

Influence of the amino acid and fractional composition of dry milk of mammals on the digestibility of dry milk mixtures

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Abstract

Keywords:

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Introduction. The aim of the study was to determine the influence of the amino acid and fractional composition of dry milk of mammals on the digestibility of dry milk mixtures.

Materials and methods. Use of goat, mare, sheep dry milk and dry milk mixtures for baby food were studied. Amino acid composition was determined by ion exchange chromatography; fractional composition by electrophoresis; digestibility of dry milk mixtures was studied *in vitro*.

Results and discussion. The limiting amino acid of dry cow and sheep's milk proteins is cystine, the amino acid scores of which are 46.0% and 47.0%, respectively. The limiting amino acid of mare and goat milk proteins is tyrosine, the amino acid scores of which are 52.0% and 57.0%, respectively. Mare milk protein belongs to the albumin type as well as protein of human milk. Mare and sheep's milks is known to contain various protective proteins including immunoglobulin and lactoferrin. Mare and sheep's milks contain large amount of immunoglobulin A close to human milk, namely, 515% and 6.92%, respectively. The ratio of casein to whey proteins in mare milk is 1:1, in cow's milk is 4:1, and in goat and sheep's milks is 3:2. Differences in composition are due to different genotypes of mammals and protein losses during drying process. The highest degree of digestibility in human milk and the mixture based on mare's milk 'Ligans' is 717 mg and 780 mg of amino acids per 100 g of product, respectively. The lowest degree of digestibility of proteins is in the mixture based on cow's milk 'Malyutka hypoallergenic'. A low degree of digestibility can be caused by the reaction between amino acids during drying, and the formation of casein complexes with denatured whey proteins.

Conclusions. The amino acid and fractional composition of proteins of dry mare milk is close to composition of human milk proteins, so the digestibility of products based on mare milk is similar to that of human milk.

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Introduction

Artificial feeding has a negative impact on the infant's health. 72% of formula-fed infants suffer from dysbacteriosis, and the number of children suffering from an allergy to cow's milk proteins is 2–7.5% (Mousan et al., 2016). Meanwhile, for artificial feeding of children, mixtures based on cow's milk are used. Allergic reactions can be caused by differences in the protein composition of human and cow's milk.

Mass fraction of casein is the largest in the protein composition of cow's milk (about 80%), and in human milk, which belongs to the albumin type, whey proteins predominate (about 65%). At the same time, there are differences in the fractional composition of casein – α -casein predominates in cow's milk, and β -casein is the main protein in human milk. The main serum protein of cow's milk is β -lactoglobulin, and α -lactalbumin and immunoglobulins are main serum proteins in human milk. Casein and β -lactoglobulin are the main allergens of milk, while immunoglobulins have protective function in the child's body (Zeng et al., 2019). Human milk proteins contain a larger number of essential amino acids compared to cow's milk proteins. In addition, the amino acids of human milk are in the most favorable ratio for children (Barreto et al., 2019).

There are three classes of immunoglobulins (A, G and M) in cow's milk, and four (A, E, G, M) in human milk. Human milk contains large amount of lactoferrin, so iron absorption for newborns during breast feeding is 80%, while iron absorption is only 20% during artificial feeding (Avershyna, 2012). The amount of iron ions in the child's body is extremely important, since iron is involved in many metabolic processes (El Amrousy et al., 2022). Lactoferrin acts as a factor that limits the amount of iron available to microorganisms in the body. Binding excess iron ions and other metals, lactoferrin deprives pathogenic flora of vital trace elements (Griffin, 2020; Lonnerdal et al., 2021).

The source of proteins for feeding babies is the milk of domestic animals, especially goat, mare, sheep, the use of which is not widespread in the production of baby food products. Therefore, the study of the amino acid and fractional composition of goat, mare, and sheep's milk proteins, as well as their influence on the digestibility of baby food products based on them, is an important task. A study of the amino acid composition of native mare's milk was conducted, but the question of the amino acid composition of dry mare's and sheep's milk remains insufficiently studied (Mazhitova et al., 2015). The results of the study of the fractional composition of proteins of native sheep's and mare's milk are also given, but the issue of the fractional composition of proteins of mare's and sheep's milk powder needs to be studied more carefully (Barreto et al., 2019; Navarro et al., 2018).

