

Research Article**Amino acid Profile of Porcine Lean Tissue in the Context
of Man-Made Pollution**

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ABSTRACT:

The article gives data on the composition of proteinogenic amino acids contained in a number of porcine muscles, namely, the longissimus, the iliac muscle and the set of thigh muscles of animals bred under the conditions of man-made pollution. It also gives data on changes in the amount and ratio of amino acids in the porcine muscles after 14-day administration of two different biologically active complexes. It describes long-term effects of these complexes expressed in changes of the aminoacid profile. The article evaluates the metabolic effects of the complexes used expressed in qualitative compositional changes of the longissimus, the ileac muscle and the set of thigh muscles. The paper gives reasons for deterioration in the aminoacid profile of the muscles of pigs from an experimental group and for improvement in the nitric composition of the muscles of pigs from the second experimental group compared with the control group. Also, conclusions were made about the uptake role of the longissimus and the iliac muscle.

Keywords: pigs, man-made pollution, irreplaceable amino acids, muscles, iliac muscle, longissimus

INTRODUCTION

Swine rearing is an intensive and effective branch of livestock breeding, and under the present economic conditions it plays a special role in food security of the countries (1,2).

Adequate feeding of swine requires awareness of the needs for nutrients and biologically active substances. That is why improving the feeding system of swine remains one of the research priorities that provide efficiency improvement of pork production. Nutrition relevant to the physiological needs of the animals under specific technological conditions promotes full

implementation of the meat productivity potential at minimum feed efficiency ratios.

The updated protein nutrition systems for growing pigs provide dietary balancing of the protein available for uptake by the animal organism, taking into account meeting the needs for irreplaceable amino acids. Substantial knowledge about the effect of the protein level in the diet of monogastric animals and of added synthetic amino acids on disposal of feeding-stuffs, metabolic processes and productivity has been accumulated (3-6).

One of the most important nutritional issues concerning farm animals is shortage of the native protein in the feeding-stuffs. The main protein sources for pigs are the grains and bean cultures. Feed protein must meet certain requirements to its completeness and the optimal ratio of the productivity-restricting amino acids, their availability for absorption and use in the metabolic processes of the organism. A solution to the problem of relevant feeding to a large extent lies in specifying the needs of pigs for nutrients and biologically active substances. Protein nutrition must be optimized through use of scientifically based protein level reduction in the diets and through addition of irreplaceable amino acids.

Besides, the problems with intake of irreplaceable amino acids and some vitamins are often related to oppression of normal intestinal microflora providing the organisms of farm animals with the amino acids and vitamins produced. This oppression is related to the intake of the feeding-stuffs with heavy metals and other xenobiotics which in turn inactivate the enzymes of the microorganisms participating in transformation of organic acids. Shortage of the carbohydrates necessary for synthesis of some organic acids, leads to disrupted synthesis of proteinogenic amino acids that are derivatives of α -aminocarbonic acids.

Animal organisms need individual amino acids to fulfil detoxification mechanisms occurring in the liver (the first and the second phases of transformation of xenobiotics with participation of sulphur containing amino acids and tripeptide - glutathione). Consumption of proteinogenic amino acids for operation of protective mechanisms leads to shortage of some amino acids as well to an upset amino acid balance of the organism, which in turn triggers development of the general amino acid deficiency state, decrease of protein synthesis in the animal organisms reared under the conditions of man-made pollution.

At present, more importance is given to maintenance of normal catabolic and anabolic processes in the organisms of productive animals by additional dietary administration of

biologically active complexes intended for protection of the animal organisms from exposure to the factors of man-made agroecosystem pollution. This will provide for fulfilment of the animal genetic potential for production of quality livestock products even in the context of man-made pollution.

