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Ewes fed high-concentrate diets containing flint corn and increasing levels of exogenous amylolytic enzyme: effects on nutrient intake and digestibility

Ovelhas alimentadas com dietas de alto teor de concentrado contendo milho flint e níveis crescentes de enzima amilolítica exógena: efeitos sobre o consumo e a digestibilidade dos nutrientes

Luciano Fernandes Sousa[1]*; Jhone Thallison Lira de Sousa[2]; Erica Beatriz Schultz[3]; Tays Raniellen Miranda Feitosa[4]; Vinicius Carreteiro Gomes[5]; Robert Emilio Mora Luna[6]; Gilberto de Lima Macedo Júnior[7]

Highlights

Increased levels of amylolytic enzymes in the diet did not change ewe's comsumption.
Changes in nutrients digestibility were observed.
Inclusion of exogenous amylolytic enzyme can improve dry matter digestibility.

Abstract

The aim was to evaluate if the inclusion of exogenous amylolytic enzyme affect the nutrient intake and digestibility in ewes fed high-concentrate diets containing flint corn. Five Santa Inês × Dorper crossbred ewes (54.04 ± 4.5 kg and aged 8 months) were used in a 5 x 5 Latin square design. All animals were housed in individual metabolic cages for 60 days. The treatments consisted of a control diet (without amylolytic enzyme) and four inclusion levels of an amylolytic enzyme (3,000, 6,000, 9,000, and 12,000 α-amylase dextrinizing units [DU] kg^{-1} dry matter [DM]). The enzyme was mixed into the feed at the time of supply to the animals. Data were analyzed by ANOVA, and orthogonal polynomial contrasts were used. Nutrient intake was not influenced by amylolytic enzyme inclusion. The digestibility of DM, organic matter, neutral detergent fiber, total carbohydrates, non-fibrous carbohydrates, and gross energy showed a quadratic

[1] Prof. Dr., Universidade Federal do Norte do Tocantins, UFNT, Araguaína, TO, Brazil. E-mail: luciano.sousa@mail.uft.edu.br
[2] Animal Science Doctor, UFNT, Araguaína, TO, Brazil. E-mail: jhonelira@hotmail.com
[3] Profª Drª, Universidade Federal de Viçosa, UFV, Viçosa, MG, Brazil. E-mail: ericabeatrizschultz@gmail.com
[4] PhD Student in the Programa de Pós-graduação Integrado em Zootecnia nos Trópicos, UFNT, Araguaína, TO, Brazil. E-mail: tays_raniellen@mail.uft.edu.br
[5] Marketing Development Specialist, Mosaic Fertilizantes, São Paulo, SP, Brazil. E-mail: vinicius2gomes@gmail.com
[6] Prof. Dr. at Faculdad de Agronomia e Ingienieria Forestal, Pontificia Universidad Católica de Chile, Santiago, Macul, Chile. E-mail: robertmora78@yahoo.com
[7] Prof. Dr., Universidade Federal de Uberlândia, UFU, Uberlândia, MG, Brazil. E-mail: gilbertomacedojr@gmail.com
* Author for correspondence

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increase with enzyme inclusion (P<0.05), with maximum values at levels of 7,600, 7,500, 6,300, 7,500, 7,400, and 7,800 DU kg^{-1} DM, respectively. Total digestible nutrients of diets also showed a quadratic increase, with a maximum value of 894 g kg^{-1} at a level of α-amylase activity of 7,786 DU kg^{-1} DM. The inclusion of the exogenous amylolytic enzyme from 6,300 to 7,800 DU kg^{-1} DM doesn't alter nutrient intake and improves the digestibility in ewes fed high-concentrate diets.
Key words: Alpha-amylase. Nutrition. Sheep. Starch.

Resumo

Objetivou-se avaliar se a inclusão de enzima amilolítica exógena afeta a ingestão e a digestibilidade dos nutrientes em ovelhas alimentadas com dietas de alto teor de concentrado contendo milho flint. Foram usadas cinco ovelhas mestiças Santa Inês × Dorper (54,04 ± 4,5 kg e 8 meses de idade) em um quadrado latino 5x5. Todos os animais foram alojados em gaiolas metabólicas individuais por 60 dias. Os tratamentos consistiram em uma dieta controle (sem enzima amilolítica) e quatro níveis de enzima amilolítica (3.000; 6.000; 9.000 e 12.000 unidades de dextrinização [UD] de α-amilase kg^{-1} de matéria seca [MS]). A enzima foi misturada com o alimento no momento do fornecimento aos animais. Os dados foram analisados por ANOVA e contrates ortogonais polinomiais. O consumo de nutrientes não foi influenciado pela inclusão da enzima amilolítica. A digestibilidade da MS, matéria orgânica, fibra em detergente neutro, carboidratos totais, carboidratos não fibrosos e energia bruta apresentaram efeito quadrático com a inclusão de enzima (P<0,05), com valores máximos nos níveis de inclusão da enzima 7.600; 7.500; 6.300; 7.500; 7.400 e 7.800 UD kg^{-1} MS, respectivamente. Os nutrientes digestíveis totais também apresentaram efeito quadrático, com máximo valor de 894 g kg^{-1} no nível de atividade de α-amilase de 7.786 UD kg^{-1} MS. A inclusão da enzima amilolítica exógena entre 6.300 e 7.800 UD kg^{-1} MS não modifica o consumo de nutrientes e melhora a digestibilidade de ovelhas alimentadas com dietas de alto teor de concentrado.
Palavras-chave: Alfa-amilase. Amido. Nutrição. Ovelhas.

