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Evaluation of extruded roughage with different additives in sheep diet

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ABSTRACT. The purpose was to evaluate the effect of extruded roughage Foragge® with different additives on intake, digestibility nutrients and nitrogen balance of sheep. Twenty adults, non-pregnant ewes with average weight 68 kg were used. The treatments were extruded roughage with additives (essential oil, virginiamycin, unpurified inactive yeast, tannin and purified inactive yeast). The design was in randomized blocks. The means were contrasted by SNK test, and the fecal score was tested by the Kruskal Wallis test (1952), at 5% significance. There was no difference in the intake of dry matter, crude protein, nitrogen, water, water in relation to dry matter, neutral detergent fiber (NDF), and acid detergent fiber ($p > 0.05$). As well as the digestibility of dry matter, crude protein and neutral detergent fiber, the fecal weight, fecal nitrogen, retained nitrogen and nitrogen retained in relation to nitrogen ingested ($p > 0.05$). However, dry matter intake as a function of body weight and metabolic weight, urinary nitrogen, hemicellulose intake, and hemicellulose as a function of NDF, were higher in the Foragge Factor® treatment ($p < 0.05$). The inclusion of different additives in the extruded roughage improved nutritional parameters, without causing disturbances.

Keywords: essential oils; tannin; virginiamycin; yeasts.

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Introduction

In an attempt to improve animal performance, farmers are increasingly applying technologies in animal breeding and feeding. In this context, additives have drawn attention because their use promises to improve animal performance, besides reducing production costs. Additives most used include ionophores (i.e: monensin) and non-ionophores, such as virginiamycin, tannins, yeasts, or essential oils.

Extruded feed has been used in ruminant diet, in order to increase access of ruminal microorganisms to feed, and consequently the digestibility and, also facilitate handling. Thus, extruded roughage produced from the aerial part of grasses is an alternative fiber feed for ruminants, able to improve digestibility, reduce waste, and increase animal production efficiency.

Therefore, it is expected that the use of additives in the extruded feed for ruminants has better use of energy consumed due to increasing the fermentation; improving feed conversion; increasing daily weight gain; and, in some cases, avoid the occurrence of metabolic disorders, such as acidosis and others. This because, according to Souza et al. (2016), additives act by controlling ruminal populations involved in fermentation.

The intake, digestibility nutrients and nitrogen balance are influenced by several factors such as nutrient content, associative effects between feeds, roughage to concentrate ratio, and processed feed (Gomes et al., 2012). Inclusion of additives improves the rumen environment and consequently affects the use of nutrients. The use of additives increases in vitro nutrient digestibility and dry matter intake (Figueroa et al., 2015). In addition, extrusion can contribute to the factors mentioned above, as it improves digestibility by increasing the availability of nutrients to ruminal microorganisms.

Although the positive aspects of extrusion and additives are known, there is little information about this combination in sheep diet. Thus, the goal was to evaluate the effect of extruded roughage Foragge® with different additives on intake and digestibility nutrients, and nitrogen balance of sheep.

Material and methods

The study was conducted between October 24, 2017 to November 25, 2017, at the Federal University of Uberlândia, in Uberlândia (UFU), State of Minas Gerais, Brazil. All animal procedures were approved by the ethics committee on animal use of UFU, according to protocol 094/17.

The experiment was divided into two phases of 15 days. In the phases, the first ten days was for adaptation and the other five days for data collection. At the end of the first phase, the animals changed treatment, and, in the second phase, a new adaptation and data collection were made.

Twenty adults, non-pregnant ewes (Santa Inês x Dorper) with an average weight of 68 kg and age over four years, were housed in individual stalls with slatted floors provided with feeders and drinkers. In each period, they were dewormed with monepantel, using 1 mL for 10 kg body weight, weighed to obtain the average body weight and determine intake as a function of body and metabolic weight.

