

# PHYSIOLOGICAL BASIS FOR NORMALIZATION OF PROTEIN-AMINO ACID NUTRITION FOR DAIRY COWS\*\*

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**Abstract:** Based on a range of experiments we show the need and recommend practical way for optimization of amino acid nutrition for dairy cows. We calculated the requirements in available amino acids and the supply of these amino acids by feeding rations.

## Introduction

Last 20 years of research of new feeding systems mainly focused on the problems of protein nutrition. The index of decay of raw protein of the feed in rumen was the basis for all these systems. Based on this index many countries developed and brought into practice progressive systems of protein nutrition of cattle with high-productivity (ARC, NRC, INRA). The similar system was created in Russia as well (1,2). But after the development of these systems the intensity of research significantly decreased and lots of questions stayed poorly investigated. Nevertheless, during this period a lot of data were accumulated, which allow to improve the existing systems.

For highly productive cows questions of amino-acid nutrition as well as protein nutrition are of a special importance. With equal content of protein and its fractions (dissociated and non-dissociated protein) in ration, dairy productivity and efficiency of use of metabolic protein will depend on the balance of amino-acids mix which enters into the blood from digestion. If in nutrition of monogastric animals (poultry, pigs) the problem of amino-acid nutrition is successfully resolved by the use of synthetic acids in compound feeds, for ruminants the only amino acids coming from fore stomachs with microbe mass or protein that was not degraded hold nutritional value while "non-protected" synthetic amino acids are destroyed by rumen micro organisms. The attempts of transferring of ration balancing for amino-acids from monogastric animals

to ruminants can not be successful because of a range of conversions of amino acids in rumen (different dissociation speed and desamination of separate amino acids). Thus, the speed of dissociation of separate amino acids in fraction of insoluble dissociated protein of sunflower meal may be 3 times different (ex., arginine-2,0%/hour, lysine 6,2%/hour). At the same time at the speed of dissociation of methionine 2,5%/hour, desamination of arginine takes place nearly 30% faster. But till the present moment in our country the development of norms of amino acid nutrition for dairy cows their transformation in Gastro Intestinal Tract (GIT) and bioavailability was not taken into account in our country though these types of investigation are on-going. That is why the existing norms of the content of certain essential amino acids do not have sufficient physiological substantiation and enough of practical meaning.

Requirements in amino acids are provided by the digestion of proteins which enter into the gut from fore stomachs. For calculation and forecasting of the amino acid input from the gut into the blood one should have data about the digestion and amino acid content of all the fractions of protein. The supply of feed protein into the gut depends on its dissociation in the rumen, and the absorption depends on the digestion and amino acid content of non-dissociated protein of separate individual foods. The investigation of this problem did not solve it satisfactory. In the world literature there are contradictory data about amino acid content of non dissociated protein and about the degree of digestion of individual amino acids in the gut.

In cows nutrition there is an unresolved problem of ration balance for ruminants with available essential amino acids. There were some attempts to develop appropriate approach were made but were not fully developed (3). Insufficient development of these approaches and absence of calculations to satisfy the requirements by rations often cause ambiguous results when one takes attempts to balance amino acids. Protected amino acids appeared on the market, but with insufficiently developed schemes of calculations, they do not provide the optimum result for consumers. Thus the use of these preparations is not financially beneficial for consumers after all. So, for the correct use of these preparations one should know the requirements of animals in amino acids and the content of amino acids in the feeds of rations and feed additives with regards to their availability. And only on this basis one can provide the satisfaction of the needs in amino acids. For example, the determination of bioavailability of "protected" methionine of 8 commercial preparations showed that in 6 of them its availability was from 0 to 75% and only in 2 preparations it was in accordance with the regulations of manufacturer (4).

The goal of our research was the experimental proof of the method of calculation of requirements of dairy cows in metabolic amino acids and determination of the availability of amino acids for absorption.

## Methods and materials

Experiments were performed on lactating cows with cannulas of rumen and a duodenum, vessel cannulas and blood flow sensor installation. Cows were kept on different typical rations. Main stages of transformation of amino acids in organism of dairy cows were investigated: absorption, income into the blood in a free form and with the mass of erythrocytes and the use of milk proteins in synthesis with the purpose of examination of supply of animals with limiting amino acids. Dissociation of protein of different feeds was determined by "in sacco" method (5). The mobile nylon bag technique (6) has made it possible to evaluate the digestibility of non-degraded protein and amino acids for the variety of feeds. The content of amino acids of protein fractions was examined on the amino acid analyser after preliminary hydrolysis.