The analysis of literature data showed that the protein composition of dry sheep, goat and mare's milk, as well as its effect on digestion under *in vitro* conditions, wasn't sufficiently studied. The aim of the study is to determine the influence of the protein composition of animal milk powder on the ability of food products based on it to be digested *in vitro*.

Materials and methods

Materials

Whole cow's milk, whole goat's milk, whole mare's milk, whole sheep's milk were used for laboratory research. Milk was obtained from farms in Donetsk, Khmelnytskyi, Chernivtsi and Zakarpattia regions in Ukraine. Research was conducted in different periods of animal lactation. Human milk samples were collected during the lactation period of 1–8 weeks at Children's Clinical Hospital No. 8, Kyiv, Ukraine.

Dry milk mixtures based on sheep's "Agnus" and mare's milk "Ligans" were used to investigate digestion. The mixtures are developed at the Department of bakery and confectionery goods technology of the National University of Food Technologies (Kyiv, Ukraine). The recipes of the mixtures are presented in Table 1.

Table 1
Recipes of dry milk mixtures for children from birth to 6 months

Raw material, %	Mixture 'Ligans'	Mixture 'Agnus'	Mixture 'Malyutka hypoallergenic'
Dry demineralized whey	—	—	48.20
Cow's milk	—	—	26.40
Sheep powder milk	—	56.00	—
Milk powder mare	84.65	—	—
Mixture of vegetable oils	—	—	13.30
Soybean oil	—	4.00	—
Sunflower oil	7.00	2.00	—
Olive oil	8.00	—	—
Lactose	—	36.30	3.00
Lactulose	—	1.00	—
Fat-soluble vitamins, mg/100 g	0.03	0.03	—
Water-soluble vitamins, mg / 100 g	0.23	0.31	—
Minerals:	0.21	0.56	—
Sodium citrate salts, mg / 100 g	—	4.63	—
Citrate potassium salts, mg / 100 g	—	13.88	—
Taurine	0.03	0.03	—
Inositol	0.11	0.11	—
Total:	100.00	100.00	

Methods

Drying milk

Semi-industrial spray dryer "Nyro-Atomizer" (Denmark) with a working volume of the chamber of 0.9 m³ and a productivity of evaporated moisture up to 5.0 kg/h was used for milk drying. Drying agent speed was 0.5 m/s and a drying agent relative humidity was 25%. The size of the droplets of the sprayed product was 40-50 µm, the mass fraction of dry substances in the product was 40-43%. The temperature of the drying agent for goat milk was 160–170 °C, for mare's milk was 150–160 °C, for sheep's milk was 170–180 °C.

Amino acid composition

The amino acid composition of milk proteins was determined by ion exchange chromatography using an automatic T 339 amino acid analyser (Carta et al., 2010). The elution of amino acids from the column was conducted, in turn, by Li-citrate buffers from pH 2.75±0.01; pH 2.95±0.01; pH 3.2±0.02; pH 3.8±0.02; pH 5.0±0.2. Amino acids were detected at a wavelength of 560 nm by rectification with a ninhydrin solution on a photometer. The results of detection were registered by a variplotter in the form of the peaks of absorption of light of ninhydrin positive substances in an eluent that number the direct ratio concentrations

of this substance in solution. The correlation of the solution of ninhydrin reagent and eluents was 12; the temperature of thermostatic T1 = 38.5 °C; T2 = 65 °C. The prototype was diluted in Li-citrate buffer by pH 2.2±0.02 and inflicted on an ion exchange column. The quantitative estimation of chromatograms of the pre-production model settled in relation to the Bio-Rad standard mixture of amino acids. The mass of every amino acid, expressed as g per 100 g protein (Ai), in the investigated solution was calculated by the following formula:

$$Ai = \frac{M_i \cdot S_i}{S_i^3}$$

where M_i is molecular mass of each amino acid; S_i is an area of peak of each amino acid on an aminogram from the investigated solution; S_i^3 is an area of peak of each amino acid on an aminogram from the solution of the standard mixture of amino acids which accords to one micromole (Litvynchuk et al., 2022).

