

**Research Article****Special Features of Complete Amino Acid Profile Formation of Porcine  
Organs when using Biologically Active Substances****Donnik I. M.<sup>1</sup>, Bepamyatnykh E. N.<sup>1,2</sup>, Isaeva A. G.<sup>1,2</sup>,****Krivotogova A. S.<sup>1,2</sup>, Shkuratova I. A.<sup>1,2</sup>, Loretts O. G.<sup>1</sup>,****Musikhina N. B.<sup>1</sup> and Dudkina N. N. H. H.<sup>2</sup>**<sup>1</sup>Federal State Budgetary educational institution of higher education  
"Ural State Agrarian University" (FSBEI HE Ural SAU),  
42 Karla Libknekhta St. Yekaterinburg, Russian Federation<sup>2</sup>Federal state budgetary scientific institution «Ural Federal Agrarian Scientific Centre,  
Ural Branch of the Russian Academy of Science» (FSBSI UrFASC, UrB RAS),  
112 A Belinskogo St., Yekaterinburg, Russian Federation

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**ABSTRACT:**

The article gives data on the composition of proteinogenic amino acids in the parenchymal organs of pigs grown under the conditions of man-made pollution. It also gives data on the changes in the amino acid profile of the porcine parenchymal organs after 14-day administration of two biologically active complexes different in composition. The article evaluates the positive and negative effects of the complexes on the amino acid metabolism in the porcine heart, liver and kidneys. The study was supported by the Russian Science Foundation (project No. 16-16-00071).

**Key words:** pigs, industrial pollution, irreplaceable amino acids, parenchymal organs, liver, kidneys, heart, biologically active complexes

**INTRODUCTION**

Under the conditions of man-made pollution, specific technologies are necessary to manufacture quality animal products with a complete amino acid profile. Special meaning is given to irreplaceable proteinogenic amino acids and vitamins that the animal organism must uptake with the feeding-stuffs or synthesize by their friendly intestinal microflora.

Vegetable feeding-stuffs produced in a climatic parameter of an area of risk farming are characterized by low biological value. This is caused, first and foremost, by insufficient exposure to sunlight and by a shortage of some essential micronutrients in the agrobiocoenosis, it reduces the productivity of feed plant cultures,

reducing their value in respect of proteinogenic amino acids, vitamins and minerals. Besides, an excess of some man-made elements in the environment leads to their accumulation in feed cultures, therefore, to contamination of the feeding-stuffs with these man-made pollutants. All these factors affect the organisms of productive animals, namely, the metabolism, physiology of the individual organs and systems, the composition and the activity of their intestinal microflora. Oppression of the normal intestinal microflora leads to a reduced uptake of irreplaceable amino acids and some vitamins by the organism. For example, heavy metals and other xenobiotics uptaken with the feed

inactivate the enzymes of the microorganisms participating in transformation of organic acids necessary for synthesis of many derivatives of  $\alpha$ -aminocarbonic acids.

Besides, in the animals suffering from a chronic toxic load caused by an excess amount of heavy metals in the feed, aminoacid consumption for operation of detoxification mechanisms of the liver (the glutathione system) increases. A depleted supply of individual amino acids leads to upsetting of the general aminoacid balance, to development of the total aminoacid deficiency and to protein oppression in the animal organisms under the conditions of man-made pollution.

That is why such importance is given to maintenance of normal balance between catabolic and anabolic processes in the organisms of productive animals, which is achieved by administration of biologically active complexes (BAC) that protect the organism from the effect of the negative factors. Normalization of the animal metabolism promotes fulfillment of the genetic productivity potential even in the context of man-made pollution of agrobiocoenoses.

