Effect of degradable protein in cow diet on body systems' physiological status

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Abstract. The relationship between indicators of enzymatic and microbiological processes in the rumen and metabolism in the body was studied in lactating cows when the content of soluble protein in the diet was changed. The change in digestible protein content was provided by different ratios of natural feed and synthetic protein additives. The study was conducted on 3 lactating first-calf Holstein cows with rumen and 12 intestinal cannulas. The rate of fractional efflux from the complex stomach was evaluated by chromium oxide and microbial synthesis by purine bases. Experimental data were obtained to improve feeding rates, evaluate diets for high-yielding dairy cattle and develop rates of readily available carbohydrates in diets. At increase of share of soluble and degradable protein fractions in feed there is a natural increase of ammonia formation in rumen and urea content in blood and at achievement of ratio of degradable protein fractions and easily available carbohydrates in feed in the ratio of 0.44, there is maximum efficiency of microbial synthesis and supply with available amino acids of synthesis of milk components, which is proved by increase of concentration of amino acids in blood and formation of milk protein and promotes more complete realization of productive capacity of dairy cattle. Application to lactating cows of a ration with a higher ratio of degradable protein and DHC (higher than 0.44) reduces the efficiency of feed nitrogen utilization by reducing the efficiency of microbial protein synthesis by 10%.

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1 Introduction

Modern highly productive dairy farming is based on the use of highly concentrated diets. The main negative aspect of this is the acidification of the rumen environment and, as a consequence, changes in the composition of microbiota and the direction of rumen metabolism [1]. Maintaining the optimal pH range in the rumen by feed factors is a key factor in ensuring the required milk fat content [2, 3, 4].

Improvement of ruminant feeding systems involves the use of knowledge of quantitative parameters of fermentation of feed nutrients in the rumen to optimize microbial protein synthesis in the rumen. The problem of increasing the intake of amino acids into the body of animals can be solved not only by increasing the intake of feed amino acids into the duodenum, but also by microbial protein. The most important indicator quantifying the

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microbial protein supply from the complex stomach to the intestine is microbial synthesis. The key indicator in this case is the efficiency of microbial synthesis or the amount of microbial nitrogen or protein synthesized per unit of fermentable organic matter of feed in the pre-gastric gut. The dependence of microbial synthesis in pre-gastric digestion increases when expressed from apparently digestible CB, OM, hexoses, VFA formation, and ATP formation [5]. One of the most important features of ruminant pre-gastric digestion is microbial protein synthesis, which provides an opportunity to increase the supply of amino acids to the organism by using non-protein sources of nitrogen and improving the quality composition of feed amino acids. Depending on the composition of the diet, 50 to 80% of feed nitrogen is converted into microbial nitrogen [6, 7]. However, synthesis of microbial CP from protein feed is not efficient due to partial losses with ammonia and conversion of amino acid nitrogen into purine and pyrimidine bases, the nitrogen of which reaches 15% of the total microbial nitrogen [8]. Currently, there are two main ways to increase the amount and improve the composition of amino acids entering the duodenum: protection of feed high value protein from decay in the rumen and increasing the efficiency of microbial synthesis from decayed compounds in the rumen. Therefore, the objective of our research was to investigate the factors affecting the efficiency of microbial protein synthesis in pre-stomachs. A large number of researches on determination of microbial protein synthesis in the rumen of ruminants showed that the decisive condition determining the level of protein synthesis by microorganisms is the composition of the ration. According to the data of different authors, fluctuations in synthesis efficiency range from 10 to 45 g of nitrogen per kg of digestible organic matter [9]. Some share of variability in the results is attributed to the use of different methods of microbial protein determination.

At present, norms for the content of breakdown protein in diets are established, but without taking into account the optimal content of soluble protein and the rate of breakdown of crude protein (CP) and carbohydrates. According to some authors [10], an increase in the proportion of soluble CP in the composition of decomposable CP is often accompanied by a decrease in the efficiency of microbial synthesis. The importance of synchronization of protein and carbohydrate breakdown rate in the rumen for efficient microbial synthesis was also shown. The ratio of crude fiber and the sum of easily digestible carbohydrates, which provides effective microbial synthesis of protein, was determined [11]. However, due to the introduction of a new standardized indicator characterizing the fiber content in diets - Neutral Detergent Fiber (NDF) and the introduction of characteristics of feed nutrients by availability for digestion in the pre-gastric tract, it is necessary to specify the optimal ratio of soluble and degradable CP in diets and the optimal release of nitrogenous substances per g of fermentable carbohydrates (NDF, sugars and starch).