Fractional composition of milk proteins

Determination of the fractional composition of milk proteins was carried out by the electrophoresis method. Electrophoresis was performed on a Hoefer Mighty Small apparatus (Amersham Biosciences, USA) at an amperage of 19 mA for concentrating and 35 mA for separating gels (Massouras et al., 2017, Yukalo et al., 2019).

Processing of the obtained data by the method of disk electrophoresis of electrophoregrams was carried out using the ImageMaster TotalLab v.2.01 program (Amersham Biosciences) (Massouras et al., 2017, Yukalo et al., 2019).

Protein digestibility

Protein digestibility was determined under *in vitro* conditions. Sequential enzymatic hydrolysis of samples of dry milk mixtures was carried out with the complex of proteinases pepsin-chymotrypsin. Digestion efficiency was estimated by accumulation of the products of hydrolysis (Tagliazucchi et al., 2018).

Statistical analysis

The statistical processing of the results was performed by sequential regression analysis using the Microsoft Excel XP and Origin Pro8 software calculating correlation coefficients (Hinkle et al., 2003).

Results and discussions

Amino acid composition of dry milk samples

The results of the study of the amino acid composition of samples of dry milk are presented in the Table 2.

Dry cow's milk and human milk were selected as control samples. The obtained results showed significant differences in the protein composition of the studied animal milk and its difference from human milk. This is explained by the fact that milk differs in the content of total protein and in the content of individual fractions. The milk of mammals is intended to ensure the rapid growth and strengthening of the skeleton of their offspring.

Table 2

Amino acid composition of dry animal milk

Amino acid	Cow's milk		Mare's milk		Goat's milk		Sheep's milk		Breast milk	
	% to protein	AA score, %	% to protein	AA score, %	% to protein	AA score, %	% to protein	AA score, %	% to protein	AA score, %
Mass fraction of protein, %	23.0		16.1		29.6		25.2		0.8–1.5	
Lysine	7.80	153	6.67	131	7.29	143	8.4	153	7.40	155
Histidine	2.50	86	2.23	76	2.59	89	2.11	73	2.20	96
Arginine	3.50	80	4.33	96	2.67	61	2.54	58	6.70	128
Aspartic acid	5.10	—	5.32	—	7.42	—	8.67	—	9.90	—
Threonine	4.30	123	3.89	111	4.88	140	4.10	117	4.80	138
Serine	4.90	—	8.17	—	5.24	—	5.37	—	5.50	—
Glutamic acid	20.6	—	19.31	—	20.40	—	22.30	—	16.4	—
Proline	7.4	—	9.73	—	14.43	—	7.72	—	6.40	—
Glycine	—	—	1.71	—	2.12	—	2.61	—	—	—
Alanine	2.40	—	3.31	—	2.98	—	5.40	—	2.96	—
Cystine	1.20	46	1.39	64	1.74	67	1.23	47	2.96	100
Valine	6.60	138	4.60	96	5.05	105	4.79	100	5.20	116
Methionine	2.50	96	3.09	119	2.10	81	2.82	108	2.50	120
Isoleucine	5.50	131	3.80	91	4.18	100	3.59	86	6.30	160
Leucine	9.60	137	14.39	206	8.45	121	9.08	130	17.00	220
Tyrosine	4.30	59	3.75	52	4.18	57	4.45	61	5.50	107
Phenylalanine	5.2	71	4.03	55	4.25	58	4.82	66	6.30	120

The limiting amino acid in cow's and sheep's milk is cystine, the amino acid ratio of which is 46 and 47%, respectively. In goat's and mare's milk cystine content is also quite low – 67% and 64%, respectively, though it is not the limiting amino acid. The limiting amino acid for dry mare's and goat's milk is tyrosine, the content of which is also insufficient in cow's and sheep's milk due to the low amino acid score. Lysine, threonine, valine and leucine are essential amino acids, and they are contained in the proteins of dry milk of animals in sufficient amounts, which even exceed their amount in an ideal protein. The histidine content is low in the protein composition of all types of milk.

