the season. The data is presented in the Table 4.

Tabular data (Table 4) indicate that there are no significant fluctuations in the quantitative content of individual fractions of whey proteins in the milk of the Kyrgyz yak in the warm (spring) and hot (July) seasons. Similar results were obtained by scientists for Chinese yak milk (Maiwa) [11,19]. α-LA and β-LG are the two major fractions of whey proteins, accounting for 20% and 50% of the total, respectively [20,21].

Table 3. **Seasonal changes in the protein composition of yak milk**

Таблица 3. **Сезонные изменения белкового состава молока ячих, обитающих в Кыргызской Республике**

Variables, %	Milk from Kyrgyz yak (own data)			Cow milk [22]
	Spring (May) (n=2)	Summer (July) (n=2)	Average value	
Protein	5.66 ± 0.06*a	4.20 ± 0.06b	4.93 ± 0.06	3.37 ± 0.09
Whey protein	2.11 ± 0.3a	1.04 ± 0.1b	1.57 ± 0.2	0.82 ± 0.05
Casein protein	3.52 ± 0.033a	3.13 ± 0.033b	3.32 ± 0.033	2.57 ± 0.04
Total nitrogen	0.887 ± 0.004a	0.659 ± 0.004b	0.773 ± 0.004	0.528 ± 0.006
Non-protein nitrogen	0.0349 ± 0.003a	0.0348 ± 0.003a	0.03485 ± 0.003	0.0320 ± 0.0030

* Results are presented as arithmetic mean ±SD.
both a and b occurring in the same line means that the results are significantly different ($p < 0.05$); n: the number of samples analyzed and computed in the statistical analysis.

Table 4. **Fractional composition of whey proteins in yak milk depending on the season (mg/L of milk)**

Таблица 4. **Фракционный состав сывороточных белков ячьего молока в зависимости от сезона года (мг/л молока)**

Whey proteins factions	Milk from Kyrgyz yak (own data)			Yak milk [19]	Cow milk [19]
	Spring (May) (n=2)	Summer (July) (n=2)	Average value		
Serum albumin (BSA)	1.27 ± 0.06*a	1.06 ± 0.005b	1.165 ± 0.005	1.49	0.41
α-Lactalbumin (α-LA)	1.65 ± 0.008a	1.51 ± 0.007b	1.58 ± 0.007	0.72	1.24
β-Lactoglobulin A (β-LG A)	0.747 ± 0.003a	0.576 ± 0.002b	0.661 ± 0.002	0.74	3.30
β-Lactoglobulin B (β-LG B)	1.36 ± 0.006a	1.28 ± 0.006a	1.32 ± 0.006	5.49	
Lactoferrin	0.09 ± 0.0004a	0.07 ± 0.0003a	0.08 ± 0.0004	—	—

* The results are presented as the arithmetic mean ±SD.
both a and b occurring in the same line means that the results are significantly different ($p < 0.05$); n: the number of samples analyzed and computed in the statistical analysis.

β-Lactoglobulin is the main whey protein in the milk of ruminant animals such as cow and goat [23]. β-Lactoglobulin also dominates in the overall aggregation and gelation of whey proteins (WP) [24].

It is also known that at different periods of the year the proportion of β-lactoglobulin in the composition of WP may fluctuate. Thus, in the work of Ostroumov [25] showed that the maximum amount of β-lactoglobulin is observed in milk of the winter period: January — 61.9%, December — 61.0%. In the summer months (June, July) this fraction was 57.5–57.8%. A slight decrease in the proportion of the β-lactoglobulin fraction is noted in the WP of spring milk (March — 50.1%, April — 41.4%, May — 47.7%).

In the milk of Kyrgyz yaks, the average total content of two genetic variants of β-lactoglobulin (β-LG A and β-LG B) is 1.98 mg/cm^3 or 41.25% of all WP (Table 4). In spring these values reach 2.1 mg/cm^3 or 41.02%, and in summer — 1.86 mg/cm^3 or 41.4%. That is, in contrast to cow's milk, in Kyrgyz yak milk there are no noticeable fluctuations in the content of β-lactoglobulin according to the seasons of the year.

At the same time, a significant difference was found between the content of β-LG B in the WP of Kyrgyz and Chinese yak milk (Table 4). The milk under study contains approximately 3.15 times less of this fraction than Chinese yak milk. Further research is needed to explain this phenomenon.

