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Research Article

Productive performance and economic profitable of weaned lambs supplemented with a *Trichoderma longibrachiatum* strain isolated from sheep

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Abstract

In this study, we evaluated the productive performance and economic profitably of weaned lambs fed low quality hay and supplemented with a *Trichoderma longibrachiatum* strain isolated from sheep (ICA/UFMG LT 001). Twelve early-weaned Santa Inês x Dooper lambs were housed in individual pens during 63 days, and randomly split into two groups: (1) lambs receiving the medium without the fungi and (2) lambs receiving 30 ml of culture medium containing the fungal strain. The average daily gain (ADG), dry matter intake (DMI), feed conversion (FC) and feed efficiency (FE) were analyzed in split plots of three periods of 21days and the total weight gain and final body weight were evaluated. The lambs fed the fungal strain showed higher ADG between 21 and 42 days of experiment for (P<0.05) and the increase in total weight gain (1.86 kg) comparing with untreated lambs. However, no significant differences were detected for the DMI, FC and FE. The fungal supplementation reduced into 13.35 % in the cost/kg of live weight and increased the gross and liquid profits by 9.28 % and 13.32 %, respectively. This study was aim evaluate the direct addition of a fungus isolated from the digestive tract of sheep in the diet of recently weaned lambs. We concluded that this supplementation improves the productive performance and economic feasibility until the 42 days of feedlot these young ruminants.

Keywords: Autochthonous fungus, cellulolytic fungus, microbial additive, diet adaptation, *Urochloa decumbens*

Introduction

The low quality of the roughage tropical pastures results in lower nutrient utilization and lower productivity, generating high feeding costs [1,2]. Therefore, when considering this high cost, nutritional alternatives should be evaluated to improve the economic and productive efficiency of ruminants [3].

The use of fungi and their enzymes have been used as additives for ruminant diets improving the use of low-quality fiber and dry matter intake as well as performance and profitability [3-6]. However, there is inconsistency in the results with the use of these additives, especially because type of the fungus species or strains, characteristics of individual animals, ruminant species and age, characteristics of the diets and type of nutritional management [7,4,8].

Ruminants grown in tropical pastures have show a relevant diversity of facultative anaerobic fungi in the rumen [9], which may be important in the degradation of complex food structures by the production of enzymes [10,11]. In a previous study we reported the occurrence of the genera *Aspergillus* spp., *Paecilomyces* spp., *Acremonium* spp., and *Trichoderma* spp. isolated from the digestive tract of lambs raised on pasture *Megathyrsus maximum* cv. Tanzania. These fungi may be important in the normal microbiota of this ecosystem and show probiotic or biotechnological potentials [12].

The inclusion of selected fungi from the ruminal environment will improve the utilization of foods containing low quality fiber, resulting in increased economic profitable in the feedlot or pasture creation. In this study we analyzed the productive performance and economic profitable of lambs weaned and the supplemented with a selected fungal strain isolated of sheep raised in tropical pasture.

Material and methods

Location and animals

The trial was carried in north of Minas Gerais, Brazil. The climate of the region is classified as tropical (AW) according to the classification of Köppen [13], marked by a long dry season from April to October. Twelve lambs Santa Inês x Dooper crossbreed, males, uncastrated with 3.5 months and initial body

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weight (BW) of 18.80 ± 2.34 kg were used. After identification and weighing, the animals were dewormed with 1% ivermectina (0.5 ml / 25 kg bw, Vallée, Minas Gerais Brazil) and Albendazole (1 ml/10 kg bw by oral route, Vallée, Minas Gerais, Brazil), and vaccinated against Clostridiosis (2 ml/animal, Poli-Start Vallée, Minas Gerais, Brazil). The animals were housed in individual stalls with 1.20 m wide, 2 m long and 1.30 m high, equipped with water trough, and provided with troughs to provide the diet.