An irreplaceable amino acid for the child's body is also arginine, the content of which in mare's milk corresponds to its content in ideal protein. Egg protein is considered to be ideal protein due to the fact that amino acid score of all its essential amino acids is more than 100% (Toghyani et al., 2020). The amino acid score of arginine in cow's milk is 80%, which is 14% less than in mare's milk. Arginine amino acid scores of goat and sheep's milk are 61% and 58%, respectively, which are 19 and 22% lower compared to cow's milk.

The authors (Hodgkinson et al., 2018) claim that native mare's milk contains all 9 essential amino acids in the levels of need established by the World Health Organization. The content of the amino acid methionine is sufficient in the proteins of mare's and sheep's milk, the scores of which are 119% and 108%, respectively. The content of methionine in cow's milk approaches its content in ideal protein. Cow's and sheep's milk protein contain 29–35% less phenylalanine than the ideal protein, amino acid score is 71 and 66%, respectively.

Fractional composition of proteins of dry milk samples

The fractional composition of milk, as well as the amino acid composition, is also determined for different types of milk. The fractional composition of proteins is presented on the electrophoregram (Figure 1) and in quantitative form in the Table 3.

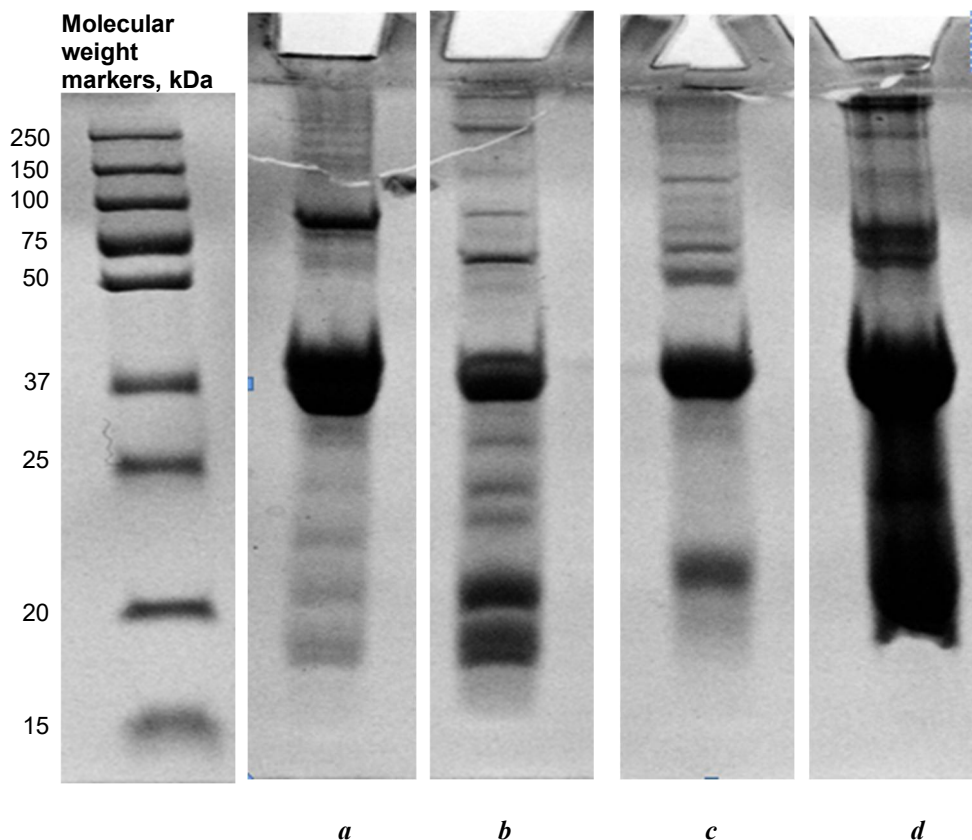


Figure 1. Electrophoregram of dry milk:
a – cow's milk; *b* – goat's milk; *c* – sheep's milk;
d – mare's milk
 (molecular weight markers: 250, 150, 100, 75, 50, 37, 25, 20, 15 kDa)