Introduction

The use of high-concentrate diets (71-100%) is a tool to achieve higher productivity rates in sheep and beef cattle production systems (Cirne et al., 2013; Costa et al., 2013) due to the alteration of the fermentation process, maximizing the efficiency of microbial synthesis and nutrient use (Arrigoni et al., 2013). In high-concentrate diets, corn is the primary source of grain to meet the energy requirements of beef cattle (Costa et al., 2013) and sheep (Cirne et al., 2013), despite the fact that large quantities of starch or other rapidly fermented carbohydrate produce ruminal acidosis (Zhang et al., 2020).

On the other hand, in the grain corn, starch ruminal degradation depends on the texture of the endosperm, which is higher in dent-endosperm cultivars than in flint-endosperm cultivars, and the variation depends strongly on the vitreouness; flint corn has a greater proportion of vitreous endosperm than dent corn (Philippeau et al., 1999; Philippeau & Michalet-Doreau, 1997). In floury endosperm, starch granules are more accessible to ruminal bacteria because starch granules are loosely associated with the protein matrix (McAllister & Ribeiro, 2013). Conversely, in the vitreous endosperm, starch granules are surrounded by protein bodies and embedded in a dense matrix, which limits the action of hydrolytic enzymes (Philippeau & Michalet-Doreau, 1997). In a previous study, flint corn showed lesser ruminal degradability than dent corn (Rossi et al., 2016), and vitreousness was negatively correlated with the starch A fraction of ruminal degradation and with the fractional rate of starch degradation (Correa et al., 2002).

In Brazil, the corn seed industry has opted for flint hybrids (Rossi et al., 2016) because of price and adaptability , high vitreousness between 64.2 and 80% and with starch ruminal degradation values of 46-52%, lower when compared to dent hybrids (> 75%) (Correa et al., 2002). Ferraretto et al. (2013) concluded that to increase starch digestibility in the total tract, ruminal starch degradation must be increased, and post-ruminal starch digestion does not fully compensate for starch that escapes ruminal degradation. The in-vitro ruminal starch digestion of corn can be increased using amylolytic enzyme (Amaro et al., 2021), and Klingerman et al. (2009) found that the addition of increasing doses resulted in a linear increase in apparent VFA production after 6 h of in-vitro ruminal fermentation of flint corn. Oliveira et al. (2019) showed amylolytic enzyme increase dry matter, protein, and starch in vitro digestibility. Therefore, a strategy for increasing the starch ruminal degradation of flint corn could be the use of exogenous amylolytic enzymes.

Our hypothesis is that the inclusion of amylolytic enzyme in high-concentrate diets containing mainly flint corn may increase nutrient intake and digestibility. So, the aim of this study was to evaluate the nutrient intake and digestibility of high-concentrate diets containing flint corn in ewes fed increasing levels of exogenous amylolytic enzyme.

Material and Methods

Experimental site, animals, feed, and experimental diets

The experiment was carried out at the sheep and goat sector at the facilities of Capim Branco Farm, belonging to the Federal University of Uberlândia, located in Uberlândia, Minas Gerais State, Brazil. All procedures were approved by the Committee of Ethics on Animal Studies at the Federal University of Uberlândia (case nº 017/16).

Five Santa Inês × Dorper crossbred ewes with an average body weight (BW) of 54.04 ± 4.5 kg and an average body condition score of 4.25, 8 months of age, were used with 5 × 5 Latin square design. During the adaptation period, all animals were dewormed, labelled, weighed, and randomly distributed in metabolic cages. The experiment lasted for 60 days, shared into five periods of 12 days: seven days for adaptation of the animals to experimental diets and metabolic cages and five days for sampling and data recording.

