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BY-PRODUCTS OF THE OIL INDUSTRY AS SOURCES OF AMINO ACIDS IN FEED

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Abstract: A global increase in the demand for livestock products suggests that there will be a consequent rise in demand for feed, not only of cereals but of other feeds and particularly proteins. In the present study, oil industry by-products such as soybean meal, soybean cake and sunflower meal were analysed as sources of amino acids in animal nutrition. From among oilseed by-products, the soybean meal content the most of crude protein up to 44% and the best of amino acid composition, while content of crude cellulose (about 6%) is lower in comparison to other oilseed meals. The results showed that the total amino acids in the examined samples ranged from 31.87 to 41.01%, and the total essential and nonessential amino acids varied from 13.41 to 17.38% and from 18.46 to 23.76%, respectively. Generally, the protein contained in soybean meal and cake was rich in essential amino acids. However, because of the lowest amino acid score, methionine was considered as a limiting amino acid in both soybean by-products. On the other hand, soya's meal contained higher level of lysine than other protein-based vegetable alternative to soya like sunflower meals examined in this study. Glutamic acid, aspartic acid, leucine and valine were the most abundant amino acids in all tested by-products of the oil industry. Therefore, partial substitution of protein sources in feeds with proteins from the oil industry by-products may improve feed quality.

Key words: *amino acids, soybean meal, soybean cake, sunflower meal*

INTRODUCTION

The global growth in demand for livestock products has led to an increase in demand for protein based animal feed, which poses certain challenges especially in terms of the agricultural land use (Dei, 2011; van Zanten et al., 2016). Of the vegetable protein sources currently utilized for animal feed, soybean is the most widely used due to its high levels of protein and a favourable amino acid profiles. Many countries depend on soybean and cakes imports because soybeans only grow productively in certain regions of the world. Increasing concerns are emerging from developing countries on the cost of importing soybeans for animal feed. Therefore, efforts are being put into the utilization of diverse local sources of feed ingredients, in particular as protein sources (Manceron et al., 2014). The use of alter-

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native, more sustainable protein feeds/supplements seems to be necessary to replace, at least partly, the current supply chains in order to meet the growing needs. Because of the rising cost of conventional feedstuffs, it is critical for producers to look for alternative feedstuffs in order to reduce the cost of producing animal meat. Animal nutrition is one of the greatest challenges in modern animal husbandry, therefore the knowledge of nutritional values as well as food processing technologies of these alternative feedstuffs play an important role in optimizing their use in animal diet (Beuković et al., 2016; Henchion et al., 2017; Patsios et al., 2020). Furthermore, information on which amino acids are limiting in natural feedstuff and those that contain an excellent balance of amino acids is important in formulating animal diet, and an important task of the feed industry (Ivković et al., 2013; Jajić et al., 2012).

Oilseed crops are grown worldwide and the relatively high protein content (18 – 49%) of different solvent-extracted oilseed meals make them suitable sources of plant proteins (Sarv, 2017). According to the Food and Agriculture Organization of the United Nations in 2017, the world production of oilseeds amounted to 479 million tons, whereas consumption amounted to 492 million tons (FAOSTAT, 2018; OECD-FAO, 2018). Soybean had the largest share of oilseed production in the world in 2017 with 73%, followed by rapeseed (18%), cotton seeds (12%) and sunflower (9%). The main function of dietary protein is to supply amino acids for the growth and maintenance of body tissue. The balance of amino acids found within the seed of oilseed crops compares favourably with that required for human and animal nutrition (Naczk et al., 1998; Sarv, 2017). Oilseeds are subjected to various processing, so the oil industry supplies many kinds of by-products (cakes, expellers, oilseed meal), which are used in animal nutrition. Soybean meal, which is the by-product of oil extraction, is the best vegetable protein source considering on quantity as well its quality (Banaszkiewicz, 2011). According to Dale (1996), soybean meal is the highest quality nutrient of plant origin and the most common meal in animal nutrition, for these reasons, soybean meal is the ‘standard’ to which other sources of protein and amino acids must be compared. The sharp rise in the use of soybean meal in animal nutrition as a substitutable source of protein for feed has been a major driving force in soybean production (Dei, 2011).

There are many different potential oilseeds in addition to soybean, each with strengths and weaknesses in the protein meal supply. Global projections show increasing demands for vegetable oils in the next decades, and a significant increase in demand for oil meals and cakes. Predictions of future land use suggest that the area of oil crops will increase substantially in some developing countries. Oil palm, sunflower and oilseed rape, in addition to soybeans, will dominate and provide much of the future increase (Henchion et al., 2017; Wilson, 2012). Therefore, the objective of this study was to analyse chemical composition and amino acid profiles of the oil industry by-products which is important for nutritional purposes and for acceptance of these products in animal feed.