The molecular weight of the proteins of the detected fractions ranges from 10 to 250 kDa. The molecular mass of 19–24 kDa is typical for caseins, 18 kDa for lactoglobulin, 14 kDa for lactalbumin, 69 kDa for albumin, and 150–1000 kDa for immunoglobulins. Cow's milk proteins have the lowest content of immunoglobulins among all samples. Mare's and goat's milk contain them 12 and 3 times more. Sheep's milk contains 10 times more immunoglobulins than cow's milk. Immunoglobulin M is contained in small amounts in cow's milk, and is absent in goat's milk. The content of immunoglobulin M in mare's and

sheep's milk is 20 times higher than in human milk. The smallest amount of lactoferrin is in cow's milk. Other types of milk contain 3-7 times more lactoferrin.

In the casein fraction of human milk, β -casein predominates (Zeng et al., 2019). The same is observed in mare's, goat's and sheep's milk. In cow's milk casein composition, α -casein predominates. The obtained data are similar to the other studies of the fractional composition of native milks. Minor differences are observed, which are explained by the fact that the chemical composition of milk depends on the genetic characteristics and breed of the mammal, geographical and climatic conditions, nutrition, lactation period, season, and ecological condition of the area (Kala et al., 2019, Markevich-Kenshycka et al., 2013).

Table 3

Fractional composition of proteins of dry milk

Milk proteins	Cow's milk (control)	Mare's milk	Goat's milk	Sheep's milk	Human milk
Casein, %	83.0 $\pm$ 2.2	45.0 $\pm$ 1.2	70.0 $\pm$ 1.9	65.0 $\pm$ 1.7	24.0 $\pm$ 0.8
α -casein	53.56 $\pm$ 1.4	7.5 $\pm$ 0.3	23.24 $\pm$ 0.7	20.4 $\pm$ 0.7	not found
β -casein	26.68 $\pm$ 1.0	24.48 $\pm$ 1.0	33.94 $\pm$ 1.1	35.94 $\pm$ 1.1	23.0 $\pm$ 0.7
κ -casein	0.66 $\pm$ 0.01	2.56 $\pm$ 0.2	2.76 $\pm$ 0.3	3.46 $\pm$ 0.3	0.96 $\pm$ 0.01
γ -casein	2.46 $\pm$ 0.2	8.1 $\pm$ 0.3	9.18 $\pm$ 0.4	2.17 $\pm$ 0.2	not found
Whey proteins, %	17.00 $\pm$ 0.7	55.00 $\pm$ 1.4	30.0 $\pm$ 1.1	35.0 $\pm$ 1.1	76.0 $\pm$ 1.9
β -lactoglobulin	10.35 $\pm$ 0.4	20.61 $\pm$ 0.7	0.99 $\pm$ 0.01	7.2 $\pm$ 0.4	not found
α -lactalbumin	2.33 $\pm$ 0.2	7.76 $\pm$ 0.3	12.88 $\pm$ 0.4	4.89 $\pm$ 0.3	35.0 $\pm$ 1.1
Albumin	1.7 $\pm$ 0.1	6.72 $\pm$ 0.3	4.9 $\pm$ 0.3	4.19 $\pm$ 0.3	6.1 $\pm$ 0.3
Immunoglobulins:					
Ig G	1.2 $\pm$ 0.1	13.3 $\pm$ 0.5	4.72 $\pm$ 0.3	7.95 $\pm$ 0.4	0.65 $\pm$ 0.01
Ig A	0.30 $\pm$ 0.01	5.15 $\pm$ 0.4	0.26 $\pm$ 0.01	6.92 $\pm$ 0.3	4.8 $\pm$ 0.3
Ig M	0.0001 $\pm$ 0.00001	0.02 $\pm$ 0.001	—	0.02 $\pm$ 0.001	0.001 $\pm$ 0.00001
Ig E	0.01 $\pm$ 0.001	0.02 $\pm$ 0.001	—	0.02 $\pm$ 0.001	0.30 $\pm$ 0.01
<i>Total:</i>	1.51 $\pm$ 0.1	18.49 $\pm$ 0.7	4.98 $\pm$ 0.3	14.91 $\pm$ 0.5	5.75 $\pm$ 0.3
Lactoferrin	0.68 $\pm$ 0.01	1.82 $\pm$ 0.01	4.98 $\pm$ 0.3	2.9 $\pm$ 0.2	14.5 $\pm$ 0.5