The treatments consisted of roughage extruded with different additives: Foragge Essential® (essential oils), Foragge Max® (Virginiamycin®), Foragge AA® (unpurified inactive yeast), Foragge Bypro® (Tannin), and Foragge Factor® (purified inactive yeast). Foragge® feed is an extruded product from *Urochloa* forages, enriched with minerals, vitamins, starch, and the tested additives. Diet composition is listed in Table 1.

Table 1. Chemical composition of the experimental diets.

Diet composition (g kg^{-1})*	Foragge Essential®	Foragge Max®	Foragge AA®	Foragge Bypro®	Foragge Factor®
Dry matter	900.0	900.0	900.0	900.0	900.0
Crude Protein	72.0	71.0	72.0	70.0	72.0
Neutral detergent fiber	422.0	423.0	423.0	447.0	423.0
Acid detergent fiber	263.0	263.0	263.0	298.0	263.0
Ethereal extract	19.0	20.0	19.0	16.0	20.0
Ash	37.0	37.0	37.0	39.0	37.0
Total digestible nutrients	557.0	558.0	558.0	612.0	557.0
Starch	255.0	255.0	254.0	232.0	255.0
Additive	5.5	3.0	2.0	2.0	2.0

*Data provided by Nutratta®.

It was offered about 3.5% body weight, as complete mixed ration, the extruded roughage Foragge® with different additives at 8:00am and 5:00pm. The mineral mix Masterfós® and water were supplied *ad libitum*. The amount of supplied feed was corrected to produce 10% leftovers in dry matter.

Total feces was collected in plastic collectors below each individual stall, weighed and daily sampled in each experimental period, in the same way, samples of feed and leftovers were stored in plastic bags at −18°C. Samples of feed, leftovers and feces were analyzed to determine the concentrations of dry matter (DM), ash, crude protein (CP), neutral detergent fiber (NDF) and, acid detergent fiber (NDA).

Samples of feed, leftovers and feces were pre-dried in a forced ventilation oven at 55°C for 72h and ground to 1-mm particles (Wiley mill, Marconi, MA- 580, Piracicaba, São Paulo State, Brazil). Samples were analyzed for DM, ash, nitrogen (N) and CP (AOAC, 1990/ 942.05; AOAC, 1990/ 954.01). NDF and ADF were determined according to Van Soest, Robertson, and Lewis (1991). Hemicellulose was determined by the difference between NDF and ADF.

For intake and digestibility measurements, the food supplied, leftovers and feces were daily weighed on a scale accurate to five grams. Nutrients in take were calculated by the difference between offered and leftovers, and nutrients digestibility were calculated by the difference between consumed nutrient and feces nutrient divided by consumed nutrient (Maynard, Stewart, & Bettany, 1984). The consumption of water was measured daily. The offered water placed in 20-liter buckets that replacement when necessary and, one bucket for evaporation control. So, leftovers in all buckets were measured by a graduated test tube to estimate drinking water intake by the difference between water supplied, evaporated and leftovers.

Fecal scores were determined according to Gomes et al. (2012), with observations of feces on a scale 1 to 6, being: 1 - dry and dull feces, 2 - normal feces, 3 - slightly softened feces, 4 - soft feces, losing their shape, 5 - soft feces and without the normal shape, and 6 - diarrheal feces.

Total urine was collected in a bucket with 100 mL sulfuric acid (H2SO4 5%) to prevent nitrogen (N) volatilization, as well as possible fermentation. Samples of 20% total urine were stored in a plastic bottle, duly identified per animal, in each experimental period, and stored at -18°C. Urine nitrogen was determined by AOAC (1990/ 942.05).

Nitrogen (N) balance or retained was calculated by the difference between nitrogen consumed to nitrogen concentration in feces and urine (Zeoula et al., 2006). Consequently, the relation between N consumed and N retained was calculated.

