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Optimization of cattle by-products amino acid composition formula

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Abstract. The aim of this research was to develop optimal formulations of by-product mixtures in terms of biological value using MS Excel Solver standard software application. The objects of study were underutilized cattle by-products as tripe, ears, lips, lungs, and heart. Physical and chemical studies were carried out to compile a database of the by-products used. As a result, the protein content was 14.3% in tripe, 24.6% in lips, 24.9% in ears, 15.2% in lungs, and 16.8% in heart ($P < 0.05$). The content of essential amino acids in various by-products, determined by high-performance liquid chromatography, did not have significant differences compared with the results obtained by other researchers. While conducting optimization of the by-product formulation, focused on the physiologically-based content of the essential amino acids in the 'ideal' protein according to the Food and Agriculture Organization of the United Nations and the World Health Organization (FAO/WHO). Essential amino acids index (EAAI) was chosen as the goal function. In the process of optimization, indicators such as chemical score, EAAI, biological value, and coefficient of amino acid score differences (CAASD) were calculated. Several variants of the formulations with high biological value were obtained as a result of the optimization. According to the results of the research it was found that more balanced ratio of the essential amino acids was in the following formulations: 1 – tripe (4.9%), ears (28.4%) and heart (66.7%) or 2 – ears (25.4%), lips (8.9%) and heart (65.7%). According to the results, the highest *in vitro* protein digestibility was in compositions number 1 and 2 (78.2% and 76.8%), which correlated with the calculated biological value. Thus, the use of computer modeling allowed obtaining the formulations of the by-products composition with the highest possible biological value by varying the content of the various by-products.

Key words: biological value, amino acid, optimization, by-product.

INTRODUCTION

During the slaughter of farm animals, large quantities of by-products such as bone, blood, skin, offal, etc., are produced. According to various scientific data, on average, the output of by-products is about 40% of cattle live weight, 50% of sheep and goats, 30% of pigs and poultry live weight, and 35% of lambs live weight (Smith, 1993;

Vernooij, 2012). According to Ockerman & Basu (2004) the yield of offal ranges from 10 to 30% of the live weight of pigs and cattle, respectively. Processing and recycling of by-products possess a problem from an economic and environmental point of view (Jayathilakan et al., 2012; Toldrá et al., 2016). However, these can be an additional source of food and make up for the deficit of animal protein. One of the main problems in feeding the world's growing population is a lack of protein foods, which is seen as especially urgent in developing countries (Subba, 2002). Due to the high cost of meat, meat organs, referred to as 'offal' or the 'fifth quarter', are an alternative source of animal origin nutrients (Bester et al., 2018). Therefore, more attention should be paid to the possibility of using animal proteins, including those which are contained in by-products (Van Heerden & Morey, 2014; Bester et al., 2018).

By-products are of high value as a source of cheap protein. They are important for the majority of developing countries which are very poor. Here, offal is a staple food in the diets of many people (Jayathilakan et al., 2012). For instance, the consumption of offal in Turkey and India is higher than in most other countries (Jayathilakan et al., 2012; Coskuntuna et al., 2015). Offal is widely used in food in South Africa (Van Heerden & Morey, 2014). Offal is quite varied in regards to composition and functionality, most of it contains a good amount of nutrients such as essential amino acids, minerals and vitamins (Aristoy, 2011; Honikel, 2011). Most by-products are characterized by good digestibility of proteins. Spleen, kidney, lungs and tripe proteins have the highest rate of digestibility (in vitro); hearts, udders and tongue proteins have a medium rate; meat heads and lips proteins have the lowest rate (Anonymous, 1985). Despite high nutritional value, usage of by-products in the composition of meat products is often limited due to variations in composition or functionality and unattractive organoleptic qualities (Smith, 1993). Offal consumption and utilization in meat processing sausage-type products or traditional dishes may be increased (Florek et al., 2012).

