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Evaluation of heat treatment in soy products

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Abstract: *The objective of this study was to evaluate the degree of heat treatment in soy products using the KOH protein solubility method. For the analysis, eight samples of soybean products were collected, specifically raw soybeans (2 samples), soybean extracted meal (2 samples), extruded soy (2 samples), and soybean cakes (2 samples). Evaluation of nutritional contents, the obtained results generally correlated with the recommended values. In both samples of raw soybean (no. 1, no. 2), one sample of extruded soy (no. 2), and one sample of soybean meal (no. 2), we confirmed protein solubility values in KOH that exceeded the recommended limit for sufficiently heat-treated feeds. Animals consuming inadequately processed soybean feed may be at higher risk of intestinal mucosal disorders, impaired growth performance, and reduced protein digestibility.*

Keywords: *soy, heat, trypsin inhibitor, protein*

I. INTRODUCTION

The most used raw materials in farm animals' nutrition include soybeans, which are considered one of the most important plant sources of protein worldwide. Soybeans contain approximately 40% high-quality proteins with a suitable amino acid profile, making them an indispensable part of feed mixtures and rations for monogastric and ruminant animals. In soybeans, fats (approximately 20%) and proteins (approximately 40%) account for an average of about 60% of the dry matter. The remaining content is mainly carbohydrates (approximately 30%) and ash (approximately 10%). The water content in stored mature beans is usually about 13% to ensure storage stability [1]. Soybeans contain several antinutritional substances such as trypsin inhibitors, phytic acid, tannins, saponins, phytoestrogens, alkaloids, lectins, and antivitamins [2]. According to the comprehensive database Matrix Enzyme Resource of Protease Specificity, protease inhibitors are classified into 38 clans and divided into 78 families. Among them are various groups of inhibitors, including serpins, phytocystatins, Kunitz-type inhibitors, Bowman-Birk inhibitors, bifunctional α-amylase-trypsin inhibitors, mustard-type, potato-type-I and potato-type-II inhibitors, potato metallo carboxypeptidase inhibitors, and pumpkin and cyclotide inhibitors [3]. High levels of protease inhibitors in soybeans cause a decrease in protein digestion by forming a trypsin inhibitor bond with trypsin. These protease inhibitors stimulate cholecystokinin, which promotes trypsinogen synthesis in the pancreas with subsequent pancreatic hypertrophy.

The production of trypsinogen with an increased methionine content represents its limit at the blood level, thereby reducing protein synthesis. Soybean trypsin inhibitors are thermolabile, and nutritional prevention consists of heat treatment of the feed at 120 °C [4–5]. Feeding raw soybeans poses a health risk to animals and, therefore, soybeans must be heat-treated before feeding. The aim of heat treatment of soybeans is to reduce the content of antinutritional substances, increase the availability of nutrients, reduce the content of undesirable microorganisms, and improve the dietary properties of feeds. The optimal temperature for inactivation of trypsin inhibitor for monogastrics is 120 °C, and the critical value is 130 °C, at which point protein availability decreases [5].

On the contrary, in ruminants, higher protein denaturation is welcome due to the increase in the content of rumen undegradable proteins, which is the task of heat treatment of soybeans in ruminants, and therefore it is recommended to heat treat soybeans at a temperature of 140 °C [6].

The aim of this study was to evaluate the degree of heat treatment of soy products using the KOH protein solubility method.

II. Material and Methods

Sample collection and preparation

For the analysis, eight samples of soybean products were collected, specifically raw soybeans (2 samples), soybean extracted meal (2 samples), extruded soy (2 samples), and soybean cakes (2 samples). Each sample was analyzed three times. The samples were obtained through random sampling from farms located in the eastern part of Slovakia. After collection, all samples were properly labelled, stored under appropriate conditions, and transported to the laboratory to prevent any deterioration.

