



## THE METHOD OF CORRECTION OF AMINO ACID METABOLISM OF COWS IN THE AREA OF MICRONUTRIENT DEFICIENCY IN UKRAINE

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**ABSTRACT.** Using the original method the new effective "Pankorm" feed supplement was developed, made from a pig's pancreas after extraction of insulin from it. The aim of the study was establishment of preventive action effectiveness of the new "Pankorm" feed supplement for cows in case of amino acid metabolism disorder in their bodies in the area of micronutrient deficiency. For the purpose of conduct of the research a group of 30 cows was formed on the basis of similarity. The control group consisted of animals that received the basic ration. The cows received the feed supplement daily during two months of winter housing season in the amount of 20 percent of the total amount of concentrated feed. The animals that received the "Pankorm" feed supplement during 1.5 months demonstrated a significant reduction of signs of iodine, cobalt and copper deficiency, and normalisation of pulse and breathing rate.

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### Introduction

Protein is an extremely important component of nutrients necessary for a body. Its exceptional role in nutrition of animals is explained not only by the fact that it is a source of nutrients and energy, but also by the fact that the basis of biological process of any cell is protein synthesis that is possible only using amino acids, which in turn are the product of protein breakdown. It should be pointed out that a certain part of amino acids is not synthesised in the body of an animal, and their supply with feed is vitally important.

Solving the problem of amino acid nutrition of animals using only feed of animal origin and high-protein feed of plant origin is not possible. For example, analysis of the balance of concentrated feed shows that 64% of lysine is obtained from grain protein, 9.5% is obtained from plant protein concentrate (cakes, peas), 7.5% from protein of animal origin, and 5.5% of lysine is obtained by animal breeding industry from feed yeast, which in total amounts to 86.5% of the required amount. That's why the most promising method is balancing of rations by adding to them synthetic amino acids, first and foremost lysine and methionine, in order to reach the required standard (Oksamitny, 2004; Chernyshov, 2002; Dostoevsky, 2007; Korobko, 2003).

In the future the deficit of protein will constantly grow. The EU has already adopted fishing quota limits which many lead to reduction of production of fish flour and increase of its prices. The veterinary and sanitary requirements make the use of meat and bone meal problematic. That's why it is necessary to switch to the use of alternative sources of protein more actively. During the past decade the interest towards this issue has considerably increased.

The main conditions that determine effectiveness of the use of amino acids are the type and content of the ration, its caloric value, content and quality of protein, balance and amount of amino acids in the feed, provision of vitamins, especially group B vitamins, minerals, regularity and speed of intake of amino acids in combination with other agents of the feed (Livshin, 1961; Kurilov *et al.*, 1983; Georgiev, 1986; Khokhlov, Dotsenko, 2003; Medvedev, Kalantar, 1972; Kalnitsky, 1999).

The aim of the study was establishment of preventive action effectiveness of the new "Pankorm" feed supplement for cows in case of amino acid metabolism disorder in their bodies in the area of micronutrient deficiency.

## Materials and method

Scientific and production trials was conducted during the years 2009 – 2011 in the farm units of the Volyn and Rivne oblasts of Ukraine on black-motley breed cattle. The soil, water sources and rations of cows are characterised by deficiency of macro- and micronutrients and lack of balance of amino acid content.

For the purpose of conduct of the research a group of 30 cows was formed on the basis of similarity. The control group consisted of animals that received the basic ration. The cows received the feed supplement daily during two months of winter housing season in the amount of 20 percent of the total amount of concentrated feed 3.5-4.2 kg per day per cow. During the final stage of the experiment clinical examination of the animals according to the scheme proposed by M.O. Sudakov, and biochemical research according to standard methods were performed.