To form an optimal rumen biocenosis, information on the effect on the microflora of the ratio of different fractions of readily available carbohydrates in the diets is necessary. It is known that feed sources of starch have different fermentation rates, so rationing should be carried out not on its total content, but on the fraction undergoing decomposition in the rumen and the rate of fermentation of simple sugars. It should also take into account the fact that the energy of readily available carbohydrates obtained by microflora can be used for microbial protein synthesis, and the limiting factor during this period is the availability of available forms of nitrogen.

The problem of providing the need of animals in available amino acids can be solved not only by increasing the intake of feed amino acids of the feed into the duodenum, but also by microbial protein [12]. The conducted research has shown that shifts in one or another direction in the ratio of digestive carbonhydrates (DHC) and degradable protein (DP) have a significant effect on the processes of rumen fermentation, which is manifested not only at the level of microbial protein synthesis, but also in disorders of rumen digestion in general. In this regard, the use of a constant value of microbial synthesis efficiency does not allow us to

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adequately assess the availability of both available protein and volatile fatty acids (VFA) in the cow's body supply due to fiber digestion.

Research Objective is to study the influence of shifts in the DP/DHC (degradable protein/easily digestible carbohydrates) ratio in the diet on the dynamics of ammonia and VFA content in the rumen fluid, the degree of synchronization of fermentation of protein and carbohydrate fractions in the rumen with the indicators of microbial synthesis and physiological state of lactating cows.

2 Methods and Materials

The research was conducted at the end of the first period of lactation on 3 Holstein cows with rumen and 12-intestine cannulation with an average live weight of 610 kg and an average daily milk yield of 18 liters. The experiments were conducted by the method of periods of 21 days each [13,14]. In the studied diets, the content of all components except breakdown crude protein was fixed, and different levels of the ratio of easily digestible carbohydrates (DHC) and breakdown protein (DP) were formed by combinations of natural and "protected" urea and sunflower meal fed as a supplement to the basic diet (BD) (Table 1). Protected protein supplements were obtained by fusing urea with zeolite, and sunflower meal was heat-treated in a microwave unit.

The volume of chyme entering the duodenum was calculated from the chromium oxide content of the average daily sample [14]. Based on the nutrient intake into the duodenum, their apparent digestibility in the compound stomach was estimated. The true digestibility of feed crude protein was we calculated taking into account microbial crude protein synthesis, which was estimated by the content of purine bases in duodenal chyme [15].

Table 1. Research design

Additive components	Experiment periods			
	1	2	3	4
Unprotected sunflower meal	+	+		
Protected sunflower meal			+	+
Unprotected urea	+		+	
Protected urea		+		+

Table 2 shows data on ration composition and its characterization. Some difference between the diets in the level of metabolizable energy is due to the fact that its calculation is based on the data on the digestibility of nutrients of the diet on the basis of the balance experiment. In other indicators the diets coincided, except for: soluble protein content and, related to this, DP/DHC ratio.

Table 2. Composition and characteristics of diet

Indicators	Diet			
	1	2	3	4
Corn silage, kg	25.0	25.0	25.0	25.0
Variegated grass hay, kg	1.0	1.0	1.0	1.0
Wheat*, kg	2.73	2.73	2.73	2.73
Barley*, kg	3.0	3.0	3.0	3.0
Sunflower meal*, kg	1.5	1.5	0	0
Sunflower meal protected**, kg	0	0	1.5	1.5
Feed urea, kg	0.05	0	0.05	0
Protected feed urea***	0	0.09	0	0.09
Premix, g	230	230	230	230
Dicalcium phosphate, g	345	345	345	345