Animals were weighed on a mechanical scale with an accuracy of 100 g on the first day of the experiment and on the last day of each collection period. Diets were calculated to meet the nutritional requirements of medium-sized ewes, with an average gain of 200 g animal^{-1} day^{-1} (National Reerach Council [NRC], 2007). Feed was supplied twice a day, at 08h00 and 16h00, as a total mixed ration, and intake was adjusted according to the leftovers from the previous day, allowing 5 to 10% leftovers from the total provided. Water was offered *ad libitum.*

The amylolytic enzyme used in this experiment (AMAIZE™, Alltech Inc., Nicholasville, KY, USA) was powdered *Aspergillus oryzae* extract, which mainly contains α-amylase or 1,4-α-D-glucan glucanohydrolase (Tricarico et al., 2008) and is resistant to degradation by rumen microbes (Klingerman et al., 2009). One α-amylase dextrinizing unit (DU) is defined as the quantity of enzyme required to dextrinize soluble starch at the rate of 1 g h^{-1} at 30ºC and pH 4.8. The α-amylase concentration in the *A. oryzae*-based supplement was 600 DU g^{-1} (Tricarico et al., 2008).

The treatments consisted of five inclusion levels of exogenous amylolytic enzyme in the diet: 0, 0.5, 1.0, 1.5, and 2.0%, representing 3,000, 6,000, 9,000, and 12,000 DU kg^{-1} DM, respectively. The enzyme was mixed into the fed and supplied in the two meals. Experimental diets were 20:80 corn silage: concentrate (dry matter [DM] basis). The corn silage chemical composition was 340, 65, and 566 g kg^{-1} of DM, crude protein (CP), and neutral detergent fiber (NDF), respectively. The concentrate consisted of (g kg^{-1}) ground corn (605), soybean meal (360), mineral salt (25), and urea (10) and contained 897, 245, and 248 g kg^{-1} of DM, CP, and NDF, respectively.

Obtaining experimental parameters

Samples of silage, concentrate, and leftovers were taken and weighed daily, and at the end of the 5 days of collection, a composite sample for each animal and each period was taken. Over a period of 5 days, total feces collection was performed. The feces of each day were weighed, and 20% was stored. At the end of each period, composite samples were prepared, per animal, and stored at -10ºC.

Samples of feeds, leftover, and feces were pre-dried in a forced-air oven (55ºC) to constant weight and subsequently ground in a 1-mm pass sieve for chemical analysis. Samples were analyzed for DM, ash, ether extract (EE), CP, according to the Association of Official Analytical Chemists [AOAC] (2016), and NDF (Mertens, 2002) were assessed in the ANKOM™ fiber analyzer.

Nutrient intake was calculated by subtracting the amounts in the leftovers from the daily amounts offered. Nutrient digestibility was calculated as the difference between consumption and excretion in feces, divided by the amount of nutrients consumed. Total carbohydrates (TC) and total digestible nutrients (TDN) were calculated according to Sniffen et al. (1992), whereas non-fibrous carbohydrates (NFC) were estimated according to Detmann et al. (2012). Energy contained in feeds, feces, and leftovers was measured in an adiabatic calorimeter (Parr®, 6200 model, Moline, Illinois, USA). Gross energy (GE) of diets was obtained, and digestible energy was calculated.

Design and statistical analysis

Data were analyzed by ANOVA according to a 5 × 5 Latin square design, and orthogonal polynomial contrasts were used to determine linear and quadratic effects. The significance level was set at 0.05 probability of type I error by the F-test. The statistical model was as follows:

$$Y_{ijk} = \mu + T_i + P_j + A_k + E_{ijk},$$

where Y_{ijk} is a dependent variable, μ is the mean for all observations, T_i is the effect of diet i, P_j is the effect of period j, Ak is the effect of animal k, and $E_{ijk} \sim N(0, \sigma^2_e)$ represents the residual error.

Results and Discussion

The DM intake (DMI) was not influenced by the enzyme inclusion in the diet (Table 1). Mendoza et al. (2013) also observed no difference on DMI when glucoamylase was added to high-grain diets in lambs. The same behavior was observed when amylolytic enzyme was included in dairy cow diets (Nozière et al., 2014; Zilio et al., 2019). The average DMI of 1.78% BW was lower than that recommended (2.2% BW) by the NRC (2007). Since the inclusion of amylolytic enzyme does not influence the molar ratio of propionic acid (Kong et al., 2021; Sosa et al., 2020; Zilio et al., 2019), the lower DMI may be due to the high-concentrate diet, which increases the ruminal propionate concentration (Homem et al., 2019; Mao et al., 2013). Consequently, propionate uptake by the liver rapidly produces ATP, stimulating satiety (Allen et al., 2009). Nutrient intakes were not influenced (Table 1) by the enzyme inclusion in the diet. The lack of response of the nutrient intake is in line with the findings of other authors (Nozière et al., 2014; Zilio et al., 2019) when amylolytic enzyme was added in dairy cow diets.

