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## Bromatological and mycotoxin analysis on soybean meal before and after the industrial process of micronization

### Análise bromatológica e micotoxicológica do farelo de soja antes e após processo industrial de micronização

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#### ABSTRACT

Aflatoxins, fumonisins and zearalenone take part of the most studied mycotoxin groups due to their toxic effects on animal and human health. This research evaluated samples of soybeans meal used in animal food industry. A hundred and twenty one soybean meal samples were analyzed, so that 66 were analyzed before the industrial processing of micronization and 55 after it. The bromatological average of samples before micronization showed the following answers: 12.4% moisture; 46.4% protein; 79.5% protein solubility; 5.9% ash content; 2.2% fat; 4.3% fiber and 0.02 ( $\Delta$ pH) of urease activity. The samples of micronization soybean meal showed 7.0% average values for moisture and 48.6% for crude protein. The mycotoxin levels were low in natura soybean meal; therefore, average values were 0.5  $\mu$ g kg<sup>-1</sup>, 29.6  $\mu$ g kg<sup>-1</sup> and 56.8  $\mu$ g kg<sup>-1</sup> for aflatoxin, zearalenone and fumonisin, respectively. After micronization, the average values for the studied samples were 1.3  $\mu$ g kg<sup>-1</sup>, 67.5  $\mu$ g kg<sup>-1</sup> and 89.1  $\mu$ g kg<sup>-1</sup>, respectively for the same mycotoxins. The results for bromatological and mycotoxin analyses indicate similarity with the established patterns according to the Brazilian Compendium for Animal feed and reference literature. However, at least one of the three studied mycotoxin was detected in all of the analyzed samples and there was greater contamination of soybeans meal after the micronization process.

**Key words:** mycotoxins, micronization soybean meal, animal feedstuff.

#### RESUMO

Aflatoxinas, fumonisinas e zearalenona pertencem aos grupos de micotoxinas mais estudados, devido aos efeitos tóxicos provocados na saúde animal e humana. O presente estudo avaliou amostras de farelo de soja utilizado em uma indústria de nutrição animal. Foram analisadas 121 amostras de farelo de soja, sendo 66 antes do processo industrial de micronização

e 55 após o processamento. A composição bromatológica média das amostras antes da micronização apresentou 12,4% de umidade; 46,4% de proteína; 79,5% de solubilidade proteica; 5,9% de cinzas; 2,2% de gordura; 4,3% de fibra e 0,02 ( $\Delta$ pH) de atividade ureásica. As amostras de farelo de soja micronizado apresentaram valores médios de 7,0% para umidade e 48,6% para proteína bruta. Os resultados apresentaram baixos níveis de micotoxinas no farelo de soja in natura, detectando valores médios para aflatoxina, zearalenona e fumonisina de 0,5  $\mu$ g kg<sup>-1</sup>, 29,6  $\mu$ g kg<sup>-1</sup> e 56,8  $\mu$ g kg<sup>-1</sup>, respectivamente. Após a micronização, as amostras apresentaram valores médios de 1,3  $\mu$ g kg<sup>-1</sup>, 67,5  $\mu$ g kg<sup>-1</sup> e 89,1  $\mu$ g kg<sup>-1</sup>, respectivamente, para as mesmas micotoxinas. Os resultados das análises bromatológicas e de micotoxinas evidenciaram conformidade com os padrões estabelecidos pelo Compendio Brasileiro de Alimentação Animal e literatura de referência. Entretanto, detectou-se a presença de, no mínimo, uma das três micotoxinas pesquisadas em todas as amostras analisadas e uma maior contaminação no farelo de soja após o processo de micronização.

**Palavras-chave:** micotoxinas, farelo de soja micronizado, alimentação animal.

#### INTRODUCTION

Companies responsible for the animal feed have worked hard to produce adequate and free of contaminants food in order to improve a great nutrient absorption. The adequate offer of nutrients which will meet the animals' needs allows better food conversion and greater production (TININI et al., 2012). The essential raw matters to produce animal feed are corn and soybean meal, which when

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associated to minerals, vitamins, amino acids and additives meet the nutrients need for vital functions of the animal (BELLAYER & SNIZEK JR, 2012).

Micronization is a grinding process that transforms soybean meal into very fine particles (microns). Due to the increase of the superficial area of a raw matter generated through this process, micronization enables an increase in solubility and bioavailability, which improves powder uniformity and mixture quality (CAMPOS & SILVA, 1986).