Experimental design

The experiment was carried out in a completely randomized design to evaluate the groups of lambs fed with the fungi strain and another without the fungi (control) using six replicates (lambs). As a source of bulky food was used only *U. decumbens* hay wrapped after the seed drop during the dry season and ground (± 19 mm)

The composition of the diets was analyzed by the Combs - Goeser and CNF (NIRS) [14] and balanced for a gain of 200 g/day according to the NRC recommendations [15] in 70% concentrated and 30% voluminous (Table 1). The microbial additive used consisted of a fungi strain (ICA/UFMG LT 001), isolated from the digestive tract of sheep raised on pastures of *Megathyrsus maximum* cv. Tanzania. One strain of *Trichoderma* genus was selected because showed higher potential of degradation of crystalline cellulose (Avicel) as described by [12].

For identification, this strain was grown on Sabouraud agar for seven days, and DNA was extracted according to the method of [16]. The ITS region of rDNA was amplified from the extracted DNA by polymerase chain reaction (PCR) using primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC), according to the method of [17]. The amplified product was quantified with a NanoDrop 1000ND (NanoDrop Technologies), and the concentration was adjusted to 100 ng μL^{-1} for use in sequencing reactions.

Sequencing was performed with DYEnamic™ (Amersham Biosciences, USA) in a MegaBACE™ 1000 automated sequencing system at the Genome Analysis Center and Gene Expression of UFMG. The obtained DNA sequences were analyzed using BLASTn (v.2.215) of BLAST 2.0 at the NCBI website [18]. The species *Trichoderma longibrachiatum* was identified with 100% sequence similarity (1136 dp evaluated, BLAST result, n° acc. GenBank KX463453.1 for *T. longibrachiatum* SKF-3).

Experimental management

The adaptation of the lambs to the environment and diet occurred in 15 days and 63 days were of data collection. Feeding was administrated at 7:00 a.m. and 3:00 p.m., adjusted to keep 15 % of the offered. At the moment of feeding, a group of animals was supplemented with 30 ml of the culture medium containing the fungal strain (10^9 colony-forming unit (CFU)/ ml and mixed with 100 g of the concentrate, which allowed total intake of the supplement. The lambs of the control group received the same the culture medium without the fungal strain. Water was supplied ad libitum to animals in individual water trough.

The total food provided and the leftovers were weighed daily to evaluate the individual intake of the animals. Weight gain assessments were performed weekly by weighing in the morning before the first meal of the day.

Evaluation of dry matter intake and productive performance

The dry matter intake (DMI) g/animal/day was evaluated in three feeding periods with 21 days each (1st period of 0-21 days, 2nd period of 21-42 days and 3rd period of 42-63 days). The productive performance was evaluated by measuring the final body weight (FBW, kg), average daily gain (ADG) g/animal/day considering the three periods of analysis and total weight gain (TWG, kg). The DMI/DWG ratio was calculated for feed conversion (FC g/g) evaluation.

Economic profitable

The economic profitable was expressed in gross profit (GP) and net profit (NP) according to [19]. The price of the stuffs used in the diets was used to estimate the cost of the diet (CD) based on the dry matter. Considering the dietary parameters, the costs of kg of live weight (CLW) = CD/total feedlot weight gain; gross

Table 1 Diet and nutritional composition feed to feedlot lambs supplemented with fungal strain ICA/UFMG LT 001 for 63 days.

Ingredient	DM%	EE%	TDN%	CP%	NDF%
Maize	89	7.9	83.18	9	16.52
Soybean meal	88.8	1.76	80.68	46	15.54
Ureia + ammonuim sulfate	100	0	0	277	0
Minerals	100	0	0	0	0
Urochloa decumbens hay*	95.38	1.02	30.94	3.06	82.26
Inclusion %, dry matter					
Maize	59.1	4.7	49.2	5.3	9.8
Soybean meal	8.3	0.1	6.7	3.8	1.3
Ureia + ammonuim sulfate	0.5	0.0	0.0	1.4	0.0
Minerals	2.0	0.0	0.0	0.0	0.0
Urochloa decumbens hay	30.0	0.3	30.9	3.1	24.7

* Additional bromatological composition: ADF = 53.04 % DM; Lignin = 7.50 % DM; Cellulose = 45.54 % DM; Hemicellulose = 29.22 % MS.

profit (GP) = FBW x price of kg of live weight and net profit (NP) = GP – CD, were calculated.

