

CHEMICAL COMPOSITION OF THE PROTEIN COMPLEX OF LINSEED CAKE OBTAINED FROM SEEDS OF A NEW VARIETY OF KAZAKH SELECTION

Alma Yermukanova¹, Saule Zhiyenbayeva¹, Georgii Stankevych²,
Eva Mrkvicová³ , Eva Slezáková³, Dinara Kultaeva⁴

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¹ Almaty Technological University, Department of Bread Technology and Processing Industries, Furkat Street 348/4, 050012 Almaty, Kazakhstan

² Department of Grain and Feed Technology, Odesa National University of Technology, Kanatna Street 112, 65039 Odesa, Ukraine

³ Department of Animal Nutrition and Forage Production, Mendel University in Brno, 613 00 Brno, Zemědělská 1, Czech Republic

⁴ Department of Agronomy, Kostanay Regional University “A. Baitursynov”, Baytursynov Street 47, 110000 Kostanay, Kazakhstan

Abstract

The aim of this study was to evaluate the composition of the protein complex and some other selected values (e.g. the representation and proportion of amino acids in crude protein) of linseed cake from the flax (*Linum sativum*) variety ‘Kostanay 11’, which is widely grown in the Republic of Kazakhstan. The values were chosen to assess the suitability and benefits of including ‘Kostanay 11’ in livestock and poultry feed. This cake composition of the ‘Kostanay 11’ variety was then compared with the ‘Northern’ and ‘Golden’ flax varieties, which are more widely grown in the area. The amino acid composition of linseed cake was further compared with other cakes used for feeding livestock and poultry. For example, soya and sunflower products, which are the most popular worldwide.

Only for this new variety of linseed – Kostanay11 (and only for linseed cake) was the chemical analysis carried out directly in the Institute's laboratory. The values obtained were then compared with those given in the standards used by the feed industry.

All the research and subsequent data comparison leads us to the conclusion that the linseed cakes of the new Kostanay 11 variety are fully sufficient as one of the components of feed for animal nutrition. According to our findings, its nutritional value is not only equal to that of other flax varieties grown in Kazakhstan, but in many parameters, it even reaches the quality of the most desirable soybean cakes.

Keywords: flax, Kazakhstan variety, protein, linseed cake, fractional composition of protein, amino acid composition

INTRODUCTION

The matter of adequate animal nutrition in the Republic of Kazakhstan is problematic, both because of the poor availability of feed and its price. One of the reasons preventing the optimal production

of compound feeds for maximum performance of poultry and livestock is the insufficient supply of protein and energy raw materials.

Another obstacle to the proper and sufficient production of compound feed for livestock and



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poultry is the inadequate supply of protein and energy raw materials. Kazakhstan's own resources cover only 30–40% of its needs. It is therefore necessary to find new ways to reduce the shortage of feed raw materials, in particular by using by-products and waste from food production. Much of the by-products from the food industry contain high levels of nutrients and biologically active substances and can therefore be used as alternative feed sources. (Vastolo *et al.*, 2022). This is where the possibility of using flaxseed-processing residues comes in. Considering the high nutritional value and low price of linseed cake, research aimed at partial replacement and reduction of expensive soybean cake in the diets of farm animals and poultry is important (Lei *et al.*, 2022).

Kazakhstan is one of the five largest flaxseed growing countries (Baigenews, 2018). Flaxseed is a promising crop for this country; its plantings have been constantly growing in recent years and have already reached more than 1.3 million hectares (tass.com/society) and the successes of Kazakhstani selection have made it possible to obtain new, promising varieties of oilseed flax with large seeds and high oil content. This variety was called “Kostanay 11”, the weight of 1000 seeds is 6.5–7 g, and the oil content in the seeds exceeds 42.6%, and it's designed to produce high-quality oil and short fiber.

Flaxseed proteins are characterized by a balanced amino acid composition and high nutritional value (Kaushik *et al.*, 2016; Gutte *et al.*, 2015). The protein complex of flax seeds contains all amino acids essential for the human body and meets the Food standard and Agricultural Organization of the United Nation (UN food and agriculture organization, FAO food and nutrition paper, 2013). Flaxseed proteins have not only high biological value, but also functional and technological properties comparable to those of the widely used soy protein (Xin-Pei *et al.*, 2022).

