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LYSINE CONTENT IN DIFERENT HYBRIDS OF MAIZE IN PIG'S DIETS

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SUMMARY: The aim of this study was to examine the content of lysine in maize, as one of the main compound of feeding mixtures for pigs. Sixteen commercial samples of maize hybrids, were analyzed for lysine content which was highly correlated with the level of protein, r = 0.86. Obtained results were compared to ones established by EVONIK. Analytical methods based on acid hydrolysis and detection by liquid chromatography was used by applying previously validated conditions. Limit of detection (LOD) for lysine in maize was 0.035%, while the limit of quantification (LOQ) was 0.106%. Lysine content was ranged from 0.142% to 0.297% with the average value of 0.223% and standard deviation of 0.049%.

Key words: *lysine, maize hybrids, pigs.*

INTRODUCTION

Dietary amino acids have crucial signification for health, development, growth, reproduction and lactation in animals. Some animals, like non-ruminants cannot synthesize essential amino acid and they must be taken in with food. Inadequate settlement for pig with protein and amino acids is particularly harmful if it occurred in early stages (Kovčin et al., 2001).

Pig diets mostly involve a portion of amino acid that is not biologically available to the animal. This is because most proteins are not fully digested and the amino acids are not fully absorbed, and also because not all absorbed amino acids are metabolically available. Diets vary considerably in the proportions of their amino acids that are biologically available. The amino acids in some proteins such as milk products are almost fully bioavailable, whereas those in other proteins such as certain plant seeds are much

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less so (Southern, 1991; Lewis and Bayley, 1995; Žilić, 2010). A few essential amino acids tend to be limiting in typical swine diets. Lysine is usually the most-limiting (or first-limiting) amino acid. It means that if a diet is formulated to supply the correct amount of lysine, then generally the levels of other essential amino acids will be adequate (Myer et al., 1996). If diet does not insure the proper amount of lysine and other essential amino acids, pigs will grow slower than the pigs that receive the appropriate concentration of amino acids, also will develop less muscle and more fat. Higher amino acid levels allow the animal to deposit greater amounts of lean tissue rather than fat. Estimation of the proportions of amino acids in ideal protein for growing swine have been derived from an examination of various types of data, including the composition of pig tissue, the composition of sows' milk, and combinations of individual estimation of amino acid requirements. Therefore, the overall ideal pattern will change as the proportions of maintenance and new tissue synthesis change. In addition, there are changes in the amino acid content of pig tissue as the pig grow from birth to market weight (Kyriazakis et al., 1993; Susenbeth, 1995; Mahan and Shields, 1998).

Pigs require a diet with a balanced pattern on necessary amino acids in which protein supplements usually represents 20% of diet, but make up approximately up to 35% of the cost of diet. Lower cost of protein reduced quantity or concentration of amino acids in diet which reduce the margin of safety. Information about quantity of amino acids and quantity required by the pig is mandatory for efficiently amino acids optimization (Mosenthin et al., 2000). In theory, any deviation of pattern of amino acids in ideal protein will lead to a reduction in animal performance, at least in terms of the efficiency of dietary protein utilization. In practice, however, swine seem to be relatively tolerant to quite wide variations in the pattern of amino acids, as long as all amino acid requirements are met. Knowing the composition of amino acids in proteins is used to assess the feeding value of dietary proteins. In order to meet the protein requirements, such as amino acids requirements, the diet must include enough essential amino acids in the right proportion. The fast and accurate determination of the amino acids from the raw materials used in animal feeding is necessary for a correct estimation of the feeding value of the protein (Maros et al., 2011). Requirements vary depending on the species and age of animals. The effects of maize lysine content would be most evident in a swine finisher diet because of the relatively high proportion of maize typically used.

According to Serbian regulations governing the quality of animal feed, complete feed mixture for pigs are divided into several categories and for each lysine and protein content is specified. For a compete mixture for (feeding) piglets lysine content ranges from 1% - 1.3%, and 18% - 22% of protein content. In a complete mixture for pigs these values are from 0.55% - 0.8% for lysine, and 13% - 16% for protein. In pigs it requires extra protein and lysine (Službeni glasnik Republike Srbije, 2010).