* Results given as: M $\pm$ SD (mean $\pm$ standard deviation) of triplicate trials.

The predominant whey protein in goat's milk, as well as in human milk, is α -lactalbumin, and in mare's, sheep's and cow's milk it is β -lactoglobulin, which causes food allergies. It was established that immunoglobulin A in mare's and sheep's milk is 17–23 times more than in cow's milk.

β -lactoglobulin was poorly absorbed under all conditions of gastric digestion. Caseins responded differently to pH change than whey proteins, with lower casein digestion at pH 3.0 than at pH 5.0. Goat milk caseins are generally absorbed more efficiently compared to cow's milk caseins, and the peptide profiles of goat milk differ from cow's milk (Hodgkinson et al., 2018). The drying mode has a significant effect on the amino acid composition of dry milk proteins. Heating milk proteins can lead to the formation of cross-links between different amino acids within the protein (Lieshout et al., 2015).

The formation of lysinoalanine in α -lactalbumin lead to a decrease in protein digestibility (Lieshout et al., 2020). Cross-linking of amino acids may or may not affect protein digestibility, thus demonstrating the need and relevance of the conducted research.

The ratio of casein to whey proteins in mare's milk is 1:1. In cow's milk, the casein to whey protein ratio is 4:1. In goat's and sheep's milk, the ratio between the fractions is 3:2. Thus, it can be concluded that mare's milk belongs to milk of the albumin group, as well as human milk. So, the composition of mare's milk contains significant amounts of finely dispersed proteins (albumins), the composition of cow's, goat's and sheep's milk has large amounts of caseins.

Protein digestibility of milk mixtures

At high temperatures of milk drying casein interacts with denatured whey proteins, which can cause reduced hydrolysis of caseins. It is also indicated that at high temperatures, cross-linking of amino acids with the formation of lysinoalanine is observed in milk. Cross-linking could affect protein digestibility by altering enzyme availability (Lieshout et al., 2020, Roy et al., 2021).

Milk mixtures based on mare ("Ligans") and sheep ("Agnus") milk were studied for the ability to digest protein substances *in vitro*. Human milk and "Malyutka hypoallergenic" (Khorolsky milk-canned plant for children's products, Ukraine) mixtures were selected as controls.

The results of the study of the digestion of protein substances are presented in the Figure 2.

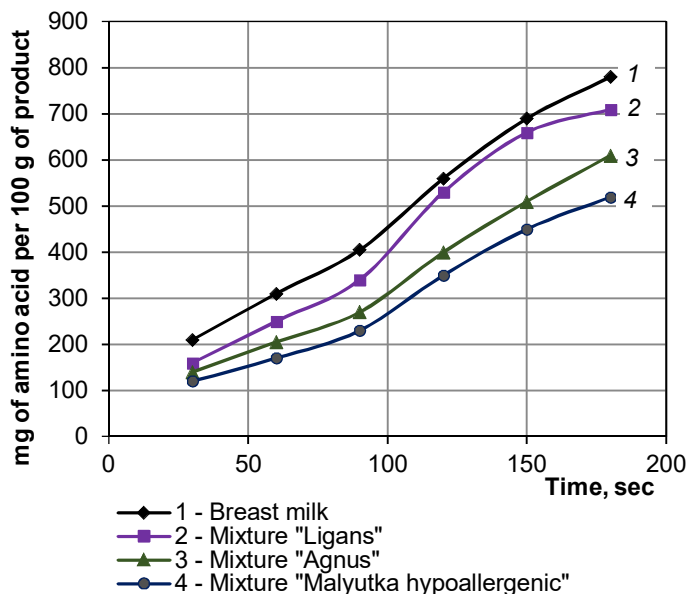


Figure 2. Digestibility of protein substances of the studied products

* Data are given as an average result of triplicate trials.