Statistical analysis

After normality and homogeneity verification, the ADG, DMI, FC and FE characteristics were analyzed in a split plot considering the two treatments, 3 periods (as measures repeated in time) and the interaction between the treatments and periods, by the statistical model:

$$Y_{ijk} = m + A_i + B_j + C_k + (A \times C)\, i_k + eb_{ijk}$$

where: Yijk is the observation; m is the overall mean; Ai is the effect of treatments (i = 2); Bj is the random effect of the animals (n = 6); Ck is the effect of the periods (k = 3); (A x C) ik is the fixed effect of the interaction between treatments and periods and ebijk is the effect of experimental error.

The TWG and FBW characteristics were submitted to simple analysis of variance by the statistical model:

$$Yijk = m + Ai + eaij$$

where Yijk is the observation; m is the overall mean, Ai is the effect of the treatments, Bj is the random effect of the animals and eaij is the experimental error. Differences were considered significant statistically when (P <0.05) or with tendency when (P <0.1) by the t test. Regression analysis was promoted to model weight gain during the experimental period and, the determination coefficient were used to select the model. Descriptive statistics were applied for the economic profitable evaluation.

Results

Productive performance and dry matter intake

The initial body weight (IBW) for both groups was similar, but the final body weight (FBW) tended to be higher (6.8 %) in animals supplemented with the fungal strain (P <0.1), which presented higher ADG (226.45 g/day x 196.82 g/day) (Table 2, P<0,001). However, no differences were observed between treatments for DMI, FC and FE (Table 2, P> 0.05).

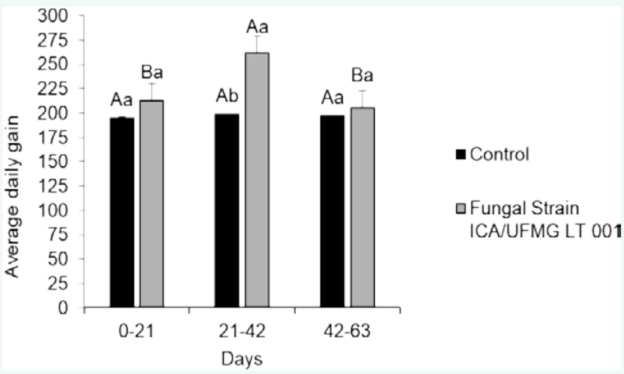

Figure 1 Average daily gain in three periods of 21 day for lambs weaned and supplemented or not with fungal strain ICA/UFMG LT 001
Values with similar case-capitalized letters between the periods and lowercase between treatments within each period do not differ at P <0.05.

Significant interaction was observed between the treatments and the periods evaluated for ADG (P <0.031), with the hug growth observed in the period between 21 and 42 days of feedlot (Figure 1) for the group supplemented with the fungal strain (P <0.05). In the control group, the ADG did not present significant changes in the evaluated periods. For DMI, FC and FE, the interaction between the treatments and the periods evaluated was not significant, although it presented differences (P <0.05) between the periods evaluated for DMI and a tendency for better FE in the group supplemented with the fungal strain (P <0.1) (Table 2).

By regression analysis, the third-degree polynomial model explained 64.9 % of the ADG variation for the group supplemented with the fungal strain during the evaluation period (*P <0.05, $Y=361.28^{*}-148.93x^{ns} + 40.83x^{2ns}-3.03x^{3*}$)

However, none of the mathematical models evaluated explained the variation for ADG of the group that did not receive the fungal strain since it remained constant during the experiment.

Economic profitable

The group of animals supplemented with the fungal strain presented higher TWG (14.26 kg), which resulted in a reduction of cost/kg of weight produced and consequently, there was an increase of 9.28 % and 13.32 % in gross and net profit, respectively, of the animals supplemented with the fungal strain (Table 3).