## MATERIALS AND METHODS

An experiment in formation of complete proteinogenic composition of porcine parenchymal organ tissues was conducted in three groups of animals. The pigs were selected on the principle of analogues and were kept in the same technological conditions and fed a similar diet at a pig-breeding farm located in a region with pronounced man-made pollution. Each group consisted of 25 heads with the mean weight of 60 kg at the experiment start. The pigs of experimental group #1 received a biologically active complex that included cholecalciferol, pyridoxine, niacin, ascorbic acid, magnesium citrate, a complex of calcium and methionine hydroxy analogue (MHA) and lysine. The animals of experimental group #2 received a complex consisting of vitamin/mineral, oil and vegetable components. This complex included pyridoxine, niacin, ascorbic acid, magnesium citrate, a complex of calcium and methionine hydroxy analogue

(MHA), soy isolate, lysine, threonine, valine, tryptophan, flax, olive and sesame oil and the leaves of *Urtica dioica*. The complex was administered for 14 days. The animal blood specimens for clinical and biochemical tests were collected prior to the experiment start, and then on days 7, 14 and 34. After the experiment was completed, the pigs were slaughtered, and specimens of the parenchymal organs – the heart, the liver and the kidneys – were collected from all the animals.

The organs were homogenized and tested for the aminoacid profile using a LC-20 liquid chromatograph Prominence manufactured by firm Shimadzu with a spectrophotometric detector (254 nm); a C18 stationary reverse-phase column (Analysentechnik 250 x 4.6 mm, 5  $\mu$ m, Germany) and a relevant precolumn; the mobile phase was a mixture of 0.06 Mol/dm<sup>3</sup> of sodium acetate solution, pH=5.5 and 4.05 and 1% solution of propanol-2 in acetonitrile. The chromatographic test was performed in the gradient elution mode at the mobile phase flow velocity of 1.2 cm<sup>3</sup>/min. The reagents were standard amino acid specimens (Sigma), phenyl isothiocyanate (Sigma), acetonitrile (grade 0, CryoChrome), isopropyl alcohol (ACS), sodium acetate (ACS) hydrochloric acid (ACS.), sodium hydroxide (CP). The sample preparation for determination of the total amino acids included acidic hydrolysis with 6 Mol/dm<sup>3</sup> of hydrochloric acid solution at 110°C for 18 h. After cooling, the hydrolysates were filtered. The aliquots of hydrolysates were passed through the same test stages as the standard solutions. All the measurements were performed in duplicate (Dudkina N.N., et al, 2017). The obtained data were assessed with averaging and standard deviation calculation. Normality of sampling was determined using the Shapiro-Wilk test. The statistical significance of the differences among the groups in various indexes was evaluated using the ANOVA procedure (Gaussian distribution) and the Mann-Whitney test.

## RESULTS AND DISCUSSION

Analysis of the data obtained in the course of the experiment established that there were no

statistically significant changes in the composition of proteinogenic amino acids in the liver of the animals from experimental group #1. That said, a general trend toward reduction in the amount of amino acids in the liver was noted. The trend was not pronounced, which might have been related to stimulation of the metabolic processes in the liver occurring on the background of dietary protein deficiency and

for energy delivery to the cells. A statistically significant increase in the content of such amino acids as threonine by 74.8% and lysine by 60.9% in the liver was expectative and was determined by the composition of the complex (Table 1).

