VALUATION OF AGROINDUSTRIAL RESIDUALS USING SOLID STATE FERMENTATION WITH *Aspergillus niger*

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RESUMO – A produção de biomassa microbiana em processos fermentativos é utilizada para aumentar o teor proteico do substrato e/ou agregar valores aos resíduos agroindustriais, como a palha de soja e o feno de azevém, os quais possuem de baixa qualidade nutricional. A utilização destes volumosos na dieta dos ruminantes poderá afetar o desempenho destes, sendo preciso agregar valor nutricional. Uma forma de se realizar esse processo é a incorporação de biomassa microbiana, como a do fungo *Aspergillus niger*, sendo este considerado seguro para a alimentação e boa fonte de proteínas. O objetivo deste trabalho foi agregar valor nutricional à palha de soja e ao feno de avevém através da fermentação em estado sólido com o *Aspergillus niger* para uso na alimentação de ruminantes. A fermentação fúngica mostrou-se uma boa alternativa para o uso dos volumosos, principalmente a palha de soja, visto que grandes volumes desta são desperdiçados anualmente devido ao baixo valor nutritivo.

ABSTRACT –The production of microbial biomass in solid state fermentations is used to increase the protein content of substrates and to add value to agro-industrial wastes, such as straw and soybean hay, considered of low nutritional quality ryegrass. The use of roughage in the diet of ruminants may affect the performance of these, and need to add nutritional value. The incorporation of microbial biomass can be used to this proposal, such as the fungus *Aspergillus niger*, which is considered safe for food and good source of protein. The aim of this work was to add nutritional value to soybean straw and ryegrass hay by solid state fermentation with *Aspergillus niger* for use in ruminant feed. Fungal fermentation proved to be a good alternative to the use of roughage, mainly soybean straw, as large volumes of this are wasted annually due to low nutritional value.

PALAVRAS-CHAVE: *Aspergillus niger*; palha de soja; feno de azevém.

KEYWORDS: *Aspergillus niger*; soybean straw; ryegrass hay.

1. INTRODUCTION.

Filamentous fungi are microorganisms able to grow at low water activities (Pitt e Hocking, 2009) and the microbial type that best suits in solid state fermentation, due to its form of growth through hyphae that favor the colonization of means (Pinto et al., 2005).

The solid state fermentation is much employed in protein enrichment of agro-industrial wastes in order to being used in animal feed, the production of enzymes and different metabolites, for example (Pandey, 2003).

Microorganisms are used as food or protein supplement due to the ability to grow in different types of industrial by-products, producing many cells, denominated single cell protein or protein
microbial (Nasseri et al., 2011). The microbial biomass is considered a natural protein concentrate, containing complete protein with all essential amino acids, being defined as dried cells of microorganisms that develop on different sources of carbon and that are used in food and in animal feed (Suman et al., 2015). The Aspergillus genus is some of the fungi cultivated for obtaining this protein (Anupama and Ravindra, 2001).

The production of microbial protein from agro-industrial wastes has advantages, as the short generation time of microorganisms, providing rapid increase in cellular mass. The protein content of microorganisms is greater than most vegetables, it requires little water availability and space, among others (Oliveira et al., 2005). In solid state fermentation the substrate provides nutrients and also serves to support the growth of microorganisms (Pandey, 2003).

Therefore, straw soybean and ryegrass hay are good alternatives to the fungus Aspergillus niger development, being this fungus a secure microorganism for use in the food area, according to the FDA (Food and Drug Administration) which is the regulatory agency responsible for the control of food and medicine in the United States of America (Schuster et al., 2002).

Despite the soy straw is abundant and inexpensive, it is characterized by its little significant nutritional value with low protein content. Therefore, the bovines feed exclusive of soybean straw may result in weight loss for the animal.

Annually tons of soybean straw remain in the field, being that a part of this straw could be used in cattle feed (Carvalho, 1992). Brazil is the second largest world producer soybean (Embrapa, 2016). Large part of the straw produced after harvesting is wasted and the use in ruminant feed is too small. There is little information on the results of its use for feeding to ruminants.