Scientific and economic experiment was carried out on the lactation cows of black-partycoloured breed with yield of milk 8000 and 6000 kg. 4 groups of 10-15 animals were formed at the beginning of lactation by the method of pair analogues. The ration was hay-silage-concentrate.

## Results of investigation

### *Requirements in essential amino acids for main physiological functions.*

Requirements of ruminants in protein are considered as requirements in necessary quantity of available for assimilation amino acids. It is satisfied by their joint supply with the protein of microorganisms of rumen, non-dissociated protein and endogenous protein. Lately requirements of ruminants in protein were considered as requirements in essential amino acids. Taking this into account, we carried out the analysis of distribution and efficiency of each essential amino acid in offspring, uterus with pericarp waters, milk production, and thermo production.

The analysis of amino acid content of milk received from cows of different productivity and different lactation phases (beginning, middle and end phase of lactation period) and different breeds showed that the differences are not significant and in calculating the requirements one should use the average data of amino acid content (table 1). The amino acid content of weight gain of cows in different lactation phases is also rather constant.

**Table 1. Amino acid content in weight gain and milk gain as well as regular maintenance of dairy cows, g/1000g****Tabela 1. Sadržaj amino kiselina i prirastu mase i mlečnosti kao i redovnog održavanja mlečnih krava, g/1000g**

Amino acid/Amino kiselina	Gain/Prirast	Maintenance/Održavanje	Milk/Mleko
Lys	10,8	0,21	2,6
His	3,7	0,08	1
Arg	9,6	0,15	1,3
Try	1,5	0,02	0,5
Thr	7,3	0,11	1,6
Cys	1,9	0,03	0,5
Val	7,6	0,12	2,2
Met	3,1	0,05	0,9
Ile	6	0,09	2,5
Leu	10,6	0,16	3,5
Phe	5,8	0,09	2,2

The investigation of amino acid content of embryo and uterus with amniotic waters in cows different pregnancy stages showed that with growth the amino acid content of embryo is changing and because of that the requirements of the animal in separate individual amino acids may also change according to the phase of pregnancy (Table 2).

As a result of accumulation of data received earlier and from literature review we determined the pattern of deposition of irreplaceable amino acids in embryo according to stages of pregnancy. To determine the requirements of cows in individual amino acids one should take into consideration the milk productivity, protein content in milk, body gain and the phase of pregnancy on the basis of given data.

Requirements of cows in essential amino acids are determined taking into account the content and efficiency of the use of every essential amino acid for the deposition in gain, embryo, uterus with amniotic waters, milk production and heat production.

**Table 2. Deposition of amino acids in the embryo, g/1000g**  
**Tabela 2. Deponovanje amino kiselina u embrionu, g/1000g**

Amino acids/ Amino kiseline	The phase pregnancy, month/				
	2	3	6	7	8
Lys	4,5	5,4	7	7,4	7,3
His	1,5	1,7	2,1	2,5	3
Arg	4,3	4,4	6,6	6,7	6,9
Try	3,4	3,2	3,7	4,9	5,1
Thr	0,8	1,2	0,7	1,2	2,8
Cys	2,5	3	3,1	3,3	4,5
Val	0,6	1,2	0,7	1,6	3
Met	1,8	2	2,2	3,1	4,7
Ile	5	6,5	5,78	8,4	9,2
Leu	3	3,2	3,4	5	5
Weight of em- bryo, g	120	530	2550	7630	20000

To verify the requirements in amino acids calculated with the factorial method the physiological experiments were carried out on cows with the productivity of 25 kg of milk in order to evaluate the supply of the amino acids from the digestion and their consumption by milk gland while changing the supply of metabolic processes by individual amino acids (lysine, methionine, histidine, leucine, phenylalanine) by the way of infusion of different doses into duodenum during 5 days. Supply of synthesis of milk protein was evaluated on the basis of a complex of data on concentration and absorption of key metabolites of milk gland, milk protein production and the efficiency of its appearance. On the basis of obtained data we have specified the requirements of metabolic amino acids for the synthesis of 1 kg of milk with the protein content of 3.4 % (Table 3). On the whole the data coincided very well with the data received from the previous investigations (1).

So, the factorial method of calculation of the requirements of cows in available amino acids precisely reflects the true requirements of animals and on its basis one can introduce the practical norm of protein and amino acid nutrition of highly productive dairy cows.

