ORIGINAL ARTICLE

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Cellulolytic activity of anaerobic facultative fungi from the digestive tract of sheep fed with banana leaf hay

Atividade celulolítica de fungos aeróbios facultativos do trato digestório de ovinos alimentados com feno da folha de bananeira

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ABSTRACT: The addition of cellulolytic fungi, or their enzymes, in diets containing high levels of fiber are promising strategy for improving the performance. In this study, the aims were to select cellulolytic fungi from the digestive tract of sheep fed different concentrations of banana leaf hay. Thirty lambs raised in a feedlot were evaluated, distributed in a completely randomized design, with diets containing 0, 125, 250, 375, or 500 g/Kg of dry matter and six replications. Approximately 15 mL of ruminal fluid and swabs from the rectal ampulla were collected. The cultures were carried out in a medium containing microcrystalline cellulose (C medium). The mycelial fungi isolates were identified through the microculture technique. Among the fungi from the ruminal fluid, 23 isolates corresponded to the genus *Aspergillus* and three to *Paecilomyces* spp. Among the isolates from the rectal ampulla, seven were A. spp., and three were P. spp. The A. genus predominated among the isolates from both evaluated sites ($p < 0.05$). Fragments of these fungi were inoculated in triplicate in medium C at 37 °C and the cellulolytic activity index (CAI) was determined after 24, 48, and 72 hours of incubation. There was no difference in the CAI of *Aspergillus* spp. from animals fed different diets or of different evaluated sites ($P > 0.05$). However, 22 isolates of *Aspergillus* spp. and three of *Paecilomyces* spp. showed a CAI > 1, indicating biotechnological potential for cellulase production. These selected isolates could be selected for the elaboration of microbial additives in ruminant diets.

KEY WORDS: Sheep production; alternative food; *Musa paradisica*; cellulases; ruminal microbiota.

RESUMO: A adição de fungos celulolíticos, ou suas enzimas, em dietas contendo elevados teores de fibras são estratégias promissoras para melhorar o desempenho. Neste estudo os objetivos foram selecionar fungos celulolíticos do trato digestório de ovinos alimentados com diferentes concentrações do feno da folha da bananeira (FBH). Foram avaliados 30 borregos criados em sistema intensivo, distribuídos em delineamento inteiramente ao acaso, com cinco dietas contendo 0, 125, 250, 375 ou 500 g/KG de matéria seca em seis repetições. Foram coletados aproximadamente 15 mL de fluido ruminal e swabs da ampola retal. Os cultivos foram realizados em meio de cultura contendo celulose microcristalina (meio C). Os fungos micelianos foram identificados após a técnica de microcultivo. Entre os fungos provenientes do fluido ruminal, 23 isolados corresponderam ao gênero *Aspergillus* e três a *Paecilomyces* spp.. Foram identificados nas fezes dos animais sete *Aspergillus* spp. e três *Paecilomyces* spp.. O gênero *Aspergillus* predominou entre os isolados de ambos os sítios avaliados ($p = 0{,}013$). Fragmentos desses fungos foram inoculados em triplicada em meio C a 37 °C e determinou-se o índice de atividade celulolítica(IAC) após 24, 48 e 72 horas de incubação. Não houve diferença entre CAI de isolados de *Aspergillus* spp. provenientes dos animais em diferentes dietas ou sítios avaliados ($P > 0.05$). Entretanto, 22 isolados de *Aspergillus* spp. e três de *Paecilomyces* spp. apresentaram IAC >1, indicando potencial biotecnológico para produção de celulases.

PALAVRAS-CHAVE: ovinocultura; alimento alternativo; *Musa paradisaca*; celulases; microbiota ruminal.

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INTRODUCTION

Ruminants have the notorious ability to use fibrous foods; however, the components of plant cell walls are too essential for the maintenance of the ruminal microbiota (RUSSEL; RYCHLIK 2001). The relations among the populations of bacteria, protozoa, and fungi in the rumen are intense and necessarily symbiotic for the use of structural carbohydrates, with emphasis for the participation of the cellulolytic bacteria and fungi (LIU et al., 2020; PESSOA et al., 2017).

The population of anaerobic bacteria is predominant in the rumen environment; however, studies conducted with ruminants fed tropical forages have demonstrated the presence of aerobic and facultative anaerobic fungi in the rumen fluid (ABRÃO et al., 2014; ALMEIDA et al., 2014; ABRÃO et al., 2017). Anaerobic rumen bacteria and fungi exhibit laborious and costly growth under laboratory and industrial conditions (LIU et al., 2020; HARTINGER; ZEBELI, 2021). Alternatively, facultative anaerobic fungi may produce enzymes that facilitate polysaccharide degradation in ruminants. Analyses of the cellulolytic activity index (CAI) of these fungi from the digestive tract of dairy cattle, raised in semi-arid conditions, revealed that isolates of *Aspergillus* spp. and *Paecilomyces* spp. showed expressive ability to degrade microcrystalline cellulose, with a CAI > 1 (ALMEIDA et al., 2014). In beef cattle raised on lignified pastures, *Aspergillus* ssp. isolates also produced significant levels of carboxymethylcellulase (ABRÃO et al., 2017).

