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Ruminal degradability of high moisture triticale (X. *Triticosecale Wittmack*) silage with chemical and biological additives

Degradabilidade ruminal da silagem de grão úmido de triticale (X. *Triticosecale Wittmack*) com aditivos químico e biológico

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Highlights:

The addition of urea increases the soluble fraction of crude silage protein.

Urea in high moisture triticale silage is recommended for sheep feeding at a low level of consumption.

The addition of enzyme-bacterial inoculant accelerate the ruminal passage rate of dry matter and crude protein.

Abstract

The objective of this study was to evaluate the ruminal degradability of dry matter and crude protein of high moisture triticale silage ensiled with different chemical and biological additives. Urea, sodium benzoate and an enzyme-bacterial inoculant were used as treatments. Four samples from each treatment were incubated in rumen on four sheep. Effective degradability was estimated for ruminal passage rate of 2%, 5% and 8% hour^{-1}. Bayesian procedures were used to estimate potential degradation parameters *in situ*. The high moisture triticale silage with urea showed highest value for the soluble fraction (70.46%) and the best effective dry matter degradability, with a passing rate of 2% h^{-1} (90.63%), of control silage at other rates of passage. In relation to control silage, the addition of sodium benzoate and enzyme-bacterial inoculant decreased the effective degradability of dry matter, regardless of rate passage evaluated. Due to high solubility of urea, the silage added with this additive had the highest soluble fraction of crude protein (76.42%). The addition of enzyme-bacterial inoculant accelerated the ruminal passage rate of dry matter and protein to 0.26 and 0.20% h^{-1}, respectively, providing less potential degradability of both in relation to other silages. As enzyme-bacterial inoculation reduces rumen degradability of crude protein, it tends to increase the availability of amino acids for intestinal absorption. The addition of urea to high moisture triticale silage may be recommended for sheep feeding at a low level of consumption, as it improves the effective dry matter degradability.

Key words: Effective degradability. Dry matter. Crude protein. Ruminant. Urea.

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Resumo

O objetivo do trabalho foi avaliar a degradabilidade ruminal da matéria seca e da proteína bruta de silagens de grãos úmidos de triticale ensilados com diferentes aditivos químicos e biológico. Ureia, benzoato de sódio e um inoculante enzimo-bacteriano foram utilizados como tratamentos. Quatro amostras de cada tratamento foram incubadas in situ no rúmen de quatro ovinos. A degradabilidade efetiva foi estimada para taxa de passagem ruminal de 2%, 5% e 8% hora^{-1}. Foi utilizado procedimento Bayesiano para estimar parâmetros de degradação potencial *in situ*. A silagem de grãos úmidos de triticale com ureia apresentou o maior valor para a fração solúvel (70,46%) e melhor degradabilidade efetiva da matéria seca, em taxa de passagem de 2% h^{-1} (90,63%), não diferindo da silagem controle nas demais taxas de passagens. Em relação a silagem controle, a adição de benzoato de sódio e de inoculante enzimo-bacteriano diminuíram a degradabilidade efetiva da matéria seca, independente da taxa de passagem avaliada. Devido à alta solubilidade da ureia, a silagem acrescentada com esse aditivo, apresentou a maior fração solúvel da proteína bruta (76,42%). A adição de inoculante enzimo-bacteriano acelerou a taxa de passagem ruminal da matéria seca e da proteína para 0,26 e 0,20% h^{-1}, respectivamente, proporcionando menor degradabilidade potencial de ambos em relação às demais silagens. Como a inoculação enzimo-bacteriana reduz a degradabilidade ruminal da proteína bruta, tende-se a aumentar a disponibilidade de aminoácidos para absorção intestinal. A adição de ureia em silagens de grãos úmidos de triticale pode ser recomendada para alimentação de ovinos em baixo nível de consumo, por melhorar a degradabilidade efetiva da matéria seca.

Palavras-chave: Degradabilidade efetiva. Matéria seca. Proteína bruta. Ruminante. Ureia.

Introduction

In order to meet the energetic requirements of ruminants, it is common for grains to be included in diets because of their high soluble carbohydrate contents (Jobim, Lombardi, Macedo, & Branco, 2008), and triticale appears as an excellent option. Despite their relatively low protein concentration, these grains may contribute significantly to the supply of crude dietary protein when they are included in high amounts in the diet.

