

# Corn silage supplementation for sheep eating annual ryegrass

## Suplementação com silagem de milho para ovinos ingerindo azevém anual

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### Abstract

Food intake is determinant in the production process and can be modified when more than one type of forage is supplied in the diet. The aim of this study was to evaluate the effects of supplementation with corn silage + soybean meal (9:1 based on DM) in the proportion of 10 g DM kg<sup>-1</sup> live weight (LW) for lambs receiving pre-dried ryegrass (*Lolium multiflorum* Lam) in two forage supply levels: ad libitum or restricted (60% of the consumption ad libitum). Eight castrated male sheep crosses Texel × Criollo (average of 31.5 ± 2.2 kg LW) were used in an experimental design of 4 × 4 Latin square. Each experimental period was performed for 19 days, with 14 days for adaptation and 5 days for collections. Animals were fed three times a day (08h00, 11h30, and 16h30). Supplemented animals received corn silage at 08h00 and ryegrass silage at 11h30 and 16h30. The substitution rates (kg DM consumed forage per kg DM consumed silage) were 0.93 for animals with a supply of ryegrass ad libitum and zero for those receiving the same in a restricted amount. The digestible OM (DOM) intake and nitrogen retention did not vary with supplementation in animals that received ryegrass ad libitum but increased in those with restricted supply. However, animals with restricted supply and supplemented had a less digestible OM intake and nitrogen retention than the average of those that received ryegrass ad libitum. Organic matter digestibility and efficiency of microbial protein synthesis were not affected by treatments, but the digestibility of NDF and ADF was lower in supplemented animals when compared to those non-supplemented and in restricted supply when compared to consumption ad libitum. Even with supplementation, feed restriction of base forage can limit the daily intake of digestible OM and the daily retention of N in sheep.

**Key words:** Consumption. *Lolium multiflorum* L. Substitution rate. *Zea mays*.

### Resumo

O consumo é determinante no processo produtivo e pode ser modificado quando é fornecido mais de um tipo de forragem na dieta. O objetivo deste trabalho foi avaliar os efeitos da suplementação com silagem de milho + farelo de soja (9:1 com base na MS) na proporção de 10 g MS kg<sup>-1</sup> PV, para cordeiros recebendo pré-secado de azevém (*Lolium multiflorum* Lam) em duas ofertas de forragem: à vontade ou restrito (60% do consumo à vontade). Oito ovinos machos castrados cruza Texel × Crioula (média de

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31,5 ± 2,2 kg de peso vivo, PV) foram usados em um delineamento experimental em Quadrado Latino 4 × 4. Cada período experimental foi realizado durante 19 dias, com 14 de adaptação e 5 de coletas. Os animais foram alimentados três vezes ao dia (08h00, 11h30 e 16h30). Animais suplementados receberam silagem de milho às 08h00 h e silagem de azevém às 11h30 e as 16h30. As taxas de substituição (kg MS forragem consumida por kg MS silagem consumida) foram 0,93 nos animais com oferta de azevém à vontade e zero nos que recebiam o mesmo em quantidade restrita. O consumo de MO digestível e a retenção nitrogenada não variaram com a suplementação nos animais que receberam o azevém à vontade, mas aumentaram nos com oferta restrita. Contudo, os animais com oferta restrita e suplementados tiveram menor consumo de MOD e retenção nitrogenada que a média dos que receberam azevém à vontade. A digestibilidade da MO e a eficiência de síntese de proteína microbiana não foram afetadas pelos tratamentos, mas a digestibilidade da FDN e FDA foi menor nos animais suplementados em comparação aos não suplementados e nos de oferta restrita em comparação ao consumo à vontade. Mesmo com a suplementação, a restrição alimentar da forragem de base pode limitar a ingestão diária de MO digestível e a retenção diária de N em ovinos.

**Palavras-chave:** Consumo. *Lolium multiflorum* L. Taxa de substituição. *Zea mays*.

## Introduction

The seasonality of forage production can compromise the efficiency of production systems, making the use of supplementation necessary in periods of low accumulation rates. Supplementation with conserved forages has stood out as an option due to its good nutritional value and lower cost and because it does not compete with food for human consumption when compared to the use of concentrated foods. However, the response to supplementation depends on the effect of supplement intake on the consumption of base forage (substitution rate), which may vary depending on factors such as the supply of base forage (PHILLIPS, 1988) and the relationship between the nutritional value of the used food (VRANIC, 2007).