Except for CP and ether extract (Table 2), the apparent digestibility of DM, organic matter, NDF, TC, NFC, GE and NDT increased quadractly (P<0.05; Table 2). When increasing levels of amylase from *Bacillus licheniformis* were added to the sheep diet. Increases in nutrient digestibility may be due to the synergism between endogenous and exogenous enzymes, increasing overall ruminal enzymatic activity and hydrolysis (Meale et al., 2014). On the other hand, when α-amylase of *A. oryzae* is used, the overall nutrient digestibility increases since the maltodextrins produced from the hydrolysis of native starch can be used by a wide variety of ruminal bacteria, including amylolytic and non-amylolytic species (oligosaccharide cross-feeding hypothesis) (Tricarico et al., 2008).

Table 1
Nutrient intake of ewes fed different levels of exogenous amylolytic enzyme in the diet

Intake (g d^{-1})	Alpha-amylase enzyme (units kg^{-1} DM)					SEM	P-value		
	0	3,000	6,000	9,000	12,000		L	Q	LFM
Dry matter	1,128	1,097	1,073	1,099	1,068	62.9	0.559	0.831	0.910
Organic matter	942	910	889	910	883	58.6	0.536	0.815	0.920
Crude protein	257	259	244	259	244	16.0	0.623	0.926	0.709
Ether extract	45.1	43.2	42.4	43.8	43.3	2.38	0.699	0.581	0.901
Neutral detergent fiber	211	200	196	192	190	12.2	0.228	0.721	0.985
Total carbohydrates	640	607	601	607	595	40.8	0.501	0.735	0.945
Non fibrous carbohydrates	583	611	563	619	572	36.3	0.909	0.739	0.465
Total digestible nutrient	936	912	891	917	889	51.9	0.607	0.840	0.894
Dry matter (% BW)	1.85	1.80	1.76	1.77	1.73	0.10	0.425	0.847	0.967

BW: body weight. SEM: Standard Error of the Mean L: Linear, Q: Quadratic. LFM: lack-of-fit to the model.

Table 2
Apparent digestibility of nutrients in ewes fed different levels of exogenous amylolytic enzyme in the diet

Digestibility (g kg^{-1})	Alpha-amylase enzyme (units kg^{-1} DM)					SEM	P-value		
	0	3,000	6,000	9,000	12,000		L	Q	LFM
Dry matter	776.7	808.0	832.4	825.7	806.4	11.2	0.048	0.008	0.889
Organic matter	843.5	872.1	889.0	883.6	872.6	9.70	0.043	0.016	0.927
Crude protein	861.3	877.7	894.4	892.8	880.8	11.0	0.141	0.088	0.911
Ether extract	901.7	918.8	928.0	923.7	919.3	7.40	0.111	0.064	0.904
Neutral detergent fiber	651.2	710.6	750.2	730.2	712.0	24.4	0.093	0.037	0.853
Total carbohydrates	833.0	866.9	884.4	877.5	866.4	10.7	0.042	0.015	0.892
Non fibrous carbohydrates	920.0	941.9	945.7	947.5	938.9	5.50	0.029	0.010	0.792
Gross energy	776.3	807.9	832.4	827.1	804.9	11.8	0.043	0.009	0.907
TDN (g kg^{-1})	850.0	880.0	890.0	890.0	880.0	8.70	0.037	0.018	0.946

TDN: total digestible nutrients. SEM: Standard Error of the Mean. L: Linear. Q: Quadratic. LFM: lack-of-fit to the model.

The quadratic response in nutrient digestibility can be explained by the above-mentioned oligosaccharide cross-feeding hypothesis, in which low exogenous enzyme concentrations would not produce sufficient oligosaccharides for effective cross-feeding, whereas high enzyme doses, or prolonged exposure to enzymes, would extensively hydrolyze polymers to di- and mono-saccharides, thereby failing to support an effective cross-feeding mechanism (Tricarico et al., 2008). Although there was no change in DMI, the increase in digestibility due to enzyme inclusion indicates that nutrients are available to the animal.

A quadratic increase in NFC digestibility was observed. However, *A. oryzae* α-amylase is inactivated by gastric digestion (Tricarico et al., 2008), and the capacity of the ruminant small intestine to digest large amounts of starch is reduced as a consequence of the low levels of pancreatic amylase in these species (Moharrery et al., 2014; Swanson, 2019). Therefore, the increase in NFC digestibility can be due to an improvement of starch ruminal degradation since diet NFC are mainly comprised of starch corn.

Conclusions

The inclusion of the exogenous amylolytic enzyme between 6,300 and 7,800 DU kg^{-1} DM improves the digestibility of high-concentrate diets containing flint corn and does not compromise the voluntary intake of nutrients in ewes.

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