Laboratory analyses

Samples of feed were analyzed for dry matter (DM), consisting of crude protein (CP), fat, fiber, and ash, using conventional methods according to Commission Regulation (EC) no. 152/2009 [7]. After drying the sample at 105±2 °C under the indicated conditions, DM was calculated by weight. The CP content was determined using a Kjeltec, type 2300 (Foss Tecator AB, Höganas, Sweden), according to the Kjeldahl method. The Soxhlet method was used to determine total fat (as ether extract). Fat was extracted in a 2-unit extractor (Det Gras J.P. Selecta S.A., Barcelona, Spain) with petroleum ether. Fiber was extracted by defatting the well-dried samples and separating the residue in a fiber extractor (Dosi-Fibre extractor, J.P. Selecta S.A., Barcelona, Spain), and ash content was determined by burning the samples at 550 °C in an AAF 1100 electric muffle furnace (Carbolite Gero, Derbyshire, United Kingdom).

To confirm the heat treatment of soybean products, the protein solubility method in potassium hydroxide (KOH) according to Araba and Dale [8] was used. This method consists of determining the percentage of protein that is solubilized in a KOH solution. The sample preparation itself before analysis was carried out as follows: 1.5 g of soy samples were placed in 75 ml of 0.2% KOH solution and stirred for 20 minutes at 22 °C. Then 50 ml were taken and immediately centrifuged for 15 minutes. A 10 ml aliquot was taken for the determination of nitrogen (N) content in the liquid fraction by the Kjeldahl method.

Data analysis

The statistical functions of the MS Excel program were used to evaluate the average value and standard deviation (SD).

III. Results and Discussion

The nutritional contents of soy products are summarized in Table 1. The assessment of the nutrient content, especially protein and fat, in soybean feeds is an important factor for the correct selection of feed ingredients with a subsequent impact on animal production indicators. A nutritional assessment was performed on eight soybean feeds, and differences in nutrient content were confirmed depending on the feed processing method used. The obtained results generally correlated with the values reported in the literature [9–11]. The CP content in the analyzed samples of raw (384.7 ± 31.96 g.kg^{-1} of DM) and extruded soy (364.0 ± 66.46 g.kg^{-1} of DM) ranged within the recommended values (350.0 – 400.0 g.kg^{-1} of DM) for these feeds [11–12]. In samples of soybean meal and soybean cake, the CP content ranged at 364.0 ± 5.80 and 447.7 ± 5.52 g.kg^{-1} of DM, respectively, which corresponds to the recommended values for these feeds at 420.0 – 500.0 g.kg^{-1} of DM [11].

Table 1. Nutritional values of soybean products (g.kg^{-1}of DM[1])

	DM[1] (g.kg^{-1})	CP[2]	Fat	Fibre	Ash
			(g.kg^{-1} of 100% DM)		
Raw soybean no. 1	860.2	362.1	197.9	101.0	57.5
Raw soybean no. 2	862.3	407.3	163.1	100.8	59.4
Extruded soy no. 1	896.7	411.0	193.4	75.6	63.5
Extruded soy no. 2	970.4	317.0	204.7	91.8	58.5
Soybean meal no. 1	908.3	482.1	9.7	51.9	72.4
Soybean meal no. 2	899.5	490.3	18.3	50.1	87.4
Soybean cake no. 1	918.3	451.6	84.2	58.2	65.6
Soybean cake no. 2	919.1	443.8	96.1	63.2	66.6