Hay is used in drier periods and during the winter to maintain the productivity of bovines, for example. The pasture, alone, does not provide an abundant food and quality, therefore an alternative food for the animals during the year (Stabra, 2014). However, the quality of hay is related to the plant, the climatic conditions during drying and storage.

The aim of this work was to add nutritional value to soybean straw and ryegrass hay by solid state fermentation with the fungus Aspergillus niger for use in ruminant feed.

2. MATERIAL AND METHODS
The fungus Aspergillus niger was used for accumulation of proteins through solid state fermentation in soybean straw and ryegrass hay.

2.1 Preparation of the inoculum
The preparation of the inoculum was made from the fungus Aspergillus niger that was kept in tubes with PDA (potato dextrose agar) at 4 °C through the addition of 10 mL of Tween 80 at 0.1% in the tube containing the fungus, followed by scraping with handle nickel/chrome to form the spore solution. Later, 2.5 mL of the spore solution was added in 1 L erlenmeyer containing 100 mL of solidified PDA (potato dextrose agar) and incubated at 30 °C for 7 days. After the fungus growth, a spore suspension was prepared through the addition of 50 mL of a 0.1% solution of Tween 80 in Erlenmeyer with agitation and filtration in sterile gauze to the retention of hyphae.

2.2 Preparation of the culture medium
The culture mediums were prepared with ground soybean straw and ryegrass hay. Distilled water was added to each medium to keep to 60% humidity. The medium was autoclaved at 103 kPa for 20 min at 121 °C and added to sterile beakers. The inoculation was performed by addition of the spore suspension in a way that the means presented in the start time 2.10^6 spores/g of medium. The beakers were capped with acrylic blanket and incubated in an incubator at 30 °C for a period of 7 days according to Figures 1 and 2 show, respectively, the milled 2013/2014 soybean straw and ryegrass hay 2014 (a) before and (b) after 7 days of fermentation with the filamentous fungus Aspergillus niger.
Fermented roughage were autoclaved and added in own inox tray for drying up to humidity of at least 14%.

Figure 1 - Milled soybean straw 2013/2014: (a) without and (b) with Aspergillus niger.

Figure 2 - Ryegrass hay 2014: (a) without and (b) with Aspergillus niger.


The influence of fungal fermentation on the physicochemical composition ryegrass hay 2014 and soybean straw 2013/2014 was compared with the control test (without the fungus Aspergillus niger).

3. RESULTS AND DISCUSSION

There was no accumulation of proteins both ryegrass hay as in soybean straw (Table 1). But the soy straw presents a greater content than ryegrass hay. The accumulation of proteins can be optimized by adding nutrients such as carbon and nitrogen sources in culture media. Glucose, for example, is a single carbon source, assimilating easy for the microbial metabolism and stimulates aerobic conditions in the first instance the growth of the microorganism. The protein content is directly proportional to the microbial growth, this justifies the use of inductors to increase the protein content (Fang and Zhong, 2002).

Table 1 - Chemical composition soybean straw 2013/2014 and ryegrass hay in 2014 with / without the fungus Aspergillus niger.