The experimental design was in randomized blocks (five treatments and two periods/blocks). The statistical model was: y_{ijkl} = μ + τ_i + P_j + ε_{ijk}. Where: y_{ijkl} = observation ijkl; μ = overall mean; τ_i = fixed effect of treatment i; P_j = fixed effect of period/block j; ε_{ijk} = random error. All data were tested for normality by Shapiro and Wilk (1965) and homoscedasticity (Levene, 1960). After checking these assumptions, data were subjected to analysis of variance, and means were compared by the SNK test (Student-Newman-Keuls) with 5% significance ($p < 0.05$) for type I error. Fecal score was analyzed by the Kruskal and Wallis (1952) test with 5% significance ($p < 0.05$).

Results and discussion

Foragge® feed was produced with the purpose of partially or totally replacing corn silage, a fact proven by chemical values close to those found in the Foragge® product in question (Table 1). According to Valadares Filho (2006), the average chemical composition of corn silage produced in Brazil has 71.8 CP, 27.9 EE, 539.8 NDF, and 294.9 ADF g kg^{-1} and dry matter digestibility of 595.8 g kg^{-1}. These values are similar to those found in Foragge® products.

There was no difference in intake of dry matter, water, water in relation to body weight, and crude protein ($p > 0.05$) with the inclusion of the additives. As well as in the digestibility of dry matter, and crude protein ($p > 0.05$) (Table 2). A factor that can change feed intake is the roughage/concentrate ratio of the diet. In this study, there was no change in these proportions between treatments, since all animals consumed Foragge®, varying only the additive.

Table 2. Intake and digestibility of nutrients in sheep fed diets with extruded roughage with different additives.

Item	Foragge Essential®	Foragge AA®	Foragge Factor®	Foragge Max®	Foragge Bypro®	General Mean	p-value	CV
DMI	2.24	1.76	2.54	1.84	1.94	2.07	0.125	31.04
DMI/BW	3.58 AB	2.49 B	4.13 A	2.81 AB	2.83 AB	3.18	0.025	33.16
DMI/MW	100.65 AB	72.34 B	115.67 A	80.05 AB	81.54 AB	90.31	0.035	32.37
DMD	574.3	539.6	534.6	524.5	523.6	539.7	0.475	11.34
WI	4.4	4.35	5.03	3.38	4.8	4.42	0.250	32.58
WI/DMI	1.95	2.66	1.96	1.77	3.12	2.3	0.222	36.45
CPI	0.153	0.147	0.2	0.155	0.215	0.174	0.107	34.66
CPD	493.4	530.0	507.5	521.6	560.7	522.7	0.588	16.28

*Different letters on the row indicate significance at 5% ($p < 0.05$) by SNK test. DMI: dry matter intake (kg day^{-1}); DMI/ BW: dry matter in relation to body weight (%); DMI/MW: dry matter in relation to metabolic weight (g kg$^{-0.75}$ day^{-1}); DMD dry matter digestibility (g kg^{-1}); WI: water intake (L day^{-1}); WI/DMI: water intake in relation to dry matter intake; CPI crude protein intake (kg day^{-1}); CPD crude protein digestibility (g kg^{-1}); CV: coefficient of variation (%).

However, DM in relation to body and metabolic weight was significantly different between treatments ($p < 0.05$). According to Hudson and Christopherson (2018) regard intake through metabolic weight (MW) is more effective, because it is a basis for expressing energy metabolism in maintenance requirement. Where, the animals that consuming Foragge Factor® had the highest DM/BW and DM/MW and the lowest values were found with Foragge AA®. The inclusion of purified inactive yeast increased by approximately 58% DM/BW compared to Foragge AA®.

Conceptually, purification allows the structural and functional characterization of the yeast, thus improving the ruminal environment, because of the absence of impurities contained in the unprocessed yeast. Another factor is that purification improves the quality of inactive yeast, by increasing the standardization and quality of the product.

According to Pires (2012), yeast improves digestion, the use of nutrients, and increases intake once it leads to an increase in the fiber degradation rate, especially in diets rich in concentrate. That is, yeasts have a stimulating function due to the presence of peptides, these are used by microorganisms with a readily available nitrogen source, together with the presence of 25% starch (Table 1).