Forage supplementation results in higher levels of substitution when compared to those observed with concentrates (MAYNE; WRIGHT, 1988; STOCKDALE, 2000), but the interactions resulting from the use of two types of roughages in the ruminant diet have not yet been completely clarified. In such a situation, it is more difficult to predict the daily nutrient consumption since feeding systems generally consider that the substitution rate of a roughage food for another is constant and 1:1 regardless of the management conditions (INRA, 2007). However, in a low forage supply for dairy cows, the substitution rate can reduce drastically

(PÉREZ-PRÍETO et al., 2011). Thus, the use of supplementation with conserved forage for sheep still needs to be better studied.

The aim of this study was to determine the effect of supplementation with corn silage on nutrient intake and nitrogen retention in sheep fed different levels of pre-dried ryegrass.

## Material and Methods

### *Animals, treatments, and experimental design*

Eight male castrated sheep were used, four of them with rumen cannula and crossbred of Texel × Criollo breeds, with an average weight of 30.8 ± 4.65 kg. The animals were distributed in an experimental design of 4 × 4 double Latin square, with four periods of 19 days (14 days of adaptation and 5 days of collection). The experimental diets consisted of pre-dried ryegrass provided ad libitum or restricted (60% of the consumption ad libitum), supplemented or not with corn silage + soybean meal in the proportion of 10 g DM kg<sup>-1</sup> LW. To calculate the amount of ryegrass DM to be offered in the treatment with restriction, the value of 3.0% of LW was used, which was the voluntary consumption measured in a previous experiment. The proportion of soybean meal was adjusted so that there was no degradable protein deficiency in the rumen (INRA, 2007).

*Food management and sample collection*

The animals were fed three times a day (08h00, 11h30, and 16h30). The supplemented animals received corn silage at 08h00 when the pre-dried ryegrass leftovers from the previous day were removed and weighed. The amount of pre-dried offered in the treatments ad libitum was calculated

by the consumption of the previous day, allowing leftovers of 20%. The animals were housed in individual metabolic cages inside a covered shed, with access to water and mineral supplementation ad libitum. The bromatological composition of the food is shown in Table 1.

**Table 1.** Chemical composition of the offered food.

	Pre-dried ryegrass	Corn silage	Soybean meal
Dry matter (g kg <sup>-1</sup> )	478	264	878
Organic matter (g kg <sup>-1</sup> DM)	879	950	934
CP (g kg <sup>-1</sup> DM)	179	59	521
NDF (g kg <sup>-1</sup> DM)	567	523	177
ADF (g kg <sup>-1</sup> DM)	327	254	89

Samples of the offered forage were collected from the fourteenth experimental day in the morning and in the afternoon. Samples of forage leftovers were collected from the fifteenth day. These samples were dried in a forced air circulation oven at 60 °C for 72 hours and stored for further analysis. Feces samples were collected from the 15th to the 19th day of each experimental period. The total of feces produced per animal daily was weighed and samples were collected at a ratio of 100 g animal<sup>-1</sup>. These samples were then dried in a forced air circulation oven at 60 °C for 72 hours and ground in a 1.0 mm sieve for laboratory analysis.

The total volume of urine produced daily per animal was quantified from the 15th to the 19th day of each experimental period. Urine was collected in flasks containing 100 mL of 20% sulfuric acid solution and 1% aliquots were taken, gauze filtered, and diluted in 100 mL volumetric flasks with distilled water. Daily urine samples consisted of a sample composed of animal per period, which was stored at -20 °C.