Process of digestion of protein substances in the developed products and in human milk proceeds with higher intensity (Figure 2) than in the mixture "Malyutka hypoallergenic"

(Khorol milk canned plant for children's products – the only Ukrainian manufacturer of dry milk mixtures). The degree of digestibility of proteins by pepsin and renin and the dynamics of this process had their own characteristics for different samples: the highest degree of digestibility was observed in the sample of human milk and mixture "Ligans" and was 717 mg and 780 mg of AA per 100 g of product, respectively. The lowest digestibility of proteins was found in the "Malyutka hypoallergenic" mixture compared to other samples. Accumulation of amino acids in this mixture is 33% less, compared to human milk. There is a peak value of amino acids during pepsin and renin hydrolysis at 90 min of the experiment in all samples, except for "Malyutka hypoallergenic" mixture. The digestion process of the "Ligans" mixture containing milk powder mare is similar to the intensity of digestion of human milk. At the end of hydrolysis with trypsin, 8% less amino acids were accumulated in the "Ligans" mixture than in human milk. This is explained by the immediate action of the enzyme on the protein molecules, which are immediately subjected to fermentation and splitting since proteins of the albumin group are more susceptible to the action of proteases (Hodgkinson et al., 2018). "Agnus" mixture containing sheep powder milk is characterized by slower protein hydrolysis compared to human milk and "Ligans" mixture (16% less than "Ligans" and 28% less than human milk).

The explanation for this is the higher content of casein in milk, as well as the processes which change the features of coagulation of milk by citric acid salts (Roy et al., 2021). The higher the amount of casein and calcium salts in milk, the faster the clotting speed and the stronger the protein clot. In case of the addition of citric acid potassium and sodium salts in milk, the coagulation of its proteins by rennet enzymes slows down (Hodgkinson et al., 2018). Sodium and calcium citric acid salts interact with free calcium ions, as a result of which calcium in the form of poorly soluble calcium citric acid is removed from the sphere of action, but does not precipitate. All these processes inhibit the rapid breakdown of proteins, the cleavage occurs more smoothly and for a longer time (Roy et al., 2021). The researchers compared the *in vitro* digestibility, selected biological activities, and digested products of proteins from skimmed cow's, camel's, goat's, and sheep's milk. It was established that goat's milk had the highest digestibility. According to our results the digestibility of products based on mare and sheep's milk is similar to the digestibility of human milk and "Malyutka hypoallergenic" mixture.

Conclusions

1. The amino acid composition of dry animal milk was determined and a comparative analysis was carried out with the amino acid composition of human milk proteins. It was shown that the limiting amino acid of cow's milk powder (46.0%) and sheep's milk powder (47.0%) is cystine. The limiting amino acid of dry mare milk (52.0%) and dry goat milk (57.0%) is tyrosine.
2. The fractional composition of proteins of different types of dry milk was studied. It was found that only mare milk belongs to the albumin type, as well as human milk. Mare and sheep's milk contain the largest amount of milk protective factors (immunoglobulins and lactoferrin), which makes it possible to consider these types of milk as hypoallergenic. It was found that mare and sheep's milk contain the amount of immunoglobulin A close to human milk (5.15 and 6.92%, respectively),
3. It was established that the digestibility of products based on mare and sheep's milk is similar to the digestibility of human milk and "Malyutka hypoallergenic" mixture.

Accumulation of amino acids in mixtures based on mare and sheep's milk occurs more intensively than in the mixture "Malyutka hypoallergenic".

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