<table>
<thead>
<tr>
<th>Batch*</th>
<th>Proteins</th>
<th>Lipids</th>
<th>Crude fibers</th>
<th>NDF</th>
<th>ADF</th>
<th>Ashes</th>
<th>Carbohydrates</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>4.16±0.08a</td>
<td>2.01±0.05a</td>
<td>37.44±0.40b</td>
<td>93.43±2.99d</td>
<td>49.67±1.24a</td>
<td>5.83±0.24a</td>
<td>88.01±0.32c</td>
</tr>
<tr>
<td>FF</td>
<td>4.39±0.85a</td>
<td>2.79±0.36ab</td>
<td>42.81±0.33c</td>
<td>84.73±0.17c</td>
<td>56.83±1.09b</td>
<td>5.62±0.22a</td>
<td>87.20±0.79c</td>
</tr>
<tr>
<td>P</td>
<td>6.59±0.11b</td>
<td>2.07±0.10a</td>
<td>31.92±0.51a</td>
<td>56.66±0.10a</td>
<td>46.24±3.24a</td>
<td>6.14±0.10a</td>
<td>85.20±0.03b</td>
</tr>
<tr>
<td>PF</td>
<td>7.87±0.04b</td>
<td>3.22±0.40b</td>
<td>43.57±1.04c</td>
<td>65.76±2.58b</td>
<td>48.52±0.14a</td>
<td>7.78±0.14b</td>
<td>81.13±0.33a</td>
</tr>
</tbody>
</table>

Legend: The columns, means followed by the same letter were not significantly different from each other at 95% confidence (mean ± SD). Results on dry basis.


The tests carried out with ryegrass hay, ryegrass hay with Aspergillus niger and soybean straw (F, FF and P) obtained equality in lipid content. Fungal fermentation in the ryegrass hay and soybean straw resulted in an increase in lipid content (Table 1). This increase gives the fungus accumulate lipids, the main lipidic component are triglycerides which are considered as storage lipids that can be used as a source of energy and carbon for growth and development (Akpinar-Bayizit, 2014).

In the fiber content, the highest was observed in trials with ryegrass hay and soybean straw.
both with the fungus *Aspergillus niger*. Increasing this content can be due to fungi (*Aspergillus spp.*) cultivated in solid state fermentation are very important for increasing fiber digestion since they are capable of degrading the lignocellulose through the production of enzymes such as cellulases, hemicellulases and ligninases (Li et al., 2013). The neutral detergent fiber (NDF) is the constituent with low degradability of the diet (Weiss, 1993), then the greater the NDF value, the lower the capacity of the cow have eat it. The straw fermented soybeans showed the lowest content in relation to fermented ryegrass hay (Table 1). The ryegrass hay had the highest contents of NDF. This increase may be due to the digestibility of the NDF fraction is reduced with the advance of the forage cutting age resulting in high cell wall percentage which restricts the attack of digestive enzymes and as a consequence there is a decrease in fiber digestibility and increased time retention of solids in the rumen (Júnior et al., 2007).

Fermented ryegrass hay showed higher acid detergent fiber content (ADF) (Table 1). The ryegrass hay, straw soy and fermented soybean straw showed ADF of equal content. The higher the ADF value, lower digestibility of the food (Van Soest, 1994), this indicates that the fungus failed to produce sufficient enzyme to degrade resistant to digestion fibrous components, such as lignin.

The ash content increased with fungal fermentation in soybean straw. However, ryegrass hay with and without fermentation and soybean straw had equal ash content (Table 1).

Ryegrass hay with and without the fungus *Aspergillus niger* present equal value and higher on carbohydrates (Table 1), because the indigestible fraction of total carbohydrates tends to increase with advances in plant maturity (Van Soest, 1994). The lower content was obtained in fermented soybean straw, followed by soybean straw.

Soybean straw compared to ryegrass hay, adding the fungus *Aspergillus niger* was the best result, as showed higher protein and lipid content. Thus, the fungal fermentation of soybean straw with *Aspergillus niger* improved the quality of that, but we need to add inductors so that they raise the protein content.

### 4. CONCLUSION

The solid state fermentation with the fungus *Aspergillus niger* increased the possibility of using soybean straw in ruminant feed, since the use of this is a good option for large volumes that are produced and wasted every year due to low nutritional value. The ryegrass hay is widely used in feed for ruminants, but the low quality due to the time of cutting and/or drying, are some of the factors that influence milk productivity and weight gain. Therefore, the fungal fermentation with the fungus *Aspergillus niger* is a good alternative to use roughage studied with the aim of improve the quality of cattle feed.

### 5. REFERENCES


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