Ruminal liquid samples were collected on the nineteenth day of each experimental period at 7h00,

9h00, 11h00, 13h00, 15h00, 17h00, 19h00, 21h00, and 23h00 for determination of ruminal fermentation parameters (pH, NH<sub>3</sub>, and volatile fatty acids), via ruminal cannula, through a pump adapted for this purpose. Immediately, the pH was determined in a digital pH meter. The fluid was filtered in six layers of gauze from which two aliquots were taken. An aliquot of 18 mL was acidified with 2 mL of 20% H<sub>2</sub>SO<sub>4</sub> solution for determination of NH<sub>3</sub> and another of 20 mL preserved with 0.4 mL of 10% NaOH solution (v/v) for determination of volatile fatty acids (VFA) (CECAVA et al., 1991). These samples were frozen at -20 °C for further analysis.

*Laboratory analyses*

In the samples of offered food, in leftovers and feces DM content was determined by oven drying at 105 °C for 20 hours and mineral matter by firing in a muffle oven at 580 °C for 4 hours. The total nitrogen content (N) was determined by the Kjeldahl method (AOAC, 1995). NDF contents were determined as proposed by Mertens (2002), except for the use of the equipment ANKOM, where samples weighed in filter bags were treated with a neutral detergent

solution. Concentrations of acid detergent fiber were analyzed according to the method No. 973.18 by AOAC (1995). In the ruminal liquid samples, the concentration of ammoniacal N was measured by the procedure described by Weatherburn (1967). The determination of VFA was performed by a high-performance liquid chromatography on a Shimadzu HPLC chromatograph model LC10-VP equipped with a quaternary gradient pump, automatic injector with thermostatic sample holder (10 oc.), column oven (40 oc.), and Ultra-Violet (UV) detector set at 210 nm in this study. The column used was Aminex HPX-87H (BioRad), associated with a pre-column. The mobile phase used was 5mM H<sub>2</sub>SO<sub>4</sub>, isocratic, with a flow of 0.6 mL min<sup>-1</sup>. The injection volume was 20 µL. A calibration curve was prepared for oxalic (0-40 ug mL<sup>-1</sup>), citric (0-375 ug mL<sup>-1</sup>), tartaric (0-25 ug mL<sup>-1</sup>), malic (0-500 ug mL<sup>-1</sup>), aconitic (0-10 ug mL<sup>-1</sup>), lactic (0-750 ug mL<sup>-1</sup>), fumaric (0-2.5 ug mL<sup>-1</sup>), acetic (0-1000 ug mL<sup>-1</sup>), propionic (0-125 ug mL<sup>-1</sup>), and butyric acid (0-2000 ug mL<sup>-1</sup>). The concentrations were chosen according to the acid responses to the UV detector. The ruminal microbial protein synthesis was estimated based on the urinary excretion of purine derivatives (allantoin and uric acid), as described by Chen and Gomes (1995).

### Calculations

Forage consumption was measured by the difference between the amount of forage offered and leftovers between the 15th and 19th days of each period. The consumptions of organic matter and constituents of organic matter of the forage were calculated from the amount of nutrient offered less the amount of that found in the leftovers.

The substitution rate (SR) was calculated by the ratio between the amount of ryegrass that was no longer ingested and the amount of supplemented forage (corn silage) consumed:

$$SR = \frac{(CR - CRs)}{CCS}$$

where CR is the average consumption of DM of ryegrass in non-supplemented animals, CRs is the average consumption of DM of ryegrass in supplemented animals, and CCS is the average consumption of DM of corn silage.

The apparent digestibility of dry matter, organic matter, and constituents of organic matter of ryegrass and silage were calculated by the proportion of the amount ingested that was not excreted in the feces. The true digestibility of organic matter (TDOM) was estimated according to Mulligan et al. (2002), considering that the excreted OM of alimentary origin is the NDF excreted in the feces, where:

$$TDOM (\%) = \frac{(\text{consumption of OM} - \text{NDF excreted}) \times 100}{\text{consumption of OM}}$$

The estimation of microbial protein synthesis as a function of the purine derivatives was estimated based on the equation described by Chen and Gomes (1995):

$$Y = 0.84X + (0.150LW^{0.75} e^{-0.25X})$$

where Y is the excretion of purine derivatives in the urine (mmol day<sup>-1</sup>) and X is the uptake of microbial purines (mmol day<sup>-1</sup>).