MATERIALS AND METHODS

To conduct an experiment in formation of a complete proteinogenic tissue composition of porcine parenchymal organs, we formed three groups of animals reared under the conditions of man-made agrosphere pollution. The number of animals in each group was 25 heads with the average 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, and magnesium citrate, a complex of calcium and methionine hydroxy analogue (MHA) and lysine with their feeding-stuffs. The animals of experimental group #2 received a complex consisting of vitamin/mineral, oil and vegetable components. This complex included soy isolate, pyridoxine, niacin, ascorbic acid, magnesium citrate, a complex of calcium and methionine hydroxy analogue (MHA), lysine, threonine, valine, tryptophan, flax, olive and sesame oil and the leaves of *Urtica dioica*. The pigs from the comparison group (the control group) were fed only on standard diet. The duration of the complex use was 14 days. Before the experiment start, blood from caudal auricular veins was collected from all the animals using vacuum blood collection systems for clinical and biochemical blood tests. Later, blood was collected on days 7, 14 and 34.

After the experiment was completed, the pigs were slaughtered in a specialized slaughterhouse.

After the slaughter, specimens of lean tissue, namely, of the longissimus (*Musculus longissimus*), the iliac muscle (*Musculus iliacus*), thigh muscles and the muscles of the exterior part of the shoulder were collected from all the animals.

The organs were homogenized and tested for the amino acid profile using a LC-20 liquid chromatograph 'Prominace' manufactured by firm 'Shimadzu' with a spectrophotometric detector (254 nm); a reverse stationary phase column C18 (Analysentechnik 250 x 4.6 mm, 5 µm, Germany) and a relevant precolumn: the mobile phase is a mixture of 0.06 Mol/dm³ solution of sodium acetate, pH≤5.5 and 4.05, and 1% solution of propanol-2 in acetonitrile.

Sample preparation for determination of total amino acids involved acid hydrolysis with 6 Mol/dm³ of hydrochloric acid solution at 110°C for 18 h. All the measurements were performed in duplicate (7).

The results obtained during the studies were analyzed using mathematical statistical techniques with averaging and standard deviation calculation.

Normality of the sampling method was determined using the Shapiro-Wilk test.

To evaluate significant differences among the groups in various indexes in case of Gaussian sampling we used the ANOVA procedure, and if the given conditions were not fulfilled, we used the Mann-Whitney test.

RESULTS AND DISCUSSION

Resulting from the analysis of the data obtained in the course of the experiment, it was established that no marked changes were observed in the total proteinogenic amino acid content in the longissimus of the pigs from experimental group #1, but a trend toward a decline in the total content of all the amino acids by 10.7% ($p \leq 0.19$) was noted. This was expressed as a decline in the total content of irreplaceable amino acids by 14.6% ($p \leq 0.19$) and that of dispensable ones by 8.3% ($p \leq 0.19$). A trend toward a decline in the content of branch-chained amino acids (BCAA) by 21.1% was observed. This trend was expressed as a decline in the valine content by 26.8%, that in the isoleucine content by 28.1% and to a lesser degree in the leucine content by 10.1%. The BCAA content decline in this muscle speaks for deficiency of the substrates necessary for provision of aerobic breathing processes

occurring in the muscle, and, consequently, consumption of valine, isoleucine and leucine for synthesis of acetyl-CoA or succinyl-CoA, which in turn enter the tricarboxylic acid cycle.

A statistically significant decline in the content of glutamic acid by 8.9% and that of histidine by 22.4% was also noticed in the longissimus of the pigs from experimental group #1. A decline in the histidine and alanine content by 15.8% might speak for a fall in the L-carnosine content, which probably led to a decline in the resistance of the muscles to the negative effect of lactate (8,9).

At the same time, a statistically significant increase in the total content of proteinogenic amino acids by 28.2% was observed in the longissimus of the pigs from experimental group #2 as against the comparison group. This increase was provided by a growth in the total content of essential amino acids by 37.1% as well by growth in the content of dispensable amino acids by 22.6%. Also, an increase of BCAA content by 52.0% compared with the control group was noted in the longissimus of the pigs from this group. The greatest contribution to this increase was made by isoleucine whose content grew by 67.6%, followed by leucine (46.6%) and by valine whose content grew by 41.1%. This fact spoke for a sufficient energy supply of this muscle in the pigs of that group.

