Influence of protein concentrate in the diet on productivity and amino acid composition of cow milk

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Abstract. The article presents experimental data on the use of different levels of protein concentrate supplement in the diets of lactating cows with a high proportion of indigestible protein in the rumen. During the course of the research it was observed that the inclusion of Agro-Matic protein concentrate in the amount of 1.0 and 1.5 kg per head per day in the diet instead of other protein feeds increased the level of indigestible (by-pass) protein by 6.3 % and 9.1 %, respectively. The introduction of the maximum amount of Agro-Matic protein concentrate into the diet has contributed to the change in the amino acid composition of milk. It was established that the yield of milk protein during the period of milking was significantly higher in animals receiving different levels of protein concentrate compared to those of the control group. Feeding lactating cows diets with different levels of protein concentrate did not have a negative impact on biochemical parameters of animal blood. All measured blood of experimental animals were within the physiological norms. The maximum content of essential amino acids in the milk of cows from 3 experimental groups was significantly higher and reached up to 1560.9 mg% versus 1480.17 mg% in the control group.

1. Introduction
The production of high-quality milk and dairy products is of great importance in the economy of Russian Federation; these products are also included in the list of basic human food and fall under the Doctrine of national food security [1-6].

In condition of market economy, the problem of low-quality milk production on farms of agricultural enterprises is one among the most relevant [7-11].

With an increase in the milk productivity of livestock, the impact of the non-digestible fraction of the feed protein has also increased. The predominant share of a high degree of cleavage protein in the rumen of cows comes from the protein of haylage, silage and most of grains (wheat, barley, oats, etc.). The optimal ratio of cleavable to non-cleavable protein in feeds for high-yielding dairy cows during the milking period is from 60-70 % to 30-40 % [8, 12, 13, 15, 16-20].
The use of complete feed and feed additives in the diets of highly productive lactating cows contributes to reimbursing the content of deficient and missing nutrients [1-4, 11, 21-22]. The aim of the current study was to investigate the influence of protein concentrate Agro-Matic supplementation to the diet of dairy cows on productivity of animals and amino acid composition of cow milk.

2. Materials and methods

The current research started in February 2018 on the farm of Agricultural Production Cooperative ‘Plemzavod Maisky’ in Vologda region, Russia.

The subjects of the research were heifers in the second half of pregnancy and Ayrshire breed cows of older age in dry period. The animals were selected by method of the pair-analogues considering their origin, sex, age, live weight, milk production in the previous lactation period, milk yield over 8200 kg per year and physiological state.

At the time of the experiment the cows and the heifers were eight – months pregnant with a live weight of 550 kg and average fatness. They were divided into 3 groups of 15 heads each –1st group a control group, 2nd and 3rd groups – experimental groups. During the course of the study all animals were kept on the same housing and feeding conditions and were clinically healthy.

The animals in the control group received the standard diet used in the farm, balanced in nutritional value according to the norms of feeding developed by All-Russia Institute of Animal Husbandry (2016) [3] and designed to receive milk yield of 39 kg per 1 day during the milking period.

The composition for the cows in 2nd and 3rd group (experimental groups) was based on standard diet with addition of different levels of Agro-Matic protein concentrate of 1.0 and 1.5 kg, respectively and simultaneous reduction of other protein-rich feeds. The content of exchange energy and the level of crude protein in protein concentrate enriched diets was equal to the control group.

The basic diet in the control group included the following: grass hay – 0,5 kg; corn silage – 7; grass haylage of the first cut – 7, grain haylage (from barley) – 12, beet molasses – 1.5, beet pulp – 1.5, soybean cake – 1.0, sunflower cake with 36 % crude protein content – 1.5, compound feed-concentrate – 11, nutrakor (bypass oil) – 0.3 kg, mineral feeds: monocalcium phosphate – 130 g, table salt – 120 g.

During the time of experiment milk quality and productivity of cows were measured 3 times a month, respectively, by the method of control milking. The percentage of milk fat was determined in accordance with GOST 5867-90 Milk and dairy products. Method of determination of fat [23] and Gerber acid method described in GOST R ISO 2446-2011 Milk. Method for determination of fat content [24].

Milk samples were taken from 3 animals from each experimental group, collected in one common container and stored in a refrigerator at + 4 ° C. The gross content of essential and non-essential amino acids in cow’s milk was determined in the laboratory ‘Evonik Chemistry’, LLC. Amino acids in the cow milk were determined on a Foss AMINONIR DS2500 spectrometer in the 3rd month of lactation.