The calculation of X retention (g day<sup>-1</sup>) based on the Y value was performed using the Newton-Raphson method, as follows:

$$X(n+1) = Xn - \frac{(0.84X + (0.150LW^{0.75} e^{-0.25X})) - Y}{0.84 - (0.038LW^{0.75} e^{-0.25X})}$$

We considered a concentration of N in the purines of 70 mg mmol<sup>-1</sup>, true digestibility of purines of 0.83 and, N purines: N microbial ratio (NM) of 0.116.

The efficiency of microbial protein synthesis in the rumen (EMPS) was calculated in relation to the consumption of digestible organic matter (CDOM):

$$EMPS = \frac{NM \text{ (g day}^{-1}\text{)}}{CDOM \text{ (kg day}^{-1}\text{)}}$$

*Statistical analysis*

The data were submitted to the analysis of variance using the procedure PROC MIXED of the statistical package SAS (SAS, 1996). The model used included the random effects of animal and period and the fixed effects of supply (ad libitum or restricted) and supplementation (with or without), in addition to the interaction supply × supplementation, as described below.

$$Y_{ijkl} = \mu + \alpha_i + \rho_j + o_k + \sigma_l + o_k \times \sigma_l + e_{ijkl}$$

where  $Y_{ijk}$  is the value observed in the  $i$ -th animal and  $j$ -th period for the  $k$ -th treatment,  $\mu$  is the general mean,  $\alpha_i$  is the random effect of the  $i$ -th animal,  $\rho_j$  is the random effect of the  $j$ -th period,  $o_k$  is the fixed effect of the  $k$ -th supply factor,  $\sigma_l$  is the fixed effect of the  $l$ -th supplementation factor,  $o_k \times \sigma_l$  is the effect of the interaction of supply and supplementation factors, and  $e_{ijkl}$  is the experimental error associated with  $Y_{ijkl}$ .

Ruminal fermentation parameters were also analyzed using the procedure PROC MIXED of the SAS. The covariance matrix model used was chosen

according to the Akaike information criterion (WOLFINGER et al., 1993). The analyses were performed considering measures repeated over time and the model took into account the random effects of animal and period, in addition to the fixed effects of supply, supplementation, collection time, and interactions supply × supplementation, supply × supplementation time × collection time, and supply × supplementation × collection time.

**Results and Discussion***Consumption and digestibility of non-nitrogen compounds*

The consumption of DM, OM, and NDF of the pre-dried ryegrass decreased when the animals receiving this forage were supplemented with corn silage but did not change when the pre-dried supply was restricted (interaction supply × supplementation:  $P < 0.001$ ) (Table 2). The total DM intake increased with supplementation in animals receiving the pre-dried in a restricted amount but did not vary in animals that received pre-dried ad libitum (interaction supply × supplementation:  $P < 0.001$ ).

**Table 2.** Nutrient intake in sheep fed with restricted supply or ad libitum of pre-dried ryegrass (*Lolium multiflorum*) when supplemented or not with corn silage (*Zea mays*) + soybean (*Glycine max*) meal at a ratio of 9:1 of dry matter.

	Restricted		Ad libitum		SEM <sup>1</sup>	P value		
	Without suppl	With suppl	Without suppl	With suppl		Supply	Suppl. <sup>2</sup>	S × S <sup>3</sup>
<i>Ryegrass intake</i>								
Dry matter (g day <sup>-1</sup> )	560	566	921	680	104	<0.001	0.006	0.005
NDF (g day <sup>-1</sup> )	314	320	519	384	57.1	<0.001	0.007	0.004
Dry matter (g kg <sup>-1</sup> LW)	16.4	16.0	25.6	19.2	2.72	<0.001	0.003	0.008
Organic matter (g kg <sup>-1</sup> LW <sup>0.75</sup> )	34.8	34.2	55.0	41.2	5.91	<0.001	0.004	0.007
<i>Corn silage intake</i>								
Dry matter (g day <sup>-1</sup> )	-	280	-	258	23.2	0.225	<0.001	0.225
NDF (g day <sup>-1</sup> )	-	131	-	117	12.7	0.164	<0.001	0.164
Dry matter (g kg <sup>-1</sup> LW)	-	7.91	-	7.31	0.372	0.038	<0.001	0.038
Organic matter (g kg <sup>-1</sup> LW <sup>0.75</sup> )	-	18.3	-	16.9	0.895	0.044	<0.001	0.044
<i>Total intake</i>								