A growth of the ratio of the amino acid tryptophan to hydroxyproline by 33.5% as against the comparison group was noted in the longissimus of the pigs from experimental group #2. The growth of this ratio was provided by an increase in the tryptophan content by 24.4% as well as by a fall in the hydroxyproline content by 9.4%. This fact spoke for a decline in the collagen content in this muscle, therefore, it meant a decrease in the content of connective tissue.

Also, increases in the levels of aspartic and glutamic acid by 42.1% and 13.5%, respectively, that of serine by 31.7%, that of glycine by 14.5%, that of arginine by 29.7%, and that of threonine by 55.8% were noticed, which, taken together with the growth in the

content of most amino acids, spoke for an increase in the protein component of the given muscle. At the same time, a co-growth of the phenylalanine level by 30.7% and that of tyrosine by 35.7% was noticed, which implied a positive endocrine profile of catechol amines and thyroid hormones (10).

A decline of the methionine content in the longissimus of the pigs from experimental group #2 by 25.1% spoke for intensive consumption of those amino acids for protein synthesis initialization in this muscle.

Table 1. Content of proteinogenic amino acids in the porcine longissimus

Tissue	Group	Units	Asp	Glu	Hyp	Ser	Gly	His	Arg	Thr	Ala	Tyr	Val
Musculus longissimus	Comparison group (Mean)	g/100g	1,68	2,89	0,04	0,64	0,71	0,83	1,04	0,85	1,04	0,54	0,68
	St. dev.	g/100g	0,20	0,10	0,01	0,08	0,08	0,09	0,13	0,02	0,21	0,05	0,15
	Exp. group #1 (Mean)	g/100g	1,73	2,64	0,06	0,60	0,66	0,64	0,91	0,78	0,88	0,49	0,50
	St. dev.	g/100g	0,15	0,05	0,02	0,06	0,01	0,08	0,12	0,05	0,14	0,08	0,04
	Ratio #1 of comparison group	±%	3,5	-8,9	59,4	-6,6	-7,0	-22,4	-11,8	-8,9	-15,8	-7,7	-26,8
	p		0,66	0,05	0,38	0,66	0,66	0,05	0,38	0,05	0,38	0,38	0,19
	Exp. group #2 (Mean)	g/100g	2,38	3,28	0,03	0,85	0,81	0,87	1,35	1,33	1,23	0,73	0,96
	St. dev.	g/100g	0,14	0,21	0,00	0,04	0,03	0,03	0,09	0,09	0,08	0,04	0,06
	Ratio #2 of comparison group	±%	42,1	13,5	-9,4	31,7	14,5	4,3	29,7	55,8	18,2	35,7	41,1
	p		0,05	0,05	0,38	0,05	0,05	1,00	0,05	0,05	0,19	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	0,56	0,76	0,84	0,57	1,55	0,13	2,28	3,38	5,93	9,40	15,33
	St. dev.	g/100g	0,09	0,20	0,09	0,07	0,17	0,06	0,42	1,75	0,66	0,51	1,17
	Exp. group #1 (Mean)	g/100g	0,50	0,55	0,75	0,51	1,36	0,12	1,80	2,42	5,07	8,62	13,69
	St. dev.	g/100g	0,04	0,05	0,02	0,10	0,05	0,02	0,09	1,63	0,18	0,25	0,42
	Ratio #1 of comparison group	±%	-9,9	-28,1	-10,1	-10,2	-12,3	-2,6	-21,1	-28,3	-14,6	-8,3	-10,7
	p		0,66	0,19	0,38	0,38	0,19	0,66	0,19	0,38	0,19	0,19	0,19
	Exp. group #2 (Mean)	g/100g	0,42	1,28	1,23	0,74	2,03	0,16	3,46	4,51	8,13	11,52	19,66
St. dev.	g/100g	0,09	0,04	0,06	0,06	0,08	0,03	0,11	0,69	0,42	0,52	0,93	
Ratio #2 of comparison group	±%	-25,1	67,6	46,6	30,7	30,7	24,4	52,0	33,5	37,1	22,6	28,2	
p		0,19	0,05	0,05	0,05	0,05	0,38	0,05	0,38	0,05	0,05	0,05	