The diet is the component that most increases production costs, and therefore alternative foods such as co-products and agro-industrial wastes are used sustainably in the feeding of ruminants (OLIVEIRA et al., 2013; NUNES et al., 2007). Tissue culture banana (Musa spp.) generates a significant amount of waste, and the leaves have significant contents of crude protein (between 12% and 17.2%) (RIBEIRO et al., 2007).

Studies have demonstrated the potential use of banana coproducts as alternative foods for sheep. In a study that evaluated sheep confined and fed banana pseudostem hay and treated with virginiamycin, there were promising results for improvement in crude protein digestibility (SILVEIRA JUNIOR et al., 2020). Similarly, replacing rye (*Lolium* spp.) hay by-products with banana (*Musa acuminata* L.) for lambs improved the feed conversion rate and raised the total daily forage intake (BARBERA et al., 2018).

The impact of the use of banana leaves on the ruminal microbiota has been poorly elucidated in the scientific literature. However, it is known that banana leaf hay (BLH) can modulate the rumen ecosystem, significantly reducing the population of protozoa, especially *Entodinium* spp. in the rumen of sheep. This reduction was possibly associated with the high tannin contents of the leaves (FREITAS et al., 2017). However, the presence of these phenolic compounds may compromise protein digestibility, and meanwhile, it can reduce methane production (RIBEIRO et al.,2007; MIN; SOLAIMAN, 2021).

However, these studies have not considered the possible effects of compounds present in banana leaf hay (BLH) or other agricultural co-products on cellulolytic fungi in the rumen (PATRA; SAXENA, 2009). BLH inclusion could modulate the population of these eukaryotes in the ruminal environment and favor the selection of fungal isolates with higher cellulolytic activity for degradation of this forage, allowing the selection of strains with higher expression of fibrolytic enzymes of interest for biotechnological purposes and animal nutrition. In this study, the objectives were to evaluate the occurrence and to select cellulolytic fungi from the digestive tract of sheep fed with BLH.

MATERIAL AND METHODS

Site, ethical considerations, and evaluated animals

The experiment was conducted on a farm, located in Montes Claros, Minas Gerais, Brazil. Before the experimental period, the animals were identified with ear tags and submitted to endo- and ectoparasites control (ivermectin 200 mcg/kg body weight, Ranger, MSD Saúde Animal, Brazil). We evaluated 30 non-castrated male sheep, of no defined breed, with a mean age of 5 months and mean weight of 24.50 ± 3.6 kg. The animals were distributed in an entirely randomized design and were kept in individual stalls with concrete floor covered with sawdust and received feed and water ad libitum.

Food preparation, feeding, and diet

The banana leaves were collected in Janaúba, Minas Gerais, Brazil, and were chopped in a stationary machine, with subsequent drying of the material in the sun for 48 to 72 hours. For the chemical analyses, this material was ground in a Willy's knife mill and sieved to a particle size of 1-3 mm. Feed and diet samples were analyzed according to the Association of Official Analytical Chemists (AOAC, 2012) for DM (method 934.01), ash (method 942.05), crude protein (CP, method 954.01), and ether extract (EE, method 920.39). Neutral detergent fiber (NDF, Van Soest et al., 1991) and acid detergent fiber (FDA) analyses (AOAC, 1997, method 973.18) were performed using an ANKOM200 Fiber Analyzer unit (ANKOM Technology Corporation, Fairport, New York, USA). Total digestible nutrient (NDT) content was obtained by an equation developed by Bolsen (1996).

Experimental management

The experimental period lasted 63 days. During the initial 30-day period, all animals received ad libitum *Cynodon*

spp. hay and BLH in equal proportions and with a volume/concentrate ratio of 50:50, based on dry matter (Table 1). The diets were formulated to meet the nutritional requirements of lambs for daily gains of 200 g, according to the recommendations of the National Research Council (2007). The bromatological compositions and percentages are described in Table 1.

Groups of six animals (repetitions) received diets containing 0, 125, 250, 375, and 500 g FBH/Kg of dry matter as a replacement for *Cynodon* spp. hay (Table 1). All procedures performed with the animals were approved by the ethics committee on animal experimentation of the Universidade Estadual of Montes Claros (protocol no. 128/2013).