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Dry matter (g day <sup>-1</sup> )	560	845	921	939	93.9	<0.001	0.001	0.001
NDF (g day <sup>-1</sup> )	314	451	519	502	51.2	<0.001	0.005	0.001
Dry matter (g kg <sup>-1</sup> LW)	16.4	23.9	25.6	26.5	2.52	<0.001	0.001	0.003
Organic matter (g kg <sup>-1</sup> LW <sup>0.75</sup> )	34.8	52.5	55.0	58.1	5.42	<0.001	<0.001	0.002

<sup>1</sup> Standard error mean; <sup>2</sup>Effect of corn silage supplementation; <sup>3</sup>Effect of the interaction between the supply of pre-dried ryegrass and supplementation with corn silage.

The variation of DM consumption of ryegrass in animals that received base forage ad libitum, when supplemented with corn silage, resulted in a substitution rate of 0.93, while the animals that received the base forage in the restricted supply had a substitution rate equal to zero. The substitution rate found for the supply of base forage ad libitum (pre-dried ryegrass) is within the range reported in the literature when forage is used as a supplement, with values ranging from 0.2 to 1.2 (PHILLIPS, 1988; MORRISON; PATTERSON, 2007; DELAGARDE et al., 2011). The substitution rate equal to zero found for the restricted supply of ryegrass can be explained by the fact that the amount of food offered was not sufficient to meet the energy requirements of the animals. This is in accordance with several researchers who reported lower or zero substitution rates when the supply of base forage decreases (BARGO et al., 2003; MAYNE; WRIGHT, 1988). Woodward et al. (2002) reported substitution rates

of 0.10 and 0.14 for cows supplemented with grass silage and corn silage, respectively, being this substitution rate lower, a reflection of the restricted supply of pasture offered in the study.

No effect was observed for the interaction between supply and supplementation for the digestibility of DM, OM, CP, and dietary fibrous fraction (Table 3). The apparent digestibility of DM and OM were similar between treatments, but the digestibility of NDF and ADF were lower ( $P < 0.05$ ) in supplemented animals when compared to those not supplemented and those that received the pre-dried in a restricted amount when compared to those that received pre-dried ad libitum. The total intake of digestible OM increased with supplementation in animals receiving the pre-dried in a restricted amount but did not vary in animals that received pre-dried ad libitum (interaction supply  $\times$  supplementation:  $P < 0.001$ ).

**Table 3.** Effect of supply and supplementation on digestibility of DM, OM, CP, and dietary fibrous fraction in sheep fed pre-dried ryegrass (*Lolium multiflorum* Lam.) and supplemented with corn silage (*Zea mays*) + soybean (*Glycine max*) meal at a ratio of 9:1 dry matter.

	Restricted		Ad libitum		SEM <sup>1</sup>	P value		
	Without suppl	With suppl	Without suppl	With suppl		Supply	Suppl. <sup>2</sup>	S $\times$ S <sup>3</sup>
<i>Apparent digestibility</i>								
Dry matter	0.636	0.658	0.668	0.678	0.033	0.040	0.186	0.629
Organic matter	0.665	0.674	0.682	0.697	0.030	0.080	0.260	0.786
NDF	0.713	0.662	0.729	0.696	0.040	0.102	0.010	0.54
ADF	0.711	0.651	0.728	0.703	0.044	0.041	0.015	0.268

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True digestibility OM	0.813	0.798	0.824	0.818	0.024	0.089	0.256	0.616
Digestible OM intake (g day <sup>-1</sup> )	328	515	552	592	69.1	<0.001	0.001	0.009
Digestible OM intake (g kg <sup>-1</sup> LW <sup>0.75</sup> )	23.1	35.4	37.6	40.5	4.39	<0.001	<0.001	0.009
Digestible true OM intake (g kg <sup>-1</sup> LW <sup>0.75</sup> )	402	610	668	693	75.6	<0.001	0.001	0.004

<sup>1</sup> Standard error mean; <sup>2</sup>Effect of corn silage supplementation; <sup>3</sup>Effect of the interaction between the supply of pre-dried ryegrass and supplementation with corn silage.