**Table 1.** Content of amino acids (%) in the feed supplement

Amino acid	%
Asparaginic	6.52
Threonine	2.90
Serine	3.48
Glutaminic	7.66
Proline	3.44
Glycine	3.94
Alanine	3.94
Cystine	0.90
Valine	3.69
Methionine	0.61
Isoleucine	2.88
Leucine	4.85
Tyrosine	2.69
Phenylalanine	2.93
Histidine	1.50
Tryptophane	1.60
Lysine	4.37
Arginine	4.77
<b>Total</b>	<b>62.67</b>

The results of the clinical study of cows and the laboratory analysis of blood and milk were processed using the variation statistics method using a personal computer ("Statistika" program). The following values were designated: the arithmetic mean value (M), statistical error of the mean value (m), the validation of the difference between the arithmetical mean values of two sets of variational values according to the validation criteria ( $p \leq$ ) and Student's tables, the mean square deviation (m). The difference between two values was considered as valid where  $p \leq 0.05$ ; 0.01 and 0.001. The correlation relationship between indications was designated according to the Pearson coefficient (r).

The study of condition of the phosphorus and calcium metabolism was performed with respect to the content in blood of the cows of total calcium (complexometric method with a reaction with murexide), inorganic phosphorus according to G. Briggs, modified by S.A. Ivanovsky, magnesium – using calmagite

indicator (a set of chemical agents of NPF "Simko Ltd.").

The content of microelements of soil and feeds was studied using the chromatography-mass spectrometry method.

## Results and discussion

Complete protein nutrition of animals is achieved by using feed enriched with protein supplements that contain the required amount of amino acids. The most widespread supplements are soybean cake, meat and bone meal, and fish flour (Kurilov, 1972; Shmanenkov, 1987; ShvakeI, 2008; Kucherenko *et al.*, 1986; Cooper, 1998; White *et al.*, 1981; Schmidt-Nielsen, 1982).

We have developed the new highly effective feed supplement obtained from a pig's pancreas after extraction of insulin from it. The feed supplement is certified with the developed and approved normative and technical documentation (TY 15.7.16308289.001.-2003). With regard to amino acid content has a higher and more balanced level of amino acids compared to conventional supplements. The total amount of essential amino acids in is 62.67% (Table 1), while their content in cake is 42.4%, meat and bone meal – 61.4%, and fish flour – 43.3%. One significant advantage of practical application of the new supplement is its price, which is much lower than the price of soybean cake, meat and bone meal or fish flour.

Clinical examination of the cows of experimental and control groups showed that the animals that received the feed supplement during 1.5 months demonstrated a significant reduction of signs of iodine, cobalt and copper deficiency, and normalisation of pulse and breathing rate. The numbers of erythrocytes and leukocytes were normal, in average amounting to 6.4 g/l and 7.6 g/l.

As a result of biochemical research of blood serum of the cows of the control group it was established that the content of total protein in average amounted to 82.4 g/l, total calcium – 2.8 mmol/l, inorganic phosphorus – 1.47 mmol/l, and the figures of alkaline reserve and activity of alkaline phosphatase amounted to, respectively, 48 vol. % CO<sub>2</sub> and 0.89 mmol/h\*1. Calcium- phosphorus balance amounted to 1.5. These figures correspond to the standard ones. There was also an evident increase in milk productivity of the cows amounting to 14-15.3 percent. The given morphological and biochemical figures of the animals of the control group were lower than the physiological parameters, and sings of biogeochemical endemia were clinically evident in case of 32.9 percent of the cows.

In order to study action of feed supplement on the main biochemical figures of blood of dairy cows, directly characterising not only amino acid metabolism, but also mineral metabolism, studies were conducted during the 60-day housing season in all biogeochemical zones of Ukraine.

At the beginning of the test (Tables 2 and 3), in all biogeochemical zones of Ukraine dairy cows had hypocalcaemia which was especially apparent with the