Offal with a high content of connective tissue proteins are promising for producing hydrolysates of these proteins and compositions for the production of the antioxidant peptides (Aristoy, 2011; Lasekan et al., 2013; Mora et al., 2014). For example, a collagen composition, protein-collagen emulsion, protein concentrate is widely used as a protein component in production of sausages and ready-to-eat products (Kurt & Zorba, 2007; Kalenik et al., 2017). Protein hydrolysates from meat by-products are an interesting alternative to soy products due to the lack of allergenic proteins and the presence of large amounts of all essential amino acids (Martínez-Alvarez et al., 2015).

The need for improving the use of food by-products to reduce food waste is noted (Government Office for Science, 2011). One of the ways to solve the problem of protein deficiency mentioned by Sun-Waterhouse et al. (2014) is to increase the economic efficiency of using proteins from raw materials, including by-products, and to improve the functionality of protein ingredients through modification. The application of appropriate processing, such as enzymatic hydrolysis, thermal treatment, dehydration, emulsification, and ultrafiltration contributes to obtaining modified substances and proteins, which are considered value-added food ingredients (Mora et al., 2014; Sun-Waterhouse et al., 2014).

The search for alternative protein sources has advanced in recent years and provides a relevant approach to meeting global protein requirements. Given the above, it can be concluded that by-products, including offal, are a source of animal protein and can be used directly in food or modified into certain protein substances.

It has been proved that offal is a good source of essential and limiting amino acids. However, some by-products such as ears, feet, or lips are rich in connective tissue, which is mainly composed by glycine, proline and alanine. This lack of essential amino acids can be overcome by blending ingredients to achieve a balanced amino acid profile in the final product (Mullen & Álvarez, 2016).

Many researchers recommend using methods of linear and experimental-statistical programming to obtain a multi-component products with a balanced composition (Musina & Lisin, 2012; Nadtochii, 2013; Musina & Lisin, 2015; Lisitsyn et al., 2016).

The problem of designing recipes with a large number of components while achieving the required quality indicators is quite difficult without using the software. Manual solution the system of linear equations and inequalities with a considerable number of variables is a significant difficulty, at which the probability of calculation errors is high (Musina & Lisin, 2012; Musina & Lisin, 2015).One of the most commonly used criteria for optimality in the development of product formulations is the biological value. Zhackslykova et al. (2014) proposed to use various indicators of the biological value of protein (amino acid score, index Osera, essential amino acid index, PDCAAS) as optimality criteria in the development of meat products formulations with the addition of by-products. Satina & Yudina (2010) developed a methodology for the designing meat products formulations based on the modeling of amino acid and fatty acid compositions. Nadtochii (2013) proposed to simulate the biological value of the protein component of a multi-component product using standard add-in Solver processor spreadsheet Microsoft Excel.

The aim of the research was to develop optimal formulations of by-product mixtures in terms of biological value using MS Excel Solver standard software application.

MATERIALS AND METHODS

Optimization of the formulation of by-product composition

Cattle by-products such as lungs, tripe, ears, lips and heart were selected as starting components of the formulation composition. These by-products were obtained after slaughtering 6 cows of Holstein-Friesian at the age of three years.

Construction of a multi-component by-product composition was produced using SOLVER standard software of Microsoft Excel 2013. The calculation of the formulation consisted of several steps: compiling a data bank and balance equations for the amino acid composition, defining the objective function to optimize formulations, solving the problem by using the tool SOLVER, and analyzing and selecting a recipe appropriate for the goal. To compile a data bank on by-product amino acid composition, the content of essential amino acids was experimentally found in the studied by-products by High Performance Liquid Chromatography (HPLC).

Essential amino acid index (EAAI), which is defined as the average geometric ratio of each amino acid in test protein to its quantity in the whole egg protein according to Oser method (1951), was selected as the goal function:

$$EAAI = \sqrt[n]{a_1 \times a_2 \times ... a_n} \quad (1)$$

where a_n is the ratio of the amount of each essential amino acid in the investigated protein to its amount in the whole egg protein and n is the amount of EAA (n = 8).

The basic indicators and criteria such as coefficient of amino acid score differences (CAASD) and biological value (BV) were used to evaluate the food adequacy and the most important protein components (Lipatov, 1995).