The lower consumption of digestible OM of non-supplemented animals in restricted supply occurred due to the lower OM consumption in this treatment since the digestibility of DM, OM, and the fibrous fraction was not affected by treatments. On the other hand, a decrease in the digestibility of the fibrous fraction (NDF and ADF) for supplemented animals may be related to a lower digestibility of corn silage. The explanation for reduced digestibility of NDF in the restricted supply without supplementation is the lower availability of energy and NH<sub>3</sub> for fibrous carbohydrate fermenting microorganisms. In addition, the results observed in supplemented animals are similar to those observed by other researchers (BROWNE et al., 2005; CAVALCANTE et al., 2004), who tested corn silage supplementation and found a decline in the apparent digestibility of NDF and ADF, with the inclusion of corn silage as a function of the higher non-fibrous carbohydrate content in this food when compared to Tifton hay. Reductions in ruminal pH are often cited as the major cause of the reduction in fiber digestibility but do not always explain decreases in digestibility (CATON; DHUYVETTER, 1997). However, the effect of ruminal pH on cellulose digestibility has been frequently confused with changes due to increased food intake or fiber concentration in the diet, which also alter its digestibility as a function

of the increase in the passage rate. The increase in the passage rate usually reduces the digestibility of dietary components, particularly the most resistant components to degradation, such as fiber (RUSSELL; WILSON, 1996).

#### *Consumption and use of nitrogen compounds*

Nitrogen consumption increased with supplementation in animals receiving the pre-dried in a restricted amount but did not vary in animals that received this forage ad libitum (interaction supply × supplementation: P<0.01) (Table 4). The N excreted in the urine (P<0.05) and N retention (P<0.001) were higher in animals receiving the base forage ad libitum when compared to those receiving this forage in a restricted amount, with the lowest observed N retention in animals with restricted supply without supplementation. The production of microbial N was lower in restricted and non-supplemented animals when compared to those of other treatments, which did not differ from each other (interaction supply × supplementation: P<0.01). The efficiency of microbial synthesis did not vary with supply but was higher in supplemented animals when compared to those non-supplemented (P<0.05).

**Table 4.** Effect of supply and supplementation on consumption, fecal and urinary excretion and retention of nitrogen compounds in sheep fed pre-dried ryegrass (*Lolium multiflorum* Lam.) as base forage and supplemented with corn silage (*Zea mays*) + soybean (*Glycine max*) meal at a ratio of 9:1 dry matter.

	Restricted		Ad libitum		SEM <sup>1</sup>	P value		
	Without suppl	With suppl	Without suppl	With suppl		Supply	Suppl. <sup>2</sup>	S × S <sup>3</sup>
N intake (g dia <sup>-1</sup> )	16.9	21.5	27.0	24.6	3.74	0	0.468	0.019
N excreted in feces (g day <sup>-1</sup> )	4.03	5.50	5.36	6.12	2.07	0.209	0.154	0.648
N excreted in urine (g day <sup>-1</sup> )	10.7	10.7	13.2	11.0	2.19	0.09	0.173	0.179
N retained (g day <sup>-1</sup> )	2.20	5.26	8.44	7.45	2.72	0.001	0.310	0.054
N microbial (g day <sup>-1</sup> )	4.45	7.90	7.88	8.80	1.55	0.002	0.002	0.036
EMPS <sup>4</sup>	10.8	12.9	11.7	13.0	2.6477	0.597	0.091	0.665

<sup>1</sup> Standard error mean; <sup>2</sup>Effect of corn silage supplementation; <sup>3</sup>Effect of the interaction between the supply of pre-dried ryegrass and supplementation with corn silage; <sup>4</sup>Efficiency of microbial protein synthesis (microbial N (g/day)/digestible OM intake (kg/day)).

Nitrogen retention did not vary in animals that had offered ad libitum, but in restricted supply, it was higher in supplemented animals. This can be explained because in the restricted diet without supplementation, the animals did not have sufficient digestible organic matter that allowed a higher microbial growth and N retention. According to Cameron et al. (1991), microbial protein synthesis and microbial growth depend on an adequate amount of energy and nitrogen for the synthesis and assimilation of amino acids. In this sense, the values of N excreted in the feces in the restricted supply varied according to the consumption of DOM. This is explained because most of the nitrogen excreted in the feces is of microbial origin, being referred to as endogenous, which increases linearly as a function of consumption (KOZLOSKI, 2011).