Discussion

Productive performance and dry matter intake

The use of microbial and probiotic additives in the ruminant's diet may improve feed utilization and consequently profit performance. In this study, the addition of the fungal strain promoted an increase at 15.05 % for ADG, and this increase was not associated with a significant increase in DMI (Table 2). The addition of the fungi strain in the diet of the young lambs could improve the fibrolytic activity in the rumen. This may be justified by the ability of this fungal specie to produce cellulase, hemicellulase, xylanase, glucanase, important enzymes in the degradation of complex constituent of the vegetal cell wall [20-22].

The variation of ADG observed during the experimental period could be the result of the physiological and behavioral modifications in the young animals (Figure 1). The lowest ADG observed in the first experimental period (0 - 21 days) (Figure 1) could be justified because the animals were early weaned and therefore had a low capacity for eating fibrous food during this period. However, the addition of the fungal strain could have contributed to the improvement of the ruminal ecosystem and favored the fibrolytic microbiota and, consequently, the use of the low-quality hay. This improvement may have contributed to better nutrient supply to animals and increased ADG up to approximately four weeks of supplementation (Figure 1). During the end period (42-63 days), the ADG averages were similar between the two lamb groups. Possibly, after 42 days, the

Table 2 Productive performance, dry matter intake and feed conversion for lambs weaned and supplemented or not with fungal strain ICA / UFMG LT 001.

Items	Treatments effects		Standard error	*P*-value		
	Control	Fungal strain		Treat[1]	Per[2]	Interaction[3]
IBW (kg)	19.05	19.44	0.978	0.501	-	-
ADG (g/day)	196.82	226.45	0.021	0.047	< 0.001	<0.031
DMI (g/day)	976.31	1036.14	44.140	0.362	< 0.001	0.319
FC	4.96	4.57	0.320	0.970	0.285	0.669

IBW = Initial body weight; ADG = Average daily gain; DMI = Dry matter intake; FC = Feed conversion. [1] Treatments; [2] Periods. [3] Treatments and periods interaction.

Table 3: Cost of the diet, gross and net profit for lambs weaned and supplemented or not with fungal strain ICA / UFMG LT 001.

Items	Treatmentes effects		SEM[a]
	Control	Fungal strain	
Cost of the diet	31.80	32.80	1.650
Seeling price (US$/kg)	2.61	2.61	--
Cost/kg of body weight	2.60	2.29	0.250
Gross profit (US$/animal)	80.30	87.75	7.670
Net profit (US$/animal)	48.49	54.95	6.170

[a] Standard error of mean

untreated lambs already could be adapted to the diet, and this fungi supplementation would not be more important.

The improvement in the ADG, without significant changes in DMI, observed in this research may be based on the improvement of the hydrolytic capacity of the rumen ecosystem as reported by [23] that showed enzyme cocktail produced by a fungal strain from South African soil improving lamb performance when used as a feed additive. However, this product showed limited potential in low forage diet. The better dry matter (DM) digestibility by producing fibrolytic enzymes may favor the growth of all fibrolytic microbiota [24] and increased production of volatile fatty acids in the rumen [25]. In dairy cow diets, the supplementation with fibrolytic enzymes from *T. longibrachiatum* may increase the population of rumen bacteria that utilize hemicelluloses and secondary products of cellulose digestion [26].

In another study, similar results were observed with the addition of commercial fibrolytic enzymes derived from *Trichoderma longibrachiatum* in lambs fed with *Cenchrus ciliaris* L. hay (300 g/kg DM). The authors observed increase of 8.80 % in ADG and 12.6 % in total weight gain for the group supplemented with these enzymes. This improvement resulted from better digestibility of the dry matter, NDF and ADF of hay, without significant effects on DMI [4].

The addition of xylanase and glucanase derived from *Aspergillus* spp. and *Trichoderma* spp. for goats fed rice straw (300 g/kg DM), resulted in improvement of fiber digestibility, providing substrates for growth and metabolism of rumen bacteria. These enzymes promoted a 34.70 % increase in ADG without changes in DMI [8].

