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# INFLUENCE OF CHANGES IN AMINO ACID COMPOSITION OF BLOOD PROTEIN HYDROLYSATE ON THE PRODUCTIVITY OF BHK-21/2-17 CELL LINEAGE AND AMOUNT OF FMDV IMMUNOGENIC COMPONENTS

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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 BHK-21/2-17 cell lineage productivity on the season of hydrolysate raw material preparation was observed. Cell growth stimulated by blood protein hydrolysate from raw material prepared in summer months 1.2–1.3 times exceeded cell growth stimulated by blood protein hydrolysate prepared in autumn-winter period (differences are significant, $p < 0,001$). The amount of FMDV immunogenic components from "summer" blood protein hydrolysate 1.3–1.6 exceeded the amount obtained during other seasons of the year (differences are significant, $p < 0,001$). There was found a direct correlation between BHK-21/2-17 cell lineage productivity, concentration of FMDV immunogenic components and seasonal dynamics of the contents of the following amino acids composing blood protein hydrolysates: alanin, asparagine, valine, histidine, glycine, isoleucine, leucine, lysine, methionine, proline, serine, tyrosine, threonine, tryptophane, and phenylalanine. To increase BHK-21/2-17 cell lineage productivity and concentration of FMDV immunogenic components it was suggested to add some additional amino acids into the culture growth medium for suspension cell culture in autumn-summer period together with blood protein hydrolysate.

Key words: blood protein hydrolysate, amino acids, months of the year, productivity, FMDV immunogenic components.

## INTRODUCTION

Over the past few decades biotechnology field related to design of nutrient media containing animal and plant protein enzyme hydrolysates as amino acid sources has been established and developed [5, 7].

It was established that 13 amino acids are needed for vertebrate cell growth outside the organism (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, triptophane, valine, arginine, glutamine, histidine, tyrosine, cysteine) [2, 10].

Protein hydrolysates are the products of protein cleavage in amino acids and simple peptides. Hydrolysates usually consist of amino acids with variable concentration as well as peptides of different molar weight [4, 8]. Blood protein hydrolysate (BPH) contains the sufficient amount of amino acids necessary for BHK-21/2-17 cell growth [6].

All amino acids participate in biosynthesis of different proteins. Amino acid performance of this or that function *in vitro* mostly depends on conditions of cell cultivation. One of the reasons for presence of amino acids not needed for the whole organism in the medium is their restricted synthesis in the culture [4, 11]

Some authors mark that the demand in these organic acids depends on different types of cells. Very often amino acids are added to compensate the inability of some cells to synthesize them or to compensate their leakage into the medium [13].

Different amino acids are consumed from culture medium by growing cells at a different speed. It was noted that regular addition of arginine (20–40 mg/dm$^3$) and increase in the number of glutamine up to 450 mg/dm$^3$ raised the intensity of suspended cultures [12]. Besides, as a rule during cultivation amino acids are utilized by 20–25%, herewith pH of the medium goes down as much as 0.4–0.7 units [7].

BPH is raw material for preparing growth media during cultivation of BHK-21/2-17 [6, 9] cell suspension, but unfortunately, there are practically no data in publications on the influence of changes in BPH amino acid composition on productivity and amount of FMDV immunogenic components.

## MATERIALS AND METHODS

*Cell line*. Continuous suspension Baby hamster kidney (BHK-21/2-17) cell culture was used.

*Cell cultivation*. The cells were grown according to the "Industrial regulation for vaccine production against different FMDV types in metal 1,800 dm$^3$ cultivators.

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

*FMD virus*. Culture FMDV A, O, Asia-2 was used for infection.

*Determination of cell concentration and viability*. BHK-21/2-17 cell concentration in suspension was determined using Gorjaev's chamber for counting blood corpuscles. The viability was assessed by calculating the amount of live cells using 1% trypan blue water solution.

*Determination of* BHK-*21/2-17 cell line productivity*. Productivity was calculated as the ratio between the final cell concentration and original one in one passage.

*Measurement of total virus protein concentration and FMDV components* was performed according to the "Methods of determining virus specific protein and FMDV composition using quantitative complement fixation test" [1].