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Exchanged energy, MJ	138.1	137.4	139.7	138.8
Dry matter, kg	15.0	15.0	15.0	15.0
Crude protein, g	2209	2209	2209	2209
Degradable protein, g	1660	1551	1376	1303
Soluble protein (DP), g	896	780	736	619
Metabolisabl protein, g	1079	1096	1276	1269
Crude fat, g	795	795	795	795
NDF, g	4340	4340	4340	4340
Crude fiber, g	2304	2304	2304	2304
Starch, g	3576	3576	3576	3576
Degradable starch+, g	3157	3157	3157	3157
Sugar, g	379	379	379	379
Nitrogen balance in rumen	0.69	0.73	0.8	0.84
Amount of digestible carbohydrates (DHC), g	3536	3536	3536	3536
DHC, %	23.5	23.5	23.5	23.5
Soluble protein, %	5.9	5.2	4.9	4.1
Degradable protein (DP), %	11.1	10.3	9.1	8.7
DP/ DHC	0.47	0.438	0.39	0.37

*- as part of mixed fodder
** - thermally treated in microwave unit
*** - fused with zeolite

To evaluate the parameters of digestive system functioning and substrate formation, the pH, ammonia, VFA and their composition were determined in rumen contents taken from cows through the rumen cannula before feeding, 1, 3 and 5 hours after morning feeding. The number of bacteria and infusoria, amylolytic and cellulosolytic activity of microflora were determined in the contents obtained after 3 hours. [16, 17].

To assess the level of carbohydrate and lipid nutrition, the concentration of glucose, VFA, triglycerides, AEFA, ketone bodies were determined in the tail blood. To assess the state of protein metabolism, the content of urea and free amino acids was determined. The general physiological state was evaluated by enzymatic and hematologic blood parameters. The level of lipids, protein, lactose, urea was determined in milk.

Kjeltec apparatus for nitrogen determination, ABK-1 calorimeter for determination of caloric content of feed and milk samples, Milko-Star milk analyzer (Italy), VFA analysis of rumen fluid, blood analyses - on biochemical semi-automatic analyzer Screen Master LIHD113 (Italy) were used in research. Statistical processing was carried out using the software package "Excel" by methods of dispersion, regression [18, 19].

3 Results and Discussion

Average daily indices of rumen digestion generally corresponded to the ration characteristics and were at the level of physiological norms (Table 3). Reduction of DP content in diets led to a natural decrease in ammonia level, while other indicators did not change significantly, which indicates normal microbiological processes in the rumen. At the same time, diet 2 had the lowest concentration of ammonia nitrogen and propionate, as well as the highest VFA content and cellulosolytic activity, which indicates the best conditions for the incorporation of ammonia nitrogen into microbial crude protein at the expense of energy from fiber digestion.

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Table 3. Daily averages of enzymatic and microbiological processes in the rumen of cows (M±m, n=3)

Indicators	Diet 1	Diet 2	Diet 3	Diet 4
pH	6.52±0.27	6.50±0.16	6.39±0.50	6.59±0.25
Ammonia nitrogen, mg%	18.9±5.16	14.36±1.36	15.5±3.20	15.82±3.93
VFA, mMol/100ml	15.1±1.31	20.06±2.07	13.3±4.70	13.5±4.16
Acetate, %	67.5±3.35	67.9±0.28	65.9±2.41	68.7±0.17
Propionate, %	20.6±2.40	18.7±0.36	19.0±1.23	19.5±0.68
Butyrate, %	11.8±0.95	13.3±0.65	15.1±1.18	11.8±0.51
Bacteria, 10^9/mL	5.9±0.92	5.75±0.45	6.1±0.47	6.5±0.55
Infusoria, 10^3/mL	257±102	240±55	312±17.5	165±45
Amylolytic activity, U/mL	22.7±2.65	21.7±2.64	18.2±3.47	24.1±2.37
Cellulosolytic activity, %	4.8±2.96	5.9±2.72	3.7±4.01	4.81±0.64

Analysis of changes in fermentation parameters in the rumen of cows for the first five hours after morning feeding showed that on all diets with a significant variation of absolute values there is a regular reaction of rumen microflora population in the form of synchronous, oppositely directed shifts in the concentration of ammonia and VFA. In the first hour after feeding, ammonia concentration decreases, and VFA concentration increases; in subsequent periods of time, the reverse trend is evident (Table 4). At the same time, the composition of VFA is also synchronized in time: the proportion of acetate decreases after feeding, while propionate and butyrate increases.