The amino acid profile of the porcine iliac muscle from experimental group #1 pigs had a negative trend toward a decline in the total amount of proteinogenic amino acids whose content decreased by 14.6% compared with the control group. The decrease of the total amino acid content in the iliac muscle of the experimental pigs was caused by a decrease in the content of both essential amino acids by 13.5% and in the total amount of dispensable proteinogenic amino acids by 15.2%. A lower amount of irreplaceable branch-chain amino acids with by 19.5%, expressed in the isoleucine

content decrease by 26.7% and by that of valine by 23.7%. This, as we said above, speaks for their intensive consumption as energetic substrates. Along with that, a fall in the level of alanine which is in turn synthesized from piruval and BCAAs was recorded in the iliac muscle, and that also spoke for shortage of the substrates used as an energysource. Namely, it is glucose, which in turn is transformed into alanine in the course of gluconeogenesis (11).

The tryptophan/hydroxyproline ratio in the iliac muscle did not undergo any significant changes compared with the control group. A decrease in the level of glutamic acid by 10.1% and that of

arginine by 25.6% were probably related to enhanced destruction of amino acids accompanied by ammonia formation, which resulted in involvement of glutamic acid and arginine in the ornithine cycle (12). A decrease in the amount of histidine was supposedly related to a decrease in the amount of carnosine in this muscle (Table 2).

At the same time, when the second biologically active complex was administered, an increase of the total proteinogenic amino acid content by 26.4% was noted in the iliac muscle of the pigs from the second experimental group. It was

accompanied by an increase in the amount of essential amino acids by 35.9%, and also in that of dispensable amino acids by 20.7%.

Besides, a rise in the amount of branch-chain amino acids by 55.0% was noted. in the iliac muscle of the pigs from this group. The highest contribution belonged to isoleucine whose content grew by 69.0%, followed by a valine increase by 61.9% and by that of leucine by 38.8%. This fact spoke for sufficient energy supply of this muscle from experimental group # 2 pigs. At this content the alanine level grew only by 10.3%.

Also. an increase in the phenylalanine level by 43.7% and that in the tyrosine level by 42.7%, were also observed in the iliac muscle of the pigs from experimental group #2, which spoke for improvement in the metabolic processes in this muscle.

Besides, a trend toward an increase of the tryptophan/hydroxyproline ratio that was 5.12 in the in the iliac muscle of the pigs from experimental group #2 was discovered, which was 21.1% higher than in the control group.

Summing the metabolic effects of the biologically active complex used in this group, one can conclude that this complex causes a general improvement of the nitrogen balance in the iliac muscle of the pigs from experimental group #2.