animals in the north-eastern and southern biogeochemical zones. Concentration of inorganic phosphorus and magnesium in blood of the cows was also reduced or remained at the level of the lower limit of physiological values. It should be noted that the content of the bone tissue matrix includes a high amount of organic acids, among which citric acid has an important place. The mechanism of calcification of bone tissue depends on the level of citric acid in it, which can be an indicator of sufficient amount of mineral elements in bodies of the animals (Fedorovich et al., 2013). In particular, the concentration of citric acid in blood of the cows kept in the western and north-eastern biogeochemical zones of Ukraine was  $152 \pm 4.30$  and  $175 \pm 2.65$   $\mu\text{mol/l}$  respectively, which is evidently 40.6% and 43.7% lower ( $p < 0.001$ ) than the corresponding figure of blood of the cows kept in the central and southern biogeochemical zones of Ukraine. This indicates that by the end of the test the content of citric acid in blood serum of the cows of the control groups in the western and south-eastern biogeochemical zones of Ukraine was typical for subclinical and clinical course of osteodystrophy. Increase in concentration of chondroitin sulfate and sialoglycoproteins was also established in blood of the cows of the control group. However, in blood serum of the cows of the southern biogeochemical zone of Ukraine these figures were within physiological fluctuations. Glycoproteins include sialoglycoprotein – a protein related to sialic acid which in turn is a part of composition of the connective tissue. Chondroitin sulfates together with hyaluronic acid serve as the basis of intercellular substance of organic matrix of bone and cartilaginous tissue.

It is for this reason that the concentration of chondroitin sulfate and sialoglycoproteins in blood serum is an objective and informative indicator of changes in bone tissue.

On the basis of content of general glycosaminoglycans it is possible to claim that there are disorganisation processes present in the organic matrix of bone tissue, as these biopolymers together with collagen are indispensable components of bone tissue that facilitate its ossification and structural organisation (Levtchenko et al., 2010).

Therefore, changes in content of general glycosaminoglycans and their fractions indicate clinical or subclinical course of osteodystrophy in dairy cows, and it can serve as an objective diagnostic test of mineral metabolism disorder in their bodies.

In case of dairy cows of the experimental group that consumed designated doses of feed supplement, positive changes in biogeochemical figures of blood serum that characterise processes of mineralisation and ossification of bone tissue were established.

Therefore, resulting from action of feed supplement in blood of the dairy cows of the experimental group, by the end of the test the figures of content of total calcium normalised from  $1.81 \pm 0.02$  to  $2.92 \pm 0.01$   $\text{mmol/l}$  in the southern biogeochemical zone;

from  $1.91 \pm 0.18$  to  $2.07 \pm 0.03$   $\text{mmol/l}$  in the northern-eastern biogeochemical zone of Ukraine. Concentration of inorganic phosphorus in blood serum of the cows of the experimental group normalised in all biogeochemical zones of Ukraine, and the most optimal level was registered in case of animals of the southern biogeochemical zone of Ukraine –  $1.65 \pm 0.02$   $\text{mmol/l}$ , which is 35% higher compared to the beginning of the test. Concentration of magnesium in blood serum of the cows of the western and south-eastern biogeochemical zones of Ukraine did not show evident changes and remained within the limits of physiological values. The most optimal level and evident increase in concentration of magnesium was established in blood serum of the cows in the central and southern biogeochemical zones of Ukraine –  $0.98 \pm 0.02$  and  $1.04 \pm 0.02$  respectively. The figures of content of citric acid by the end of the test were 4–4.5% lower in case of blood of the cows of the central and southern biogeochemical zones and evidently lower ( $p < 0.05$ ) in case of the cows of the western and northern-eastern biogeochemical zones of Ukraine.

In case of dairy cows of the experimental group that were using feed supplement in the amount of 20% of the total amount of concentrated feed, positive changes in biochemical figures of blood serum that characterise processes of mineralisation and ossification of bone tissue were established.

In particular, resulting from action of feed supplement in blood of the dairy cows of the experimental group, by the end of the test the figures of total content of calcium normalised from  $1.81 \pm 0.02$  to  $2.92 \pm 0.01$   $\text{mmol/l}$  in the southern biogeochemical zone; from  $1.91 \pm 0.18$  to  $2.07 \pm 0.03$   $\text{mmol/l}$  in the northern-eastern biogeochemical zone of Ukraine. Concentration of inorganic phosphorus in blood serum of the cows of the experimental group normalised in all biogeochemical zones of Ukraine, and the most optimal level was registered in case of animals of the southern biogeochemical zone of Ukraine –  $1.65 \pm 0.02$   $\text{mmol/l}$ , which is 35% higher compared to the beginning of the test. Concentration of magnesium in blood serum of the cows of the western and south-eastern biogeochemical zones of Ukraine did not show evident changes and remained within the limits of physiological values. The most optimal level and evident increase in concentration of magnesium was established in blood serum of the cows in the central and southern biogeochemical zones of Ukraine –  $0.98 \pm 0.02$  and  $1.04 \pm 0.02$  respectively. The figures of content of citric acid by the end of the test were 4–4.5% lower in case of blood of the cows of the central and southern biogeochemical zones and evidently lower ( $p < 0.05$ ) in case of the dairy cows of the western and northern-eastern biogeochemical zones of Ukraine.