The aim of this study was to examine the content of lysine in different hybrids of maize used for making diets for pigs. Different maize hybrids were tested for protein content and that content was correlated to the lysine content.

MATERIAL AND METHODS

Material. Acetonitrile (LC grade) and methanol (LC grade) were purchased from Sigma-Aldrich (St. Louis, MO). Borate buffer, OPA and FMOC reagents and amino acid mixture standard solutions (10, 25, 100, 250 and 1000 pmol μl^{-1}) were obtained

from Agilent Technologies (Waldbronn, Germany). Hydrochloric acid, used for preparation of 6 M and 0.1 M HCl, was obtained by Lach-Ner (Neratovice, Czech Republic). Sodium phosphate monobasic was purchased from Acros Organics (New Jersey, USA). LC grade water was produced by Heming ID-3 system (Belgrade, Serbia), while cellulose membrane filters (pore size 0.22 μm) were purchased from Agilent Technologies (Waldbronn, Germany).

Equipment. The analysis was performed using the Agilent 1260 Infinity Liquid Chromatography System, equipped with μ-degasser binary pump, standard autosampler, ZORBAX Eclipse-AAA column and DAD detector.

Samples. There were 16 commercial maize hybrid samples of known producer in order to determine lysine content. *Sample preparation:* Samples milled in a laboratory mill. After that, 0.6 g to 0.7 g of sample was measured (equivalent to the nitrogen content of 10 mg) in a vacuum hydrolysis tube, and then hydrolyzed with 6 M HCl. Hydrolysis was carried out with Reacti-Therm™ apparatus with constant stirring for 6 hours at 150 °C. After hydrolysis, samples were allowed to reach the room temperature and then evaporated to dryness using the Reacti-Therm™ at 70 °C in a stream of nitrogen. The residue was quantitatively transferred into a 50 ml volumetric flask with 0.1 M HCl. The solution was filtered through quantitative filter paper, and then additionally filtered using a cellulose membrane filter (pore size 0.22 μm). This method was previously validated (Jajić et al., 2012).

HPLC determination. Chromatography conditions were in accordance with the Agilent method (Henderson et al., 2000) with the exception of mobile phase A preparation where 5.678 g Na_2HPO_4 was dissolved in 1 L water and then adjusted with 6 M HCl to pH 7.8. Hydrolyzed samples and standard mixtures of amino acids were automatically derivatized with OPA and FMOC reagents using the appropriate injector program. After derivatization, sample or standard mixture was injected on ZORBAX Eclipse-AAA column (5 μm, 150 × 4.6 mm) at 40 °C. Mobile phase A was 40 mM Na_2HPO_4, adjusted with NaOH to pH 7.8, and mobile phase B acetonitrile-methanol-water (45:45:10, v/v/v). Separation was performed at a flow rate of 2 ml min^{-1} with a gradient program, during the 26 min period of analysis. All data were analyzed to test the correlation.

RESULTS AND DISCUTION

Lysine is usually used as a reference for other essential amino acids in the ideal protein concept due to its simplicity of analysis in feedstuffs.

Table 1. Lysine content in maize samples

Sample	Lysine (%)	Protein (%)
1	0.241	7.49
2	0.188	6.47
3	0.235	6.67
4	0.255	7.54
5	0.257	7.22
6	0.196	6.25
7	0.256	8.28
8	0.242	7.54
9	0.194	7.03
10	0.279	8.78
11	0.198	6.62
12	0.241	7.60
13	0.237	7.60
14	0.231	7.50
15	0.234	7.91
16	0.290	8.83
Average	0.236	7.46
Standard deviation (%)	0.030	0.796
Coefficient of variation (%)	12.57	10.67

Therefore, estimating accurately the requirement of lysine is essential for the setting of other indispensable amino acids (Baker, 1997).

In this paper there were 16 samples of hybrid maize, which further will be used for purpose of making swine mixture, and was controlled by the proportion of lysine as an important parameter for feeding gilts. In complete mixtures it ranges from 0.55% to 1.3% and in supplementary mixtures lysine value is 2.0% - 3.3% (Službeni glasnik Republike Srbije, 2010).