Table 2. Content of proteinogenic amino acids in the porcine iliac muscle

Tissue	Group	Units	Asp	Glu	Hyp	Ser	Gly	His	Arg	Thr	Ala	Tyr	Val
Musculus iliacus	Comparison group (Mean)	g/100g	1,92	2,91	0,03	0,69	0,69	0,71	1,03	0,84	1,07	0,55	0,63
	St. dev.	g/100g	0,32	0,18	0,01	0,11	0,03	0,07	0,26	0,27	0,11	0,08	0,06
	Exp. group #1 (Mean)	g/100g	1,70	2,61	0,03	0,56	0,59	0,55	0,77	0,73	0,83	0,49	0,48
	St. dev.	g/100g	0,06	0,09	0,01	0,03	0,03	0,08	0,09	0,05	0,15	0,06	0,06
	Ratio #1 of comparison group	±%	-11,5	-10,1	4,2	-19,0	-13,8	-22,5	-25,6	-12,8	-22,4	-10,6	-23,7
	p		0,66	0,05	1,00	0,05	0,05	0,05	0,19	1,00	0,05	0,38	0,05
	Exp. group #2 (Mean)	g/100g	2,20	3,31	0,03	0,88	0,82	0,79	1,58	1,34	1,18	0,79	1,02
	St. dev.	g/100g	0,14	0,18	0,01	0,07	0,05	0,05	0,32	0,07	0,20	0,09	0,12
	Ratio #2 of comparison group	±%	14,9	13,8	9,5	28,2	19,0	10,7	53,0	59,9	10,3	42,7	61,9
	p		0,19	0,05	1,00	0,05	0,05	0,19	0,19	0,05	0,38	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	0,51	0,76	0,88	0,54	1,50	0,13	2,27	4,23	5,78	9,60	15,38
	St. dev.	g/100g	0,09	0,10	0,15	0,13	0,14	0,06	0,21	0,77	0,52	0,89	1,37
	Exp. group #1 (Mean)	g/100g	0,44	0,56	0,79	0,54	1,34	0,13	1,83	4,59	5,00	8,14	13,14
	St. dev.	g/100g	0,11	0,05	0,05	0,07	0,10	0,01	0,15	1,57	0,33	0,31	0,64
	Ratio #1 of comparison group	±%	-14,6	-26,7	-10,3	-0,6	-10,4	2,2	-19,5	8,5	-13,5	-15,2	-14,6
	p		0,38	0,05	0,66	1,00	0,38	0,66	0,05	1,00	0,05	0,05	0,05
	Exp. group #2 (Mean)	g/100g	0,43	1,28	1,22	0,78	1,64	0,16	3,52	5,12	7,86	11,58	19,44
	St. dev.	g/100g	0,06	0,14	0,08	0,09	0,66	0,03	0,33	1,15	0,28	0,78	0,57
	Ratio #2 of comparison group	±%	-16,6	69,0	38,8	43,7	9,2	26,0	55,3	21,1	35,9	20,7	26,4
p		0,38	0,05	0,05	0,05	0,66	0,66	0,05	0,38	0,05	0,05	0,05	

In thigh muscles of the pigs from experimental group #1, a decline in the total amount of proteinogenic amino acids by 15.1% was detected. It was accompanied by a decline in the amount of essential amino acids by 15.6% and in these muscles correlated with a decrease in the branch-chain amino acid content by 23.9%. This was expressed in a fall of the leucine level by 12.0%, in that of isoleucine by 34.8% and in that of valine by 25.8%. Simultaneously with the BCAA content fall, a decrease in the amount of alanine by 43.3% was noted. As described above, this fact can speak for shortage of the substrates used as energy sources. Also, a decline in the amount of tryptophan by 18.8% was observed, which caused a decline in the tryptophan/hydroxyproline ratio (2.1 c. u.) by 14.5% as against the comparison group. That said, the content of phenylalanine and tyrosine in the thigh muscles did not undergo any significant changes as against the comparison group (Table 3).

In thigh muscles of the pigs from experimental group #2, no statistically significant increase in the total proteinogenic amino acid content was noted, anyway, in percentage terms there was a trend toward an increase in their content. An increase in the total amount of amino acids is provided by an increase in the total amount of irreplaceable proteinogenic amino acids by 30.0%. As in the previous case, this growth was

related to an increase in the content of branch-chain amino acids by 38.2% ($p \leq 0.05$). The main contribution to the BCAA content growth increase was made by isoleucine (by 46.5%, $p \leq 0.05$) and leucine (by 35.0%, $p \leq 0.05$). Also, a decline in the hydroxyproline content by 31.3% was noted in this group of pigs. This in turn led to growth of the tryptophan/hydroxyproline ratio (3.5 c. u.) by 42.9% as against the comparison group. The amount of phenylalanine and tyrosine in thigh muscles of this group of pigs did not undergo any significant changes compared with the control group, though in percentage terms the phenylalanine content was 26.9%, and that of tyrosine was 33.2% higher than in the comparison group.