In the milk we studied, the ratio of the amounts of β-LG A and β-LG B in spring and summer was 1:1.8 and 1:2.2, respectively. The ratio of the amount of β-lactoglobulin and α-lactalbumin was on average 1:1.25. The work [26] presents the results of a study of seasonal fluctuations in the amount of β-lactoglobulin in milk of khainak (hybrid of yak and cattle). It has been established that the ratio β-LG A/β-LG B in spring, summer and autumn is 1:2.5, 1:1.9 and 1:1.5, respectively, which indicates the dependence of the content of these fractions in milk on the season. Similar changes also occur in cow milk depending on the milk protein genotype, stage of lactation, feeding, etc. [24,26–29].

Lactoferrin is a minor protein and it has bactericidal properties against *E. coli*. In the milk studied, its content averages 0.08 mg/cm^3 (Table 4), which is comparable to cow milk [25]. In the milk of the Chinese yak Maiwa, the content of lactoferrin ranges from 0.144 to 0.180 mg/cm^3 [30], which is 1.8–2.3 times higher than in the milk of the Kyrgyz yak, which is most likely due to differences in the feeding diet of the animals.

The identified seasonal fluctuations in protein content in Kyrgyz yak milk may also affect its biological value, which is determined by the amino acid profile. In this regard, we studied seasonal changes (qualitative and quantitative) in the amino acid composition of milk proteins of yak animals living in the mountainous regions of Kyrgyzstan. The obtained data (in comparison with known ones) are given in the Table 5 and in Figure 1.

The results showed (Table 5) that the total content of amino acids (essential and non-essential) in the spring is slightly higher ($p < 0.05$) than in the summer, which is consistent with the protein content in the studied time periods. Moreover, among the essential amino acids, the sum of leucine-isoleucine has the highest content, the average value of which was 927.45 mg/100 g of milk, which is 1.4 times more than in Chinese yak milk, and 2.15 times higher than in cow milk. Among the non-essential amino acids, we can highlight proline and glutamic acid, the content of which is also quite high in the spring and summer. Moreover, in spring there is almost 2 times more glutamic acid than in summer. The increased content in spring milk compared to the summer period is also typical for other amino acids (Table 5). Thus, the average content of amino acids in the milk of yak from Kyrgyzstan (our own research) is 5850.81 mg/100 g of milk, while the milk of yak grazing on the highland pastures of China contains 4700 mg of amino acids per 100 g of milk [11], and in cow milk —

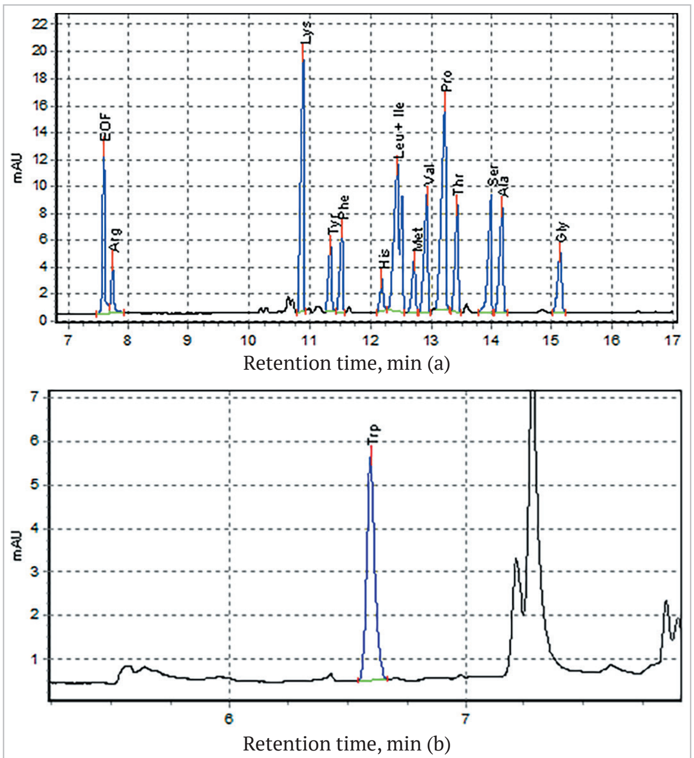

Figure 1. **Electropherograms of amino acid analysis of yak milk proteins in Spring: a — *Arg, Lys, Tyr, Phe, His, Leu+Ile, Met, Val, Pro, Thr, Ser, Ala, Gly;* b — *Trp***