Marcondes et al. (2009) assert that metabolizable protein requirements in ruminants are met by both dietary protein and microbial protein production, and this source accounts for more than half of the amino acids available for absorption. However, for optimal adaptation of diets to ruminants, information about the fractionation of foods and their digestion rates is necessary, only then will it be possible to synchronize the availability of energy and nitrogen at ruminal level.

The fermentative benefits of the inclusion of chemical and biological additives in high moisture silages are described by several authors (Jobim et al., 2008; Knicky & Spörndly, 2011; Oliveira et al., 2018). Other studies evaluate the effective degradability of dry grains of triticale (Krieg, Seifried, Steingass, & Rodehutscord, 2017a,b), but trials with high moisture triticale silage are still scarce.

According to Krieg et al. (2017a), there is a lack of information on protein value characteristics for ruminants, particularly on triticale. In addition, the possibility of using different additives in high moisture silages increases the variability of possible results, and studies on this area are necessary.

The objective of this study was to determine ruminal degradation kinetic of dry matter and crude protein of high moisture triticale silages with different chemical and biological additives.

Material and Methods

The experiment was performed at Fazenda Escola (Fazesc) at the State University of Londrina (UEL) and at Laboratory of Food and Animal Nutrition (LANA-UEL), located at coordinates 23° 23 ‘S and 51° 11’ W and 566 m altitude.

Harvesting of triticale grains (X. *Triticosecale Wittmack* cv. IPR 111) for silage production occurred when they reached approximately 30% humidity. Soon after the harvest the grains were crushed in sieves with 8 mm sieve, adding the additives according to defined treatments. The treatments were: high moisture triticale silage without additive (HMTC); high moisture triticale silage with 0.5% urea in natural matter (HMTU); high moisture triticale silage with 1.5% sodium benzoate in natural matter (HMTSB); and high moisture triticale silage with enzyme-bacterial inoculant (HMTEB).

The silages were conditioned in concrete silos with a capacity of 250 L and stored for 11 months until opening. The enzyme-bacterial inoculant used was Silotrato®, composed of *Lactobacillus curvatus*, *L. acidophilus*, *L. plantarum*, *L. buchneri*, *L. lactis*, *Pediococcus acidilactici* and *Enterococcus faecium*, in concentrations of 10^9 CFU g^{-1} and 4% of enzymatic complex based on cellulase. The application was made in order to obtain maximum uniformity of the grain mass in the form of spray, with the additive diluted in non-chlorinated water at the concentration of 4.3 g L^{-1}, according to the manufacturer's recommendation. The chemical additives, urea and sodium benzoate, were homogenized to the triticale grains manually after processing. In order to exclude the effect of the addition of water in the silage with enzyme-bacterial inoculant, in the others also it was included pure water in the same volume.

In evaluation of ruminal degradability, four castrated male sheep with a mean weight of 50 kg were used, carrying ruminal cannula. The animals were housed in a covered place, with a slatted floor and equipped with feed troughs and drinking fountains. Procedures were adopted in accordance with the ethical principles of animal experimentation, approved by the Committee of Ethics in Animal Experimentation of the State University of Londrina, according to document number 26014.2012.79. The experimental design used was 4x4 Latin square, being four periods and four treatments.

The animals were adapted to diet for 10 days prior to ruminal incubation, and fed twice a day at 8:00 a.m. and 4:00 p.m. Samples of the silages were collected and subjected to drying in a forced ventilation oven at 55ºC for 72 hours. Afterwards, they were milled in a Willey mill with a 1 mm harrow for chemical-bromatological analysis (Table 1).

The ruminal degradability of dry matter and crude protein, and their respective degradation rates, were estimated by *in situ* technique using nylon bags (Ankom - Bar Diamond, Inc., Parma Idaho - USA) with dimensions of 10 x 20 cm, and pores of 53 μm containing 5 g of ground sample (Nocek, 1988). The incubation times were 0h, 1,5h, 3h, 6h, 9h, 12h, 24h, 36h and 48h.

After each respective incubation time, the bags were removed and immediately washed in running water, frozen, and washed again according to Vanzant, Cochran and Titgemeyer (1998). After washing, the bags were subjected to forced ventilation drying at 55 ºC for 72 hours for determination of dry matter and crude protein using the Kjeldahl method. The percentage of disappearance of dry matter and crude protein was calculated by the difference between input and output content of each nutrient.