Table 3. Content of proteinogenic amino acids in porcine thigh muscles

Tissue	Group	Units	Asp	Glu	Hyp	Ser	Gly	His	Arg	Thr	Ala	Tyr	Val
Thigh muscle group	Comparison group (Mean)	g/100g	1,77	2,89	0,07	0,65	0,71	0,69	1,13	0,85	1,50	0,53	0,70
	St. dev.	g/100g	0,14	0,36	0,02	0,03	0,05	0,15	0,14	0,11	0,85	0,05	0,14
	Exp. group #1 (Mean)	g/100g	1,59	2,71	0,07	0,59	0,68	0,59	0,89	0,72	0,85	0,49	0,52
	St. dev.	g/100g	0,25	0,62	0,02	0,03	0,07	0,04	0,14	0,09	0,12	0,01	0,13
	Ratio #1 of comparison group	±%	-10,4	-6,3	-2,2	-9,3	-4,2	-13,7	-20,9	-15,1	-43,3	-7,9	-25,8
	p		0,66	0,66	1,00	0,19	0,38	0,66	0,19	0,19	0,05	0,38	0,19
	Exp. group #2 (Mean)	g/100g	2,07	3,04	0,05	0,81	0,80	0,78	1,33	1,23	1,12	0,71	0,93
	St. dev.	g/100g	0,24	0,48	0,01	0,16	0,15	0,19	0,42	0,28	0,15	0,17	0,18
	Ratio #2 of comparison group	±%	16,7	5,1	-31,3	24,4	13,3	13,7	18,0	44,8	-25,3	33,2	32,5
	p		0,19	1,00	0,05	0,38	0,38	0,66	1,00	0,05	1,00	0,19	0,38
	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	0,47	0,82	0,86	0,59	1,56	0,16	2,38	2,41	6,01	9,93	15,94
	St. dev.	g/100g	0,15	0,18	0,02	0,02	0,13	0,02	0,33	0,50	0,55	0,47	1,00
	Exp. group #1 (Mean)	g/100g	0,45	0,53	0,76	0,59	1,37	0,13	1,81	2,06	5,08	8,45	13,53
	St. dev.	g/100g	0,01	0,13	0,06	0,14	0,18	0,00	0,31	0,53	0,68	1,11	1,77
	Ratio #1 of comparison group	±%	-4,2	-34,8	-12,0	0,7	-12,3	-18,8	-23,9	-14,5	-15,6	-14,9	-15,1
	p		0,66	0,19	0,05	0,66	0,19	0,05	0,19	0,66	0,19	0,19	0,19
	Exp. group #2 (Mean)	g/100g	0,43	1,20	1,16	0,75	1,96	0,16	3,29	3,45	7,82	10,70	18,52
	St. dev.	g/100g	0,13	0,17	0,17	0,19	0,44	0,02	0,53	0,87	1,53	1,85	3,37
	Ratio #2 of comparison group	±%	-8,9	46,5	35,0	26,9	25,7	-2,1	38,2	42,9	30,0	7,7	16,2
p		0,66	0,05	0,05	0,38	0,38	1,00	0,05	0,19	0,19	0,66	0,38	

acids in all the studied muscle groups. In the first place, this depletion affected the longissimus and the iliac muscle. So, application of this complex has a number of restrictions. In the first place, it must be used only in combination with feeding-stuffs that have the key amino acid balance.

Administration of a biologically active complex #2 topigs under the conditions of man-made pollution is efficient and appropriate, as our studies showed its high efficiency in replenishment and improvement of the amino acid profile of the porcine lean tissue even 30 days after its application. The most significant effects of the amino acid profile improvement were demonstrated on the longissimus and the iliac muscle, which is probably related to their lower exercise stress

CONCLUSION

Analysis of amino acid profile changes in the lean tissue of swine reared under the conditions of man-made pollution after application of two biologically active complexes differing in their composition makes it possible to arrive at the following conclusions.

Administration of biologically active complex #1 to pigs under the conditions of man-made pollution and incompleteness of the protein composition of the feeding-stuffs carries certain negative consequences expressed in intensification of the metabolic processes with depletion of irreplaceable and dispensable amino

and to use of their protein components as the depot of proteinogenic amino acids.

CONFLICT OF INTEREST

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

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