Depending on the method used to extract linseed oil from flaxseeds, there are two types of linseed oil by-product. It is linseed press cake (expeller cake), which is the basic solid residue *after the pressing of linseed oil*. Linseed cakes are produced by cold or hot mechanical pressing. They contain residual oil (6–15%, depending on the method of pressing). They are coarser in texture and may take the form of sheets or crumbs. Linseed meal (*linseed meal, oilseed meal*) is formed after pressing and subsequent extraction of the residual oil (often with solvents). They have a lower fat content than pomace (typically up to 3–5% fat).

Both products are a good source of nutrients for feed and diets for all types of animals and poultry (Hall *et al.*, 2006; Lei *et al.*, 2022).

In the Republic of Kazakhstan, the most common practice is the pressing of linseed, therefore the linseed cake is more common than the linseed meal.

Linseed meal is a protein supplement that can seriously compete in nutritional value and

productivity with traditional high-protein components in poultry feed (Chen *et al.*, 2014) and it is used in animal feeding to balance concentrates of protein, fat and essential amino acids. Several studies noted that the introduction of this product into diets led to high concentrations of α -linolenic, linoleic acid in the milk of ruminants (Tsai, 2020).

Linseed cake is more nutritious in terms of energy content than meal but contains slightly less protein. 1 kg of linseed cake contains about 15,485 MJ of metabolic energy and 33–35% crude protein. It contains residual oil that is rich in alpha linolenic acid, which positively contributes to good animal health (Olomu *et al.*, 1991). After pressing, up to 19% of crude fat remains in it, 50% of which is linolenic (omega3) acid. For this reason, the accumulation of linoleic acid in the cake is reduced to 12%, and oleic acid to 25%. Consequently, linseed cake is more suitable for poultry diets in terms of fatty acid composition than sunflower and soybean cakes, in which linolenic acid is minimized and the level of linoleic acid is excessively high (Podobed, 2021a). Inclusion of linseed cake in the diet is it possible to correct the diet according to the ratio of n-3 to n-6 and obtain the expected effect of increasing productivity, improving the reproductive capacity and quality of products from growing farm animals and poultry. Linseed cake can be used to produce n-3 enriched eggs (Khan, 2019; Nain *et al.*, 2012; Zelenka *et al.*, 2008). Linseed cake ensures the accumulation of n-3 fatty acids in meat, improving its taste and dietary properties. Linseed cake is not much different from other types of cake in terms of the content of calcium, phosphorus, selenium, zinc and iron. Linseed cake is a valuable source of protein and essential amino acids, with arginine being the most important (Attia *et al.*, 2024). In addition to everything, linseed cake can be considered as a means of normalizing the electrolyte balance of the diet, which ensures the maintenance of a normal ratio of electrolytes in the blood and stimulates the growth of animals and poultry.

The protein complex in different plant species is represented by different fractions - groups of similar proteins that differ in different physicochemical properties of the components. One of the features of proteins is their unequal solubility in solutions of salts, alkalis and organic compounds. The principle of protein solubility is based on the conditional classification of simple plant proteins into albumins, globulins, prolamins and glutelin. Determining the composition and ratio of protein fractions is an important qualitative indicator of the plant protein complex (Polyakov *et al.*, 2011).

Different groups of plants are characterized by unequal accumulation of individual protein fractions. Linseed cake from flax variety “Kostanay 11” in its amino acid value is equal to the known cakes common in feed production and is second only to soybean cake.

Osborne (1907) proved that there are no two-plant species that contain identical proteins in their seeds,

and changes in the ratio of protein fractions are a sign that most clearly reveals the degree of response of crops, species, and varieties to changes in the external environment. According to the classification proposed by Osborne (1907), the types of protein fractions by solubility can be conditionally divided into water-soluble - albumins, soluble in salt solutions - globulins, soluble in alcohol - prolamins, soluble in alkalis - glutelin, insoluble - scleroproteins. This classification of proteins according to their solubility is not strict but has not lost its relevance today.

As for the information on the fractional composition of flax proteins, it is extremely contradictory. From the literature it is known that the protein fractions of flax seeds are represented mainly by albumins, globulins and glutelin.

Prolamins, an alcohol-soluble fraction that makes up a significant part of the protein complex of grain crops, are absent in flaxseeds or are found in trace quantities, like proteins in oilseeds. Data on the fractional composition of flaxseed proteins varies widely, which is associated with varietal characteristics, growing region, and seed processing conditions.