The indicators of biological value were calculated in the following sequence:

4. Chemical score (amino acid score (AAS) was calculated using the following formula [FAO/WHO, 1990]

$$AAS = \frac{EAA\ intest\ protein}{total EAA\ intest\ protein} \times \frac{total EAA\ inegg}{EAA\ inegg} \times 100 \quad (2)$$

5. The CAASD (%) shows that the average value of EAA amino acid score is excessive as compared to the lowest level of any essential amino acid.

CAASD was calculated, %, applying the formula:

$$CAASD = \sum \frac{\Delta AASD}{n} \quad (3)$$

where *n* is the amount of the essential amino acid (n = 8).

Amino acid score difference (AASD), %, was calculated according to the formula:

$$\Delta AASD = C_i - C_{\min} \quad (4)$$

where C_i – amino acid excess and C_{min} is the minimal amino acid score of the test protein against the ideal protein, %.

6. Biological value (BV), %, was calculated according to the formula:

$$BV = 100 - CAASD, \quad (5)$$

7. Nutritional index (NI) NI was calculated using the formula:

$$NI = \frac{EAAI \times \%\ protein}{100} \quad (6)$$

8. Computed protein efficiency ratio (C-PER) was calculated according to the formula:

$$C - PER = -2.107 + 7.1312 \times SPC - 2.5188 \times SPC^2 \quad (7)$$

where SPC is the EAA score ratio of sample to casein.

Preparation of samples for hydrolysis

The by-products were removed through 30 min after slaughtering. The by-products were washed under running tap water to remove blood clots and trimmed of the visible fatty and connective tissue. The treated by-products were packed individually in polyethylene bags, transported to the laboratory and stored at 4 °C during 48 h and then were chopped in a meat grinder (Fimar 32/RS Unger, Italy) with a plate having 3 mm diameter holes. After that, an average sample for each by-product was formed. To carry out hydrolysis, 100 mg of the by-product was taken from the sample and placed in glass ampoules with a tapered end. Then, 10 mL of a 6 M solution of hydrochloric acid was added. Subsequently, the mixture was thoroughly stirred and blown with a stream of nitrogen for 2 min. The glass ampoules were sealed and placed into a thermostat. Hydrolysis was carried out at 110 °C for 24 h. After cooling, the hydrolysates were filtered through membrane filters with 0.45 μm pore diameter, and 0.5 mL aliquots were taken. The aliquots were dried at 65 °C in a stream of air. Afterwards, 0.10 mL of 0.15 M NaOH solution was added to the dried aliquots and thoroughly mixed. Then, 0.35 mL of

phenyl isothiocyanate solution in isopropanol was added to the resulting mixture and stirred. The solution obtained after filtration was subjected to chromatographic analysis. The concentration of amino acids in the samples was calculated depending on the protein content in gram per 100 g of the product. The protein content of the by-products was determined using the Kjeldahl method (AOAC 2000, Method No. 988.05).

Determination of by-product amino acid composition

Determination of amino acids was carried out on a HPLC SHIMADZU LC-20 Prominence (Japan) with fluorimetric and spectrophotometric detectors. We used the chromatographic column 25 cm × 4.6 mm SUPELCO C18, 5 μm (USA) with the precolumn to protect the main column from impurities. The chromatographic analysis was carried out in eluent gradient mode at a flow rate of 1.2 mL min^{-1} and the column thermostat temperature of 40 °C. The measurement was performed by HPLC on a reversed phase column with fluorimetric and spectrophotometric detectors at wavelengths of 246 and 260 nm using acid hydrolysis and amino acid modification by phenylisothiocyanate solution in isopropanol to obtain phenylthiohydantoins. A mixture of 6.0 mM CH_3COONa solution at pH 5.5 (component A), 1% isopropanol in an acetonitrile solution (component B), and a 6.0 mM CH_3COONa solution at pH 4.05 (component C) was used as a mobile phase. We used standard samples of amino acids produced by Sigma Aldrich (Germany).