**Table 1.** Content of proteinogenic amino acids in porcine liver

Organ	Group	Units	Asp	Glu	Hyp	Ser	Gly	His	Arg	Thr	Ala	Tyr	Val
Liver	Comparison group (Mean)	g/100g	1,21	1,81	0,08	0,63	0,87	0,53	0,94	0,60	0,75	0,56	0,65
	St. dev.	g/100g	0,92	1,29	0,02	0,17	0,21	0,21	0,08	0,14	0,29	0,10	0,08
	Exp. group #1 (Mean)	g/100g	1,63	2,24	0,08	0,66	0,83	0,54	0,89	0,60	0,78	0,48	0,60
	St. dev.	g/100g	0,13	0,24	0,02	0,01	0,01	0,09	0,10	0,02	0,12	0,06	0,14
	Ratio #1 of comparison group	±%	34,8	24,3	5,3	3,8	-4,7	2,6	-5,0	0,7	4,5	-14,4	-7,4
	p		1,00	1,00	1,00	0,66	0,66	0,66	0,66	0,66	1,00	0,38	1,00
	Exp. group #2 (Mean)	g/100g	2,09	2,52	0,09	0,93	1,06	0,45	1,28	1,04	1,10	0,68	1,01
	St. dev.	g/100g	0,29	0,52	0,01	0,15	0,09	0,07	0,52	0,17	0,26	0,18	0,16
	Ratio #2 of comparison group	±%	73,3	39,6	17,5	46,1	21,5	-14,2	35,9	74,8	46,5	20,9	56,4
	p		0,19	1,00	0,38	0,19	0,38	1,00	0,38	0,05	0,38	0,38	0,05
	Group	Units	Met	Ile	Leu	Phe	Lys	Trp	BCAA	Trp/Hyp	Total essen. amino acids	Total nonessen. amino acid	Total amino acid
	Comparison group (Mean)	g/100g	0,43	0,57	0,79	0,81	1,00	0,14	2,01	1,85	4,99	7,37	12,36
	St. dev.	g/100g	0,09	0,07	0,11	0,12	0,41	0,01	0,24	0,28	0,65	3,01	3,65
	Exp. group #1 (Mean)	g/100g	0,43	0,49	0,77	0,75	1,11	0,13	1,85	1,59	4,86	8,14	13,00
	St. dev.	g/100g	0,06	0,09	0,07	0,12	0,15	0,05	0,29	0,80	0,60	0,52	1,11
	Ratio #1 of comparison group	±%	-0,1	-15,5	-3,2	-7,8	11,8	-11,7	-8,0	-13,9	-2,4	10,4	5,2
	p		1,00	0,38	1,00	0,66	1,00	1,00	0,66	1,00	1,00	1,00	1,00
	Exp. group #2 (Mean)	g/100g	0,32	1,04	1,21	0,87	1,59	0,18	3,26	1,93	7,27	10,19	17,46
	St. dev.	g/100g	0,12	0,08	0,12	0,23	0,27	0,04	0,36	0,50	1,12	2,01	3,09
	Ratio #2 of comparison group	±%	-24,8	81,8	52,3	7,6	60,0	24,8	62,1	4,6	45,8	38,3	41,3
p		0,38	0,05	0,05	1,00	0,05	0,38	0,05	1,00	0,05	0,38	0,19	

contamination of the feed with heavy metals. A trend toward a decline of the branch-chain amino acid amount in the liver was apparently related to an increase in their consumption for energy delivery to the cells under the conditions of nutritious substrates. Also, the expectative dynamics of methionine and lysine was absent in the liver of the animals from experimental group #1.

In contrast, a statistically significant increase of the total essential amino acid content by 45.8% as against the comparison group was detected in the liver of the pigs from experimental group #2. This fact is in the first place preconditioned by an increase in the sum of essential branch-chain amino acids (BCAA) by 62.1%. An increase of the valine content by 56.4%, that of the leucine content by 52.3% and that of isoleucine by 81.8% was detected. This effect was probably determined by administration into the diet of the pigs of a high-valine BAC whose consumption decreased leucine and isoleucine consumption

A trend toward a decline in the concentration of all the amino acids by 16.3% (including a statistically insignificant decline of dispensable amino acids by 10.4% and a significant decline in irreplaceable amino acids by 25.2%) was detected in the cardiac muscle of the pigs from experimental group #1. This fall of the concentration was caused by BCAA content decrease by 31.8%, all the three amino acids being in a significantly lower amount. A decrease in the concentration of valine was 39.3%, in that of leucine was 18.7% and in that of isoleucine was 41.7%. A decrease in the content of most amino acids in the heart of the animals from experimental group #1 was apparently conditioned by dietary protein deficiency and enhancement of its consumption. Protein consumption for the energetic supply of the cells increased against the background of eliminated restriction of enzyme synthesis for consumption of the vitamins in BAC as a cofactor. A decline in the amount of arginine by