Flaxseed proteins have not only high biological value, but also functional and technological properties comparable to those of the widely used soy protein. Proteins with high functional properties are highly soluble in water and form strong gels, stable emulsions and foams. In this regard, the attention of researchers and technologists to obtaining protein products from flaxseed is growing (Kaushik *et al.*, 2016).

The high content of nutrients in flaxseed cake, primarily high-quality protein, was the reason that contributed to the expansion of research abroad to study the feed value and efficiency of using flaxseed processing products, mainly cake, in feeding farm animals and poultry.

Thus, it can be argued that the biochemical characteristics of the protein complex of flaxseed have not been sufficiently studied, which, in our opinion, limits the use of protein products obtained from flax seeds such as flaxseed cake in the production of feed for farm animals and poultry.

The zoning and widespread distribution of the flax variety "Kostanay 11" and some others on the territory of the Republic of Kazakhstan has led to great interest in them from processors. Nevertheless, the features of the chemical composition and nutritional value of cakes and cakes obtained from such varieties remain unstudied. This hinders the activity in their use as feed and does not allow us to establish their true effectiveness in feeding poultry.

The purpose of our work is to study the fractional composition of the protein complex of flaxseed cake from flax variety "Kostanay 11".

MATERIALS AND METHODS

In this work, physicochemical studies were carried out on a laboratory sample of linseed cake from

flax variety "Kostanay 11". In accordance with the methodology described below, only selected values for the "Kostanay 11" flax variety (linseeds and linseed cakes) were determined in the laboratory; other data were taken from commonly used feed tables for Republic of Kazakhstan. All laboratory values relating to Kostanay 11 were performed in the laboratories of the Technological Institute of Kazakhstan in accordance with GOST standards. Each ground sample was analyzed in two replicates and the average was calculated and presented in the results tables.

GOST methodologies were used for individual analyses, which are almost identical to the required ISO standards, but there are still differences between the two systems that may have slightly influenced the result. The similarities and differences between the GOST system and the ISO system are summarized in the appendix to this article, including any differences in the analysis of the monitored nutritional components.

Similarly, the values shown in Tab. II and Tab. III (part Results and discussion) were measured in the laboratory only for the flax variety "Kostanay 11"; the other values were selected from standards commonly used by feed manufacturers in Kazakhstan and are shown in the tables only for clarity and ease of comparison.

A basic nutrient comparison is given in Tab. I, Tab. II and in Tab. III.

By-products arising from oil production, in particular the production of cakes (expellers). This samples were produced by cold pressing at the "Dynasty Agro" (LLP enterprise) during the flax harvest in 2023 from the production of the Kostanay 11 flax variety in northern Kazakhstan.

GOST standards (originating from the former Soviet Union and used in countries such as Russia, Kazakhstan, and others in the Commonwealth of Independent States) and ISO standards (developed by the International Organization for Standardization) both aim to ensure quality, safety, and consistency in the analysis of animal feed. However, they differ in approach, structure, international recognition, and technical methods. Here's a breakdown of their similarities and differences:

- Linseed cake from flax variety "Kostanay 11" is presented in Fig. 1. According to organoleptic characteristics and the current GOST 10974–95, flaxseed cake is produced in the form of shells or ground to flour, uniformly colored and has a wide range of color shades from light gray to light brown, characteristic linen smell without signs of mustiness and burning.
- The color of linseed cake is mainly determined by the characteristics of the seed varieties, and if different varieties are mixed during processing, the cake will not have a uniform consistency and color. However, this does not have a negative effect on its nutritional value.



1: Linseed cake from flax variety "Kostanay 11"

Research Methods

- The mass fraction of crude protein of linseed cake was determined according to GOST 13496.4-93 "Feed, compound feed, compound feed raw materials".
- The mass fraction of fat in raw materials was determined according to GOST 13496.15 – 97 "Feed, compound feed, compound feed raw materials".
- The content of dietary fiber in raw materials was determined according to GOST 13496.2-91. The method is based on removing acid-base-soluble substances from the product and determining the mass of the residue, which is conventionally taken as fiber.
- The amino acid composition of linseed cake was determined according to GOST 32195-2013.

Amino acids are separated by Sykam ion exchange chromatography S-433- DS, react with ninhydrin and determine their content by photometric detection at a wavelength of 570 nm.