The diet was fed twice a day, adjusted to maintain leftovers between 10% and 15% of what was offered, based on dry matter. The amount offered and the leftovers were recorded, collected, and weighed every day. On the last day of the experiment, rumen fluid was collected after a 12-hour fast. For collection, trichotomy and asepsis with iodine-PVP solution (1%) was performed in an area of approximately 5 cm^2, located at ventral part of the left abdomen, below the paralumbar fossa and cranial to the knee joint (DIRKSEN, 1993). Using a human catheter (Solidor ®, 14.2, BioMed Health Care Products, Haryana - India) attached to sterile syringes, 15 mL of rumen fluid were collected (ABRÃO et al. 2014). Each syringe was sealed, labeled, and stored in an isothermal box with ice. After pre-sterilization of the perianal region with PVP iodine, feces were collected directly from the rectum of the animals with sterile swabs and sent for analysis.

Table 1. Proportion of ingredients and chemical composition of experimental diets (g/kg of dry matter) for sheep fed banana leaf hay.

Ingredients/ Diets	0 FBH	125 FBH	250 FBH	375 FBH	500 FBH
Cynodon spp. hay	500.0	375.0	250.0	125.0	0.0
Banana leaf hay	0.0	125.0	250.0	375.0	500.0
Corn grain	387.9	403.7	421.0	436.0	451.2
Soybean meal	80.3	62.5	43.2	26.0	7.8
Dicalcium phosphate	0.0	1.0	2.2	3.7	5.0
Limestone	19.2	20.3	21.1	21.8	23.5
Bicarbonate	7.6	7.5	7.5	7.5	7.5
mineral premix 1	5.0	5.0	5.0	5.0	5.0
Item	**Diets (g/Kg of dry matter)**				
	0% FBH	125 FBH	250 FBH	375 FBH	500 FBH
MS	918.7	919.7	917.0	914.2	916.0
MM	97.5	93.4	82.9	81.7	93.1
PB	137.9	134.2	129.1	126.4	120.8
NDT	726.7	716.0	705.2	694.3	683.0
FDN	484.5	491.2	487.7	477.7	458.5
FDA	257.3	260.8	256.3	249.6	244.3

DM= dry matter, MM = mineral matter, CP= crude protein, NDT=total digestible nutrients, FDN= neutral detergent fiber, and FDA= acid detergent fiber. 1 Premix composition: 150 g calcium (max.), 130 g calcium (min.), 65 g phosphorus (min.), 130 g sodium (min.), 650 mg fluoride (max.), 12 g sulfur (min.), 10 g magnesium (min.), 1. 000 mg iron (min), 3,000 mg manganese (min), 80 mg cobalt (min), 5,000 mg zinc (min), 60 mg iodine (min), 10 mg selenium (min), 50,000 IU vitamin A (min), and 312 IU vitamin E (min).

Isolation, culture, and cellulolytic activity of the fungi

For the culture, sterile swabs were used to inoculate by depletion the rumen fluid and stool samples into petri plates containing medium C (1% microcrystalline cellulose, 0.5% ammonium sulfate, 0.05% magnesium sulfate heptahydrate, and 2% agar). After inoculation, the plates were inverted and incubated at 37 °C and monitored for growth of fungal colonies for up to 21 days (LACAZ et al. 2002). A microculture technique was performed to identify the mycelial fungi isolates (LACAZ et al. 1998). Micromorphological characteristics, shown by optical microscopy, were associated with those described for fungi of biotechnological and veterinary interest (LACAZ et al. 1998; LACAZ et al. 2002).

Twenty-six mycelial fungal isolates selected from rumen fluid and 10 isolates from feces were recovered, and each colony was re-inoculated in medium C. After incubation for seven days, each isolate was seeded again, in triplicate, in the center of petri plates (90 mm x 90 mm) containing 15 mL of the same medium. They were then incubated in a BOD oven at 37°C and cellulolytic activity readings were taken at 24, 48, and 72 hours, according to the adaptation of the methodology described by TEATHER; WOOD (1982).

At the end of each incubation period, 15 mL of Congo red dye solution (1 mg mL-1) were added and the plates were incubated for 15 minutes. Subsequently, these plates were washed with 15 mL of 1M NaCl solution three consecutive times, and the diameters of the clear halos that indicated cellulose degradation and the diameters of the colonies were measured using a digital pachymeter (Mitutoyo®). The CAI of each fungal isolate was calculated by dividing the diameter of the hydrolysis halos by the diameter of the colonies evaluation period for each (ABRÃO et al., 2017).

The detection rates of the fungal genera of the two sites evaluated were compared using the chi-square test. To compare the cellulolytic activity and the incubation times of the evaluated groups of fungi, an analysis of variance was conducted with the non-parametric Kruskal Wallis test. The tests were performed in the statistical package SAEG - System

Analysis Statistics and Genetics. Statistical significance was set at P <0.05.