Table 4. Dynamics of fermentation indices in cow rumen

Time after morning feeding, hour	Diet 1	Diet 2	Diet 3	Diet 4
pH				
0	6.9±0.22	6.515±0.06	6.245±0.48	6.445±0.56
1	6.56±0.1	6.56±0.41	6.27±0.26	6.615±0.25
3	6.765±0.1	6.405±0.19	5.695±0.215	6.6±0.01
5	6.92±0.1	6.66±0.31	6.475±0.055	6.32±0.55
Ammonia, mg %				
0	14.7±1.2	21.78±1.4	15.4±2.1	13.8±0.56
1	13.8±1.18	15.54±0.56	12.67±4.13	15.4±0.7
3	13.3±1.3	20.3±3.7	14.56±1.26	14±2.1
5	17.3±2.1	21.41±1.68	12.88±4.62	15.68±2.38
VFA, mmol/100ml				
0	13.75±5.25	16.35±1.15	16.1±1.87	14.25±4.75
1	23±1.44	20±0.5	18.4±0.125	18.8±0.37
3	9±1.57	10.3±3.87	19.5±2.2	14.25±.25
5	7.5±1.1	16.5±3.0	13.8±4.37	16.1±3.12
Acetate, %				
0	71.4±2.09	70.7±1.3	69.6±2.08	71.3±1.28
1	66.9±3.74	65.90±0.79	64.9±2.83	66.2±0.34
3	66.3±5.25	67.7±0.79	64.3±1.95	68.6±0.55
5	65.5±6.52	67.3±0.44	64.6±2.79	66.3±1.60
Propionate, %				
0	18.1±0.11	18.2±0.58	17.8±2.2	17.9±1.45
1	21.3±3.12	21.0±0.99	22.2±1.71	21.9±0.71
3	21.7±3.31	17.8±0.17	18.7±0.22	18.4±0.13
5	21.3±3.28	17.8±0.65	17.25±0.81	19.6±1.87
Butyrate, %				
0	10.5±1.98	11.0±1.25	12.5±0.11	10.8±1.25
1	11.6±0.61	13.0±1.78	12.8±1.11	11.8±1.05
3	11.9±1.94	14.3±0.62	16.9±1.73	12.9±0.68
5	13.2±3.24	14.7±0.21	18.0±1.98	14.0±0.27

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The decrease in ammonia concentration observed in the first hour cannot be a consequence of dilution of rumen contents by consumed food, because in this case the effect would be manifested for other metabolites of rumen fluid. In our opinion, in the first hour after food intake there is intensive digestion of available carbohydrates of concentrated feeds and active involvement in microbial synthesis of ammonium nitrogen, which contributed to a slight decrease in its level in rumen fluid. In favor of this version is evidenced by the established change in the VFA ratio during this period - an increase in the proportion of propionate and a decrease in acetate.

The ability of the diet to maintain the necessary level of available nitrogen for microbial crude protein synthesis can be expressed in the ratio of ammonia and VFA concentrations in rumen fluid (Table 5). The highest values of this index were observed in the second diet in all studied time intervals after morning feeding. This may indicate that the DP/DHC ratio in the diet of these animals was close to optimal in terms of the efficiency of ammonium nitrogen involvement in microbial synthesis. At the same time, the energy supply of rumen microflora for protein synthesis from ammonia nitrogen during a long period between feedings depends on the availability of fiber for digestion, which is reflected in the dynamics of acetate content in rumen fluid. The average acetate content in VFA was the highest in the second group 67.9%.

Table 5. Ratio of ammonia and VFA concentrations in cow rumen

Period after morning feeding, hour	Diet 1	Diet 2	Diet 3	Diet 4
0-1	0.83	1.05	0.82	0.89
1-3	1.04	1.37	0.72	0.90
3-5	1.63	1.63	0.84	0.98
Average	1.17	1.35	0.79	0.92

The study of nitrogen fractions entering the 12-peritoneum of cows showed (Table 6) that the decrease in DP level at the same level of DHC in the diets during the periods of the experiment led to an increase in feed and microbial nitrogen entering the intestine, the efficiency of microbial synthesis only up to the level 3 of the diet. Further decrease in decomposability was accompanied by an increase in the intestinal intake of feed nitrogen, but a sharp decrease in microbial nitrogen. As a result, the sum of microbial and feed nitrogen approached the levels of the first diet. This shows an insufficient supply of available forms of nitrogen to microbial synthesis due to lower ATPH generation during amino acid fermentation compared to hexoses formed from carbohydrates. Therefore, exceeding DP rates in diets not only leads to overconsumption of feed protein, but also reduces metabolic protein formation, due to a decrease in microbial protein. In such cases, the efficiency of microbial synthesis in calculations should be reduced by 10%.