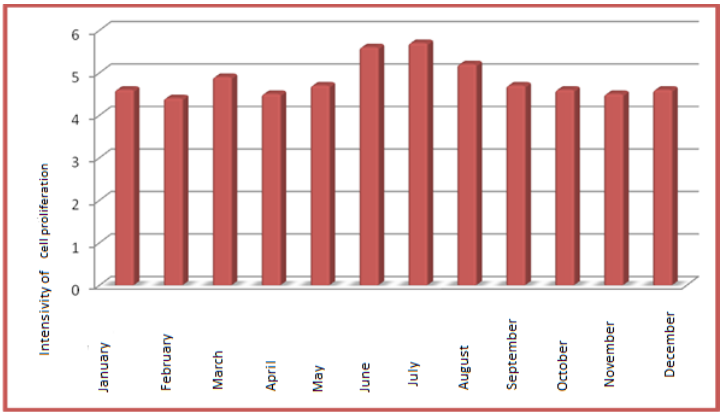
*BPH amino acid composition assessment was performed* according to Methodical Guidance M-04-38-2009 "Feed, feed-stuff and raw material for their production. Methods for measuring mass fraction of amino acids by capillary electrophoresis using the capillary electrophoresis "Kapel" system.

*Statistical data processing*. For statistical processing of obtained results the arithmetic mean and its standard deviation were calculated. 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. To define the linear correlation between the two characteristics correlation coefficient (R) was calculated, which can have value from +1 to –1 depending on the strength of relationship. In case of total direct correlation – $R = 1$, in case of total reverse correlation – $R = -1$. In case there is no correlation the coefficient is close to 0. As a rule, *R* value in interval 0.20–0.30 is indicative of weak (0.50–0.60) – moderate (0.80–0.90) and strong correlation between the characteristics [3].

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%) [3].

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

**Fig. 1. Productivity of suspension BHK-21/2-17 cell culture depending on the month of the BPH raw material preparation**




## RESULTS AND DISCUSSION

Previously performed tests studied the dependence of the BPH amino acid composition on the raw material obtained in different seasons of the year.

It was determined 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

**Table 1**
**Productivity of suspension BHK-21/2-17cell culture depending on the season of BPH raw material preparation**

| Parameter | January | February | March | April | May | June | July | August | September | October | November | December |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average productivity (M) | 4.6 | 4.4 | 4.9 | 4.5 | 4.7 | 5.6 | 5.7 | 5.2 | 4.7 | 4.6 | 4.5 | 4.6 |
| Standard error (±*m*) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Number of tests (*n*) | 119 | 72 | 115 | 95 | 88 | 107 | 80 | 24 | 85 | 89 | 85 | 84 |

was 1.4 times higher then during the previous months ($p < 0,01$).

So, there was determined a direct dependence of the dynamics of amino acid composition in BPH and physiological and biochemical cattle blood values (as cattle whole blood, its clots and other wastes of serum production were the primary material for BPH preparation) from the seasons.

At this stage of the research the productivity of the BHK-21/2-17 cell line in suspension with the tested BPH. Blood protein hydrolysate obtained from raw material prepared in summer stimulated the suspension cell culture growth by 1.2–1.3 times more in comparison with other seasons (the difference is considerable, $p < 0,001$) (Table 1, Fig. 1).

There was detected correlation between productivity and dynamics of the seasonal variation of the amino acid composition in BPH (Table. 2). The correlation coefficient varied within the following limits: –0.367 up to 0,947, which was indicative of the weak, moderate and strong dependence between the characteristics. Correlation relationship between the content of arginine, asparagine acid and cell productivity was not detected. Glutamine acid demonstrated reverse weak correlation with productivity ($R = –0.367$). Proline, asparagine, isoleucine, methionine, serine, tyrosine demonstrated a moderate degree of correlation with productivity, herewith, R equaled 0.774–0.947. The rest of amino acids, such as alanine, valine, histidine, glycine, leucine, lysine, threonine, tryptophane, phenylalanine, demonstrated high level of direct correlation with cell productivity ($R$ was within 0.774–0.947).

There was detected correlation between the number of FMDV immunogenic components and dynamics of the seasonal variation of amino acid composition in BPH. Herewith the coefficient of $R$ correlation varied within –0,356–0,772, which is indicative of the weak and moderate correlation between the characteristics. Correlation between arginine content and concentration of FMDV immunogenic components was not detected. Dependence of 146S + 75S-component yield from the amount of asparagine and glutamine acids demonstrated weak reverse correlation – $R$ was –0,356 and –0,465 respectively. The weak direct correlation between the characteristics was observed in case of tyrosine, isoleucine, proline, serine, ($R$ is within 0.313–0.461). The rest of aminoacids, such as alanine, asparagine, valine, histidine, glycine, leucine, lysine, methionine, threonine, triptophane, phenylalanine, demonstrated direct moderate correlation with FMDV immunogenic component yield ($R$ was 0.566–0.772).

Blood protein hydrolysate from raw material prepared in summer stimulated increase in the concentration of FMDV immunogenic component concentration during its reproduction in cell suspension by 1.3–1.6 times more in comparison with other seasons (differences are considerable, $p < 0,001$) (Fig. 2, Table 2).