1 - dry matter, 2 - crude protein

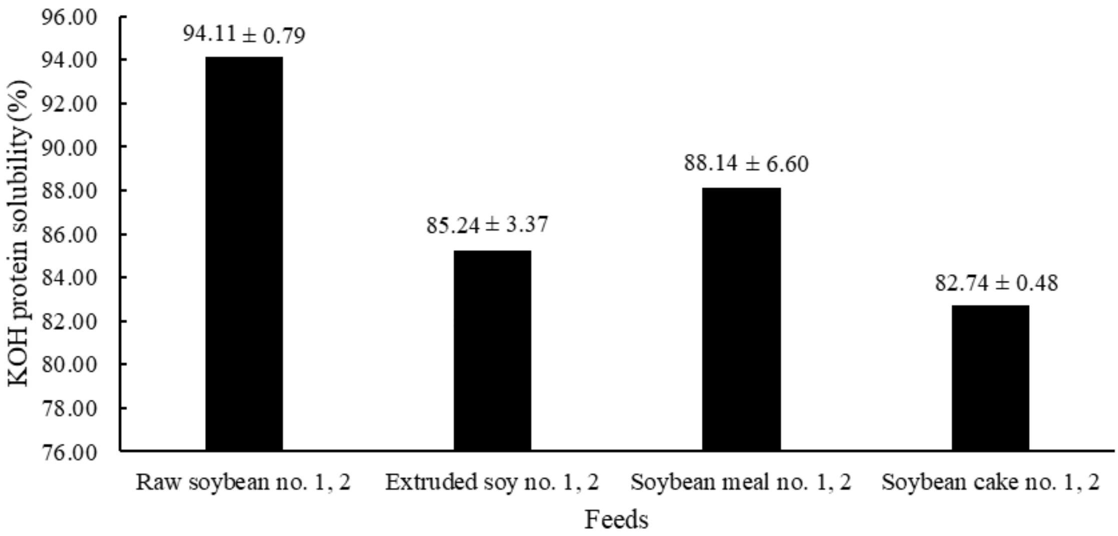

Figure 1. Evaluation of the heat treatment of soy products

Evaluation of the heat treatment of soy products is summarized in Figure 1. The average value of KOH protein solubility for the raw soybean (sample no.1 and 2) was 94.11±0.79% but individually, sample 1 showed 93.50±0.64% and sample 2 showed 94.72±0.20%. For extruded soy (sample no.1 and 2), the average value was 85.24±3.37% with sample 1 at 82.37±0.20% and sample 2 at 88.13±0.86%. In soybean meal, the average KOH protein solubility was 88.14±6.60%, individually, sample 1 had 82.51±1.83% and sample 2 had 93.77±0.42%. For soybean cake (samples no. 1 and 2), the average value was 82.74±0.48%, with sample 1 at 82.37±0.19% and sample 2 at 83.12±0.16%. Correctly heat-processed soybean feeds should have a protein solubility of around 78-85% and raw soybeans should have a solubility in KOH solution of more than 90% [13]. In both samples of raw soybean (no. 1, no. 2), one sample of extruded soy (no. 2), and one sample of soybean meal (no. 2), we confirmed protein solubility values in KOH that exceeded the recommended limit for sufficiently heat-treated feeds, indicating that these feeds were not sufficiently heat-treated. Feeding heat-untreated soy can, in addition to negatively affecting protein digestion, digestibility, and utilization in animals, cause intestinal mucosal disorders and increase susceptibility to diseases, as trypsin inhibitors disrupt normal enzyme function and digestion, ultimately impacting animal growth [14–15]. Among the most effective preventive measures, in addition to heat treatment, are radiofrequency heating, selecting soybean cultivars with lower trypsin inhibitor expression, and verifying feed quality before inclusion in diets for sensitive animal species, which allows for risk management and ensures feed safety [16–17].

IV. Conclusion

In this study, we evaluated the degree of heat treatment of various soybean products using the KOH protein solubility method. In both samples of raw soybean (no. 1, no. 2), one sample of extruded soy (no. 2), and one sample of soybean meal (no. 2), we confirmed protein solubility values in KOH that exceeded the recommended limit for sufficiently heat-treated feeds, indicating that these feeds were not sufficiently heat-treated. This insufficient processing suggests the presence of active anti-nutritional factors, such as trypsin inhibitors, which can negatively affect protein digestibility, nutrient utilization, and overall animal health. Animals consuming inadequately processed soybean feed may be at higher risk of intestinal mucosal disorders and impaired growth performance.

These findings highlight the critical importance of proper heat treatment during soybean processing and the need for routine quality control measures, including protein solubility testing, before inclusion in animal diets.

V. Acknowledgements

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