Biochemical parameters of blood samples of cows were taken from 9 animals 2 days after the end of the physiological experiment were determined in ‘Vologda regional veterinary laboratory’.

The following parameters were measured in animal blood serum: total protein – by refractometric method using IRV – 22 Refractometer with the help of The Reis table; urea – by the Michonne and Arnault method by the reaction with paradiethylaminobenzaldehyde; calcium – by spectrometric titration with the murexin indicator; phosphorus – by colorimetric method based on the reduction of phosphoric-molybdenum acid.

In the blood samples the following parameters were evaluated: glucose – by colorimetric method, color reaction with orthotoluidine reagent (Hultman method in Hyvarinen Nickel modification); carotene – by calorimetric gasoline extraction.

Processing of biometric data was performed by the methods of mathematical statistics (N.A. Plokhinsky, 1969 and E.K. Merkuryeva, 1970) using modern computer programs (standard package of statistical analysis Microsoft Excel 2007).
3. Results and Discussion

3.1. Productivity and amino acid composition of cow milk

Increasing the productivity of dairy cattle breeding and quality of the final dairy products in the Russian Federation at lower production costs is set among the priority objectives of the State Program until 2022 in the field of modernization of agriculture and industrial safety under conditions of foreign sanctions [1, 2, 4, 5, 6, 22].

In the course of current study, milk production was determined during 120 days of lactation from the moment of calving onwards. The following indicators were considered: average daily milk yield and gross milk yield with natural fat content and 4% fat content, mass fraction of milk protein and fat, milk protein and fat yield (table 1).

**Table 1. Milk quality and milk yield during 120 days of lactation, kg.**

| Indicator | The 1st - control | The 2nd - experimental | The 3rd - experimental |
|-----------|-------------------|------------------------|------------------------|
| ADY** milk with natural fat content, kg | 32.6±0.82 | 34.4±0.70 | 35.8±0.75* |
| Gross yield milk with natural fat content, kg | 3910.1±98.56 | 4123.4±83.92 | 4297.5±90.27* |
| ADY milk with 4% fat content, kg | 32.6±0.90 | 34.5±1.71 | 35.8±0.82* |
| Gross yield of milk with 4% fat content, kg | 3910.1±107.81 | 4142.0±205.03 | 4297.5±98.79* |
| Mass fraction of milk fat, % | 4.00±0.033 | 4.03±0.043 | 4.00±0.034 |
| Gross yield of milk fat, kg | 156.4±4.62 | 166.2±4.16 | 171.9±4.27* |
| Mass fraction of milk protein, % | 3.26±0.015 | 3.34±0.021* | 3.29±0.028 |
| Gross yield of milk protein, kg | 127.5±3.24 | 137.7±3.11* | 141.4±3.33* |

Note: * – the difference is significant with respect to the control group at P > 0.95; **ADY – average daily yield.

Analysis of the experimental data shows that the supplementation of standard diet with 1.0 and 1.5 kg of Agro-Matic protein concentrate has increased the gross milk yield with 4% fat content by 5.93 % and 9.91 %, respectively, compared to animals from control group. There was a marked tendency of increase of gross yield milk with natural fat content and milk with 4% basic in the experimental groups.

In the 3rd experimental group gross yield with natural fat content was 4297.5 kg, which was significantly higher than in the control group. It should be noted that the gross yield of milk in the 2nd experimental group and the 3rd experimental group was 213.3 kg higher than in the control group and 387.4 kg higher compared to animals receiving sunflower cake in the diet. The amount of milk protein in the experimental groups was increased in comparison with animal analogues from the control group. It should be noted that during the experiment the highest content of fat and protein in milk was in the 2nd experimental group, with the values of 4.03 % and 3.34 %, respectively. Thus, it became evident that supplementation of 1 kg Agro-Matic protein concentrate to standard diets positively impacts milk productivity of cows and significantly increases the mass fraction of milk protein and fat compared to the control group.

During the reference period, the gross yield of milk fat and protein in the experimental groups of animals receiving Agro-Matic protein concentrate in the diet were significantly higher than in animals-analogues from the first control group. The highest yield of milk fat was observed in cows of the 3rd group, which was 10.9 % higher compared to the animals from the control group. Although at the time of the experiment the values of the mass fraction of fat and protein were generally the highest due to the high milk productivity of cows receiving Agro-Matic protein concentrate in the diet, the maximum content of protein and fat was found in cows from the 3rd experimental group, which was respectively 10.90 % and 9.91 % higher than in the control group.
The biological value of milk protein is based on the content of its specific amino acids. It is known that more than 70 % of overall amino acid amount that enters the body is involved in milk formation. The studies conducted on lactating cows with milk yield of 6-8 thousand kg per annum and milk fat content of 3.4 % raised on diets with a balanced composition of exchange protein revealed the deficit of some essential amino acids such as methionine and leucine [5, 13, 14, 15, 20, 26]. According to Yu.V. Sizova the amount of amino acids to support life are: lysine – 36.9 g/day, methionine – 6.9, phenylalanine – 10.7, and leucine – 24.7 g/day [11, 21].