In another study evaluating buffalo performance, the addition of xylanase and cellulase derived from *Trichoderma reesei,* in the diet containing *Avena sativa* (50 %) silage, resulted in significantly increase of DWG and FC without changes for DMI [27]. In calves, the increase in 15.37 % of ADG with addition of the anaerobic fungus *Orpinomyces* sp. also was not related to increment in DMI in diets containing 50 % of rice straw [28].

Changes in DMI were also not observed with the addition of enzymes derived from *Aspergillus niger*, *Trichoderma longibrachiatum* in diets (40 % voluminous) of cows [5] Mohamed *et al*., 2013), and for cows supplemented with enzymes derived from *Trichoderma reesei* and fed with 60 % of voluminous [29]. The supplementation with enzymatic mixture produced by *Aspergillus awamori* in sheep fed with 50 % of seed promoted a significant increase for ADG without significant changes in DMI [3], which was also found in the present study.

The low quality of the roughage as the used in this study is an important factor influencing the food passage rate in the rumen and the stimulus to intake. These factors may directly interfere with the effects of microbial additives on ruminal degradation and food passage rate [30,4]. In this research, high levels of NDF (82.26 %), ADF (53.04 %) and lignin (7.50 %) of *U. decumbens* hay, used as an only roughage may have resulted in a longer stay period of its fiber in the rumen.

In contrast to that observed in this study, a study indicated a significant increase in DMI by the addition of 1 and 3 μl/g of dry matter of the commercial(xylanase in lambs fed with corn straw (30 % in DM) [6]. In another research, an increase of 11 % in DMI was observed in goats fed with corn straw (8 % in DM), alfalfa hay (32 % in DM) and supplemented with commercial cellulase [31]. However, the addition of increasing amounts of *T. longibrachiatum* enzyme complex in the diet of sheep fed with barley (62.9 % in DM) promoted a quadratic response in DMI without significant changes to ADG [32].

In the present study, we used a (70:30) concentrate: voluminous ratio and even so it was observed an increase in ADG with the inclusion of the fungal strain in the supplementation of lambs. The dietary fiber content may also directly influence the action of fibrolytic enzymes or fungal cells in the ruminal environment [27]. Differently from that observed in this research, when using diet with 70 % of concentrate in DM, no significant increase was observed in ADG and DMI with the addition of cellulase produced by the fungus *Trichoderma* spp. in sheep [33]. However, high fiber diets in animals supplemented with enzymatic coquetel from the ABO 374 strain soil isolate resulted in a significant reduction in ADG, DMI and FC [23].

These divergent results among these studies could be related on the chemical and structural composition of the voluminous, the concentration of the form of microbial additive and the different ratios of concentrate: voluminous. These may represent important factors in the action of the fungi additives and in the composition of the ruminal ecosystem and, therefore, should be considered in future researches.

Economic profitable

The improvement of the productive indicators and the food efficiency result in the better economic efficiency. The increase in ADG for lambs fed with the fungi strain resulted in a reduction in 13.35 % for cost/kg of BW produced and, therefore, provided higher gross and net profits with commercialization of the animals (Table 3).

Although significant differences for DMI, FC and FE between the lambs groups were not detected (Table 2), the animals supplemented with the fungal strain presented numerically higher DMI values, which may have promoted a small increase in cost of diet (Table 3). However, the addition of the fungal strain resulted in the improved economic profitable expressed in NP (Table 3). Further larger-scale studies and using other fungal additive inclusion strategies would determine better economic gains.

Few studies have reported the economic profitable of adding fungal strains and their enzymes to the diet of lambs. In the research that evaluated the diet containing seeds of crushed date pits supplemented with enzymes produced by *Aspergillus awamori* in substitution of barley in the diet of sheep, no differences were observed in the relative economic efficiency with the addition of up to 50 % of the seed of date (99.73 %) in relation to the control group (100 %). However, a 14.25 % reduction in cost/kg of body weight was observed [3], which was similar to that observed in this present study (13.35 %).

Conclusion

Administration of autochthonous fungal strain ICA/UFMG LT 001 promotes better average daily gain of weaned lambs fed with low quality hay up to 42 days of feedlot. In addition, the use of this fungal strain results in a better economic profitable of the supplementation of weaned lambs.

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