The data of disappearance for the constituents of dry matter and crude protein were adjusted by nonlinear regression, which predicts the potential degradability ($\bar{Y}$ = SD) of the food using the model proposed by Mehrez and Orskov (1977), as follows: $y_{ijk} = a_{ik} + b_{ik} (1 - e^{-cik\ tj})$ [1], where, i-animal: 1, 2, ..., N; j-time: 1, 2, ..., J; k-treatment: 1, 2, ..., K; "y" is the fraction (%) of the nutrient degraded after time t (in hours); "a" is the soluble fraction (%) of the material contained in the nylon bag (the curve intercept); "b" is the potentially degradable fraction (%) of the material contained in the nylon bag after time zero; "c" is the constant fractional rate (%) of degradation of the potentially degradable fraction (% h^{-1}); "t" is the incubation time in the rumen (in hours).

Table 1
Chemical-bromatological composition of high moisture triticale silage with different additives

Parameter	Methodology for analysis	Silage			
		HMTC	HMTU	HMTSB	HMTEB
DM, % FM	Association of Official Analytical Chemists [AOAC] (1995)	68.69	70.20	70.49	71.41
OM, % DM	AOAC (1995)	98.04	98.10	97.60	97.95
CP, % DM	AOAC (1995)	17.26	18.97	16.40	16.52
EE, % DM	AOAC (1995)	1.60	1.64	1.49	1.85
NDF, % DM	Van Soest, Robertson and Lewis (1991)	10.93	11.45	12.45	12.60
ADF, % DM	Goering and Van Soest (1970)	3.91	4.36	4.33	4.90
MM, % DM	AOAC (1995)	1.96	1.90	2.40	2.05
CHO, % DM	Sniffen, O'Connor, Van Soest, Fox and Russell (1992)	79.18	77.49	79.71	79.58
NFC, % DM	Sniffen et al. (1992)	68.25	66.04	67.26	66.98
pH, index	Cherney and Cherney (2003)	4.50	4.84	5.67	4.35
$N\text{-}NH_3$, % TN	Playne and McDonald (1966)	58.7	106.2	42.1	46.4
BC, meq NaOH % DM	Playne and McDonald (1966)	32.33	36.42	21.67	29.41

HMTC: High moisture triticale silage without additive; HMTU: High moisture triticale silage with 0.5% urea; HMTSB: High moisture triticale silage with 1.5% sodium benzoate; HMTEB: High moisture triticale silage with enzyme-bacterial additive; DM: Dry matter; FM: Fresh matter; OM: Organic matter; CP: crude protein; EE: Ethereal extract; NDF: Neutral detergent fiber; ADF: Acid detergent fiber; MM: Mineral matter; CHO: Total carbohydrates; NFC: Non-fibrous carbohydrates; pH: Hydrogenation potential; $N\text{-}NH_3$: Ammoniacal nitrogen; TN: Total nitrogen; BC: Buffer capacity.

To estimate effective degradability (ED), the Orskov and McDonald (1979) model was used: ED = a + bc / c + rate [2], where the rate of passage of solids in rumen was set at values set at 2 %, 5% and 8% hour^{-1}, due to the low, medium and high levels of food consumption, respectively.

The modeling follows the suggestion of a Bayesian procedure (Rossi, Guedes, Martins, & Jobim, 2010; Rossi, 2011), where it was considered that the observations follow normal distribution, that is, $y_i \sim N\ (f\ (t_i);\ \sigma^2_e)$, where "f(ti) "is the nonlinear function proposed in [1]. For parameters "a" and "b", they were considered "*a priori*" non-informative normal distributions, that is: a ~ N(0,10-6)I(0,100) and b ~ N(0,10-6)I(0,Lsup) (parameterization OpenBUGS), with the restriction Lsup = 100 - ā to ensure the generation of values "*a posteriori*" such that a+b≤ 100. A non-informative gamma distribution restricted in the interval (0,1) was considered in the model for "c", that is: c ~ Gamma (10-3,10-3) I (0,1). For $\tau = 1/\sigma^2_e$ (precision) a gamma distribution was assumed, that is, Gamma (10-3,10-3). Obtaining the "a posteriori" marginal distributions for all parameters was done through the BRugs package of the R (R Development Core Team, 2014) program.