Amino acid composition as evaluated in the course of the current study indicates that the inclusion of protein concentrate in the diet positively affected the content of essential amino acids in milk (table 2).

| Indicator       | Group of animals (n=3)                |
|-----------------|---------------------------------------|
|                 | 1st control  | 2nd experimental | 3rd experimental |
| Methionine      | 85.2±3.35   | 95.4±5.20        | 93.37±3.60       |
| Lysine          | 234.0±8.44  | 262.8±11.27      | 263.6±8.60       |
| Threonine       | 115.8±115.8 | 130.3±6.72       | 125.7±4.47       |
| Tryptophan      | 46.6±1.27   | 52.0±2.09        | 49.8±2.13        |
| Arginine        | 105.7±3.67  | 121.0±6.75       | 120.1±5.59       |
| Isoleucine      | 182.0±2.14  | 186.7±10.94      | 193.8±10.71      |
| Leucine         | 291.8±5.00  | 273.6±18.10      | 284.5±15.77      |
| Valine          | 183.8±2.27  | 194.1±17.37      | 187.8±15.16      |
| Histidine       | 84.9±3.16   | 81.5±4.47        | 84.4±3.23        |
| Phenylalanine   | 150.3±1.28  | 149.0±6.04       | 157.8±1.81*      |
| Amount essential| 1480.2±5.85 | 1546.5±5.59      | 1560.9±16.90*    |
| amino acids     | 100         | 104.5            | 105.8            |

Note: * - the difference is significant with respect to the control group at P > 0.95

There are few main functions of lysine in the animal organism. In unchanged form lysine is a structural component of organs, tissues, and a part of the muscle fibers. Lysine actively participates in the synthesis of collagen and elastin; it is starter molecule in the process of hematopoiesis – synthesis of blood hemoglobin and it also stimulates the development of bone tissue. Lysine provides calcium absorption; it participates in the regulation of synthesis and ratio of nucleic acids. Lysine provides synthesis of some hormones and enzymes. Lysine also has a function – in immune system, process of building antibodies. Lysine optimizes the renewal of epithelial tissues and provides normal wool pigmentation. In regard to vitamin metabolism, lysine controls the conversion of carotene into vitamin A.

Ruminants are able to cover up the need for cystine and methionine by 30-40 % by in vivo synthesis of protein by the specific microbiota of the rumen. Amino acids methionine, proline, glycine and glutamic acid are constantly present in the rumen in amount of 0.1-1.4 mg per 100 ml of the content. Methionine has an important role in the protein synthesis in cattle and other animals, as it is a starting molecule in the biosynthetic pathways of protein in the body of animals.

Analyzing the data obtained in the current study, it should be noted that the maximum content of methionine in milk was found in animals from the 2nd experimental group and its content was 10.2 mg% higher compared to the control group.

The content of lysine in milk was the highest in the group of animals receiving 1.5 kg Agro-Matic protein concentrate in their diet and it was 29.6 % and 0.8 % higher compared to the control group and 2nd experimental group, respectively.
The output of the total amount of essential amino acids in the milk of cows of the 3rd experimental group was evidently higher – 5.8 % higher compared to the animals from the control group.

3.2. Biochemical parameters measured in blood of experimental cows

The process of metabolism in the body of cows is a series of complex biochemical transformations of nutrients, which are broken down to monomers during the process of digestion and enter the blood and lymph from the digestive system [2, 10, 22, 27].

Biochemical indicators of the whole serum and blood plasma are one of the main indicators of health and physiological state of animals, which fully reflect normal homeostasis and deviations if any. Biochemical indicators can rate diseases and reflect the level of metabolism in the body of dairy cattle. Any deviations in the metabolism of dairy cattle away from normal values can mainly result in decrease in milk production, poor health status and negative effect on reproductive function in general. The composition of biological body fluids (whole serum and blood plasma) reflects the intensity and direction of metabolic processes that occur in animals [1, 4, 6, 28].