**Table 3. The requirements in individual available amino acids for the synthesis of 1 kg of milk (3.4 % of protein) with the balanced content of metabolic protein of lactation cows (g/kg)**

**Tabela 3. Potrebe pojedinačnih amino kiselina za sintezu 1 kg mleka (3.4 % proteina) sa balansiranim sadržajem metaboličkog proteina krava u laktaciji (g/kg)**

Amino acids/Amino kiseline	Norm/Norma (1)	Own data/ Sopstveni podaci
Met	1,04	0,93
His	1,07	1,19
Lys	2,95	3,34
Leu	3,64	3,30

*Supply of animals by the metabolic protein and essential amino acids.*

Calculation of the supply by amino acids during the investigation is based on the data about the supply of amino acids into blood from the digestion, which are received from the experiment on the operated animals by the difference of entered amino acids into the gut and exited with excrements. But, with this method one can only evaluate the total quantity of metabolic protein and amino acids in ration, and for practical ration balancing the data about their content in individual feeds should be used.

Nowadays there are 2 approaches to the determination of bioavailability of amino acids from individual feeds or preparations:

- by the escape of amino acid from the digestion (in vivo, in sacco), or
- by its appearance in blood (4).

We have compared these two approaches. In the first case we have incubated the samples of the preparation "Smartamine" (methionine preprotected from the dissociation in rumen) in rumen in nylon kits (sacks) and further determined its digestion in gut by the method of mobile bag. The availability was calculated by the following formula: availability = after-rumen digestion (%) x (100-rumen digestion in sacco (%))/100.

In the second case we have used the method based on the changes in blood concentration of the investigated amino acid during feeding of preparation and calculation by the calibrating curve of amino acid concentration in blood with the infusion of different doses of the investigated amino acid into the thin gut. The investigations were carried out on the lactating cows in the second lactation phase with daily yield of milk 15-17 kg, kept on hay-silage-concentrate ration.

Calibrating curve was linear in the range of doses from 0 to 30 g of methionine and regressive equation was the following: The quantity of methionine

income =  $29,74(\pm 4,61) \times$  concentrated methionine (mg%) -  $7,35 (\pm 3,94)$ ;  $R=0,988$ .

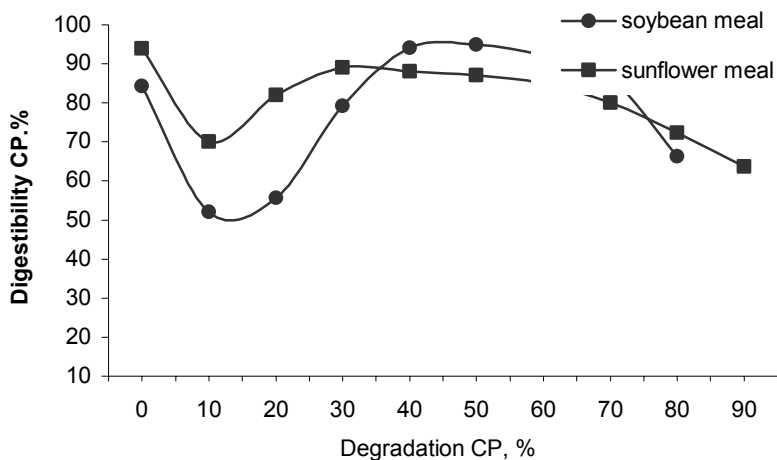
The calculation of availability in the first variant was 79,2% (with 3% of dissociation in rumen and 81,7% of digestion in gut), and in the second variant it was - 79,7%. In the experiments of other researchers the results were higher in the use of in sacco method in comparison with the second method.

The approach of determination of bioavailability on the basis of rumen and gut incubation was used also for preparations of vitamin A. The investigation of three commercial preparations showed that the availability of absorption of vitamin A differed several times (Table 4).

**Table 4. Availability of preparations of vitamin A for absorption in the gut.**  
**Tabela 4. Dostupnost preparata sa vitaminom A za apsorpciju u crevima**