Used as initial values for the parameters "a", "b" and "c", frequentist estimates and then by an iterative process MCMC (Monte Carlo Markov Chain), 210,000 values were generated in a process, considering a period of sample discharge of 10,000 initial values, so the final sample, obtained in jumps every 10 values, contains 20,000 values generated. The convergence of chains was verified through the package coda of program R, by criterion of Heidelberger and Welch (1983).

Multiple comparisons were made between the "*a posteriori*" distributions of averages of interest parameters, of analyzed treatments. They were considered as different, at the level of 5% of significance, the treatments whose confidence intervals for the mean differences do not include zero.

Results and Discussion

The high moisture triticale silage with urea presented highest value for the soluble fraction (a) of the dry matter, corroborating with the findings of Calixto, Jobim, Osmari and Tres (2017). According to same authors, the addition of urea in high moisture silages increases the crude protein content, favoring the ruminal microbial development and improving the utilization of fibrous fraction. Under the same view, Diaz, Ouellet, Amyot, Berthiaume and Thivierge (2013) observed that high levels of ammonia in high moisture silages with urea increase their concentration of soluble carbohydrates, which comprise a combination of preserved original material and the final fermentation products. Table 1 shows the crude protein and $N\text{-}NH_3$ values of evaluated silages.

The inclusion of sodium benzoate and enzyme-bacterial inoculant reduced the soluble fraction of dry matter in comparison to control silage (Table 2). The potentially degradable fraction (b) of dry matter followed an inverse behavior, which is an expected trend, in which silage with sodium benzoate presented the highest value (30.68%) and silages with control and with addition of urea values, not differing from each other.

Table 2
Bayesian estimates for kinetic parameters of *in situ* degradation of dry matter from high moisture triticale silage

Silage	Parameter*			ED		
	a, %	b, %	c, % h^{-1}	2% h^{-1}	5% h^{-1}	8% h^{-1}
HMTC	69.66^{b}	23.12^{c}	0.19^{b}	90.30^{b}	87.56^{a}	85.49^{a}
HMTU	70.46^{a}	22.96^{c}	0.16^{c}	90.63^{a}	87.64^{a}	85.46^{a}
HMTSB	62.21^{d}	30.68^{a}	0.18^{bc}	89.32^{c}	85.45^{c}	82.61^{c}
HMTEB	66.12^{c}	24.98^{b}	0.26^{a}	89.08^{c}	86.65^{b}	84.72^{b}

*a: soluble fraction; b: potentially degradable fraction; c: fraction degradation rate b.
[a, b, c, d] Differential letters in the column indicate significant differences between the means of the treatments, through Bayesian comparisons at the 95% confidence level.
HMTC: High moisture triticale silage without additive; HMTU: High moisture triticale silage with 0.5% urea; HMTSB: High moisture triticale silage with 1.5% sodium benzoate; HMTEB: High moisture triticale silage with enzyme-bacterial additive; ED: Effective degradability.

The rate of degradation of potentially degradable fraction (c) was significantly higher for silage with enzyme-bacterial inoculant. This effect may be related to the greater digestibility of fiber caused by action of enzymes present in inoculant. This improvement has already been proven in other studies (Alvarez et al., 2009; Gallardo et al., 2010).

The silage with addition of urea obtained the lowest rate of degradation of the "b" fraction (0.16% h^{-1}), which is a compensatory behavior due to the high soluble fraction. The high moisture triticale silage with sodium benzoate showed an intermediate value, and did not differ from the control and urea silages.

In comparison to control silage, the addition of sodium benzoate and enzyme-bacterial inoculant worsened the effective degradability of dry matter, regardless of rate of passage evaluated. Between both, there was no difference in rate of passage of 2% h^{-1}, but on average (5% h^{-1}) and high (8% h^{-1}) passage rate, high moisture triticale silage with inoculant showed a better effective dry matter degradability.

The inclusion of urea showed an improvement in rate of passage of 2%, h^{-1}, with no differences in rates of 5 and 8% h^{-1} when compared to control silage. Calixto et al. (2017) observed no difference in the effective dry matter degradability of high moisture corn silage pure or with urea added at the same passage rates. The authors of the present study are not aware of similar trials investigating high moisture triticale silage.

Krieg et al. (2017b) investigated the effective degradability of dry grains of triticale and found values of 85.0% under passage rate of 8% h^{-1}. Liu, Mckinnon, Thacker and Yu (2012) described effective degradability of 82.7%, assuming a rate of passage of 6% h^{-1}. The values of effective degradability of dry matter are superior to those described by Jobim et al. (2008) and similar to those found by Calixto et al. (2017) for high moisture corn, which demonstrates the nutritional potential for use of triticale grains for ruminants.