To study the biochemical parameters of the blood of cows from the experimental groups 3 cows were selected from each experimental group (table 3). Blood samples were taken 3 hours after morning feeding.

Table 3. Biochemical blood parameters of experimental cows when feeding protein concentrate.

| Indicator             | 1st control | 2nd experimental | 3rd experimental | Physiological standard |
|-----------------------|-------------|------------------|------------------|------------------------|
| Glucose, mmol/l       | 2.17±0.039  | 2.68±0.051*      | 2.22±0.025       | 2.22-3.33              |
| Total protein, g/l    | 77.8±0.61   | 86.9±1.68*       | 82.4±2.82        | 72-86                  |
| Urea, mmol/l          | 6.34±0.118  | 5.61±0.212       | 6.53±0.647       | 3.3-6.7                |
| Calcium, mmol/l       | 2.44±0.435  | 2.42±0.102       | 2.52±0.243       | 2.5-3.13               |
| Phosphorus, mmol/l    | 1.66±0.174  | 1.60±0.105       | 1.71±0.276       | 1.45-1.94              |
| Carotene, milligram/% | 0.60±0.029  | 0.65±0.051       | 1.00±0.149       | 0.4-1.0                |

Note: * - the difference is significant with respect to the control group at P > 0.95

One among the main indicators of the level of carbohydrate metabolism in the body of farm animals is the blood glucose concentration. In animals from the 2nd experimental group, receiving 1.0 kg of protein concentrate in the diet, high blood glucose levels were observed. They were 0.51 mol/l higher compared to the control group.

The main indicator of protein supply in the body is the total protein content measured in the blood serum. Protein is a building material for cells and tissues of the body and is actively involved in milk formation.

Analyzing the data obtained, it should be noted that the total protein content in the blood serum of the animals from the 3rd experimental group was slightly changed, but during the time of the experiment a significant increase by 9.1 g/l was observed in cows receiving 1.0 kg Agro-Matic protein concentrate in comparison with the control group.

It should be noted that the level of total protein in the blood serum of the experimental cows receiving 1.5 kg of protein concentrate in the diet, has increased by 4.6 g/l (5.9 %) in comparison with the control group, which in turn is a consequence of increased efficiency of the nitrogen utilization in the biosynthesis of milk protein in the body of lactating cows.

To assess the mineral metabolism in the blood serum of animals the level of calcium and phosphorus should be determined. Calcium ions are involved in the process of blood clotting, contribute to the interaction of actin with myosin in skeletal muscle tissue and to the release of neuronal mediator acetylcholine. The calcium level depends on vitamin D and calcium and phosphorus content in the diet of cows. Being the basic element of phosphoric acid, phosphorus participates in all major metabolic pathways in the body of animals. Reactions of phosphorylation are one among main parts in amino acid metabolism and glycolysis. According to data obtained in the current study, animals of the 3rd
experimental group had the highest content of calcium (2.52 mmol/l) and phosphorus (1.71 mmol/l) in blood serum and these values were within normal physiological levels.

Based on the results of this investigation it can be stated that that supplementation of 1.5 kg of Agro-Matic protein concentrate to the diet of dairy cows provides an optimally balanced diet and sufficient mineral nutrition.

4. Conclusions
Results obtained in the current complex studies of the inclusion of different levels of Agro-Matic protein concentrate instead of other protein feed in the diet of the cows on the farm of Agricultural production cooperative ‘Plemzavod Maisky’ allow to draw the following conclusions:

1. The inclusion of Agro-Matic protein concentrate in the amount of 1.5 kg per head per day has contributed to a significant increase in the daily milk yield with natural fat content. The milk yield of cows from the experimental group over the period of the milking (120 days of lactation) reached up to 4297.5 kg, which is 9.82 % higher than in the group of cows who were fed the diet without Agro-Matic protein concentrate.

2. It was established that the yield of milk protein in animals receiving different amounts of protein concentrate during the period of milking was reaching up to 141.1 kg, which is significantly higher than in animals from the control group.

3. Supplementation of the maximum amount of protein concentrate into the diet has contributed to the change in the amino acid composition of milk. The maximum amount of essential amino acids in the milk of cows from the 3rd experimental group was significantly higher and reached up to 1560.9 mg% versus 1480.17 mg% in the group of animals who were fed the diet without Agro-Matic protein concentrate.

4. Feeding to lactating cows diets with different levels of protein concentrate did not have any negative impact on biochemical parameters of blood parameters. Values of measured blood parameters of animals from all three experimental groups were within the physiological norms.

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