Table 6. Nitrogen metabolism and evacuation of nitrogen fractions from the antrum in cows

Indicators	Diet 1	Diet 2	Diet 3	Diet 4
Nitrogen intake with feed, g	353.1±21,6	352,4±58,1	352,0±44,3	352,8±25,8
Nitrogen intake into the intestine, g	337.2±9,27	347,2±65,3	384,4±3,08	335,8±52,5
including:				
ammonia nitrogen, g	7.2±1,81	5,1±1,82	4,1±0,17	3,03±1,32

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nonammonium nitrogen, g	329.9±7,45	342,4±3,26	380,3±22,3	332,7±46,2
endogenous nitrogen, g	49,4	51,3	57,0	49,9
microbial nitrogen, g	176,7±10,57	181,9±28,2	200,6±3,33	122,8±24,2
by the sum of digestible OM (DOM in sacco) and input at k synthesis of 25g:				
total, g	256	259	249	256
feed nitrogen, g	103,7±16,91	109,1±25,8	122,6±6,10	160,0±0,96
microbial + feed, g	280,4	291	323,2	282,8
Synthesis efficiency: g nitrogen/kg DOM	27,2±1,56	29,5±3,85	30,8±2,57	26,3±2,43
Degradable of SP rations:				
by nitrogen balance, %	71,3±4,67	71,2±1,6	66,1±1,68	57,8±0,6
by incubation and evacuation, %	73,5	70,2	63,4	58,9

Ammonia can be a major source of nitrogen for bacterial protein synthesis and this capacity allows the use of non-protein sources of nitrogen such as urea-an inexpensive supplemental source of nitrogen in cattle diets. However, bacteria that ferment nonstructural hydrates, the major bacterial population in carbohydrate-fed cattle diets, preferentially obtain nitrogen from peptides and amino acids rather than ammonia [20]. The growth of starch fermenting bacteria is enhanced in the presence of peptides and amino acids [21]. Therefore, the source of degradable protein for microbial synthesis in the form of urea was inferior to native sunflower meal in our experiments in terms of efficiency of incorporation into microbial protein. Similar patterns were observed when studying the efficiency of microbial synthesis in the experiments of other researchers. Thus, isonitrogenous replacement of urea nitrogen by rapeseed meal led to an increase in synthesis efficiency from 25.8 to 29.39 g/kg of digestible organic matter [10].

Analysis of blood biochemical parameters showed that the level of blood metabolites, the main precursors of milk, corresponded to the stage of lactation and productivity. Biochemical and hematologic blood parameters of experimental animals were within acceptable deviations of the physiological norm.

Increase in the share of DHC to breakdown protein in diets was accompanied by a decrease in urea concentration at the constancy of carbohydrate components and did not lead to metabolic disorders in the body of cows (Table 7).

Table 7. Biochemical and hematological parameters of cow blood, 3 hours after feeding (M±m, n=3)

Indicators	Diet 1	Diet 2	Diet 3	Diet 4
Biochemical				
Total protein g/l	75.8±5.8	66.45±3.95	73.4±0.2	70.10±3.40
Albumin g/L	22.45±0.15	23.05±0.95	22.3±0.9	22.85±0.25
Creatinine mmol/L	66.9±3.52	80.53±1.04	68.7±6.6	40.75±7.83
Uric acid µmol/L	79.49±1.23	81.79±1.58	80.0±0.1	79.78±3.20
Urea mmol/L	3.11±0.4	2.60±0.235	2.3±0.01	2.45±0.24
Lactate dehydrogenase	3.09±2.13	3.605±0.035	4.5±0.6	5.21±1.26
Glucose mmol/L	3.6±0.185	3.34±0.39	3.5±0.2	3.70±0.01
Triglyceride mmol/L	0.16±0.01	0.18±0.025	0.1±0.0	0.17±0.04