RESULTS AND DISCUSSION

After analysis of the microcultures of 26 isolates of mycelial fungi from the ruminal fluid, 88.46% isolates corresponded to the genus *Aspergillus* and 11.54% to *Paecilomyces* spp. Among the 10 isolates from the rectal ampulla, 70% were identified as *Aspergillus* spp. and 30% as *Paecilomyces* spp. (Table 2). The results indicated predominance of the genus *Aspergillus* among the isolates from rumen fluid and feces (P =0.013).

Research on the occurrence of cellulolytic fungi in the ruminal fluid of five cows, five sheep, and five goats showed a higher proportion of these microorganisms in samples from cows. The genus *Fusarium, Penicillium, Aspergillus* and *Mucor* were the most frequent and isolates of these fungi showed proven cellulolytic activity. For samples isolated from sheep rumen fluid, the genus *Mucor* was the most frequent, accounting for 40% of isolates, followed by *Penicillium* sp. which was identified for 30% of isolates, and only 10% for the genus *Aspergillus* (OYELEKE; OKUSANMI, 2008), different from what was detected in this present study.

The fungal isolates of the genus *Aspergillus* identified in the present study could also present positive interactions with other microorganisms of the rumen autochthonous microbiota and also play a favorable role in cellulose degradation, since studies have demonstrated high cellulolytic activity of this genus (RUEGGER; TAUK-TORSNISIIELO, 2004).

When evaluating the mean CAI after 24, 48, and 72 hours of *Aspergillus* spp. isolated from the ruminal fluid of sheep fed with BLH as a substitute for *C.* ssp. hay (Table 3), no differences were observed between the medians of CAI for the three incubation times and between diet types (P > 0.05). However, it was observed that the median growth of the isolates in the culture medium was significantly higher for the 48 and 72 hour incubation periods (P < 0.05).

In this study, it was possible to observe variability among CAIs for 23 isolates of *Aspergillus* spp. from rumen fluid, at different periods (Table 3). This variation could be explained by genetic differences between species and strains of this fungus genus. For the three time periods, it was observed that 16 *Aspergillus* isolates presented CAI >1, which is considered a good index for cellulolytic enzyme production (TEATHER, WOOD 1982). *Aspergillus* strain T3J12 isolates corresponded to fungi with the highest index (3.56) after 24 hours of incubation, revealing potential for the development of probiotics or prebiotics and for industrial microbiology for cellulase production (Table 3).

Table 2. Genus distribution of mycelial fungi in the gastrointestinal tract of sheep fed with banana leaf hay.

Genus	Strains		
	Rectal Ampulla	Ruminal Fluid	Total
Aspergillus spp.	7 a	23 a	30 a
Paecilomyces spp.	3 b	3 b	6 b
Total	10	26	36

Different letters in the column indicate significant difference with P values <0.05 in the chi-square test.

These higher CAI values detected reveal intra-species specific genetic variability for *A. terreus* and highlight the importance of the present study in identifying which isolates could express higher cellulolytic activity. However, future research should identify and genetically characterize these isolates and other isolates tested in this study to provide better applicability of these microorganisms and to avoid possible toxic or pathogenic effects, such as mycotoxin production and respiratory tract infections (CASADEVALL, 2007).

When evaluating isolates for selection as good producers of cellulolytic enzyme, both the CAI and the growth of the isolate in the culture medium should be considered as indicators. When analyzing CAI of *Penicillium herquei*, Ruegger and Tauk-Tornisielo (2004) observed colony diameter of 2 mm and CAI 6.0, while *Trichoderma harzianum* showed colony diameter of 67 mm and CAI 1.1. The authors indicated that isolates with a lower CAI, however, showing expressive growth on medium containing cellulose could be selected for biotechnological and industrial potential.

When comparing the CAI *Aspergillus* isolates, there was no difference in the median between isolates from animals fed different diets (P > 0.05). However, for the incubation at 72 hours, there was a higher average growth of the culture (P<0.05), indicating adaptation to the culture medium.

When analyzing the CAI of *Aspergillus* spp. from feces during the three incubation periods, six isolates showed a CAI > 1 (Table 4). These isolates may represent potential for biotechnological production of enzymes and for the development of probiotics and prebiotics (PINTO; LEITE; TERZI, 2001). However, the environmental or pathogenic role of these microorganisms must also be considered to improve sheep health.

When evaluating the cellulolytic activity of aerobic fungi of the genera *Aspergillus, Gliocladium, Paecilomyces, Rhizophus,* and *Scedosporium* in rumen fluid from different categories of dairy cattle fed different sources of tropical forages, Almeida et al., (2014) observed that isolates of the genus *Aspergillus* presented higher CAI when compared with the genus *Rhizophus*, and *Aspergillus* spp. and *Paecilomyces* spp. the isolates showed CAI >1, which indicates good cellulose degradation capacity and high potential for the development of probiotics or prebiotics in the ruminant diet.