Thus, yeast can promote an increase in the number of cellulolytic bacteria, optimizing the rumen environment, increasing the fiber digestibility, and the microbial protein flow to the small intestine (Yuan et al., 2015). It appears that the Foragge Factor® favored the colonization of feed particles, accelerating the growth of cellulolytic bacteria, because it improved the efficiency of nutrient degradability by ruminal bacteria, increasing the intake rate. This result is very important, as it shows that all additives have increased the intake by animals (Table 2).

Regardless of the additive, DMI was 1.02 kg day^{-1} (97%) higher than that recommended by the NRC (2007) for this category (1.05 kg day^{-1}). According to Mertens (1997), intake control mechanisms are energy density and fiber amount of diets. Zanine and Macedo Junior (2006) showed that the presence of low-quality fiber can limit the DMI.

Therefore, it is possible to infer that the fiber content of Foragge® was not the limiting factor for DMI, since the animals consumed more than the recommended amount. Importantly, all treatments aim to improve the rumen environment and may also favor intake. Oliveira et al. (2018) studied the effect of using extruded roughage, and showed an improvement in the nutritional parameters of sheep, increasing DMI (3.39 kg day^{-1}) and DMD (666.6 g kg^{-1}).

As for water, Forbes (1968) recommended the intake of approximately 7 liters per day, that is, the animals ingested an insufficient amount of water (Table 2). However, as the DMI was 97% above the recommended level, this fact can show that the animals did not suffer hydric stress.

The CP (crude protein) recommended by the NRC (2007) is 0.075 kg day^{-1}, but the CP intake was 232% higher than recommended (0.174 kg day^{-1}), also justified by the increase of 97% in DMI (Table 2). Crude protein digestibility showed a behavior similar to dry matter digestibility, close to DCP of corn silage, which is 562.4 g kg^{-1} according to Valadares (2006). The similar response of crude protein intake and digestibility was because, according to Camerom, Klusmeyer, Lynch, Clark, and Nelson (1991), the crude protein digestibility and intake increased with the amount of crude protein in the feed but, the amount of protein is similar between the treatments (Table 1).

There were no differences for weight feces fresh and dry, as well as fecal dry matter ($p > 0.05$) (Table 3). Fecal weight is related to diet composition, rate of passage, and digestibility. According to Santos and Nogueira (2012), volume of feces in ruminants is affected by the ambient temperature, quality, and quantity of feed, management, in addition to characteristics of the animal, such as breed and ages. The diets had the same roughage: concentrate ratio, there was no effect of the use of additives on feces weight (Table 2).

Table 3. Feces and urine parameters in sheep fed diets with extruded roughage with different additives.

Item	Foragge Essential®	Foragge AA®	Foragge Factor®	Foragge Max®	Foragge Bypro®	General Mean	CV	p-value
FF	3.14	2.47	3.12	2.33	2.71	2.76	32.69	0.2946
DF	1.07	0.83	1.13	0.79	0.95	0.96	30.08	0.1208
DMF	345.5	347.6	369.9	356.1	360.7	356.0	157.1	0.9056
FE	2.17	2.2	2.37	2.2	2.2	2.23	33.76	0.7835
UV	0.994	1.17	0.909	0.956	1.411	1.01	33.76	0.6037
UD	1.023	1.016	1.024	1.019	1.019	1.02	0.91	0.5176

FF: fresh feces (kg day^{-1}); DF: dry feces (kg day^{-1}); DMF: dry matter of feces (g kg^{-1}). FE: fecal score 1 to 6. UV: urine volume (L day^{-1}); UD: urine density (mL dL^{-1}); CV: coefficient of variation (%).

Sheep produce between 0.8 and 1.5 kg of fresh feces per day (Vieira, 2008). However, sheep had a production of 1.2 kg feces per day, higher than recommended (Table 3), without a change in digestibility when compared to, for example, corn silage as a roughage source. The dry matter of feces was lower than recommended by Van Clef, Ezequiel, D'Aurea, Fávaro, and Sancanari (2010), from 370 to 440 g per kg.