The presence of mycotoxin in animal feed also affects the product quality. This factor may cause problems to the industry and farming since it may increase the incidence of diseases and lessen yield efficiency (SASSAHARA et al., 2003).

There are over four hundred types of different toxins that are produced by over 350 types of fungi. The well-known mycotoxins in grains and processed products in soybeans are: Aflatoxins (B1, B2, G1 and G2), deoxynivalenol, nivalenol, ochratoxin A and zearalenone. The aflatoxins and ochratoxins are produced by fungi of *Aspergillus* genus, while deoxynivalenol, nivalenol and zearalenone are produced by fungi of *Fusarium* genus (SALINAS, 2006).

The fumonisins are produced by *Fusarium verticillioides* and *F. proliferatum* under high humidity conditions and high temperature. These fungi are widely distributed in a global scale and found in soil and on plants surface and they contaminate grains in both field and storage process. Fumonisin B1 (FB1) prevails in nature and it is the most toxic fungus. It represents almost 70% of total contamination on food and on animal food naturally contaminated (KOBASHIGAWA, 2010).

Legal mechanisms have been adopted in many countries in the attempt to avoid the harmful effects caused by mycotoxins in ingredients that compose animals' feedstuffs. The most well-known laws are the ones that regulate aflatoxin levels this also happens to the one established by MERCOSUL and the other one adopted by countries in America which establish laws for mycotoxins in such products. Nevertheless, laws for other mycotoxins are under implementation in the same way they already are for different animal species in European Union (FREIRE et al., 2007).

Although there are legal standards for aflatoxins or, in some countries, for other mycotoxins, these micotoxins rarely appear isolated. There is usually simultaneous interaction among different mycotoxins, and this may decrease the safety limits established by law.

In Brazil, research on food quality for animal consumption has shown increasing problems caused by mycotoxins. The tropical and subtropical climates in specific Brazilian regions are appropriate fungi development and consequently mycotoxins. The demands of the consumer market plus this climate feature and the excessive control employed by the national and international governmental agencies point to the need of implementing programs to monitor food quality (SASSAHARA et al., 2003).

Due to the importance of quality control in the production of animal feed in all steps of this process, this study aimed at evaluating soybean meal samples before and after the industrial process of micronization according to bromatological and mycotoxin analyses.

## MATERIAL AND METHODS

### Soybean meal sampling collection

Samples of almost 1,000 grams were weekly collected and analyzed in a manufacturing unit in an industry for animal nutrition from Santa Catarina state, from September, 2010 to November, 2011. A total of 121 soybean meal samples were analyzed and 66 of them were obtained before the industrial micronization process while 55 were collected after it.

The soybean meal samples were collected in the arrival of trucks and trailer with the aid of a depth probe. After sampling, were sent to the laboratory in identified plastic bags. Then, the samples were grinded and homogenized in a Retsch® ZM 200 mill (Retsch, Inc.- Germany) at 18,000 rpm speed in order to obtain particles smaller than 0.5mm.

The samples of micronized soybean meal were collected by a scoop in the storage silo or during the product weighting. Since the particles of micronized soybean meal samples were less than 212 µm the end of the processing, were analyzed without the milling step.

### The analyses were performed in triplicate Bromatological analyses

The bromatological analyses of soybean meal samples before micronization were: moisture (MC), soluble protein in KOH (SP), ashes content (AC), lipids (L), crude fiber (CF) and urease activity according to Analytical Methods for Animal Feed Control (BRASIL, 1991). Crude protein (CP) was determined by DUMAS method, according to the Analytical Methods Guide of the Brazilian Compendium of Animal Nutrition (CBAA, 2009).

The analyses for micronized soybean meal were moisture (MC) and crude protein (CP), according to the above mentioned methods.

#### Mycotoxin analyses

The mycotoxin analyses were carried out based on enzyme-linked immunosorbent assay (ELISA) using NEOGEN VERATOX® kits, which determine the total aflatoxin amount ( $B_1 + B_2 + G_1 + G_2$ ), total fumonisin ( $FB_1 + FB_2 + FB_3$ ) and zearalenone.