- The fractional composition of seed proteins based on solubility was determined according to Osborne (1907). The cake was ground and water-, salt-, alcohol- and alkali-soluble proteins were successively extracted. To isolate albumins from seeds, distilled water was used, 0.5 M NaCl was used to isolate globulins, 70% ethanol was used to isolate prolamins, and 0.2% NaOH solution was used to isolate glutelin. From the obtained extracts, proteins were concentrated by precipitation with ammonium

sulfate and dissolved in a small volume of solution. The amount of protein was determined spectrophotometrically using the Warburg-Christian method on an SF-46 spectrophotometer.

RESULTS AND DISCUSSION

Analysis of the chemical composition of linseed cake indicates that it can be classified as a highly digestible feed protein concentrate that can provide a significant correction of the protein and amino acid nutrition of farm animals and poultry.

Linseed cake of the "Kostanay 11" variety is characterized by an increased level of protein and fat compared with the values given in the tables. The fats that remain in the linseed cake after the oil has been separated have the same beneficial properties as the linseed oil itself. Linseed oil is rich in linolenic acid, which belongs to the omega-3 group (Dosen *et al.*, 1991). However, an increased accumulation of crude fibre is observed in linseed cake, although the fibre content remains lower than in hempseed cake and sunflower cake. This means that the digestibility of such a protein supplement will be maximum and it can be included in the diets of almost all sex and age groups of animals and poultry, at higher doses than sunflower cake. The results of our research are similar to those of results published by Podobed (2019), in whose works linseed cake is compared with sunflower and soya cake, but unfortunately the flax variety from which linseed cake is obtained is not specified. Podobed (2021a) concludes that the

1: Selected basic nutritional indicators of by-products from the production of the most common oilseeds (oil-bearing seeds), in particular oilseed cakes (expellers)

Index	Linseed cake		Hempseed cake	Sunflower cake	Soybean cake
	"Kostanay 11"	Tabular data			
Crude protein, %	36.5	33.30	34	36	36.0
Crude fat, %	7.5	6.46	6.97	18.5	5.8
Crude fiber, %	11.30	9.8	24.7	17.0	7.3

energy value of linseed cake is as close as possible to that of soya cake, and that its crude protein content is almost the same. Linseed cake differs positively from sunflower cake in its low concentration of crude fibre.

Different groups of proteins differ in amino acid composition and other properties – solubility, resistance to proteolytic enzymes, localisation. For example, proteins of the prolamin and glutelin groups are inferior in terms of amino acid composition. They contain none or only a small amount of essential amino acids. At the same time, it is these proteins that are mainly used by a young plant during germination, and the more of them there are, the better the chances of the plant surviving the early stages of ontogenesis. Albumins and globulin have a more complete amino acid composition. The quality of protein for poultry is determined by the amino acid composition and the physico-chemical properties, in particular the solubility. The content of soluble fractions in protein, especially those soluble in water and salt solutions, as well as amino acids, depends on the degree of moisture heat treatment. It is assumed that as the water – and salt-soluble fractions in the feed composition increase, the digestibility of the total protein also increases (Podobed, 2021b). Therefore, the ratio of individual fractions in the protein of the diet can indirectly be used to assess its ability to be effectively digested.

In our studies we compared the data on the solubility of flax proteins grown on the territory of the Republic of Kazakhstan (variety “Northern” and “Golden”) with the solubility of protein fractions of linseed cake obtained from the processing of flax variety “Kostanay 11” (Tab. II). The data indicate the predominance of the albumin fraction in the linseed protein complex.

A characteristic feature of the linseed protein complex is the high total content of water – and salt-soluble proteins - albumins and globulins, which are considered to be highly digestible (especially

albumins). During aqueous and low-salt extraction, carried out at low temperatures $\leq 60^{\circ}\text{C}$, the protein enters the solution in its native state, which ensures its high biological activity.

Hardly digestible prolamins (alcohol soluble fraction), which constitute a significant part of the protein complex of cereals, are either absent or minimised in linseed of the varieties compared. This indicates that the indigestible fraction is minimised in the compared linseed varieties used as feed, and that, regardless of the linseed variety, they contain a sum of water – and salt-soluble fractions close to 50%, which indicates the high nutritional value of the proteins in the feed product studied.