In another study, the production of xylanase and cellulases from *Aspergillus japonicus* using agricultural residues

Table 3. Colony diameter (mm) and cellulolytic activity of *Aspergillus* spp. isolated from the rumen of sheep fed with banana leaf hay after cultivation in medium containing microcrystalline cellulose (1%) at 24, 48, and 72 hours of incubation.

Isolated	24 hours		48 hours		72 hours	
	Colony (mm)	CAI*	Colony (mm)	CAI*	Colony (mm)	CAI*
Aspergillus T1J1	11.0	1.00	22.0	1.00	24.0	1.00
Aspergillus T1J2	6.0	1.00	13.0	1.00	15.0	2.17**
Aspergillus T1J3	11.0	1.00	22.0	1.77**	30.0	1.47**
Aspergillus T1J4	14.0	1.00	40.0	1.23	32.0	1.25**
Aspergillus T1J5	7.0	1.00	22.0	1.18**	29.0	1.03
Aspergillus T2J1	7.0	1.00	44.0	1.16**	52.0	1.13**
Aspergillus T2J2	13.0	1.69**	46.0	1.15	52.0	1.21**
Aspergillus T2J3	1.0	1.00	22.0	1.27	28.0	1.18**
Aspergillus T2J4	14.0	1.29	21.0	1.67**	27.0	1.22**
Aspergillus T2J5	8.0	1.00	15.0	1.00	19.0	1.00
Aspergillus T2J6	8.0	1.00	9.0	1.00	12.0	1.00
Aspergillus T3J1	9.0	3.56**	12.0	1.00	26.0	1.00
Aspergillus T3J2	6.0	1.00	18.0	1.17	25.0	1.20**
Aspergillus T3J3	11.0	1.00	22.0	1.55	23.0	1.74**
Aspergillus T3J4	28.0	1.14	45.0	1.38**	52.0	1.37**
Aspergillus T3J5	27.0	1.11	49.0	1.16	56.0	1.21**
Aspergillus T3 J6	10.0	1.00	23.0	1.00	34.0	1.00
Aspergillus T3J7	9.0	1.00	10.0	1.00	25.0	1.00
Aspergillus T4J1	16.0	1.00	32.0	1.00	54.0	1.00
Aspergillus T5J1	10.0	1.00	20.0	1.45**	27.0	1.22**
Aspergillus T5J2	11.0	1.00	15.0	2.33**	59.0	1.00
Aspergillus T5J3	9.0	1.00	31.0	1.13	45.0	1.53**
*Aspergillus*T5J4	11.0	1.00	22.0	1.00	41.0	1.20**
Medianas	11.17 c	1.13 A	25.0b	1.24 A	34.22 a	1.22 A

*The cellulolytic activity index measures the ratio of the diameter of the microcrystalline cellulose degradation halo to the diameter of the fungal colony. ** Indicates cellulolytic activity index (CAI) greater than one. Medians of CAI followed by the same letter in the same row do not differ by Kruskal-Wallis nonparametric test with P > 0.05. T1, T2, T3, T4, and T5 containing 0, 125, 250, 375 and 500 g FBH/Kg dry matter, respectively.

Table 4. Colony diameter (mm) and cellulolytic activity index (CAI) of *Aspergillus* spp. from the rectal ampulla of sheep fed with banana leaf hay.

Isolates	24 hours		48 hours		72 hours	
	Colony diameter	CAI*	Colony diameter	CAI*	Colony diameter	CAI*
Aspergillus T1F1	12.0	1.00	18.0	1.00	43.0	1.12**
Aspergillus T1F2	1.0	1.00	5.0	1.00	46.0	1.39**
Aspergillus T4F1	10.0	1.00	15.0	2.00**	23.0	1.52
Aspergillus T4F2	7.0	1.00	33.0	1.06	43.0	1.07**
Aspergillus T5F1	5.0	1.00	22.0	1.18**	30.0	1.13
Aspergillus T5F2	7.0	1.00	9.0	1.00	18.0	1.00
Aspergillus T5F3	10.0	1.20	21.0	1.67**	32.0	1.28
Medianas	6.67 c	1.03 A	17.57 b	1.27 A	33.57 a	1.22 A

CAI = cellular activity index, which measures the ratio of the diameter of the microcrystalline cellulose degradation halo to the diameter of the fungus colony. ** Indicates cellulolytic activity index greater than one. T1, T2, T3, T4 and T5 containing 0, 125, 250, 375 and 500 g of FBH/Kg of dry matter, respectively. Median CAI following the same letter on the same line do not differ by the nonparametric Kruskal-Wallis test with P > 0.05.

was evaluated, and broad pH stability for both enzymes was observed. The crude extract of the fungus used in this study showed good rumen stability when compared with commercial preparations and can be obtained from cheaper procedures available with agro-industrial residues. According to the authors, the use of this strain is promising, as it grows rapidly under simple conditions, secreting enzymes that have properties necessary for use in ruminant diets (FACCHINI et al., 2011a).