Each toxin was extracted using 5 grams of sample in 25mL methanol 70% that was stirred for 3 minutes. Later, they were filtered in a filter paper Quanty® JP 41black label and used for the immunoassay. Quantitative determination was obtained by a Stat Fax 321 Plus (Awareness technology, Inc.- EUA) plate reader at a 650nm wavelength.

#### Calibration curve preparation

Neogen Veratox® patterns were inserted to prepare the calibration curve of each mycotoxin. For the aflatoxins, the used patterns varied from 0 to 50µg kg<sup>-1</sup> concentrations; while for fumonisin, this range was from 0 to 6,000µg kg<sup>-1</sup> and for zearalenone, from 0 to 500µg kg<sup>-1</sup>. The absorbance graph versus wavelength of each toxin analyzed was drawn and the linearity of calibration curve was in  $r^2 \geq 0.99$ . The limit of detection (LD) and quantification (LQ) of the method used for aflatoxins, zearalenone and fumonisin was 1,4µg kg<sup>-1</sup> and 5,0µg kg<sup>-1</sup>; 200µg kg<sup>-1</sup> and 500µg kg<sup>-1</sup>; 10µg kg<sup>-1</sup> and 25µg kg<sup>-1</sup>, respectively. Based on the calibration curve, the amount of aflatoxins, fumonisins and zearalenone was calculated by the line equation through the linear regression.

## RESULTS AND DISCUSSION

The bromatological averages of the studied 66 soybean meal samples before micronization are shown on table 1. This study recorded an average result of 0.02 (ΔpH) for urease activity in soybean meal samples (Table 1), which it is under the minimum answer established by the Brazilian Compendium of

Animal Feed, whose reference values for soybean meal with protein vary from 44% to 48% and for maximum urease activity is 0.15 (ΔpH) (CBAA, 2009). The urease activity of crude grain varies from 2.0 to 2.5 (ΔpH) (BUTOLO, 2002).

Soybean meal is obtained from the soybeans grains milling to extract oil, which is used for human consumption as it is an important ingredient for animal feed (BUTOLO, 2002). Due to the presence of anti-nutritional factors in soybeans, it's *in natura* use in diets formulation is not recommended since there may show some deleterious effects to health, especially in swine and poultry. Soybeans grains have some inhibitors of trypsin, chymotrypsin, lectins, among others (BELLAYER & SNIZEK Jr., 2012). The determination of urease activity aims at evaluating whether soybean meal has received the right thermal processing to inactivate the anti-nutritional factors present in soybeans (LIMA et al., 2011). Thus, the results obtained in this study have indicated an appropriate process as well as the studied presented soybean meal with excellent classification pattern concerning urea activity, between 0.01 to 0.05, according to LIMA et al. (2011).

Protein solubility is recognized as one of the best methods to evaluate sub or over-processing (BELLAYER & SNIZEK JR., 2012) and indicates the available protein percentage to be absorbed by the animal. According to MENDES et al. (2004), the ideal range for solubility concerning animal feed is 73-85%. Values below 70% have indicated overheating and over 85%, they are associated to the sub-processed soybeans.

Thus, the results of protein solubility to 79.5% soybean meal samples tested in this work, showed that are in accordance to the allowed variation, so, there was no sub or overheating in the processing.

The lipid content variable is influenced by the oil extraction process (OST et al., 2005). The values obtained for soybean meal samples before micronization showed a 2.2% average (Table 1). Similar results were observed by HENZ et al. (2009), who recorded the same average (2.2%). On the other

Table-1 - Bromatological averages of the 66 soybean meal samples before micronization.

SM	MC (%)	CP (%)	SP (%)	AC (%)	L (%)	CF (%)	Urease
Before micronization	12,4	46,4	79,5	5,9	2,2	4,3	0,02

\* (SM) Soybean meal, (MC) moisture, (CP) protein, (SP) soluble protein, (AC) ashes content, (L) lipids, (CF) crude fiber and urease activity (ΔpH).

hand, RIEGER et al. (2008) evaluated soybean meal in two regions of Paraná and found out 1.8% of average. Such answer indicated that the changes in lipid values are due to the kind of processing to extract oil.

The value average of crude protein in soybean meal was 46.4% and similar values were also found out by other authors. For POZZA et al. (2006), protein values ranged from 46.9 to 48.4%, while RIEGER et al. (2008) recorded 45.4%, GENEROSO et al. (2008) obtained 44.4% and ROSTAGNO et al. (2005) showed two protein values (45 and 48%). The soybean meal presented, approximately 43.5 to 48.5% of crude protein (OST et al., 2005).