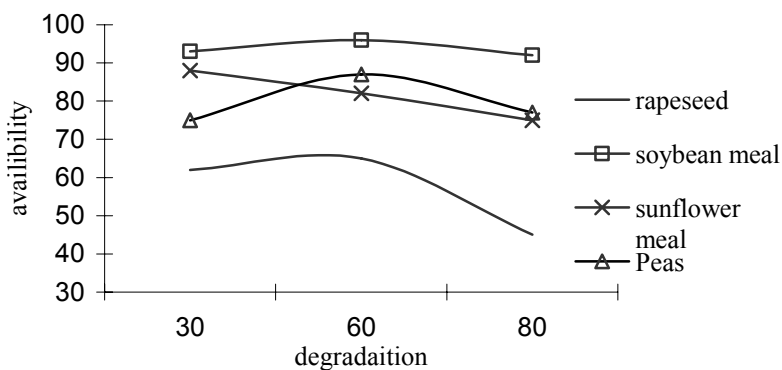
Preparations/ Preparati	Degradation in rumen/ Razgradnja u buragu, %	Intestinal digestibility/ Svarljivost u crevima, %	Availability for absorption/ Dostupno za apsorpciju %
1	94,5±0,53	98,3±0,11	5,3
2	93,4±0,48	67,5±0,25	4,4
3	16,2±0,89	99,1±0,01	83,0

Only knowing the bioavailability of amino acids from the preparations of protected amino acids one can use them directly to determine the rates of the amino acid nutrition norms of ruminants.



**Figure 1. Dependence of the digestion of protein feeds from the degree of their dissociation in rumen**

**Slika 1. Zavisnost varenja proteinskih hraniva od nivoa njihove razgradnje u buragu**



**Figure 2. Availability of Val for absorption in depending of degrees degradation in rumen.**

**Slika 2. Dostupnost Val za apsorpciju zavisno od nivoa razgradnje u buragu**

*Digestion of feed protein and microbe protein in the gut of ruminants.*

For the calculation of the absorption of feed amino acids it is known the dependence of their digestion in the thin gut from the degree of preliminary dissociation in rumen. We have established the parabolic dependence of protein digestion in the gut and the degree of its dissociation in rumen (Figure 1) and this dependence is also true for separate individual amino acids (Figure 2).



Using the method of feeds incubation in rumen and gut we have developed a scheme of calculation of any amino acid income from the given feed of any typical ration. Data of protein and amino acid availability received from the calculation of protein and amino acid income absorbed from the gut are shown in the Table 5. In rations caused lower protein dissociation in rumen, protein and amino acid availability for absorption in the gut increases for 2-3 times. At the same time it is evident that pears and raps even with low dissociation of their protein are not good sources of protein and amino acid availability for the organism of ruminant, because only 15 % of their protein may be absorbed in the gut and from soybean meal more than 50 %.

On the basis of this method we have determined the availability for the absorption of main essential amino acids from different protein feeds (Table 6), which allows to balance rations by amino acids taking into account their availability and to increase the use of protein of feeds for milk formation.

**Table 5. Availability of protein and amino acids (in %) for absorption in the gut in the rations which provide low and high dissociation**

**Tabela 5. Dostupnost proteina i amino kiselina (u %) za apsorpciju u crevima u obrocima koji obezbeđuju slabu i jaku disocijaciju**

Parameters/ Parametri	Peas/Grašak		Sunflower meal/ Suncokretova sačma		Soybean meal/ Sojina sačma		Rapeseed/ Uljana repica	
	degradation, %							
Availability/ Dostupnost	95	79	80	74	68	44	82	67
Essential amino acids/ Esencijalne amino kiseline	3,5	17,2	10,9	22,3	31,4	56,5	4,3	13,4
Non essential amino acids/ Neesencijalne masne kiseline	3,7	11,7	10,1	21,5	31,9	55,9	3,6	12,6
The sum amino acids/ Ukupne amino kiseline	3,6	14,3	10,5	21,8	31,6	56,2	3,9	12,9
Protein/Protein	3,1	16,2	13,1	17,7	29,6	51,4	3,1	15,0

**Table 6. The content of available amino acids in protein feeds (g/kg)**  
**Tabela 6. Sadržaj dostupnih amino kiselina u proteinskim hranivima (g/kg)**

Feeds/Hraniva	Amino acids/Amino kiseline			
	Met	His	Lys	Leu
soybean meal, dried/ sojima sačma, sušena	3,2	6,3	16,5	12,3
sunflower meal/ suncokretova sačma	1,9	2,5	1,7	5,4
sunflower meal, pro- tected/suncokretova sačma, protektirana	3,1	8,6	14	16
corn gluten/kukuruzni gluten	4,7	5,1	4,5	55,9
blood meal/krvno brašno	5,8	19,2	37,6	24,3

*Practical realisation of the approaches which allow to fix rates protein-amino acid nutrition of dairy cows.*