According to Gomes et al. (2012), the normal fecal score is 2, so the means observed are similar the reference values (2.23). Fecal score scale indicates changes in the gastrointestinal tract and its implications for the health and performance of animals (Ferreira, Lima, Pessoa, Paz, & Jesus, 2013). Therefore, this indicates that the animals were given an adequate diet, in addition to not having gastrointestinal disorders. The fecal score has a high correlation with fecal dry matter (Ferreira et al., 2013) and with dietary NDF; and the extruded roughage Foragge® together with the action of the additives maintained the fecal score at adequate levels, as well as the fecal dry matter (Table 3).

In sheep, urine excretion should be between 100 and 400 mL for 10 kg weight, therefore with an average weight of 68 kg, normal values for urine volume are between 679 and 2,719 mL (Reece, 2006). Thus, the average urine excretion of 1,009 mL day^{-1} was within the recommended range. Likewise, the urine density values 1.0206 g mL^{-1} remained within the normal range for sheep (1.015 - 1.045 g mL^{-1}), as described by Reece (2006). As previously shown, water intake was not limiting for these animals (Table 2), since the urinary parameters assessed here remained within the recommended.

There was no difference between treatments for nitrogen intake (NI), fecal nitrogen (FN), retained nitrogen (RN), and relation to RN to NI ($p > 0.05$). This behavior occurred due to the fact that the experimental diets had the same level of CP (7.2%) in all treatments with the difference only in the inclusion of additives (Table 4).

Table 4. Intake, loss and efficiency of nitrogen utilization in sheep fed extruded roughage with different additives.

Item	Foragge Essential®	Foragge AA®	Foragge Factor®	Foragge Max®	Foragge Bypro®	General Mean	CV	p-value
NI	25.74	23.91	32.02	25.51	34.34	28.37	32.62	0.1276
FN	14.47	10.95	16.22	11.35	11.52	12.94	36.4	0.1311
UN	2.76 B	3.06 B	5.08 A	3.36 B	5.45 A	3.96	41.08	0.0058
RN	10.85	10.05	11.81	11.94	17.94	12.53	30.68	0.2728
RN/NI	0.417	0.411	0.357	0.472	0.462	0.423	35.81	0.5893

*Different letters on the row indicate significance at 5% ($p < 0.05$) by SNK test. NI: nitrogen intake (g day^{-1}); FN: fecal nitrogen (g day^{-1}); UN: urinary nitrogen (mL day^{-1}); RN: retained nitrogen (g day^{-1}); CV: coefficient of variation (%).

Urinary nitrogen (UN) was different between treatments ($p < 0.05$), with the highest values found in the Foragge Bypro® (tannin) and Foragge Factor® (purified yeast) treatments. Retained nitrogen (RN) was similar between treatments ($p > 0.05$). Therefore, for these treatments mentioned above, even with higher nitrogen excretion via urine, nitrogen retention was not influenced. Nitrogen excreted via urine is found as urea, which is formed in the liver from excess N, which was quickly converted to rumen ammonia (Van Soest, 1994).

The increase in levels can be justified by the increase in nitrogen intake and, also, by an increase in urinary nitrogen losses from the secondary metabolism of animals. The increase in dry matter intake (Table 2) may also have favored the increase in the rate of passage and, as a consequence, reduced the fermentation time, thus being able to cause ruminal asynchrony, favoring, again, the ruminal leakage of nitrogen as ammonia. Even with greater excretion of UN, thus representing energy loss to the animal, the use of the food remained constant, since the DMD and CPD (Table 2) were the same in all treatments.

The Foragge Bypro® and Foragge Factor® favored greater loss of rumen ammonia and consequently higher urinary nitrogen values. In the present study, Foragge Bypro® DMI was 1.94 kg day^{-1} (Table 2), and consequently, the intake of the additive's tannin increased, the ingested dose was 3.8 g day^{-1}. This dosage of tannin is 80% above the recommended amount according to the one proposed for DMI, that is, the dosage was higher than expected considering the increase in DMI.