Pleshkov (1968) and Boechko (1985) indicate that globulins are the predominant fraction in linseed. They make up at least 40–50 % of the total amount of proteins. Madhusudhan (1985) and Marcone (1998) also confirm that the major components of the storage proteins in linseed are soluble in water and salt solution and these statements are summarized, for example, in the work of OMAH and MAZZA (1993). During his research, Barbasov (2005) discovered that the protein complex in linseed consists of water, salt and proteins that are soluble in alkaline solutions. In this case, the predominant component is water-soluble proteins, and the proportion of alkali-soluble proteins is up to 10%. Sammour (1999) summarised that flax seeds contains all four protein fractions, with a predominance of albumin and globulin.

Chung (2005) reports that linseed cake is high in globulin, a protein similar to albumin, which makes up 17.7% of total protein. Flax (linseed) protein contains arginine, aspartic acid, glutamic acid and the limiting amino acids are lysine, methionine and cysteine.

Meleshkina (2016) also studied the fractional composition of flax seed proteins. In her work: proteins, which according to her work make up 24.42%, are composed of globulins (45 %), albumins (32%), glutelins (9%) and prolamins (0.6%).

II: Fractional composition of linseed cake from flax variety “Kostanay 11” according to solubility in various solvents

Name of indicator, units of measurement	Linen seed Variety “Northern”	Linen seed Variety “Golden”	Linen seed Variety “Kostanay 11”	Linseed cake variety “Kostanay 11”
Water-soluble protein fraction, % of crude protein content	43.6	41.2	43.8	29.2
Salt-soluble protein fraction, % of crude protein content	21.4	18.6	21.6	16.8
Alcohol-soluble protein fraction, % of crude protein content	-	5.4	5.6	3.7
Alkali-soluble protein fraction, % of crude protein content	13.7	9.3	26.2	24.1
Insoluble protein residue, % of crude protein content (by difference)	21.3	25.5	2.8	26.2

In our studies of linseed from the Kostanay 11 variety, the prolamin fraction are clearly predominant and the glutelin fraction exceeds 10%. Although prolamin and glutelin are thought to be mainly present in cereal seeds, they are found in very small amounts in other plants (Grigorieva *et al.*, 2004).

Prusakov *et al.* (1998), point to role of prolamin fraction proteins in plant adaptation and the link between increased prolamin content and the macroecological distribution of certain species. According to Polyakov *et al.* (2011), the presence of the prolamin fraction, which is a substrate for proteolytic enzymes, increases the adaptive potential of wild plant species to adverse environmental conditions, starting from the germination phase, and subsequently has a positive effect on plant growth and development, as mentioned earlier in this paper.

On the basis of the data obtained, we can conclude that the data from the analysis of the fractional composition of linseed proteins of different varieties vary considerably, which is related to varietal characteristics. This factor should be taken into account when evaluating the nutritional value of linseed cake as a feed ingredient.

In addition, there is no doubt that the heat treatment used was specific and significantly influenced the ratio of soluble protein fractions during the conversion of linseed to cake.

Under the influence of heat treatment of linseed meal from „Kostanay 11“ variety, the content of water – and salt-soluble fractions decreased, a small fraction of alcohol-soluble components appeared, and the concentration of alkali-soluble proteins and non-extractable residues in linseed meal increased.

Literature data (Yanova *et al.*, 2022) indicate that this is a natural process during any heat treatment. However, with the chosen method of thermal exposure in the process of obtaining linseed oil, the proportion of the water-soluble fraction in the linseed cake remained the highest in concentration among all others, and the sum of the water-soluble and salt-soluble fractions amounted to about half of all fractions.

This allows us to state that linseed cake obtained from the seeds of the „Kostanay 11“ variety remains a highly digestible and nutritious product in terms of protein absorption, suitable for inclusion in the diets of all types of animals and poultry, starting with compound feeds for young animals at an early age.

For non-ruminant animals, it is not so much the total concentration of protein substances in the form of the „crude protein“ indicator that is important, but the quality of this protein in terms of the concentration of individual amino acids and their availability to the body. It is useful to compare the amino acid composition of linseed cake protein in terms of amino acid ratio with other typical cake types traditionally used in animal and poultry feeding. A comparative analysis of the amino acid composition of the cakes is given in Tab. III.

An analysis of Tab. III shows that linseed cake occupies an intermediate position between sunflower and soya cake. It is richer in lysine than sunflower, but poorer in methionine. Linseed cake from flax of the Kazakh variety “Kostanay 11” differs slightly from the tabular averages, and to a lesser extent in the total amount of essential amino acids. However, the total amount of critical amino acids in the analysed cake is almost equal to the tabular indicator, higher than in the hemp cake and slightly different from the indicators in comparison with the sunflower cake.