FACCHINI et al., (2011b) evaluated an enzymatic pretreatment containing extract of fibrolytic enzymes from the fungus *Aspergillus japonicus* CO3 for *Cynodon C.* spp. (Tifton-85), *Urochloa brizantha*, *U. decumbens* and *Panicum maximum* cv. Tanzania (Tanzania). It was verified that the pretreatment with *A. japonicus* extract showed better enzymatic activity for *U. decumbens* and *U. brizantha*. The extract of this fungus can increase the availability of fermentable sugars, increasing ruminal fermentation with hydrolysis of cell wall polysaccharides. Similarly, application of cellulases produced by *Aspergillus* spp. from the digestive tract of sheep could also be used as a food supplement for ruminants, improving fiber digestibility, which should be evaluated in future studies.

The comparison of cellulolytic activity at 24, 48, and 72 hours, evaluated specifically for the genus *Paecilomyces*, did not indicate a significant difference between these incubation times ($P > 0.05$). A significantly higher mean growth of the isolates was observed in the culture medium for periods of 48 and 72 hours of incubation ($P<0.05$, Table 5).

In this study, among the six isolates of the genus *Paecilomyces*, three presented CAI>1, which indicates good capacity for enzymatic degradation of cellulase and indicates potential for research of these isolates as additives or probiotics, since there are no scientific reports of associated pathologies to these fungi to animals and humans (LACAZ et al., 2002).

In a study of the mycobiota of the digestive tract of mullet (*Aldrichetta forsteri*), the presence of the species *Paecilomyces lilacinus* was verified, which showed the ability to grow in both aerobic and anaerobic environments. It was verified that the isolates of this species promoted greater production of the cellulase enzyme in the presence of oxygen (MOUNTFORT; RHODES, 1991).

Another study evaluated *Paecilomyces variotti*, *Aspergillus fumigatus*, *Acremonium celulolyticus, Penicillium verruculosum* and *Trichoderma* spp. sugarcane bagasse fungi and decaying wood and the *Trichoderma reesei* QM9414 and *T. reesei* RUT C30 strains. It was observed that the fungal isolates from plant residues were good cellulase producers with international unit mL^{-1} of enzymatic extract greater than 0.04 (BASSO et al., 2010).

The results obtained in this study indicate the importance of evaluating the biotechnological potential of fungi in the digestive tract of sheep for the selection of isolates with potential for the development of microbial or probiotic additives in ruminant nutrition. However, the pathogenic and toxicological role of these fungi must also be considered for human and animal health.

CONCLUSION

Fungi of the genus *Aspegillus* are predominant among the isolates of mycelial fungi from the rumen and rectal ampulla of sheep fed with FBH. *Aspergillus terreus* is the most frequent species and presents specific intra variation regarding the cellulase production capacity. There was no difference in CAI of fungal isolates among animals fed with different levels of FBH inclusion. However, 69.44% of the isolated fungi showed cellulolytic activity >1, which indicates good cellulose degradation capacity and potential for the development of probiotics or prebiotics in the ruminant diet.

Table 5. Colony diameter (mm) and cellulolytic activity index (CAI) of *Paecilomyces* spp. isolates from the gastrointestinal tract of sheep fed banana leaf hay.

Isolates	24 hours		48 hours		72 hours	
	Colony diameter	CAI*	Colony diameter	CAI*	Colony diameter	CAI*
Paecilomyces T1 J1	2.0	1.00	13.0	1.00	21.0	1.00
Paecilomyces T2 J2	34.0	1.09*	79.0	1.00	82.0	1.00
Paecilomyces T5 J3	1.0	1.00	29.0	1.00	34.0	1.00
Paecilomyces T2 F1	3.0	1.00	7.0	1.00	15.0	1.00
Paecilomyces T4 F2	10.0	1.33	23.0	1.39	40.0	1.80*
Paecilomyces T5 F3	9.0	1.11	15.0	1.40	28.0	2.18*
Medianas	9.83 b	1.09 A	27.67 a	1.13 A	36.67 a	1.33 A

* CAI corresponds to the cellulolytic activity index, which measures the ratio of the diameter of the microcrystalline cellulose degradation halo to the diameter of the fungus colony. ** Indicates cellulolytic activity index greater than 1. F= feces. J = rumen juice. T1, T2, T3, T4, and T5 containing 0, 125, 250, 375, and 500 g of FBH/Kg of dry matter, respectively. CAI medians followed by the same letter on the same line do not differ by the nonparametric Kruskal-Wallis test with $P > 0.05$.

