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CHANGES IN AMINO ACID COMPOSITION OF BLOOD PROTEIN HYDROLYSATE DEPENDING ON THE RAW MATERIAL PREPARATION SEASON

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SUMMARY

The raw material for blood protein hydrolysate preparation is whole animal blood, its clots and other serum production wastes. The dependence of amino acid composition of blood protein hydrolysate on the season of the raw material preparation was studied. The research lasted three years. It was demonstrated that the amino acid composition changed depending on the season. The peak, as a rule, was during summer months when their amount increased by 1.2–2.3 times and during autumn and winter it went down by 1.2–1.4 times (the difference is considerable, $p < 0,05$). The peak of glutamic and asparagine acid growth was in November when their amount was 1.4 times higher then during the previous months ($p < 0,01$). The increase of alanine, asparagine, valine, lysine, methionine, histidine, proline, tyrosine, threonine, and phenylalanine by 1.3–1.8 times was observed in March (the difference is considerable, $p < 0.05$). The amount of histidine, glycine, leucine, serine, and tryptophane in the beginning of spring was at the same level and the amount of arginine, asparagine, isoleucine in March decreased by 1.2–1.6 times (the difference is considerable, $p < 0.01$). So, it was determined that the dynamics of BPH amino acid composition was directly associated with the seasonal dynamics of physiological and biochemical cattle blood values. It was noted that in case of considerable change in absolute amino acid parameters their relative amount, in general, remained constant.

Key words: blood protein hydrolysate, amino acids, physiological parameters.

INTRODUCTION

Biotechnology field related to design of nutrient media containing animal and plant protein enzyme hydrolysates as amino acid sources has been established and developed [7, 9, 10].

Protein hydrolysates are the products of protein cleavage in amino acids and simple peptides. Hydrolysates usually consist of 16-20 amino acids with variable concentration as well as peptides of different molar weight [4, 11].

Three methods of protein hydrolysis are known: acid, alkaline and enzymatic. All these types of hydrolysis induce protein cleavage into components, but the final products are different. In this study we used blood protein hydrolysate (BPH) obtained by enzymatic hydrolysis method [14].

Blood protein hydrolysates of animals, mainly cattle, are widely used in our country. Blood is the best raw material, it contains 18% of complete protein, and also mineral substances that remain in the preparation when it is produced [3, 6]. The raw material for blood protein hydrolysate (BPH) preparation is whole animal blood, its clots and other serum production wastes. [14].

The study of the morphological and biochemical composition of the blood is of great importance, because it gives a certain idea of the patterns of changes in the internal environment of the organism. Thus, the following tendency was observed: there was some decline in morphological rates in the winter period, which may be induced by decrease in the intensity of metabolic processes in animals of all breeds [2].

The season of the year is a complex factor, which includes the climatic specificity of the zone, feeding characteristics, physical exercise and insolation in summer. The season causes significant changes in the morphological pattern of blood in cows. In winter, changes in blood parameters are observed: this can be called unfavorable

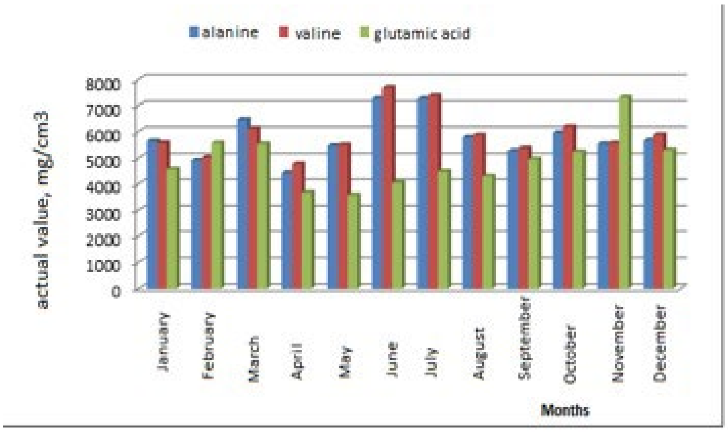


Fig. 1. Dynamics of the seasonal variation of the amino acid composition in BPH (for alanine, valine, glutamic acid)

changes in homeostasis. In winter, the number of leucocytes (in particular, phagocytic neutrophils) increases by 1-2 thousand in cattle blood, regardless of the physiological state of the cattle. At the same time, a lower hemoglobin level in the whole blood of cattle is observed. Owing to insolation, active exercise and green fodder, the general health of the animals improves, the reproductive function is normalized and milk production increases. When black-and-white cows were moved to the pasture of the farm located in Tula Oblast, the number of red blood cells and hemoglobin level increased in blood in 2 weeks, and the proportion of phagocytic leukocytes decreased [8]. Thus, the season significantly affects the cattle homeostasis, and therefore, the raw material for the preparation of blood protein hydrolysate.