Preparation of samples for *in vitro* digestibility

Compositions were prepared from the ground by-products according to the data obtained during the optimization. By-products were thoroughly mixed and formed into meatballs weighing 11 ± 1 g each. The meatballs were cooked in the air-o-steam (Rational AQ, mod. SCC 61, Germany) at 80 ± 2 °C until the core temperature reached 70 °C (Wen et al., 2015).

***In vitro* digestibility**

Cooked meatballs were ground, then 500 mg samples were taken and homogenized with 2 mL of distilled water to determine digestibility, as described by Wen et al. (2015) with slight modification. The homogenate was suspended in 15 mL 0.1 N HCl containing 8 mg pepsin and incubated for 2 h at pH 2.0, temperature 37 ± 1 °C with continuous shaking.

The resultant suspension was neutralized with 0.2 N NaOH and treated with 15 mg trypsin in 15 mL of phosphate buffer (0.2 M, pH 8.0). The mixture was shaken for 24 h at 37 ± 1 °C. After that, the enzyme was inactivated by the addition of 10 mL 10% trichloroacetic acid.

The mixture was then filtered using ashless filter paper (MN 640 m), and the precipitate was washed with distilled water (1:10, w/v), air-dried, and used for protein determination by Kjeldahl method.

In vitro protein digestibility (IVPD) was calculated using the following formula:

$$IVPD(\%) = \frac{P_b - P_a}{P_b} \times 100, \quad (8)$$

where P_b – protein content of sample before digestion, %; P_a – protein content of sample after digestion, %.

Statistical Analysis

The values are presented as the mean ± SEM. Probability values ≤ 0.05 were taken to indicate statistical significance. The data were analyzed by One-Way ANOVA using free web-based software offered by Assaad et al. (2014).

RESULTS AND DISCUSSION

Modelling and optimization of multi-component composition of food products require considerable time, so it is appropriate to use modern computer technologies. Microsoft Excel provides great opportunities for calculating recipes of multicomponent food compositions. One tool for solving optimization problems is the standard add-on SOLVER of Microsoft Excel program spreadsheets included in Microsoft Office. In terms of functionality, the added SOLVER Excel application is not inferior to analogous special mathematical programs, for example MathCAD. Other things being equal, Excel is characterized by interface simplicity (Nadtochii, 2013).

One of the most important factors in designing new food formulations is protein biological value which was determined by balanced amino acid composition. The human body is able to produce 10 out of 20 amino acids. Shortage of even one essential amino acid results in an inability to synthesize proteins and other biological substances (Feiner, 2006).

In this regard, we carried out the formulation optimization in what concerns the biological value of the feedstock, in particular the content of the essential amino acids.

Optimization of the formulation was carried out in relation to the recommended values of the essential amino acids content in the 'ideal' (standard) protein according to the Food and Agriculture Organization FAO/WHO (1990).

We entered the data on amino acid composition expressed in gram of EAA per 100 g of protein into Excel calculation sheet to get balance equations (Fig. 1). Table 1 presents the protein and amino acids contents (mg amino acid per 100 g product) obtained by chromatographic determination.

L14 =ПРОИЗВЕД(D9/D10;E9/E10;F9/F10;G9/G10;H9/H10;I9/I10;J9/J10;K9/K10)*(1/8)*100

	A	B	C	D	E	F	G	H	I	J	K	L
1												
2	Ingredients	Designation	Mass of raw materials, ppm	The content of amino acids (g amino acid/ 100 g protein)								
3				Valine	Isoleucine	Leucine	Lysine	Methionine + Cystine	Threonine	Tryptophan	Phenylalanine+Tyrosine	
4	tripe	x1	0.049	3.92	3.43	6.12	5.82	1.58	3.62	0.91	5.86	
5	lungs	x2	0.000	5.56	3.65	8.46	6.24	2.08	3.54	0.78	9.17	
6	ears	x3	0.284	3.48	2.08	4.16	4.18	1.67	2.18	0.52	3.86	
7	lips	x4	0.000	3.52	3.12	5.74	6.38	1.92	3.08	0.74	5.68	
8	heart	x5	0.667	6.12	4.86	8.82	7.94	4.42	4.92	1.36	8.24	
9			1	5.26	4.00	7.36	6.77	3.50	4.08	1.10	6.88	
10	FAO/WHO			5.00	4.00	7.00	5.50	3.50	4.00	1.00	6.00	
11	Chemical score, %			105.2	100.0	105.2	123.1	100.0	101.9	109.9	114.6	
12	Coefficient of amino acid score differences (CAASD), %											7.5
13	calculated Biological value (BV)											92.5
14	Essential amino acid index (EAAI), %											107.2

Figure 1. Example of the calculation sheet in Excel.