Accordingly, there was detected a dependence between seasonal variations of amino acid concentration in BPH, cell productivity and the amount of FMDV immunogenic virus components after reproduction in BHK-21/2-17 cell line, grown using BPH (Table 3). Herewith, in all cases there was no correlation with arginine and asparagine acid. The rest of amino acids demonstrated different level of dependence between productivity and concentration of the virus immunogenic components.

## CONCLUSION

During the performed tests influence of seasonal changes in amino acid composition of BPH on productivity of BHK-21/2-17 cell population and amount of FMDV immunogenic components.

**Fig. 2. Concentration of FMDV immunogenic components during reproduction in suspension BHK-21/2-17 cell culture depending on the month of raw material preparation for BPH**

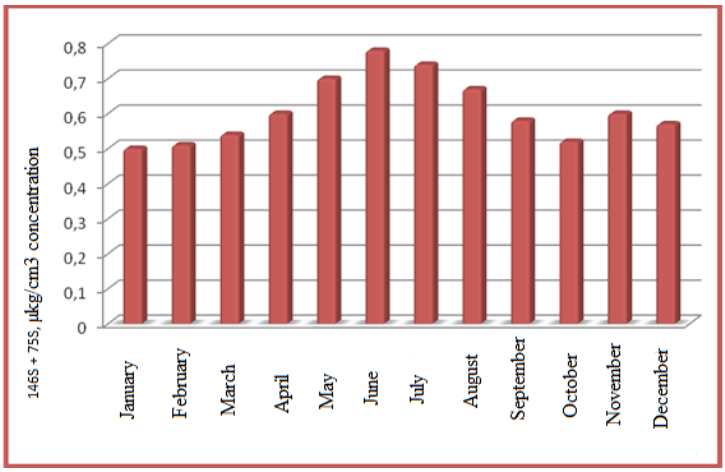



**Table 2**
**Concentration of FMDV immunogenic components during reproduction in suspension BHK-21/2-17 cell culture depending on the month of raw material preparation for BPH**

| Criteria | January | February | March | April | May | June | July | August | Sseptember | October | November | December |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 146S + 75S Concentration in 1 mln cells (µkg/cm$^3$), moderate (M) | 0.50 | 0.51 | 0.54 | 0.60 | 0.70 | 0.78 | 0.74 | 0.67 | 0.58 | 0.52 | 0.60 | 0.57 |
| Standard deviation ($\pm m$) | 0.02 | 0.03 | 0.02 | 0.13 | 0.04 | 0.03 | 0.03 | 0.05 | 0.04 | 0.02 | 0.04 | 0.04 |
| Number of experience ($n$) | 116 | 80 | 129 | 119 | 85 | 102 | 73 | 28 | 78 | 91 | 80 | 86 |

**Table 3**
**Correlation between dynamics of seasonal fluctuation of amino acid composition in PBH, BHK-21/2-17 cell line productivity and amount of FMDV immunogenic components**