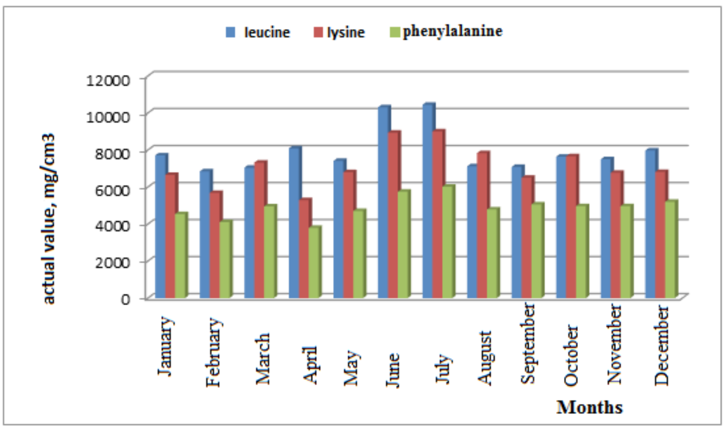
The purpose of the study was the examination of the amino acid composition of the BPH, depending on the raw materials preparation season.

MATERIALS AND METHODS

Liquid blood protein hydrolysate obtained from OOO NPP "BioChimService" was used for cell cultivation.

BPH amino acid composition assessment was performed according to Methodical Guidance M-04-38-2009 "Feed, feed-stuff and raw material for their production. Methods for measuring mass fraction of amino acids by capillary electrophoresis using the capillary electrophoresis "Kapel" system.

Fig. 2. Dynamics of the seasonal variation of the amino acid composition in BPH (for leucine, lysine and phenylalanine)



For statistical processing of obtained results, the arithmetic mean and its standard deviation were calculated monthly for each amino acid. The degree of difference between the means of two samples was assessed using Student's criteria (*t*), which is the ratio of differences between the means and the standard deviation of this difference [1].

The significance level demonstrated the confidence level of the obtained data and the probability of the random occurrence of the investigated criteria; in tests its coefficient was not more than 0.05 (5%).

Digital material was statistically processed using PC, Microsoft Excel and variation statistics standard methods.

RESULTS AND DISCUSSION

It was demonstrated that the amino acid composition of blood protein hydrolysate changed depending on the season (Table, Fig. 1–6). The peak, as a rule, was during summer months.

Monthly comparison of amino acid composition of blood protein hydrolysate demonstrated the increase of replaceable amino acids: alanine, asparagine, proline, and of nonreplaceable amino acids: valine, lysine, methionine, tyrosine, threonine, phenylalanine by 1.3-1.8 times in March (the difference is considerable, $p < 0,05$). The amount of such replaceable amino acids as glycine, serine and nonreplaceable amino acids histidine, leucine and tryptophane in the beginning of spring was at the same level. The amount of arginine, asparagine, isoleucine in March decreased by 1.2-1.6 times (the difference is considerable, $p < 0,01$).

Probably, such a difference in the dynamics of amino acids amounts in early spring was associated with changes in cattle feeding and keeping conditions. Alimentary stress associated with the monotonous nutrition, as well as lack of green fodder at this time of year induces defects in adaptation processes resulting in poor animal health and reduced productivity [5, 13].

It is known that confined management of animals in the late winter period is the most stressful, which is due to the uncompensated increase in the processes of free radical oxidation during the depletion of its own system of antioxidant protection. The stress-reaction of the organism developed at this time becomes intensive and durable, and it is transformed from a component of adaptation into a component of pathogenesis. Therefore, in order to preserve animal health and productivity, anti-stress and antioxidant drugs are used, and various additives are added to the diet [5].