The average result for crude fiber analysis was 4.3%. So, when these data were compared with GENEROSO et al. (2008) results (5.2%), it was possible to correlate it with the centesimal of crude protein. According to OST et al. (2005), variation can occur as addition of the soybean hulls derived from soybean grain processing, thereby reducing the percentage protein.

After micronization process, it could be observed a moisture decrease on soybean meal from 12.4% to 7.0%. Micronization is a heat treatment of soybean meal, thus, there is greater loss of water available as well as greater concentration of nutrients

occurs. This can also be evidenced by the averaged values of crude protein (CP) that accounted for 46.4% before and 48.6% after processing step.

The results of the studied mycotoxins analyses are represented in Figures 1A and 1B. Among the analyzed samples of soybean meal before micronization, 92% of them (n=61) were contaminated with zearalenone, 55% (n=36) were contaminated with aflatoxins and 30% (n=20) with fumonisin (Figure 1A). The maximum aflatoxin contamination was  $3.8\mu\text{g kg}^{-1}$ , fumonisin  $500\mu\text{g kg}^{-1}$  and  $204\mu\text{g kg}^{-1}$  zearalenone. Besides the values variability, it is possible to observe the contamination of the same sample with different mycotoxins.

Mycotoxins determination in soybean meal before micronization reported the following average level of contamination in samples of aflatoxins, Zearalenone and Fumonisin of  $0.5\mu\text{g kg}^{-1}$ ,  $29.6\mu\text{g kg}^{-1}$  and  $56.8\mu\text{g kg}^{-1}$ , respectively.

Among micronized soybean meal samples, it was observed that 21 samples (38%) were contaminated with fumonisin, 45 (82%) had aflatoxin and 55 (100%) contained zearalenone toxin (Figure 1A). The maximum aflatoxin contamination was  $4.7\mu\text{g kg}^{-1}$ ,  $1000\mu\text{g kg}^{-1}$  fumonisin and  $159.2\mu\text{g kg}^{-1}$  zearalenone.