Table 1. By-product indicators*

Indicators	ears	heart	lips	lungs	tripe
EAA, (mg per 100 g product)					
Valine	864 ± 5.44^{bc}	1030 ± 4.35^{a}	852 ± 5.40^{c}	878 ± 7.07^{b}	558 ± 6.58^{d}
Isoleucine	516 ± 3.07^{d}	819 ± 4.41^{a}	755 ± 6.88^{b}	554 ± 3.02^{c}	487 ± 3.77^{e}
Leucine	1030 ± 6.94^{d}	1490 ± 15.10^{a}	1390 ± 19.10^{b}	1290 ± 8.86^{c}	872 ± 5.26^{e}
Lysine	1040 ± 5.39^{c}	1340 ± 8.26^{b}	1540 ± 11.00^{a}	947 ± 6.59^{d}	829 ± 5.23^{e}
Methionine + Cystine	415 ± 4.53^{c}	745 ± 5.41^{a}	464 ± 5.55^{b}	316 ± 4.60^{d}	224 ± 3.70^{e}
Threonine	541 ± 7.09^{c}	830 ± 3.03^{a}	746 ± 5.37^{b}	537 ± 6.95^{cd}	516 ± 2.52^{d}
Tryptophan	129 ± 3.05^{c}	229 ± 3.04^{a}	179 ± 5.49^{b}	118 ± 2.81^{c}	129 ± 3.27^{c}
Phenylalanine+Tyrosine	958 ± 5.57^{b}	1390 ± 8.22^{a}	1380 ± 10.10^{a}	1390 ± 5.51^{a}	836 ± 3.01^{c}
Protein, %	24.9 ± 0.314^{a}	16.8 ± 0.074^{b}	24.6 ± 0.146^{a}	15.2 ± 0.138^{c}	14.3 ± 0.200^{d}

*Values are means ± SEM, n = 5 per treatment group. Means in a row without a common superscript letter differ statistically ($P < 0.05$) as analyzed by One-Way ANOVA and the TUKEY test.

We introduced the following designations of the ingredients used: X_1 – mass fraction of tripe, X_2 – mass fraction of lungs, X_3 – mass fraction of ears, X_4 – mass fraction of lips, X_5 – mass fraction of heart.

The balance equation prepared in accordance with the established requirements:

1) $5.0 \leq (3.92 \times X_1 + 5.56 \times X_2 + 3.48 \times X_3 + 3.52 \times X_4 + 6.12 \times X_5)$ – content of Valine;

2) $4.0 \leq (3.43 \times X_1 + 3.65 \times X_2 + 2.08 \times X_3 + 3.12 \times X_4 + 4.86 \times X_5)$ – content of Isoleucine;

3) $7.0 \leq (6.12 \times X_1 + 8.46 \times X_2 + 4.16 \times X_3 + 5.74 \times X_4 + 8.82 \times X_5)$ – content of Leucine;

4) $5.5 \leq (5.82 \times X_1 + 6.24 \times X_2 + 4.18 \times X_3 + 6.38 \times X_4 + 7.94 \times X_5)$ – content of Lysine;

5) $3.5 \leq (1.58 \times X_1 + 2.08 \times X_2 + 1.67 \times X_3 + 1.92 \times X_4 + 4.42 \times X_5)$ – content of Methionine and Cystine;

6) $4.0 \leq (3.62 \times X_1 + 3.54 \times X_2 + 2.18 \times X_3 + 3.08 \times X_4 + 4.92 \times X_5)$ – content of Threonine;

7) $1.0 \leq (0.91 \times X_1 + 0.78 \times X_2 + 0.52 \times X_3 + 0.74 \times X_4 + 1.36 \times X_5)$ – content of Tryptophan;

8) $6.0 \leq (5.86 \times X_1 + 9.17 \times X_2 + 3.86 \times X_3 + 5.68 \times X_4 + 8.24 \times X_5)$ – content of Phenylalanine and Tyrosine;

9) $(X_1 + X_2 + X_3 + X_4 + X_5) = 1$ – the sum of the mass fractions of the components.