Organ	Group	Units	Asp	Glu	Hyp	Ser	Gly	His	Arg	Thr	Ala	Tyr	Val
Heart	Comparison group (Mean)	g/100g	1,28	2,16	0,06	0,52	0,60	0,40	0,81	0,56	0,76	0,46	0,58
	St. dev.	g/100g	0,57	0,82	0,02	0,09	0,11	0,18	0,15	0,05	0,17	0,03	0,06
	Exp. group #1 (Mean)	g/100g	1,29	1,94	0,06	0,48	0,58	0,38	0,65	0,46	0,60	0,36	0,35
	St. dev.	g/100g	0,07	0,20	0,01	0,02	0,01	0,14	0,07	0,06	0,10	0,06	0,08
	Ratio #1 of comparison group	±%	0,5	-10,3	0,6	-8,8	-4,6	-4,2	-20,0	-17,5	-21,3	-22,7	-39,3
	p		0,66	0,66	1,00	0,66	0,66	1,00	0,19	0,05	0,38	0,05	0,05
	Exp. group #2 (Mean)	g/100g	1,75	2,30	0,06	0,67	0,71	0,36	1,00	0,77	1,01	0,52	0,70
	St. dev.	g/100g	0,06	0,08	0,01	0,02	0,01	0,01	0,05	0,02	0,13	0,02	0,03
	Ratio #2 of comparison group	±%	36,0	6,5	5,5	28,3	16,8	-10,9	23,6	37,2	32,6	12,0	20,0
	p		0,19	1,00	0,66	0,05	0,05	0,66	0,05	0,05	0,19	0,19	0,05
	Group	Units	Met	Ile	Leu	Phe	Lys	Trp	BCAA	Trp/Hyp	Total essen. amino acids	Total nonessen. amino acid	Total amino acid
	Comparison group (Mean)	g/100g	0,31	0,56	0,75	0,63	1,20	0,10	1,88	1,84	4,68	7,07	11,75
	St. dev.	g/100g	0,11	0,08	0,07	0,25	0,06	0,03	0,20	0,43	0,30	1,89	1,60
	Exp. group #1 (Mean)	g/100g	0,31	0,32	0,61	0,41	0,91	0,11	1,28	2,00	3,50	6,33	9,83
	St. dev.	g/100g	0,08	0,05	0,05	0,11	0,08	0,03	0,17	0,40	0,46	0,49	0,93
	Ratio #1 of comparison group	±%	2,8	-41,7	-18,7	-33,8	-24,1	10,0	-31,8	9,1	-25,2	-10,4	-16,3
	p		1,00	0,05	0,05	0,19	0,05	0,66	0,05	1,00	0,05	0,66	0,38
	Exp. group #2 (Mean)	g/100g	0,20	0,86	1,00	0,57	1,37	0,13	2,56	2,23	5,60	8,37	13,97
	St. dev.	g/100g	0,08	0,04	0,04	0,03	0,04	0,08	0,11	1,45	0,14	0,23	0,36
	Ratio #2 of comparison group	±%	-33,8	54,3	33,9	-8,3	13,8	23,2	35,6	21,4	19,5	18,5	18,9
p		0,38	0,05	0,05	0,66	0,05	0,66	0,05	0,66	0,05	0,66	0,05	

20% ( $p=0.19$ ) was also noted, which was probably conditioned by utilization of this amino acid in nitrogen oxide (NO) synthesis necessary for regulation of the arterial blood pressure. Besides, there was a growth of arginine consumption for the ornithine cycle, which was confirmed by a reduction in the amount of glutamic acid by 10.3% (Colombo J.P., 1979).

Along with that, a statistically significant increase in the sum of proteinogenic amino acids by 18.9% was noted in the hearts of the pigs from experimental group #2. This is related to an increase in the sun of irreplaceable amino acids by 19.5%. The main contribution was made by branch-chain amino acids, as their amount increased by 35.6% ( $p\leq 0.05$ ). The maximum increase as against the comparison group established for isoleucine was 54.3%, a less expressed one was established for leucine, 33.9%, and the minimum one, 20%, was established for valine. These results spoke for full energy provision of the cardiac muscle with the substrates for energy delivery to the myocardial cells. Along with that, an increase in the arginine content by 23.6% ( $p\leq 0.05$ ) was noted in the cardiac muscle of the pigs from experimental group #2, which was related to a decline in its consumption for synthesis of nitrogen oxide and ornithine (Table 2).