Due to high solubility of urea, the silage added with this additive presented the highest soluble fraction of the crude protein (76.42%; Table 3). Jobim et al. (2008) and Calixto et al. (2017) also described high solubility of crude protein in high moisture corn silage with urea, but did not observe differences with the control silage.

Table 3
Bayesian estimates for kinetic parameters of *in situ* degradation of crude protein from high moisture triticale silage

Silage	Parameter*			ED		
	a, %	b, %	c, % h^{-1}	2% h^{-1}	5% h^{-1}	8% h^{-1}
HMTC	75.29^{b}	22.97^{b}	0.14^{b}	94.50^{b}	91.15^{b}	88.87^{a}
HMTU	76.42^{a}	22.41^{bc}	0.12^{b}	94.96^{a}	91.53^{a}	89.23^{a}
HMTSB	65.36^{d}	35.27^{a}	0.09^{b}	91.12^{d}	85.43^{c}	81.49^{d}
HMTEB	74.53^{c}	21.91^{c}	0.20^{a}	94.12^{c}	91.60^{a}	89.69^{a}

*a: soluble fraction; b: potentially degradable fraction; c: fraction degradation rate b.
a, b, c, d Differential letters in the column indicate significant differences between the means of the treatments, through Bayesian comparisons at the 95% confidence level.
HMTC: High moisture triticale silage without additive; HMTU: High moisture triticale silage with 0.5% urea; HMTSB: High moisture triticale silage with 1.5% sodium benzoate; HMTEB: High moisture triticale silage with enzyme-bacterial additive; ED: Effective degradability.

Associated with a high soluble protein content of the food, it is important to decrease the degradability of the protein in rumen and, therefore, to allow better availability of amino acids for intestinal digestion, and the addition of urea does not seem to affect the crude protein passage rate (0.12% h^{-1}) for the rumen, in addition to being an important extra source of crude protein.

The silage with sodium benzoate presented the lowest soluble fraction of crude protein, which may be related to the higher pH observed in this silage (5.67; Table 1), since, according to Drzymała, Prabucka and Bielawski (2012), the enzyme Carboxypeptidase I present in triticale grains hydrolyzes peptides at pH close to 4.60. In a compensatory manner, the highest value of potentially degradable fraction (b) of crude protein was observed in silage with sodium benzoate.

The silage with inoculant presented low value for fraction “a” and lowest value for “b” fraction of crude protein (74.53 and 21.91%, respectively). This low value of fraction “b”, contrary to the hypothesis of compensation, is a reflection of higher rate of ruminal passage “c” described (0.20% h^{-1}; $P < 0.05$). An association not well defined, may have been established between the inoculant enzymes with the ruminal microbiota, promoting this difference in the rate of passage with the other treatments. Similar hypothesis has already been judged with forages (Reis et al., 2016).

The addition of urea provided the best effective degradability of the crude protein in a 2% h^{-1} and 5% h^{-1} flow rate, due to the higher solubility of this nutrient in the silage. However, in a high pass rate (8% h^{-1}), no difference was observed with the control silages and with the enzyme-bacterial inoculant.

At any passage rate, the effective degradability of the crude protein was impaired by all of the additives tested in that assay. The inclusion of sodium benzoate showed the lowest effective degradability of crude protein. This additive reduces the concentration of $N\text{-}NH_3$ in the silage (Knicky & Spörndly, 2011), interfering negatively under microbial development, culminating in this low degradation.

As a positive point, high moisture triticale silages showed effective degradability of crude protein very close to those described for high moisture corn silage (Calixto et al., 2017). Krieg et al. (2017a) described effective degradability of crude protein in dried grains of triticale varying between 79 and 84%, under a rate of 8% h^{-1}, assuming that the action of acids in fermentation process can improve the effective degradability of the food.