Samples of maize were analyzed and the results are presented in Table 1. As can be seen, lysine content ranges from 0.188% in sample number 2, to 0.290% in sample number 16. Average value for lysine in maize samples, obtained from 16 samples was 0.236% with standard deviation of 0.030% and coefficient of variation of 12.57%. The results were compared with results presented by EVONIK (EVONIK Industries, 2010), which study included 918 samples.

As can be seen, in Table 2, lysine content was ranged from 0.14% to 0.37% with the average value of 0.24%. By comparing these results, it can be concluded that lysine content of maize samples in both studies was quite similar. Namely, both, the lysine content and coefficient of variation, correspond to results presented by EVONIK.

Table 2. Lysine content in maize samples (EVONIK Industries, 2010)

EVONIK	Average (%)	0.24
	RSD (%)	9.80
	Range (%)	0.14-0.37

Furthermore, in maize samples, protein content was determinated (Table 1) and then compared to lysine content. Protein content was ranged from 6.47% in sample

number 3 to 8.83% in sample number 16 with standard deviation of 0.79% which indicates the consistency of test results. The correlation coefficient (r) was 0.86 which represent strong positive correlation which is represented in figure 1.

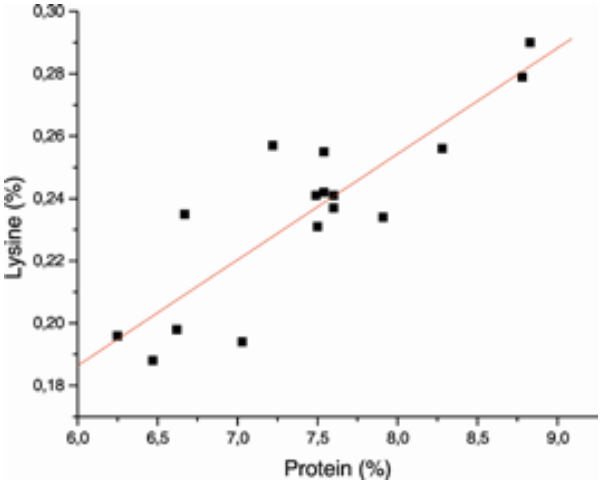

Fig. 1. Correlation between lysine and protein content

CONCLUSION

Significance of lysine in pig nutrition, as an essential amino acid is large, so it is necessary to take care of its entries, and if it is supplemented, dose it according to age. This study illustrated that lysine content corresponds with protein contents in maize hybrids. Also, it represented agreement between obtained results and results that EVONIK presented. Average value for lysine in maize samples, was 0.236% with standard deviation of 0.030% and coefficient of variation of 12.57%, and correlation coefficient (r) was 0.86 which represent strong positive correlation to protein content. The results were corresponding to results presented by EVONIK, which average is 0.24%, and value of coefficient of variation was 9.80%. Also, one of the recommendations is that in the preparation of meals for pigs as a laboratory controlled mixtures, needs to be devoted more attention to lysine ratio, than the protein content.

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SADRŽAJ LIZINA U RAZLIČITIM HIBRIDIMA KUKURUZA ZA ISHRANU SVINJA

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Izvod

Cilj ovog rada bio je ispitivanje sadržaja lizina u kukuruzu, kao veoma značajnoj aminokiselini u ishrani svinja. Sadržaj lizina je analiziran u šesnaest komercijalnih hibrida kukuruza, pri čemu je dobijena visoka korelacija (r = 0,86) u odnosu na sadržaj proteina. Dobijeni rezultati sadržaja lizina su poređeni sa Evonikovim. Analitička metoda je zasnovana na kiseloj hidrolizi, a kvantifikacija je izvršena tečnom hromatografijom, prema ranije validovanoj metodi. Granica detekcije za lizin u kukuruzu iznosi 0,035%, dok je granica određivanja 0,106%. Sadržaj lizina u analiziranim uzorcima se nalazio u opsegu od 0,142% do 0,297%, a srednja vrednost je iznosila 0,223%. Vrednost standardne devijacije je iznosila 0,049%.
Ključne reči: lizin, kukuruz, svinje.

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