Рисунок 1. **Электрофореграммы аминокислотного анализа белков ячьего молока в весенний период (а – *Arg, Lys, Tyr, Phe, His, Leu+Ile, Met, Val, Pro, Thr, Ser, Ala, Gly;* b — *Trp*)**

Table 5. **Seasonal changes in the content of amino acids in the Kyrgyz yak milk (mg of AA/ 100 g of milk)**

Таблица 5. **Сезонные изменения содержания аминокислот в молоке ячих кыргызской популяции (мг на 100 г молока)**

Variables	Milk from Kyrgyz yak (own data)			Yak milk [6]	Cow milk [6]
	Spring (May) (n=2)	Summer (July) (n=2)	Average value		
Total essential AA	3095.46	2475	2785.23	1950	1380
Threonine	326.1 ± 19.57^a	255.2 ± 15.31^b	290.65	190	150
Valine	371.0 ± 22.26^a	252.4 ± 15.14^b	311.7	260	160
Methionine	203.5 ± 12.21^a	172.7 ± 10.36^b	188.1	110	60
Lysine	593.7 ± 35.59^a	465.7 ± 27.94^b	529.7	380	270
Phenylalanine	348.9 ± 20.93^a	298.7 ± 17.92^b	323.8	220	160
Histidine	195.1 ± 11.71^a	161.2 ± 9.67^b	178.15	120	100
Leucine-Isoleucine	1010.0 ± 60.6^a	844.9 ± 50.69^b	927.45	670	430
Tryptophan	47.16 ± 2.83^a	24.2 ± 1.45^b	35.68	—	50
Total nonessential AA	**3094.5**	**2626.47**	**3065.58**	**2750**	**1950**
Arginine	265.1 ± 15.91^a	208.8 ± 12.53^b	236.95	160	110
Serine	360.8 ± 21.65^a	291.5 ± 17.49^b	326.15	230	160
Alanine	247.9 ± 14.87^a	291.5 ± 17.49^b	269.7	140	100
Proline	847.5 ± 50.85^a	745.5 ± 44.73^b	796.5	460	320
Glysine	121.2 ± 7.27^a	112.8 ± 6.77^b	117.0	120	60
Tyrosine	349.3 ± 20.96^a	293.4 ± 17.60^b	320.85	220	150
Aspartic acid	411.2 ± 24.67^a	214.8 ± 12.89^b	313.0	330	260
Cysteine	38.80 ± 2.33^a	27.97 ± 1.68^b	33.38	40	20
Glutamic acid	863.9 ± 51.83^a	440.2 ± 26.41^b	652.05	1050	770
Total AA	**6189.96**	**5101.47**	**5850.81**	**4700**	**3330**

both ^{a} and ^{b} occurring in the same line means that the results are significantly different ($p < 0.05$); n: the number of samples analyzed and computed in the statistical analysis.

3330 mg/100 g. Similar studies of khainak milk, a hybrid of yak and cattle, were carried out by Elemanova [26]. The results of the analysis of the amino acid composition of milk depending on the season of the year showed that the amount of some amino acids does not depend on the season, however, in the warm season the content of most essential amino acids was higher than in the cold season.

According to FAO/WHO recommendations, the biological value of proteins is determined by the balance of each essential amino acid in relation to their content in an ideal protein [31]. Deficiency of any amino acid in the body limits the use of all other amino acids in the process of protein biosynthesis. To determine the biological value of yak milk proteins, their amino acid composition was assessed in relation to the "ideal" protein (Table 6).

Table 6. **Assessment of the amino acid composition of Kyrgyz yak milk proteins in relation to the "Ideal" protein**

Таблица 6. **Оценка аминокислотного состава белков молока ячихи кыргызской популяции по отношению к «идеальному» белку**

Variables	Essential amino acids, g/100 g of protein							
	Threonine	Valine	Methionine	Lysine	Phenylalanine+ Tyrosine	Histidine	Leucine+ Isoleucine	Tryptophan
Ideal protein, g /100 g of protein	3.1	4.3	2.7	5.7	5.2	2.0	9.8	0.85
Average amino acid content, g /100 g of protein, (own data)	5.9	6.3	3.8	10.7	13.08	3.6	18.8	0.7
Amino acid score, %	190	146	141	188	252	180	192	82

Among the essential amino acids, phenylalanine+tyrosine and leucine+isoleucine have the highest amino acid scores — 252% and 192%, respectively. The limiting amino acid is tryptophan with a rate of 82%. The content of other essential amino acids in yak milk proteins exceeds 100% (141–190%).