**Table 2.** Content of proteinogenic amino acids in porcine heart

A reduction in the concentration of all the amino acid groups was marked in the kidneys of the animals from experimental group #1. This effect was most pronounced concerning essential amino acids (a reduction by 22.0% as against the comparison group). That said, the total BCAA content in the kidneys was significantly lower by 26.5% than in the kidneys of the animals from the control group. A decline in the leucine level by 23.1% ( $p\leq 0.05$ ) was most significant, followed by that in the isoleucine level by 33.9% ( $p=0.19$ ) and by that in the valine level by 24.6% ( $p=0.19$ ). The concentration decrease of these amino acids in the kidneys of experimental animals could have been caused by a deficiency of energetic substrates and by activation of the gluconeogenesis processes in the kidneys (Mathews C.K. et al., 2000). Also, a reduction in the phenylalanine content by 23.6% and, consequently a reduction of tyrosine content by 22.9% were noted in the kidneys (Zbarskiy B.I. et al., 1972). A co-fall in the concentration of these amino acids is supposedly related to their consumption for synthesis of catecholamines and thyroid hormones.

In the kidneys of the pigs from experimental group #1, a double increase in the hydroxyproline concentration to 0.16 g/100g was detected. This result as well as a

simultaneous decrease in the tryptophan/hydroxyproline ratio by 57.6% speaks for substitution of normal renal tissue with collagen. This fact implies a nephrotoxic effect of the BAC used under the conditions of the essential amino acid shortage and chronic intoxication with heavy metals.

In the kidneys of the animals experimental group #2, an increase in the total amino acids as against the comparison group by 41.5% was detected. This event was accompanied by an increase in the sum of the dispensable amino acids by 38.2% and that of irreplaceable ones by 47%. The increase in the sum of essential amino acids was accompanied by a statistically significant growth of the total BCAA amount by 65.4%. The level of valine grew by 54.1% that

experimental group. The amount of aspartic acid grew by 6.1%, that of serine grew by 45.4%, that of glycine grew by 31.4%, that of arginine grew by 46.3%, that of threonine grew by 53.0% and that of alanine by 34.4%.

The hydroxyproline content in the kidneys of the given group of animals was 61.6% higher as against the indexes in the comparison group. A less expressed decline in the tryptophan/hydroxyproline ratio was also noted. These two facts spoke for minimization of the negative renal effects of the BAC administration in this group of animals (Burgoon K.G., et al., 1992) (Table 3).