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Bilirubin direct µmol/L	0.84±0.01	0.99±0.02	1.0±0.03	0.98±0.01
Total bilirubin µmol/L	2.95±1.57	1.54±0.06	1.7±0.3	1.38±0.18
HDL Cholesterol mmol/l	0.51±0.04	0.55±0.09	0.6±0.01	0.64±0.03
Cholesterol mmol/L	2.01±0.03	2.82±0.33	2.5±0.04	3.27±0.41
LDL Cholesterol mmol/l	0.99±0.19	1.83±0.21	1.6±0.1	1.83±0.25
Alanine aminotransferase IU/L	19.45±3.75	23.65±7.25	19.7±2.1	27.40±3.30
Aspartataminotransferase IU/L	50.15±4.45	54.7±3.2	50.7±2.6	52.0±3.45
Alkaline phosphatase mmol/L	98.5±5.1	85±5.0	55.5±0.5	55.50±1.8
glutamyltransferase IU/L	27.85±1.15	21.1±1.1	28.2±1.0	21.55±1.35
Amylase IU/L	53±10.0	42±5.0	52.5±12.5	39.50±2.50
Creatine kinase IU/L	0.87±0.095	0.48±0.15	0.9±0.3	0.55±0.14
Chlorides µmol/L	104.5±1.55	104.55±1.75	101.9±1.9	101.6±3.35
Magnesium mmol/L	1.16±-0.185	1.16±0.21	0.9±0.1	1.04±0.05
Iron mmol/L	40.32±24.66	59.06±26.35	29.4±1.5	44.25±10.04
Calcium mmol/L	2.44±0.05	3.32±0.59	3.0±0.22	2.35±0.30
Hematologic				
Leukocytes,10^9/l	7,3±0,78	6,4±0,2	7,4±1,31	9,9±0,55
Erythrocytes,10^{12}/l	6,2±0,25	5,6±0,145	5,85±0,07	5,52±0,06
Hemoglobin, g/l	106±0,01	91±3,0	101±2,0	91±1,0
Hematocrit,%	28,5±0,03	24,6±0,65	25,7±1,1	23,8±0,3
Platelets,10^3/l	489±62,5	425±10,0	395,5±21,5	494±77,0
Lymphocytes,10^3/l	8,2±0,03	3,45±0,05	4,9±2,0	8,6±0,2

Data from balance experiments showed that a decrease in the DP/DHC ratio leads to a substantial reduction of nitrogen excretion with urine. Nitrogen excretion with urine from the accepted by periods of experience had the following values: 21.7; 15.6; 16.23; 15.6 %. The use of "protected urea had a significant effect on protein digestibility, which decreased by 7.4% in the second period and by 7.1% in the fourth period.

4 Conclusions

As a result of the work carried out on the study of the peculiarities of the functioning of the digestive processes in the pregastric of cows and substrate formation in highly productive cows at different ratios of readily available carbohydrates and degradable and soluble proteins in the feed, it was established that at the level of rumen digestion, in the studied ranges of changes, the marked dependence of microbial activity and fermentation processes on nutritional factors leads to significant changes in microbiological processes in the rumen, but little modification of the colostrum.

The experimental data obtained allowed us to draw the following conclusions:

1. It is established that with a gradual increase in the proportion of soluble and degradable protein fractions in the feed, there is a natural increase in the formation of ammonia in the

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rumen and urea content in the blood, and when the ratio of degradable protein fractions and readily available carbohydrates in the feed reaches 0. 44, there is maximum efficiency of microbial synthesis and provision of available amino acids for the synthesis of milk constituents, as evidenced by the increase in the concentration of amino acids in the blood and the formation of milk protein, and contributes to a more complete synthesis of milk constituents.

2. Feeding lactating cows' diets with a higher ratio of crude protein to DHC (above 0.44) reduces the efficiency of feed nitrogen utilization by reducing the efficiency of microbial protein synthesis by 10%.

The new experimental data obtained will be used for further development of physiological criteria of nutritional adequacy of high yielding dairy cattle diets.

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