This means that the studied protein supplement is equal in its amino acid value to the known cakes commonly used in feed production and is second only to soybean cake.

The linseed cake in question, Kostanay 11, differs from other cakes, including soya, in that it has a higher concentration of arginine. This means that when linseed cake is used to formulate feed rations, it is possible to optimise the arginine:lysine ratio in the diet without having to resort to the use of synthetic additives of this amino acid. This is especially true for meat and egg production. The use of linseed cake can therefore facilitate the optimisation of feed formulations. The results of our studies can be compared with those of Kapitonova *et al.* (2017), who investigated the amino acid composition of linseed cake, soybean cake and sunflower cake. The authors conclude that linseed cake has a relatively high availability of essential amino acids for poultry, although its amino acid composition is inferior to that of cake.

We have developed recipes for diets containing linseed cake from flax variety “Kostanay 11” for broiler chickens of the (KOB-500), studied the indicators of granule crumbliness and specific energy costs of pilot batches of granulated diets for broilers (Yermukanova *et al.*, 2024). At present, experiments are being carried out on the feeding of broiler chickens with granulated diets based on linseed cake of the variety “Kostanay 11”.

The above data indicate that different flax varieties determine a wide range of variations in the chemical composition and nutritional value of the feed product, as well as the productive effect obtained from it. Therefore, it is of scientific and practical interest to make a detailed assessment of the chemical composition of linseed cake from flax of the Kazakh variety “Kostanay 11”. The research carried out in this direction will allow us to obtain data on the nutritional value of flaxseed cake and the feasibility of its use in poultry farming practice in Kazakhstan. This, in turn, will make it possible to eliminate protein deficiency in the diet and increase the efficiency of production of livestock and poultry products in the region.

Analysis of the chemical composition of linseed cake indicates that it can be classified as a group of highly digestible feed protein concentrates, which can provide a significant correction of the protein and amino acid nutrition of animals and poultry.

III: Comparative analysis of the amino acid composition of the cakes of oil seeds

The name of indicators	Amino acid content g/100 g protein				
	Linseed cake		Hempseed cake	Sunflower cake	Soybean cake
	“Kostanay 11”	Tabular data			
Arginine	3.25	3.33	1.75	3.0	2.6
Lysine	1.32	1.42	1.35	1.27	2.26
Tyrosine	0.86	1.0	0.41	1.06	-
Aspartic acid	3.23	2.80	3.28	2.44	5.7
Glutamic acid	6.59	5.80	2.17	4.96	8.7
Phenylalanine	1.63	1.67	0.96	1.61	2.21
Histidine	0.74	0.86	0.37	1.06	0.96
Leucine	1.98	2.17	1.2	1.68	2.93
Isoleucine	0.45	1.53	0.62	1.68	2.93
Methionine	0.65	0.59	0.54	0.83	0.45
Cystine	0.66	0.61	0.66	0.62	0.49
Valin	1.76	1.78	0.37	1.9	2.13
Proline	1.22	0.27	0.40	0.22	0.26
Threonine	1.28	1.34	0.24	1.34	1.51
Serin	1.49	0.71	0.24	0.16	0.19
Alanin	1.55	0.5	0.28	0.16	0.21
Glycine	2.01	2.0	1.5	2.42	-
Tryptophan	0.59	0.55	0.39	0.49	0.55
Sum of nonessential amino acids	17.61	13.69	8.94	12.04	15.55
Total essential amino acids	13.65	15.24	7.79	14.86	18.53
Sum of critical amino acids (lysine + methionine, threonine + tryptophan)	3.84	3.9	2.52	3.93	4.77

CONCLUSION

Linseed cake obtained by processing flax of the Kazakh variety “Kostanay 11” is a complete protein supplement, a source of highly nutritious protein with a relatively low accumulation of crude fibre, which gives it a number of advantages over sunflower cake and brings it closer to soya cake. This widens the range of percentages in which it can be incorporated into compound feeds and allows it to be used in the feeding of livestock and poultry.

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Contact information

Alma Yermukanova: apple_kst@mail.ru (corresponding author)

Saule Zhiyenbayeva: sauleturgan@mail.ru

Stankevych Georgii: georgii.stn@gmail.com

Eva Mrkvicová: eva.mrkvicova@mendelu.cz,  <https://orcid.org/0000-0002-2504-5024>

Dinara Kul'taeva: dinara-kultaeva@mail.ru