**Table 3.** Content of proteinogenic amino acids in porcine kidneys

Organ	Group	Units	Asp	Glu	Hyp	Ser	Gly	His	Arg	Thr	Ala	Tyr	Val
Kidney	Comparison group (Mean)	g/100g	1,10	1,59	0,08	0,58	0,75	0,51	0,70	0,58	0,73	0,46	0,55
	St. dev.	g/100g	0,65	0,66	0,01	0,04	0,05	0,08	0,07	0,07	0,06	0,03	0,12
	Exp. group #1 (Mean)	g/100g	1,23	1,65	0,16	0,52	0,81	0,48	0,65	0,45	0,62	0,36	0,41
	St. dev.	g/100g	0,03	0,13	0,05	0,06	0,10	0,17	0,06	0,08	0,10	0,06	0,06
	Ratio #1 of comparison group	±%	12,7	3,9	114,1	-10,4	7,6	-6,6	-7,8	-23,0	-15,5	-22,9	-24,6
	p		0,66	0,66	0,05	0,19	1,00	1,00	1,00	0,19	0,38	0,05	0,19
	Exp. group #2 (Mean)	g/100g	1,85	2,18	0,12	0,85	0,99	0,42	1,03	0,88	0,99	0,56	0,85
	St. dev.	g/100g	0,20	0,26	0,01	0,11	0,11	0,05	0,10	0,10	0,15	0,05	0,07
	Ratio #2 of comparison group	±%	69,1	37,4	61,6	45,4	31,4	-17,7	46,3	53,0	34,4	21,4	54,1
	p		0,05	0,19	0,05	0,05	0,05	0,19	0,05	0,05	0,05	0,05	0,05
	Group	Units	Met	Ile	Leu	Phe	Lys	Trp	BCAA	Trp/Hyp	Total essen. amino acids	Total nonessen. amino acid	Total amino acid
	Comparison group (Mean)	g/100g	0,37	0,46	0,68	0,56	0,91	0,12	1,70	1,63	4,24	6,51	10,75
	St. dev.	g/100g	0,01	0,10	0,06	0,08	0,11	0,03	0,27	0,53	0,51	1,41	1,90
	Exp. group #1 (Mean)	g/100g	0,31	0,31	0,53	0,43	0,77	0,11	1,25	0,69	3,31	6,48	9,79
	St. dev.	g/100g	0,02	0,09	0,06	0,07	0,04	0,01	0,21	0,18	0,39	0,65	1,00
	Ratio #1 of comparison group	±%	-16,4	-33,9	-23,1	-23,6	-15,4	-12,0	-26,5	-57,6	-22,0	-0,3	-8,9
p		0,05	0,19	0,05	0,19	0,19	0,66	0,05	0,05	0,19	0,66	0,66	
Exp. group #2 (Mean)	g/100g	0,29	0,91	1,05	0,78	1,31	0,16	2,81	1,34	6,24	8,99	15,23	
St. dev.	g/100g	0,15	0,08	0,10	0,13	0,15	0,02	0,25	0,17	0,76	0,92	1,68	
Ratio #2 of comparison group	±%	-21,5	97,2	52,9	38,9	43,7	34,9	65,4	-17,8	47,0	38,2	41,7	
p		0,66	0,05	0,05	0,05	0,05	0,19	0,05	0,66	0,05	0,05	0,05	

of isoleucine grew by 97.2% and that of leucine grew by 52.9%. Along with that, a growth of lysine concentration by 43.7% and that of phenylalanine by 38.9% was observed in the kidneys of the animals from group #2. The increase in the amount of phenylalanine led to a decrease in the amount of tyrosine in the kidneys by 21.5%.

A growth in the concentration of practically all the dispensable proteinogenic amino acids was also detected in the animals of the second

It appears interesting that in all the studied parenchymal organs of the pigs from the experimental groups, a trend toward a reduction in the amount of methionine was detected. This fact may be related to administration of methionine as a methionine hydroxy analogue (MHA) in both cases. The organisms of the pigs kept under the conditions for chronic intoxication with man-made pollutants were probably unable to transform MHA into methionine efficiently, and MHA itself

interfered with the intake of the dietary methionine.

## CONCLUSION

The results obtained in the course of the experiment make it possible to arrive at the following conclusions. Administration of the first biologically active complex to pigs under the conditions of man-made pollution of the feeding-stuffs and the inadequacy of their protein profile carries certain negative effects. They are expressed in enhancement of the metabolic processes with a depletion of the irreplaceable amino acid pool in parenchymal organs. Administration of this complex to the animals in the context of protein deficiency and a chronic xenobiotic load leads to development of metabolic renal pathology. In that context, administration of the BAC containing only cholecalciferol, pyridoxine, niacin, ascorbic acid, magnesium citrate, the complex of calcium and methionine hydroxy analogue (MHA) as well as lysine has a number of restrictions. Its administration is acceptable only with the feeding-stuffs that have balance of the key amino acids.

Administration of the second biologically active complex consisting only of vitamin/mineral, oil and vegetable components is, on the contrary, appropriate and reasonable in pigs reared in regions with intensive man-made pollution. Our studies showed high efficiency of this BAC in maintenance of the aminoacid balance in the porcine parenchymal organs even 30 days after its application. However, one should take into account its potential nephrotoxic effect in case of an overdose. Under the conditions of industrial pollution, its synthetic analogue methionine hydroxy analogue (MHA) is inappropriate for administration to pigs as a methionine source.

## CONFLICT OF INTEREST

The authors confirm that the given data do not have any conflict of interest.

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