The data presented in the table and in Figure 1, demonstrate that the standard pattern of concentration change depending on the season is typical for the replaceable amino acid alanine and for nonreplaceable amino acid valine: the number of amino acids in the summer months increased by 1.3-1.4 times, in the autumn-winter period decreased by 1.2-1.5 times (the difference is considerable, $p < 0,05$).

The peak of glutamic (Fig. 1) and asparagine acid (Fig. 4) growth was in November when their amount was 1.4 times higher than during the previous months (the difference is considerable, $p < 0,01$). These compounds are replaceable amino acids.

Table 1
Changes in amino acid composition of blood protein hydrolysate by months

Amino acid	Actual value, mg/cm^3											
	January (N=27)	February (N=15)	March (N=24)	April (N=13)	May (N=20)	June (N=12)	July (N=13)	August (N=6)	September (N=16)	October (N=28)	November (N=28)	December (N=25)
Alanine	5638.9±181.8	4908.0±176.7	6463.3±659.8	4426.9±568.8	5457.0±218.3	7274.3±681.3	7272.7±508.1	5785.0±196.5	5265.9±151.7	5956.3±248.5	5531.1±170.5	5664.4±104.7
Arginine	1702.2±178.4	1867.3±160.3	1134.2±186.9	1326.2±269.6	1772.5±234.3	1768.9±231.4	1607.8±234.1	1653.3±411.2	2300.0±154.0	1366.1±171.8	2030.0±193.9	2756.0±205.0
Asparagine	2068.8±66.7	1768.6±76.7	1497.2±188.9	2458.6±133.4	2190±145.5	2967.7±302.2	2705.7±308.6	2296.7±76.7	2153.8±64.4	2258.1±33.1	2225.2±116.1	2171.4±65.5
Asparagine acid	4028.1±324.9	4677.9±828.8	6889.6±641.6	3126.9±577.3	3282.5±224.2	3898.2±289.2	4269.2±286.0	4000.0±853.8	4278.1±510.1	4391.1±542.4	6361.7±615.9	4786.1±242.6
Valine	5565.9±168.4	5022.0±187.0	6112.5±473.6	4767.7±668.5	5489.5±234.1	7689.2±693.7	7380.1±600.9	5863.3±318.4	5364.4±77.4	6184.9±282.9	5554.6±191.8	5875.2±156.2
Histidine	3525.2±92.5	3146.7±157.3	3663.3±262.2	2929.2±330.9	3496.0±138.3	4661.9±433.0	4506.7±332.3	3603.3±197.6	3295.0±95.8	3802.2±177.6	3614.6±102.9	3641.6±55.4
Glycine	2535.6±67.9	2144.0±91.8	2610.8±211.7	2153.8±277.2	2555.5±107.2	3642.4±356.5	3534.15±265.7	2990.0±112.3	2577.5±80.9	2742.1±102.1	2571.8±79.0	2548.0±64.2
Glutamic acid	4562.9±286.8	5558.7±581.6	5529.2±257.3	3673.8±594.9	3577.0±231.3	4045.5±293.2	4475.0±292.4	4295.0±989.8	4953.8±468.0	5231.4±426.0	7315.9±449.9	5286.4±274.0
Isoleutin	1152.9±61.9	1074.3±29.5	884.7±58.4	1015.6±85.3	652.7±76.3	1490.4±148.7	1570.6±121.2	1096.7±165.9	1121.3±69.7	1120.5±71.3	972.9±71.3	1177.8±39.6
Leucine	7711.7±272.0	6845.7±204.9	7046.3±181.9	8090.0±549.5	7413.3±197.7	10308.0±1132.6	10443.6±867.9	7120.0±217.8	7085.6±179.4	7640.5±141.4	7500.8±279.1	7977.4±224.6
Lysine	6655.6±323.9	5684.0±212.2	7320.4±679.2	5288.5±594.3	6803.0±303.3	8935.0±758.5	9011.5±507.1	7833.3±575.1	6504.4±146.8	7660.7±364.5	6774.3±216.4	6818.8±107.2
Methionine	1509.6±91.1	1192.7±71.3	1553.3±115.0	1538.5±67.4	1971.0±273.8	1824.5±157.9	1758.1±101.9	1458.3±58.4	1320.0±37.8	1379.3±44.2	1301.0±40.5	1396.8±50.2
Proline	2037.0±105.5	1784.1±78.7	2471.7±249.2	1957.7±229.1	2213.5±102.2	2867.8±198.4	2864.6±177.9	2630.0±145.8	2206.9±42.6	2492.5±114.1	2896.4±267.1	3163.6±750.5
Serine	3701.9±210.4	3408.7±143.1	3965.4±311.7	3071.8±423.7	3803.2±203.8	4293.3±261.0	4510.5±193.1	4491.7±420.7	3886.3±139.8	4185.0±214.8	4166.8±150.1	4285.6±99.2
Tyrosine	871.5±93.8	693.3±71.4	1248.3±206.1	640.8±46.9	930.0±136.8	1102.2±169.4	1204.7±146.8	1433.3±391.8	943.8±92.2	1238.6±186.4	1019.6±93.5	1004.8±60.8
Threonine	3671.9±197.7	3282.0±116.8	4457.1±482.5	3225.4±321.9	3987.0±234.5	5021.4±381.6	5208.5±276.8	4998.3±472.7	3772.5±119.2	4700.4±323.2	3931.8±104.6	3892.5±102.6
Tryptophane	1157.8±38.9	1112.1±31.8	1205.4±61.1	1043.8±91.5	1116.0±72.4	1528.8±114.6	1443.7±66.7	1470.0±161.3	1107.5±18.7	1395.0±89.4	1188.2±39.3	1124.0±32.3
Phenylalanine	4541.5±185.1	4107.3±178.7	4952.9±360.2	3791.5±480.1	4708.0±181.9	5748.6±436.7	6019.0±363.7	4790.0±253.6	5061.3±109.4	4967.1±171.7	4967.5±187.9	5204.4±127.5