MATERIALS AND METHODS

Amino acid analysis and chemical composition of the oil industry by-products such as sunflower meal 33%, sunflower meal 44%, soybean meal 44% and soybean cake were performed at the Institute of Food Technology in Novi Sad.

Analysis of chemical composition

Chemical composition analyzes, such as moisture content, crude protein content, crude fiber content, crude ash and crude fat content, were determined by AOAC methods in two replicates (AOAC, 1998).

Analysis of amino acid composition

The amino acids analyses of sunflower meal 33%, sunflower meal 44%, soybean meal 44% and soybean cake were performed by ion exchange chromatography using an automatic amino acid analyser Biochrom 30+ (Biochrom, Cambridge, UK), according to Spackman et al. (1958). The technique was based on amino acid separation using strong cation exchange chromatography, followed by the ninhydrin colour reaction and photometric detection at 570 nm and 440 nm (for proline). Samples of the oilseed by-products were previously hydrolysed in 6M HCl (Merck, Germany) at 110 °C for 24 h, and then cooled to room temperature. After hydrolysis, samples were filtered and made up to 25 mL in sodium citrate buffer (pH 2.2) (Biochrom, Cambridge, UK). Subsequently, prepared samples were filtered through 0.22 μm pore size PTFE filter (Plano, Texas, USA) and the filtrate was transferred to an HPLC vial (Agilent Technologies, USA). The amino acid peaks were identified by comparison of retention times with retention times of amino acid standard purchased from Sigma Aldrich (Amino Acid Standard Solution (Sigma-Aldrich, St. Louis, USA)). The results were expressed as g/100g on dry matter basis of a sample (Sakač et al., 2019).

Statistical analysis

Results were expressed as mean ± standard deviation of three independent replicates for amino acid determination. Analysis of variance (ANOVA) for comparison of sample means was used to analyse variations of the results. All data were processed statistically using the software package StatSoft Statistica, ver. 10 (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

In order to meet the nutritional requirements of livestock, a precise knowledge of the composition of animal feed is necessary. At a minimum, an understanding of the chemical composition, that it, the content of the major macronutrients, is critical for assessing the quality of animal feed as well as the presence of anti-nutritional factors. Protein content is one of the qualitative indicators for the nutritional value of seed and meal (Gizzi & Givens, 2004; Jiang et al., 2014). In this study, the content of chemical composition of soybean meal, soybean cake and sunflower meals are presented in Table 1.

Nutrients content in soybean products are the basic element to optimization diets and estimation of total quantity nutrients required by animals. Soybean meal is the best vegetable protein source in terms of quantity as well its quality, and convincingly the most commonly used oilseed meal in feed (Banaszkiewicz, 2011; Erdaw et al., 2016). However, the presence of Kunitz Trypsin Inhibitors (KTIs) and other thermolabile antinutritive factors limits the use of soybeans, which requires mandatory heat treatment to inactivate antinutritive substances with a negative effect on the digestibility and utilization of soy protein (Beuković, 2014). Quantity of protein in a given lot of soybean meal is dependent upon a number of factors, including the particular cultivar of soy, the extent to which hulls are added back to the meal, and the diluting effects of moisture and flow agents (Dale, 1996; Nahashon & Kilonzo-Nthenge, 2013). From among oilseed by-products, the soybean meal contents the most of crude protein 44.00% as presented in Table 1. In contrast, sunflower meals have lower protein levels (30 – 40%) and higher crude cellulose content (this means they have a slightly lower level of energy content) relative to soybean meal.

Of the plant protein sources currently used for animal feed, soybean seeds contain the most of crude protein and has the best amino acid profile. Amino acids are the functional subunits of proteins that, when linked together in different orders, generate the variety of proteins critical to life and are also important intermediates for many biosynthesis pathways. Amino acids in soybean meal are the key elements for proper growth and development of animals. In most poultry and swine diets soybean meal provides 80% of the dietary amino acids. Ideal protein describes the profile of dietary amino acids that are in perfect harmony with the animal's nutritional requirements (Dei, 2011; Singer et al., 2019). The determined amino acid profiles of soybean meal, soybean cake and sunflower meals are presented in Table 2. The content of total amino acids (TAA) in the tested oilseed by-products ranged from 31.87% to 41.01%. The content of essential amino acids is an important factor in assessing the nutritional value of protein (Mota et al., 2016). Soybean meal is a rich source of essential amino acids

Table 1. Chemical composition of by-products of the oil industry

CHEMICAL COMPOSITION				
Content	**Sunflower meal 33%**	**Sunflower meal 40%**	**Soybean meal 44%**	**Soybean cake**
Moisture (%)	9.04	9.12	10.01	7.54
Crude protein (%)	33.00	40.00	44.00	41.00
Crude fat (%)	1.71	0.84	0.69	4.71
Ash (%)	6.84	8.12	7.34	6.26
Crude cellulose (%)	19.70	11.65	6.35	5.03