Concentration of chondroitin sulfate and sialoglycoproteins in blood serum in case of the dairy cows that were kept in the western and north-eastern biogeochemical zones was evidently lower ( $p < 0.05$ ) by the end of the test, and in case of dairy cows that were

kept in the central and southern zones of Ukraine these figures tended to become lower, which indicates positive changes in the processes of ossification of bone tissue.

**Table 2.** Biochemical figures of blood of the dairy cows of the control group in different biogeochemical zones of Ukraine, M±m, n=10

Biogeochemical zone	Biochemical values					
	Total calcium, mmol/l	Inorganic phosphorus, mmol/l	Magnesium, mmol/l	Citric acid, mkmol/l	Chondroitin sulfate, g/l	Sialoglycoproteins, mmol/l
Western (Rivne Oblast)	2.48±0.42	1.25±0.16	0.99±0.04	152±4.30	0.38±0.02	2.54±0.07
North-eastern (Zhytomyr Oblast)	1.91±0.18	1.32±0.04	0.72±0.16	175±2.65	0.39±0.01	2.60±0.04
Central (Poltava Oblast)	2.27±0.21	1.67±0.03	0.84±0.17	256±3.47*	0.23±0.01*	2.01±0.02
Southern (Zaporizhia Oblast)	1.81±0.02*	1.22±0.05	0.84±0.08	251±2.94*	0.17±0.01*	1.93±0.02

\* p<0.001 – compared to figures of the cows in the western and south-eastern biogeochemical zones.

**Table 3.** Action of feed supplement on the main biochemical figures of blood of the dairy cows of the experimental group M±m, n=10

Biogeochemical zone	Biochemical values					
	Total calcium, mmol/l	Inorganic phosphorus, mmol/l	Magnesium, mmol/l	Citric acid, mkmol/l	Chondroitin sulfate, g/l	Sialoglycoproteins, mmol/l
Western (Rivne Oblast)	2.56±0.02	1.47±0.01	0.87±0.01	257±2.02*	0.24±0.01*	1.94±0.02*
North-eastern (Zhytomyr Oblast)	2.70±0.03*	1.55±0.02	0.91±0.01	251±2.15*	0.23±0.01*	1.92±0.02*
Central (Poltava Oblast)	2.64±0.03	1.50±0.01	0.98±0.02*	261±2.48	0.17±0.01	2.02±0.03
Southern (Zaporizhia Oblast)	2.92±0.01*	1.65±0.02*	1.04±0.02*	260±2.25	0.18±0.01	2.14±0.03

\* p<0.05 – compared to figures of blood serum of the cows of the control group

## Conclusions

It follows from the considerations presented above that the conducted research has shown highly effective preventive action of feed supplement for metabolic disorders and applying it to cows. The supplement includes 18 critical and essential amino acids. The feed supplement has a higher and more balanced level of amino acids as compared to conventional supplements. The feed supplement has optimum composition of the essential amino acids; the presence of lysine allows correcting metabolic disorders as well as to improve the amino acid metabolism, significantly improve the efficiency of nutrient use and energy intake, the economical use of feed proteins.

Resulting from action of feed supplement, by the end of the test the figures of content of total calcium, inorganic phosphorus and magnesium, as well as concentration of chondroitin sulfate and sialoglycoproteins in blood of the dairy cows of the experimental group were normalised in all biogeochemical zones of Ukraine, which indicates positive changes in mineral metabolism of the cows, including processes of ossification of bone tissue.

Application of feed supplement as a feed additive can increase body weight gain of animals by 1.5–2%. It significantly enhances the immune status of the body, identifies genetically determined productiveness of animals.

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