REFERENCES

ABRÃO, F.O. et al. Characterization of fungi from ruminal fluid of beef cattle with different ages and raised in tropical lignified pastures. **Current Microbiology,** v.69, n.5, p.649-659, 2014. Disponível em: https:/doi.org/10.1007/s00284-014-0633-5. Acesso em: 11 nov. 2019.

ABRÃO, F.O. et al. Notable fibrolytic enzyme production by *Aspergillus* spp. isolates from the gastrointestinal tract of beef cattle fed in lignified pastures. **Plos One,** v.12, n. 8, 2017. Disponível em: https:/doi.org/10.1371/journal.pone.0183628. Acesso em: 03 mar. 2019.

ALMEIDA, P.N.M. et al. Atividade celulolítica de fungos aeróbios isolados do rúmen de bovinos leiteiros alimentados com forragens tropicais. **Revista Caatinga**, v.27, n.4, p.202-207, 2014. Disponível em: http:/www.redalyc.org/articulo.oa?id=237132753024. Acesso em: 05 out. 2019.

AOAC Association of Official Analytical Chemist. Official Method of Analysis Association of Analytical Chemists. **19th Edition Washington DC**, p. 121-130, 2012..

AOAC. Association of Official Analytical Chemists. International Official Methods of Analysis. **16th Edition**, AOAC, Arlington, 1997.

BARBERA, M. et al. Effects of replacing rye-grass (*Lolium* spp.) hay by banana *(Musa acuminata* L.) by-products on feed intake, growth, and feed conversion rate of Canary hair sheep breed (Pelibuey) lambs. **Tropical Animal Health Production,** v.50, n.8, p. 1941-1945, 2018. Disponível em: https:/doi.org/10.1007/s11250-018-1618-3. Acesso em: 03 mar. 2021.

BASSO, T.P., GALLO, C.R., BASSO, L.C. Atividade celulolítica de fungos isolados de bagaço de cana-de-açúcar e madeira em decomposição. **Pesquisa Agropecuária Brasileira,** v.45, n.11, p.1282-1289, 2010. Disponível em: https:/doi.org/10.1590/S0100-204X2010001100008. Acesso em: 23 mar. 2019.

BOLSEN, K.K., ASHBELL, G., WEINBERG, Z.G. Silage fermentation and silage additives - review. **Journal Animal Science**, v.9, p.483-494, 1996.

CASADEVALL, A. Determinants of virulence in the pathogenic fungi. **Fungal Biology Reviews**, v.21, n.4, p. 130-132, 2007. Disponível em: https:/doi.org/10.1016/j.fbr.2007.02.007. Acesso em: 02 fev. 2020.

DIRKSEN, G. Sistema digestivo. In: Dirksen G, Gründer HD, Stöber M. (Eds.). **Rosenberger: exame clínico dos bovinos**. Rio de Janeiro: Guanabara Koogan, p. 167-169, 1993.

FACCHINI, F.D.A. et al. Production of fibrolytic enzymes by *Aspergillus japonicus* C03 using agro-industrial residues with potential application as additives in animal feed. **Bioprocess and Biosystems Engineering,** v.34, n.3, p.347-355, 2011a. Disponível em: https:/doi.org/10.1007/s00449-010-0477-8. Inglês. Acesso em: 02 mar. 2019.

FACCHINI, F.D.A. et al. Optimization of fibrolytic enzyme production by *Aspergillusjaponicus* C03 with potential application in ruminant feed and their effects on tropical forages hydrolysis. **Bioprocess and Biosystems Engineering**, v. 34, n.3, p. 1027-1038, 2011b. Disponível em: https:/doi.org/10.1007/s00449-011-0553-8. Acesso em: 02 mar. 2019.

FREITAS, C.E.S. et al. Sheep fed with banana leaf hay reduce ruminal protozoa population. **Tropical Animal Health Production***,* v. 49 n.4, p.807-812, 2017. Disponível em: https:/doi.org/10.1007/s11250-017-1265-0. Acesso em: 02 fev. 2020.

HARTINGER, T., ZEBELI, Q. The present role and new potentials of anaerobic fungi in ruminant nutrition. **Journal Fungi***,* v.7, n. 3, p. 200, 2021. Disponível em: https:/doi.org/10.3390/jof7030200. Acesso em: 03 abr. 2021.

LACAZ, C.S. et al. **Guia para identificação de fungos actinomicetos e algas de interesse médico.** 8. ed. São Paulo: Sarvier, p. 320, 1998.