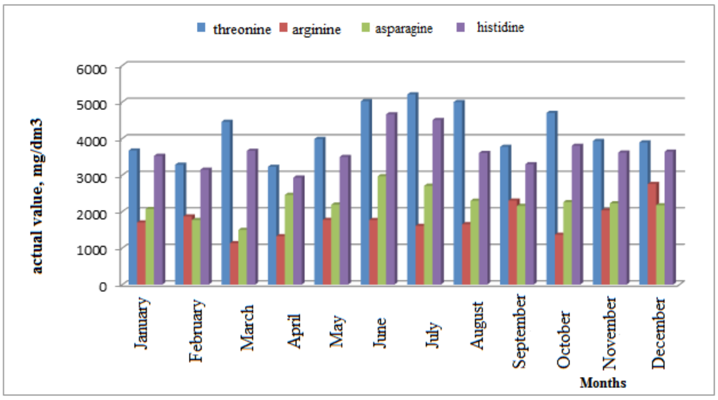


Fig. 3. Dynamics of the seasonal variation of the amino acid composition in BPH (for threonine, arginine, asparagine and histidine)

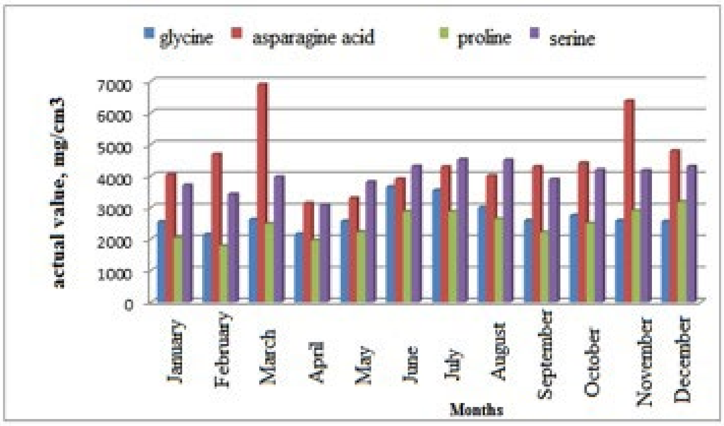


Fig. 4. Dynamics of the seasonal variation of the amino acid composition in BPH (for replaceable amino acids glycine, asparagine acid, proline, serine)