For practical use of the evaluation of protein and amino acid availability for the absorption in the gut, we have developed the scheme of calculation based on the data of their income into the gut and of the degree of release of every amino acid from fractions of protein entered into the gut (bacterial, protozoa and non-degraded feed protein). Calculation of feed amino acids income into the gut is based on the data of amino acid content of fractions of feed protein, the speed of dissociation of separate individual amino acids from insoluble dissociated fraction and the speed of evacuation of feed particles from rumen. The use of this approach is caused by the fact that the systems of calculation of income of the feed amino acids into the gut (3), which were proposed earlier were based on the data of the constant content of undissociated part of feed protein. Our data show that the content of this fraction may have different variations depending on the degree of dissociation in the rumen. With the changes of dissociation of protein of a given feed which are influenced by the rumen environment, variations appear not only in total entering, but also in correlation of amino acids.

We have made the comparative evaluation of provision of feeds by amino acids on the basis of calculated data and on data received from the direct experiences made on animals. The calculated method of the evaluation of amino acid provision of organism of lactating cows is based on the determination of quantity of amino acids, coming to the gut, from the statement about the constant of amino acid content of non-dissociated part of protein (method 1). In our modified method we take into account the amino acid content of protein fractions, the speed of dissociation of separate individual amino acids and the speed of outflow of feed particles (method 2). The results of investigation showed that

the second method allows to receive data, which are more comparable with the results of experiments carried out on animals (Tables 7, 8). This method allows to make good forecasts of amino acid dissociation in rumen (Table 7) and their general digestion (Table 8) and may be recommended for the practical use.

**Table 7. The income of feed amino acids into the gut (g/days)**

**Tabela 7. Dospevanje amino kiselina iz hraniva u creva (g/dana)**

Amino acids/Amino kiseline	Calculation/Kalkulacija		Experimental date/Eksperimentalni podaci
	method 1	method 2	
Met	13	15	15
His	31	22	20
Lys	63	56	57
Leu	101	59	53
Phe	48	43	44

**Table 8. General provision of cow organism by amino acids (g/days)**

**Tabela 8. Opšte odredbe organizma krava u odnosu na amino kiseline (g/dana)**

Amino acids/Amino kiseline	Calculation/Kalkulacija	Experimental date/Eksperimentalni podaci	Requirement/Potrebe
Met	29	32	28
His	29	27,5	32
Lys	96	94	85
Leu	94	85	96
Phe	64	58	56

The practical use of described approaches – factorial determination of requirements in protein and amino acids and the calculation of their provision from the feeds of ration was approved by us in scientific and economic experiment. On the basis of determination of methionine requirements and taking into account its income with the feeds of ration the norms of input of preparation were calculated for the ration of dairy cows, from the availability of methionine from the given preparation (20% from the requirements).

For 100 days of lactation from 40<sup>th</sup> to 140<sup>th</sup> days milk production in experimental group increased by 2,3%, protein content in milk – for 4,1, and output of milk protein – for 6,5% (Table 9).

**Table 9. Milk production of cows**  
**Tabela 9. Proizvodnja mleka**

Treatments/Tretmani	Control/Kontrola	+Met
Milk yield/Prinos mleka, kg/d	28,6±0,81	29,3±0,86*
Fat/Mast, %	3,65±0,07	3,65±0,077
Protein/Protein, %	3,13±0,05	3,26±0,042*
Fat/Mast, g/d	1045±31,89	1071±60,59
Protein/Protein, g/d	895,9±21,68	954,5±20,40*

- differences are true with the meaning of  $p < 0,05$  by the method of pair comparisons.

So, the practical ability of optimization of amino acid nutrition for dairy cows on the basis of calculation of the requirements in metabolic or available amino acids and feed provision of ration is shown.

On the basis of the researches executed by us norms of requirement of dairy cows are checked up and specified in a number of essential amino acids. It is established, that alongside with maintenance of an organism of cows with a metabolic protein, the important value should be given the contents in it of some essential amino acids. Check of an a suggested way of an estimation of availability of amino acids for absorption, their disintegration based on degradation in rumen and mastering in intestines, has shown good comparability to experimental data. The results of researches testify, that definition of availability of nutrients of forages to mastering or digestion allows to predict productive effect, messages the proved optimization of diets, establishing norms in view of this parameter.

## **Fiziološka baza za normalizaciju ishrane proteinima – aminokiselinama mlečnih krava**

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### **Rezime**

Na osnovu niza oglada pokazali smo potrebu i preporučili praktičan način za optimizaciju ishrane amino kiselinama kod mlečnih krava. Izračunali smo potrebe u dostupnim amino kiselinama i njihovo snabdevanje u obrocima.

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