10) We introduced the following requirement for the goal function: EAAI ≥ 100.

While running the tool SOLVER in a window, we entered the set of parameters. The objective of the program is to determine the optimum ratio of the components for which EAAI reaches 100% under these limitations.

The program offers multiple combinations of the ingredients (Fig. 1) which satisfy the expected requirements for the biological value of the composition and at the same time present the results of the calculated CAASD and BV.

The proposed program of formulation compositions and their biological value indices is presented in Table 2.

The content of protein in our samples of beef lungs was 15.2% ($P < 0.05$), which is similar to the content determined by Skurikhin & Volgarev (1987), but differs from the data obtained by Seong et al. (2014) in the study of Hanwoo cattle offal (17.64 ± 0.72%). The amount of protein in 100 g raw beef heart have been reported as 16.0 g (Skurikhin & Volgarev, 1987) or 14.9–28.5 g (Ockerman & Basu, 2004) and also 18.62 ± 0.53% (Seong et al., 2014). We found that beef heart contained 16.8 g of protein ($P < 0.05$). Florek et al. (2012) determined the protein content in veal calves heart is 18.74 ± 0.30 (g (100 g)$^{-1}$) ($P \leq 0.05$).

The amino acid composition of the different offal varies widely. For example, the content of valine in tripe (558 mg per100 g product) is approximately 2 times less than in heart (1,030 mg per 100 g product). The content of lysine in lips (1,540 mg per100 g product) is more than in other by-products, the content of leucine and isoleucine in lips is close to the parameters for heart (Table 1).

According to Skurikhin & Volgarev (1987), the content of valine, leucine, and threonine in the beef heart is slightly lower than that determined by us: 911 mg in 100 g product against 1,030 mg, 1,408 mg in 100 g against 1,490 mg, and 740 mg in 100 g against 830 mg. Data on the content of isoleucine and tryptophan in 100 g product are similar: 838 mg per 100 g product against 819 mg and 222 mg 100 g^{-1} product against 229 mg. The content of methionine in the Hanwoo cattle heart was 3.8 g 100 g^{-1} of protein (Seong et al., 2014), while we have determined the content of sulfur-containing amino acids methionine and cystine 4.42 g 100 g^{-1} of protein (Fig. 1). The content of methionine in the lungs determined by Cardoso-Santiago & Areas (2001) was 2.38 g 100 g^{-1} of protein.

The results of chromatographic analysis showed the highest content of essential amino acids – lysine, methionine and tryptophan in the heart and lips, slightly lower content of these amino acids noted in the ears and lungs. While Venegas Fornias (1996) identified that in the heart of cattle, these three amino acids were found in an amount of 8.2 g, 2.6 and 1.1 g 100 g^{-1} of protein, and in the lungs – 7.1, 2.0 and 0.9g 100 g^{-1} of protein, respectively. The amount of lysine and methionine in present study were slightly lower (6.24 and 2.08 g 100 g^{-1} of protein) than the values reported by Cardoso-Santiago & Areas (2001) – 7.07 and 2.38 g 100 g^{-1} of protein for boving lungs.

In determining the indicators of biological value, it is important to identify the limiting amino acid, the amino acid score of which is less than 100%. However, in compliance with the limitations in all the compositions offered by the program, there is no limiting amino acid, which indicates that they have high biological value. There is a small difference in amino acid score in relation to the ideal protein in various variants of the compositions. For variants 1, 2 and 4 – the content of isoleucine, methionine and cysteine is equal to the value of the ideal protein, threonine content slightly exceeds the value of the ideal protein. For variant 3 – methionine and cysteine content is close to the values of the ideal protein and the content of other essential amino acids exceeds the ideal protein range by 9% to 35% value. According to the results of biological value calculation, a more balanced ratio of the essential amino acids variants of the compositions 1 and 2 was achieved. The third variant of the composition is characterized by the excessive content of lysine, phenylalanine and tyrosine (Table 2).