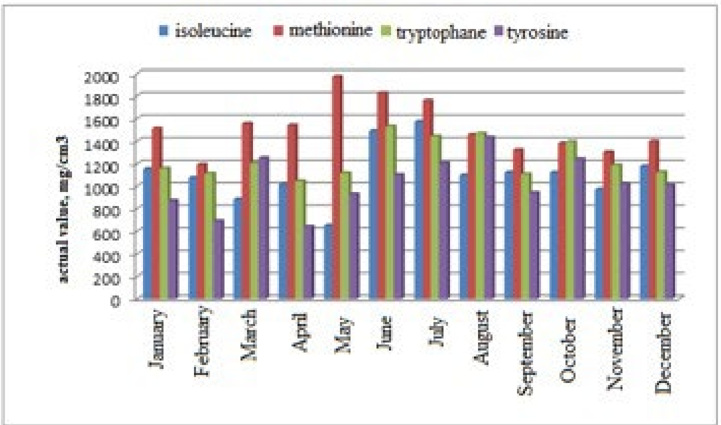


Fig. 5. Dynamics of the seasonal variation of the amino acid composition in BPH (isoleucine, methionine, tryptophane, tyrosine)

The unique character of glutamic and asparagine acids lies in the fact that, for mutual conversion into each other, all replaceable amino acids must first turn into glutamic or asparagine acid. This fact determines their integrating role in nitrogen metabolism. However, this characteristic is not limited only to compensation of amino acids that are not received with food. There is the phenomenon of «redistribution of nitrogen in the body». In case of protein deficiency in one or another organ due to disease or hyperfunction (the need for working hypertrophy), nitrogen redistribution occurs: the protein is «withdrawn» from one internal organ and sent to others [11, 12]. Thus, it is likely that when animals are transferred to the silos-concentrating type of feeding, the content of glutamine and asparagine acids increase in the body.

The data presented in Table and in Figure 2 demonstrate that dynamics of BPH nonreplaceable amino acid composition (leucine, lysine and phenylalanine) was directly associated with the seasonal dynamics: during summer months their amount increased by 1.3–1.6 times and during autumn and winter it went down by 1.2-1.5 times (the difference is considerable, $p < 0{,}05$).

It was demonstrated that the amount of arginine (Fig. 3) increased by 1.4 times at the end of the year ($p < 0{,}05$) and in March decreased by 1.6 times ($p < 0{,}01$) and remained rather stable in summer months. The amount of glycine, proline, serine (table, Fig.4) and isoleucine, methionine, tryptophane and tyrosine (table, Fig 5) increased during summer months by 1.3–2.3 times and during autumn and winter it went down by 1.2-1.4 times (the difference is considerable, $p < 0{,}05$).

When assessing the relative content of amino acids in the BPH, it was found that during all seasons of the year the percentage of the following amino acids remained at a constant level: tyrosine (1-2%), isoleucine (1-2%), tryptophane (2%), methionine (2-3%), arginine (2-4%), asparagine (2-4%), proline (3-5%), valine (8-10%), glycine (4-5%), histidine (4-6%), serine (5-7%), threonine (6-7%), phenylalanine (7-8%), alanine (8-10%), lysine (10-12%). The relative content of leucine changed by leaps in March-April from 10 to 15%, and throughout the rest of the year it remained within 11-12%. The percentage of asparagine and glutamic acid content varied within narrow limits. So, in March and November the amount of each amino acid was 10% of the total amount, the rest of the time it went down to 5-6%.

Thus, in case of considerable change in absolute amino acid parameters their relative amount, in general, remained constant.

CONCLUSION

The performed tests studied the dependence of the BPH amino acid composition on the raw material obtained in different seasons of the year and physiological and biochemical cattle blood values.

The maximum amino acid content was observed during summer months, and during autumn and winter it went down, with the exception of asparagine and glutamic acids: the peak of their growth was observed in November. Probably this occurs when animals are transferred to the silos-concentrating type of feeding.

It was noted that in case of considerable change in absolute amino acid parameters their relative amount, in general, remained constant.

The results suggest further studies on the effect of seasonal variation of the amino acid composition in BPH on growth of the cell population and the release of immunogenic components of foot and mouth disease virus.

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