| Amino acid | Contents, mg/cm³ | | | | | | | | | | | | *R*1 | *R*2 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | January | February | March | April | May | June | July | August | September | October | November | December | | |
| Alanine | 5638.9 | 4908.0 | 6463.3 | 4426.9 | 5457.0 | 7274.3 | 7272.7 | 5785.0 | 5265.9 | 5956.3 | 5531.1 | 5664.4 | 0.863 | 0.566 |
| Arginine | 1702.2 | 1867.3 | 1134.2 | 1326.2 | 1772.5 | 1768.9 | 1607.8 | 1653.3 | 2300.0 | 1366.1 | 2030.0 | 2756.0 | –0.189 | –0.006 |
| Asparagine | 2068.8 | 1768.6 | 1497.2 | 2458.6 | 2190.0 | 2967.7 | 2705.7 | 2296.7 | 2153.8 | 2258.1 | 2225.2 | 2171.4 | 0.621 | 0.772 |
| Asparagine acid | 4028.1 | 4677.9 | 6889.6 | 3126.9 | 3282.5 | 3898.2 | 4269.2 | 4000.0 | 4278.1 | 4391.1 | 6361.7 | 4786.1 | –0.124 | –0.356 |
| Valine | 5565.9 | 5022.0 | 6112.5 | 4767.7 | 5489.5 | 7689.2 | 7380.1 | 5863.3 | 5364.4 | 6184.9 | 5554.6 | 5875.2 | 0.884 | 0.646 |
| Histindine | 3525.2 | 3146.7 | 3663.3 | 2929.2 | 3496.0 | 4661.9 | 4506.7 | 3603.3 | 3295.0 | 3802.2 | 3614.6 | 3641.6 | 0.854 | 0.657 |
| Glycin | 2535.6 | 2144.0 | 2610.8 | 2153.8 | 2555.5 | 3642.4 | 3534.2 | 2990.0 | 2577.5 | 2742.1 | 2571.8 | 2548.0 | 0.947 | 0.768 |
| Glutamine acid | 4563.0 | 5558.7 | 5529.2 | 3673.8 | 3577.0 | 4045.5 | 4475.0 | 4295.0 | 4953.8 | 5231.4 | 7315.9 | 5286.4 | –0.367 | –0.465 |
| Isoleucine | 1152.9 | 1074.3 | 884.7 | 1015.6 | 652.7 | 1490.4 | 1570.6 | 1096.7 | 1121.3 | 1120.5 | 972.9 | 1177.8 | 0.681 | 0.383 |
| Leucine | 7711.7 | 6845.7 | 7046.3 | 8090.0 | 7413.3 | 10308.0 | 10443.6 | 7120.0 | 7085.6 | 7640.5 | 7500.8 | 7977.4 | 0.780 | 0.717 |
| Lysine | 6655.6 | 5684.0 | 7320.4 | 5288.5 | 6803.0 | 8935.0 | 9011.5 | 7833.3 | 6504.4 | 7660.7 | 6774.3 | 6818.8 | 0.893 | 0.651 |
| Methionine | 1509.6 | 1192.7 | 1553.3 | 1538.5 | 1971.0 | 1824.5 | 1758.1 | 1458.3 | 1320.0 | 1379.3 | 1301.1 | 1396.8 | 0.595 | 0.746 |
| Proline | 2037.0 | 1784.1 | 2471.7 | 1957.7 | 2213.5 | 2867.8 | 2864.6 | 2630.0 | 2206.9 | 2492.5 | 2896.4 | 3163.6 | 0.499 | 0.452 |
| Serine | 3701.9 | 3408.7 | 3965.4 | 3071.8 | 3803.2 | 4293.3 | 4510.5 | 4491.7 | 3886.3 | 4185.0 | 4166.8 | 4285.6 | 0.651 | 0.461 |
| Tyrosine | 871.5 | 693.3 | 1248.3 | 640.8 | 930.0 | 1102.2 | 1204.7 | 1433.3 | 943.8 | 1238.6 | 1019.6 | 1004.8 | 0.598 | 0.313 |
| Threonine | 3671.9 | 3282.0 | 4457.1 | 3225.4 | 3987.0 | 5021.4 | 5208.5 | 4998.3 | 3772.5 | 4700.4 | 3931.8 | 3892.5 | 0.855 | 0.607 |
| Tryptophane | 1157.8 | 1112.1 | 1205.4 | 1043.8 | 1116.0 | 1528.8 | 1443.7 | 1470.0 | 1107.5 | 1395.0 | 1188.2 | 1124.0 | 0.819 | 0.567 |
| Phenylalanine | 4541.5 | 4107.3 | 4952.9 | 3791.5 | 4708.0 | 5748.5 | 6019.0 | 4790.0 | 5061.3 | 4967.1 | 4967.5 | 5204.4 | 0.774 | 0.587 |
| Parameters for correlation analysis | | | | | | | | | | | | | | |
| $C_{146S+75S}$ in 1 mln cells (μkg /cm³) | 0.5 | 0.51 | 0.54 | 0.6 | 0.7 | 0.78 | 0.74 | 0.67 | 0.58 | 0.52 | 0.6 | 0.57 | – | – |
| Cell productivity | 4.6 | 4.4 | 4.9 | 4.5 | 4.7 | 5.6 | 5.7 | 5.2 | 4.7 | 4.6 | 4.5 | 4.6 | – | – |

*R*1 – Coefficient of correlation between cell productivity and seasonal amino acid content in BPH;

*R*2 – Coefficient of correlation between concentration of FMDV immunogenic components and seasonal contents of amino acids in BPH;

$C_{146S+75S}$ – concentration of immunogenic components 146S and 75S of the cultural FMDV.

Dependence of BHK-21/2-17 cell line productivity from the season of raw material preparation for BPH was identified. There was detected direct dependence between BHK-21/2-17 cell line productivity, concentration of FMDV immunogenic components and seasonal dynamics of the contents of alanine, asparagine, valine, histidine, glycine, isoleucine, leucine, lysine, methionine, proline, serine, tyrosine, threonine, tryptophane, phenylalanine, included in hydrolysate.

To increase productivity of BHK-21/2-17 cell population and concentration of FMDV immunogenic components besides BPH it is reasonable to add such amino acids as alanine, asparagine, valine, histidine, glycine, leucine, lysine, methionine, threonine, triptophane, phenylalanine into the culture growth medium in autumn and winter for growing suspension cell culture up to the level comparable with their contents in the raw material prepared in summer months.

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