LACAZ, C.S. et al. Tratado de micologia médica Lacaz. 9. ed. São Paulo: **Sarvier**. p. 1104, 2002.

LIU, K. et al. Ruminal microbiota-host interaction and its effect on nutrient metabolism. **Animal Nutrition**, v. 7, p. 49-55, 2020. Disponível em: https:/doi.org/10.1016/j.aninu.2020.12.001. Acesso em 04 mai. 2021.

MIN, B.R.; SOLAIMAN, S. Comparative aspects of plant tannins on digestive physiology, nutrition and microbial community changes in sheep and goats: A review. **Journal Animal Physiology and Animal Nutriton,** v.102, n.5, p.1181-1193, 2021. Disponível em: https:/doi.org/10.1111/jpn.12938. Acesso em: 05 set. 2021.

MOUNTFORT, D.O., RHODES, L.L. Anaerobic growth and fermentation characteristics of *Paecilomyces lilacinus* isolated from mullet gut. **Applied and Environmental Microbiology,** v.57, n.7, p.1963-1968, 1991. Disponível em: https:/doi.org/10.1128/aem.57.7.1963-1968.1991 **.** Acesso em: 02 mar. 2019.

NATIONAL RESEARCH COUNCIL. NRC. **Nutrient requirements of small ruminants**. 7th ed. Washington, D.C.: National Academy Press. p. 362, 2007.

NUNES, H. et al. Alimentos alternativos na dieta dos ovinos: uma revisão. **Associação Latinoamericana de Produção Animal**, v.15, n.4, p.147-158, 2007.

OLIVEIRA, R.L. et al. Alimentos alternativos na dieta de ruminantes. **Revista Científica de Produção Animal,** v.15, n.2, p.141-160, 2013. Disponível em: http:/dx.doi.org/10.15528/2176-4158/rcpa.v15n2p141-160. Acesso em: 10 mai. 2021.

OYELEKE, S.B., OKUSANMI, T.A. Isolation and characterization of cellulose hydrolyzing microorganisms from the rúmen of ruminants. **African Journal Biotecnology**, v.7, p.10, p. 1503-1504, 2008.

PATRA, A.K., SAXENA, J. Dietary phytochemicals as rúmen modifiers: a review of the effects on microbial populations. **Antonie Van Leeuwenhoek,** v.96, n.4, p.363-375, 2009. Disponível em: https:/doi.org/10.1007/s10482-009-9364-11. Acesso em: 08 set. 2021.

PESSOA, F.O.A. et al. A estreita relação entre a microbiota ruminal e os aditivos microbianos. **Colóquio Agrário***,* v.13, n.2, p. 1-20, 2017. Disponível em: DOI: 10.5747/ca.2017.v13.nesp2.000204. Acesso em: 07 ago. 2021.

PINTO, G.A.S., LEITE, S.G.F., TERZI, S.C. Selection of Tannase-Producing *Aspergillus niger* Strains. **Brazilian Journal of Microbiology.** v. 32, n.1, p. 24-26, 2001. Disponível em: https:/doi.org/10.1590/S1517-83822001000100006 . Acesso em: 02 mar. 2019.

RIBEIRO, A.C. Composição bromatológica e degradabilidade *in situ* de folhas de árvores frutíferas para alimentação de ruminantes. **Boletim de Medicina Veterinária**, v.3, n.3, p. 17-23, 2007. Disponível em: http:/ferramentas.unipinhal.edu.br/bolmedvet/viewarticle.php?id=28&locale=en&locale=&locale=es. Acesso em: 23 jun. 2020.

RUEGGER, M.J.S., TAUK-TORSNISIELO, S.M. Atividade da celulase de fungos isolados do solo da Estação Ecológica de Juréia-Itatins, São Paulo, Brasil. **Brazilian Journal of Botany**, v. 27, n.2, p. 205-211, 2004. Disponível em: https:/doi.org/10.1590/S0100-84042004000200001. Acesso em: 02 fev. 2019.

SILVEIRA JUNIOR J.A. et al. Use of banana (*Musa* sp.) pseudostem hay in feedlot sheep feeding. **Revista Brasileira de Zootecnia,** v.8, n.49, p. 1-15, 2020. Disponível em: https:/doi.org/10.37496/rbz4920180178. Acesso em: 02 ago. 2021.

TEATHER, R.M., WOOD, P.J. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rúmen. **Applied and Environmental Microbiology,** v. 43, n.4, p. 777-780, 1982. Disponível em: https://doi.org/10.1128/aem.43.4.777-780.1982. Acesso em: 03 abr. 2019**.**

VAN SOEST, P.J., ROBERTSON, J.B., LEWIS, B.A. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. **Journal Dairy Science,** v.74, p. 3583-3597, 1991.