Offal is a good source of essential amino acids, in particular such limiting amino acids as lysine, methionine and tryptophan, noted by Mullen et al. (2017). The amino acid composition of offal differs from that of muscle tissue due to the great quantity of connective tissue (Unsal & Aktas, 2003). Seong et al. (2014) noted that the differences in levels and quality of amino acid contents may be attributed due to the differences in protein types between the by-products. According to report, such by-products as ears, legs, lungs, and stomach contain large amounts of proline, hydroxyproline, and glycine as well as lower levels of tryptophan and tyrosine (Jayathilakan et al., 2012). However, when the by-products are combined it is possible to obtain more biologically valuable protein compositions. Aristoy (2011) reported that levels of the essential amino acids in

meat by-products is slightly reduced after cooking or heating treatment due to the low-reducing sugar content of these by-products.

Table 2. Biological value indicators of by-products compositions

Indicators	Value			
	Variant 1	Variant 2	Variant 3	Variant 4
Recipe ingredients content, %				
tripe	4.9	-	-	-
lungs	-	-	22.5	1.9
ears	28.4	25.4	0.3	26.3
lips	-	8.9	13.7	6.1
heart	66.7	65.7	63.5	65.7
Content of EAA, g per 100 g protein				
Valine	5.26	5.22	5.63	5.26
Isoleucine	4.00	4.00	4.34	4.00
Leucine	7.36	7.36	8.31	7.40
Lysine	6.77	6.85	7.33	7.82
Methionine + Cystine	3.50	3.50	3.54	3.50
Threonine	4.08	4.06	4.35	4.06
Tryptophan	1.10	1.09	1.14	1.09
Phenylalanine + Tyrosine	6.88	6.90	8.09	6.95
EAAI, %	107.5	107.5	116.5	107.7
CAASD, %	7.5	7.5	14.4	7.7
Calculated BV, %	92.5	92.5	85.6	92.3
Nutritional index	20.4	20.9	20.4	20.9
C-PER	1.986	1.986	2.095	1.667
IVPD, %	78.2	76.8	68.6	76.3

According to the results, the highest *in vitro* protein digestibility was in compositions number 1 and 2 (78.2% and 76.8%), which correlated with the calculated biological value (Table 2). The third composition was characterized by the lowest *in vitro* protein digestibility – 68.6% ($P < 0.05$), due to the higher content of collagen in this composition compared to other compositions. Wen et al. (2015) detected that the digestibility of pork by pepsin is significantly higher than of beef – 47.22% against 42.75% ($P < 0.05$). The authors explain this by the fact that raw meat contain different levels of collagen. However, digestibility of pork and beef by pepsin and trypsin was not significantly different ($P > 0.05$). However, connective tissue proteins can be hydrolyzed using an acid, alkali, or enzymes to produce hydrolysates, peptides, and amino acids containing a short chain, which are digested well by the human body (Sun-Waterhouse et al., 2014).

CONCLUSIONS

A technique of developing and optimization of by-product composition on indicators of biological value was described in the work. It is obvious that amino acid composition depends on the type of the by-products and the animal species from which they are derived. Computer modeling allowed obtaining the formulation of by-product

composition with the highest possible biological value by varying the content of the by-products in a short time.

Optimization of by-products composition using MS Excel Solver standard software applications allowed obtaining a more balanced ratio of the essential amino acids in the following formulations: 1 – tripe (4.9%), ears (28.4%) and heart (66.7%) or 2 – ears (25.4%), lips (8.9%) and heart (65.7%). According to the results, the highest biological value and *in vitro* protein digestibility were established in these compositions. The obtained by-product compositions may be used as a meat product component or as a separate product after pre-treatment (thermal, enzymatic, mechanical, and other processing).

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