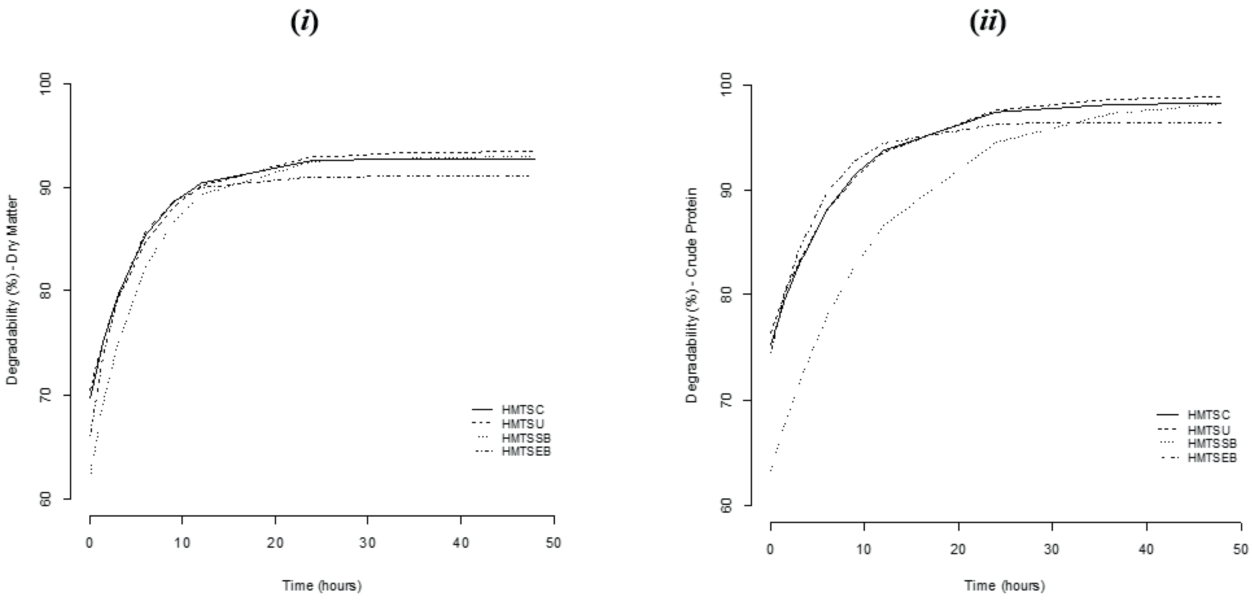

Figure 1. Adjusted simultaneous curves of total potential degradability of dry matter (*i*) and crude protein (*ii*), as a function of rumen permanence time

HMTC: High moisture triticale silage without additive; HMTU: High moisture triticale silage with 0.5% urea; HMTSB: High moisture triticale silage with 1.5% sodium benzoate; HMTEB: High moisture triticale silage with enzyme-bacterial additive.

Figure 1 shows the potential degradability curves estimated by Bayesian model, dry matter (i) and crude protein (ii). After approximately 10 hours of evaluation, the potential degradability curve of dry matter followed the same values of the soluble fractions, with sodium benzoate silage below the others (i). After this time, there was a greater similarity of the curve with the potentially degradable fractions, where the lowest potential degradability was observed for silage with enzyme-bacterial inoculant, which is a reflection of the high rate of passage of the potentially degradable fraction (c).

It is evident that silage with addition of sodium benzoate presented degradability lower to other silages in time that preceded 30 hours of incubation (ii). This initial behavior shows the reducing potential of protein solubility provided by the presence of sodium in the additive and by the higher pH of the medium (Table 1), which did not affect the potential degradability, however, since the silage with sodium benzoate did not differ from silage control at the end of the evaluation hours.

The silage with urea showed the highest potential degradability of crude protein (ii) at the end of the evaluation hours, while the addition of inoculant reduced the nutrient utilization at rumen level. Razzaghi, Larsen, Lund, & Weisbjerg (2016) showed that several types of thermal processing in corn and wheat grains impaired the *in situ* degradation of crude protein, suggesting that changes in protein matrix covering the starch may be the cause of this difference. As this was not evaluated in present study, it is not possible to assert that the chemical alterations stimulated by the additives used may interfere in the same way, however, it is a solid hypothesis.

The lower potential degradability of crude protein is seen in a positive way in some cases, especially when it is desired to increase the concentration of amino acids for intestinal absorption.

Conclusion

The addition of urea to high moisture triticale silage for sheep feeding at a low level of consumption improved the effective dry matter degradability.

High moisture triticale silage with enzymatic-bacterial inoculation reduces ruminal degradability of crude protein, and may provide a higher concentration of amino acids for intestinal absorption.

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