Essential amino acids do not only act as building blocks for tissue protein, but are also precursors to glucose, participate in urea synthesis and other metabolic processes. New physiological functions of branched chain amino acids have been discovered [32]. They are involved in the synthesis of nitrogenous compounds that regulate glucose metabolism, the synthesis of lipids and proteins. Many amino acids are the essential ingredients in the synthesis of compounds, being suppliers of nitrogen and carbon [33]. Phenylalanine is necessary for the normal functioning of the central nervous system and is involved in the formation of neurotransmitters such as norepinephrine, adrenaline and dopamine [34]. It is also necessary for the synthesis of tyrosine. And with a deficiency of the precursor substance, a corresponding deficiency of tyrosine occurs. In turn, tyrosine is a precursor of catecholamines, converted into dopamine by L-Dopa (a direct precursor of dopamine) and the enzymes tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase, and into norepinephrine by dopamine β-hydroxylase. Insufficient intake of phenylalanine and tyrosine from protein foods subsequently leads to neurocognitive disorders of the human nervous system [35]. Leucine enhances protein synthesis, so consumption of this amino acid is often recommended for people with protein deficiency. Studies have shown that supplemental leucine improves protein balance during fasting, and consumption in the fasted state improves nitrogen balance [36]. Isoleucine, like leucine, is not only a structural unit of tissue protein (which accounts for 35% of essential amino acids in muscles), but also performs other metabolic functions, in particular it is a building material for ketone bodies and glucose. Thus, this amino acid regulates blood sugar levels. Isoleucine is involved in the process of hemoglobin synthesis [37]. Together, these two amino acids (leucine and isoleucine) regulate basic metabolic processes, which is especially important in diseases such as diabetes, heart failure and cancer [38].

Thus, the nutrient composition of grasses growing in different mountain regions significantly influences the amino acid profile of yak milk. The results of the analysis of the amino acid composition of milk depending on the season of the year showed that the content of amino acids depends on the time of year, and their content is higher in spring than in summer.

4. Conclusions

It has been established that the composition of milk from yaks living in different regions of the world varies slightly. Meanwhile, the milk of Kyrgyz yak contains more protein (5.66%) compared to the milk of the yak of Tibet, Tuva, Altai and Mongolia (4.95; 4.55; 5.3 and 5.31%, respectively) with a corresponding increase in the content of all studied amino acids, which is associated with the composition of feed, with the set of flora corresponding to each region, and the phenology of a plant vegetation.

However, the mass fraction of fat in the studied milk is noticeably lower (5.4%) than in the milk of animals living in a number of other regions of the world.

The mass fraction of lactose and dry substances in the comparison objects and in the milk of Kyrgyz yaks does not differ significantly.

Research has shown that yak milk in the spring contains more proteins, including whey proteins (5.66±0.06% and 2.11±0.3%, respectively) than summer milk (4.2±0.06% and 1.04±0.1%), which is apparently due to seasonal changes in the nutrient composition of grasses.

There were no significant deviations in the fractional composition of whey proteins in yak milk obtained at different times of the year. The ratio of the amount of β-LG A and β-LG B in spring and summer was 1:1.8 and 1:2.2, respectively. The ratio of β-lactoglobulin and α-lactalbumin is 1:1.25 on average.

The total content of amino acids in the Kyrgyz yak milk is noticeably higher in a spring (6189.96 mg/100g) than in a summer (5101.47 mg/100g). The results of determining the health benefits of yak milk protein showed that the limiting amino acid is tryptophan (score 82%), the remaining essential amino acids had a score above 100% (141–190%).

One of the most important factors influencing seasonal changes in the amino acid profile of Kyrgyz yak milk is, most likely, the qualitative and quantitative content of nutrients in the feeding ration of these animals.

The results of our research serve as further evidence of the high nutritional and biological value of milk obtained from the yaks of mountainous regions of the Kyrgyz Republic, which makes it possible to produce multifunctional dairy products from such raw materials.

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