Although it did not vary significantly with the supply, the efficiency of microbial synthesis was higher in animals supplemented with corn silage when compared those non-supplemented in both forms of supplies. Because the ammonia concentrations found in the ruminal fluid were not limiting for animals of either treatment, the efficiency of microbial protein synthesis did not directly influence nitrogen retention. However, the

improvement in supplemented animals could be explained by the presence of starch in the corn silage, which improves rumen utilization of nitrogen with the increased microbial activity (HVELPLUND et al., 1987; MATSUI et al., 1998; MOSS et al., 1992). The yield of a microbial protein depends not only on the solubility of the crude protein in the diet but also on the supply of fermentable energy sources and the degree of ruminal synchronization of protein and carbohydrates (BEEVER, 1993).

#### *Parameters of ruminal fermentation*

The highest values of ruminal pH and the lowest concentrations of VFA were observed in animals without supplementation and with restricted supply, while the highest proportion of acetate was observed in animals non-supplemented with ad libitum supply (interaction supply × supplementation: P<0.001) (Table 5). Propionate concentrations decreased (P<0.001) and relations (acetate + butyrate) propionate<sup>-1</sup> increased (P<0.001) in supplemented animals when compared those non-supplemented, regardless of the supply. The average concentration of N-NH<sub>3</sub> did not change with either supply or supplementation.



**Table 5.** Rumen fermentation parameters in sheep fed pre-dried ryegrass (*Lolium multiflorum* Lam.) and supplemented with corn silage (*Zea mays*) + soybean (*Glycine max*) meal at a ratio of 9:1 dry matter.

	Restricted		Ad libitum		SEM <sup>1</sup>	Supply	P value	S × S <sup>3</sup>
	Without suppl	With suppl	Without suppl	With suppl				
pH	6.64	6.39	6.56	6.46	0.025	0.768	<0.001	0.004
Concent. NH <sub>3</sub> (mmol L <sup>-1</sup> )	8.79	9.08	9.41	8.57	0.332	0.865	0.407	0.095
Concent. VFA (mmol L <sup>-1</sup> )	89.9	104	101	95.7	1.18	0.416	0.011	<0.001
Acetate (%)	72.0	71.3	73.3	71.6	0.261	0.002	<0.001	0.047
Propionate (%)	22.6	21.4	21.3	20.7	0.204	<0.001	<0.001	0.092
(C2 + C4) C3 <sup>-1</sup> †	3.46	3.74	3.74	3.88	0.045	<0.001	<0.001	0.111

<sup>1</sup> Standard error mean; <sup>2</sup>Effect of corn silage supplementation; <sup>3</sup>Effect of the interaction between the supply of pre-dried ryegrass and supplementation with corn silage.

Variations in pH contents and ruminal VFA concentration can also be explained by the consumption of digestible OM, especially in animals with restricted supply and non-supplemented. The pH values ranged from 6.2 to 6.9, remaining within the optimum range for bacterial growth and fiber digestion, which is between 6.0 and 7.0 (WEIMER, 1996). The VFAs produced in the rumen are the final products of the fermentation of the ingested OM (ALLEN, 1997), while the balance between the production of these fermentation acids and buffer secretion is the main determinant of ruminal pH. Roughages have the capacity to stimulate the chewing that is directly related to the flow of salivary buffers inside the rumen, necessary to neutralize the fermentation acids and maintain the ruminal pH. Therefore, the higher pH values found in animals non-supplemented and with restricted supply had a fermentable OM fraction provided by the pre-dried ryegrass, which was not sufficient to cause a decrease in these values.

## Conclusions

Supplementation with corn silage does not affect digestible OM consumption and nitrogen retention when sheep receive pre-dried ryegrass ad libitum. However, even with supplementation, the imposition of long daily periods of feed restriction may limit

the intake of digestible OM and N retention when compared to animals receiving the base forage ad libitum.

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