Table 2. Amino acid profiles of by-products of the oil industry (sunflower meals, soybean meal, soybean cake) presented in g/100g of dry weight

Amino acid (g/100g)	**Sunflower meal 33%**	**Sunflower meal 40%**	**Soybean meal 44%**	**Soybean cake**
Aspartic acid	3.11 ± 0.02[a]	4.02 ± 0.03[b]	4.82 ± 0.11[c]	4.71 ± 0.15[c]
Threonine*	1.35 ± 0.10[a]	1.71 ± 0.12[b]	1.69 ± 0.02[b]	1.75 ± 0.03[bc]
Serine	1.70 ± 0.11[a]	2.02 ± 0.10[b]	2.27 ± 0.08[c]	2.14 ± 0.06[b]
Glutamic acid	6.03 ± 0.17[a]	8.05 ± 0.22[b]	8.34 ± 0.27[c]	7.85 ± 0.13[b]
Proline	1.58 ± 0.07[a]	2.06 ± 0.04[b]	2.37 ± 0.03[c]	2.43 ± 0.06[c]
Glycine	1.63 ± 0.12[a]	2.02 ± 0.03[b]	1.69 ± 0.06[a]	1.56 ± 0.04[ac]
Alanine	1.39 ± 0.09[a]	1.82 ± 0.11[b]	1.56 ± 0.05[c]	1.67 ± 0.09[bc]
Cystine	0.59 ± 0.01[a]	0.81 ± 0.03[b]	0.48 ± 0.01[c]	0.57 ± 0.02[a]
Valine*	2.32 ± 0.04[a]	2.43 ± 0.06[a]	2.19 ± 0.07[b]	2.01 ± 0.05[c]
Methionine*	1.75 ± 0.05[a]	2.02 ± 0.08[b]	0.65 ± 0.02[c]	0.43 ± 0.03[d]
Isoleucine*	1.80 ± 0.12[a]	2.04 ± 0.10[ab]	2.41 ± 0.14[c]	2.22 ± 0.12[bc]
Leucine*	2.63 ± 0.08[a]	3.06 ± 0.13[b]	3.72 ± 0.11[c]	3.35 ± 0.10[d]
Tyrosine	0.74 ± 0.06[a]	0.93 ± 0.08[ab]	1.23 ± 0.07[b]	1.42 ± 0.03[c]
Phenylalanine*	1.83 ± 0.04[a]	2.03 ± 0.07[b]	2.85 ± 0.19[c]	2.18 ± 0.05[d]
Histidine*	0.91 ± 0.03[a]	0.82 ± 0.06[a]	1.31 ± 0.04[c]	1.16 ± 0.04[d]
Lysine*	0.82 ± 0.07[a]	1.15 ± 0.04[a]	2.56 ± 0.07[b]	2.64 ± 0.06[b]
Arginine	1.69 ± 0.08[a]	1.93 ± 010[b]	0.87 ± 0.12[c]	1.41 ± 0.11[d]
TEAA	13.41 ± 0.53	15.26 ± 0.66	17.38 ± 0.66	15.74 ± 0.48
TNEAA	18.46 ± 0.73	23.66 ± 0.84	23.63 ± 0.80	23.76 ± 0.69
TAA	**31.87 ± 1.26**	**38.92 ± 1.40**	**41.01 ± 1.46**	**39.50 ± 1.17**

Values represent the average of triplicate experiments ± standard deviations. Different lowercases in the same raw represent a statistically significant difference (p < 0.05) – among tested samples. Essential amino acids are indicated by an asterisk (*). Abbreviations: TEAA, total essential amino acids: TNEAA, total nonessential amino acids; TAA, total amino acids

like lysine, threonine, isoleucine, leucine and valine, the amino acids that are seriously deficient in corn, sorghum, and other cereal grains that are commonly fed to swine and poultry.

Soybean meal can serve as the only source of supplemental protein for all types of poultry and swine at any stage of growth or production (Nahashon & Kilonzo-Nthenge, 2011). Our results indicated that content of the essential amino acids in soybean meal was 17.38%. However, the amino acids in soybean meal are not perfectly balanced, as they tend to be low in methionine and cysteine which were in line with previous research conducted by Banaszkiewicz (2011). The contents of methionine were the lowest among all other amino acids in both soybean meal and soybean cake, which could be owing to the oxidation of methionine or the lack of methionine in soybean material (Li et al, 2020; Ramachandran et al., 2007). Considerable efforts have been made to improve the methionine content of soybean seeds, where one of the main approaches is introduction of methionine rich storage protein gene (Guo et al., 2020). Further, amino acids profile of oilseed by-products presented in Table 2 show that the quality of sunflower meal 40% protein in terms of the total content of essential amino acids (15.26%) is quite good compared to soybean meal protein. Significant differences between soybean meal and sunflower meal with the same protein content are that sunflower meal contains a significantly lower amount of lysine (1.15: 2.56), and a significantly higher amount of methionine (2.02: 0.65), cystine (0.81: 0.48) and arginine (1.93: 0.87) ($p < 0.05$). Both meals contain approximately equal amounts of threonine and tyrosine.