Tannins are compounds that can reduce the ruminal degradation of protein and increase its duodenal flow when moderate doses are provided in the dry matter of forage (Min, Barry, Attwood, & Mcnabb, 2003). The use of tannin increased the excretion of ammonia in the rumen and, consequently, increased urinary N excretion. However, nitrogen balance was positive (N retained), as there was a high CPI and CPD (Table 2) within the normal range when compared to corn silage. The general average excretion of N via urine was 3.96 g kg^{-1}, below that recommended by the literature, which varies between 4.0 and 8.5 g kg^{-1} (Morgado et al., 2014).

There were no statistical differences ($p > 0.05$) between treatments for intake of neutral detergent fiber (NDF), neutral detergent fiber according to body weight (NDF BW^{-1}), neutral detergent fiber corrected for ash (NDFash), acid detergent fiber (ADF) and neutral detergent fiber digestibility NDFD (Table 5).

Table 5. Fiber intake and digestibility in sheep fed extruded roughage with different additives.

Item	Foragge Essential®	Foragge AA®	Foragge Factor®	Foragge Max®	Foragge Bypro®	General Mean	CV	p-value
NDFI	1.21	0.87	1.21	1.02	1.09	1.07	31.22	0.2816
NDFI/BW	1.79	1.23	2.03	1.56	1.59	1.64	33.74	0.0856
NDFIash	1.08	0.85	1.2	0.99	1.03	1.03	31.11	0.3007
ADFI	0.661	0.531	0.75	0.689	0.721	0.67	31.68	0.2975
HEMI	0.460 AB	0.344 B	0.497 A	0.335 C	0.374 BC	0.404	30.73	0.0499
HEM/DFN	0.419 AB	0.325 B	0.458 A	0.300 C	0.315 BC	0.365	30.76	0.0296
NDFD	449.7	453.0	440.5	494.0	443.5	455.2	15.56	0.6113

*Different letters on the row indicate significance at 5% ($p <0.05$) by SNK test. NDFI: neutral detergent fiber intake (kg day^{-1}); NDFI/BW neutral detergent fiber intake as a function of body weight (BW) (%); NDFIash: neutral detergent fiber intake corrected for ash (kg day^{-1}); ADFI: acid detergent fiber intake (kg day^{-1}); hemicelluloses intake (kg day^{-1}); NDFD: neutral detergent fiber digestibility (g kg^{-1}); CV: coefficient of variation (%).

Macedo et al. (2004) showed that the amount of NDF in the diet negatively influences DMI, due to slower fermentation and longer time in the rumen. However, the greater digestibility of the fiber can stimulate intake by increasing the rate of passage. According to Mertens (1997), the NDFI for ruminants should be from 0.8 to 1.2% BW; in this experiment the general average of NDFI/BW was 1.65%, higher than that recommended by the literature. The higher intake of NDF did not limit the DMI, in this study, since the DMI found was above the recommended level (Table 2), which can be explained by the better digestibility of extruded food enriched by additives. The digestibility values of neutral detergent fiber (NDFD) remained close to 48.77% to the reference value for corn silage (Valadares Filho, 2006).

For hemicellulose and hemicellulose due to NDF intake corrected for ash, these showed statistical difference ($p < 0.05$). The highest intake found for the treatment using Foragge Factor® is characterized by the presence of purified yeast, and the lowest, with Foragge Max®, rich in virginiamycin. According to Nagaraja, Taylor, Harmon, and Boyer (1987), the growth of cellulolytic bacteria promotes an increase in the use of fiber, maximizes the production of volatile fatty acids in the rumen, and provides more energy to the animal. According to Fereli et al. (2010), the effect of using yeast is to increase the production of microbial mass (proteolytic bacteria), which promotes a higher flow of microbial protein available to the animal.

Conclusion

The addition of different additives in the extruded roughage promoted an improvement in nutritional parameters, without causing disturbances. Since, the Foragge Factor® treatment using purified yeasts showed better results, mainly related to the dry matter intake as a function of body and metabolic weight.

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