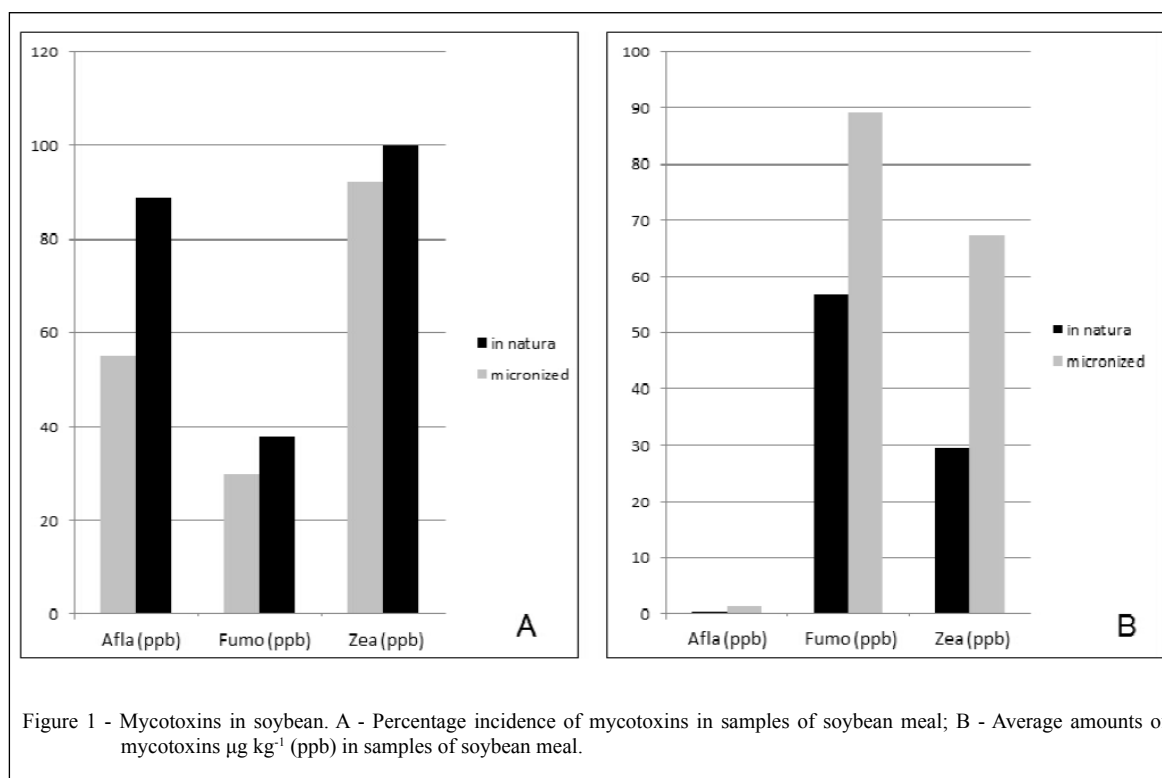


Figure 1 - Mycotoxins in soybean. A - Percentage incidence of mycotoxins in samples of soybean meal; B - Average amounts of mycotoxins  $\mu\text{g kg}^{-1}$  (ppb) in samples of soybean meal.

The contamination average level of micronized soybean meal samples of Aflatoxin, Zearalenone and Fumonisin was  $1.3\mu\text{g kg}^{-1}$ ,  $67.5\mu\text{g kg}^{-1}$  and  $89.1\mu\text{g kg}^{-1}$ , respectively (Figure 1B). Similar results to the obtained ones in this study have been described by NETTO et al. (2002), who researched various sources of mycotoxins in animal feed. Among the 21 soybeans samples analyzed by these researchers, aflatoxin was detected with 14.3% and zearalenone with 42.9% in the same samples. Moreover, TININI et al. (2012) have analyzed 34 soybean meal samples and observed the presence of mycotoxins in 44.1% of them ( $n=15$ ), but, the average values reported for aflatoxin were  $7.4\mu\text{g kg}^{-1}$  and  $2.58\mu\text{g kg}^{-1}$  for zearalenone, which was different from the average of the values obtained in this trial.

Although the fumonisin produced by *Fusarium moliniforme* strains is often found in maize (MAZIERO & BERSOT, 2010), in the present study, this mycotoxin was present in 30% *in natura* samples and in 38% micronized soybean meal (Figure 1A). Different results were reported by MALLMANN et al. (2001), Who evaluated fumonisin B1 levels in 407 samples of several cereal (maize, Rice, wheat, barley, oats and soybean meal) in Southern Brazil and did identify mycotoxin in soybean meal samples.

Average values observed for the studied mycotoxins are shown in figure 1B and there is a higher level of the studied three toxins after micronization manufacturing process. The micronization process may represent an increase in mycotoxins levels, since the external environment variations due to temperature change or humidity in silos, stores or even during grinding stage, can quicken secondary metabolites production due the stress fungus will be submitted (LAZZARI, 1997).

According to the incidence percentage, zearalenone was the most present toxin regarding a greater number of samples. It was identified in 92% samples before micronization and in 100% post-processing samples. Zearalenone is an analog of estrogen and may cause hyperestrogenism. There are also several incidents of advance puberty in children because of its presence (IAMANANKA et al., 2010).

In this research, fumonisin and aflatoxin presented lower incidence (Figure 1A). TININI et al. (2012) researched about mycotoxin presence in dairy cattle diet on family farms in western Paraná and identified that 44.1% soybean meal samples were positive for aflatoxin and zearalenone.

The presence of aflatoxin can be associated to the storage conditions of soybeans or soybean meal. Fumonisin and zearalenone are produced by field

fungi, from *Fusarium* *gender*. The possibility of the presence of these mycotoxins in products analyzed may be due to contamination of soybeans by fungi. When they find the ideal conditions of temperature and humidity for their development during storage of these grains, they just produced them. Another likely origin may be associated with the presence of impurities (OLIVEIRA et al., 2010) that have not been properly removed during the pre-cleaning step that is performed soon after harvest.

Although the obtained contamination levels are below the standard or found ones in other studies, it is of paramount importance to consider all the components on animal feed formulation because each raw matter can present different values of mycotoxins that can contribute to increased toxic levels as well as may cause acute or chronic effects.

## CONCLUSION

The results of bromatological and mycotoxin analyses have pointed out some compliance with the standards established by the Brazilian Compendium of Animal Nutrition and reference literature. However, it has been observed at least one of the three mycotoxins in all studied samples. Furthermore, the micronization process has contributed to the increased levels of all studied mycotoxins.

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