Soybean meal contains the highest amount of lysine compared to other oilseeds. The reason for using lysine as the comparator is that lysine is generally considered the first limiting amino acid for swine and most other nonruminant animals, except for poultry, where methionine is the first limiting and lysine is the second limiting amino acid. In terms of high lysine content, the soybean meal is mainly used in poultry and pig's nutrition as both of these species have a high requirement for this essential amino acid (Petterson & Pontoppidan, 2013). In previous study, high level of lysine was observed in the diets of the gilts from nine farms in Vojvodina which meets the criteria of the Regulations on the quality of the feed (Jajić et al., 2012). As shown in Table 2, the content range of essential amino acids for sunflower meal 30% and sunflower meal 40% was from 13.41 to 15.26%. Thus, sunflower meals could be considered as rich sources of almost all essential amino acids except lysine. In other words, lysine could be a limiting amino acid in sunflower meals, which is in accordance with the findings of a previous report (Ivanova et al., 2013). The content of lysine and histidine was the lowest among all other amino acids in both sunflower meals, while the main amino acids were glutamic acid, aspartic acid, valine and leucine. On the other hand, the protein of soybean cake contains considerable quantity of lysine (2.64%) but value of protein is limited by sulphur-containing amino acids methionine and cystine content (0.43% and 0.57%, respectively). The differences between lysine content were statistically significant ($p < 0.05$) between soybean and sunflower meals. Further, the dominant amino acid in all investigated oilseed by-products samples was glutamic acid approximately 6.03%, 8.05%, 8.34% and 7.85% in sunflower meal 33%, sunflower meal 40%, soybean meal 44% and soybean cake, respecttively. Glutamic acid was followed by other markedly present amino acids: aspartic acid > leucine > phenylalanine ≥ isoleucine, while sunflower meals were characterized by higher amounts of valine compared to soybean meal and soybean cake.

CONCLUSIONS

Sources of protein for animal feed are diverse with significant opportunities for further diversification and substitution. Therefore, more research on alternative sources is needed before many possibilities can be used in practice. The importance of soybean meal reflects its high level of protein, consistent availability and price competitiveness as well as higher level of lysine compared to other oil seed by-products. It is particularly attractive as an ingredient for feeds used in the pig and poultry sectors. Regarding sunflower meals, they could supplement animal diet with sulphur-containing essential amino acids which, in general, are deficient in most proteins of plant origin.

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СПОРЕДНИ ПРОИЗВОДИ ИНДУСТРИЈЕ УЉА КАО ИЗВОРИ АМИНОКИСЕЛИНА У ХРАНИ ЗА ЖИВОТИЊЕ

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Сажетак: Глобално повећање потражње за производима анималног порекла указује на значајан раст потражње за сточном храном, не само житарицама већ и другом храном за животиње, а посебно протеинима. Циљ ове студије био је да се испитају споредни производи индустрије уља, као што су сојина сачма, сојина погача и сунцокретова сачма, као извори аминокиселина у храни за животиње. Међу споредним производима индустрије уља, сојина сачма је имала највиши проценат протеина до 44% и најбољи састав аминокиселина, док је садржај сирове целулозе (око 6%) био нижи у поређењу са осталим узорцима сунцокретове сачме. Резултати су показали да се садржај укупних аминокиселина у испитиваним узорцима кретао од 31,87 до 41,01%, док су вредности есенцијалних и неесенцијалних аминокиселина варирале од 13,41 до 17,38%, односно од 18,46 до 23,76%. Поред тога, највећи садржај есенцијалних аминокиселина је примећен у сојиној сачми и погачи. Због најниже вредности, метионин се сматрао ограничавајућом аминокиселином у оба споредна производа од соје. С друге стране, садржај лизина је био виши у сојиној сачми у поређењу са испитиваним узорцима сунцокретове сачме у овој студији. Глутаминска киселина, аспарагинска киселина, леуцин и валин биле су најзаступљеније аминокиселине у свим тестираним узорцима. Стога делимична замена извора протеина у храни за животиње протеинима из споредних производа индустрије уља може побољшати квалитет хране за животиње.

Кључне речи: *аминокиселине, сојина сачма, сојина погача, сунцокретова сачма*

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