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Effects of replacing ryegrass silage with alfalfa hay on N utilisation efficiency, intake, and digestion in sheep

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ABSTRACT. Inclusion of legumes in ruminant diets increases the nutritional value and contributes to N fixation in the soil. The aim of this study was to determine the optimal level of alfalfa hay (*Medicago sativa* L.) as a partial replacement for ryegrass silage (*Lolium multiflorum* L.). Alterations in nutrient intake, N utilisation efficiency, rumen parameters, and microbial protein synthesis in sheep were investigated. Four rumen cannulated wethers, without a defined breed, in a Latin square 4×4 design, were fed four experimental diets formulated to replace ryegrass silage with alfalfa hay at a rate of 0, 15, 30, or 45%. Animals that received high levels of alfalfa showed a linear trend in dry matter intake and digestible organic matter content. Dietary N was utilised more efficiently, resulting in reduced N release into the environment when alfalfa was included in the diet. True N digestibility and retained N levels increased linearly with increasing proportion of dietary alfalfa. The efficiency of protein synthesis in the rumen remained unaffected. Furthermore, replacing ryegrass silage with alfalfa hay did not affect rumen pH. It can be concluded that such substitution enhances the intake of digestible organic matter, reduces rumen ammonia N concentrations, and increases N retention in sheep. In addition, the supply of the grass-legume mixture in the trough allows for better utilisation of the forage, thereby avoiding the setbacks and costs associated with mixed grass and legume pastures. Further studies are needed to evaluate other forage legume species because Brazil has a range of legumes that could be exploited for ruminant feed production.

Introduction

Fluctuations in the availability and nutritional value of forage throughout the year are among the factors that have the greatest impact on animal performance. Gerdes et al. (2000) evaluated tropical species throughout the year, and recorded forage production of 3.76, 2.03, 1.19 and 0.95 t dry matter (DM) ha^{-1} in spring, summer, autumn and winter, respectively. Additionally, neutral detergent fibre (NDF) levels varied, averaging 71% in the spring-summer period and 75% in the autumn-winter period, while crude protein (CP) levels ranged from 3.45% in winter to 12.9% in summer months. In southern Brazil, the inclusion of temperate grasses during the winter period is a common strategy on farms, utilised for grazing or ensiling purposes. Grass silages from temperate climates are distinguished by their good digestibility, high nitrogen content, and low neutral detergent insoluble fibre, imparting better nutritional value to the diet compared to maize silage, traditionally used in forage fields

(Alves et al., 2011). Among the most commonly utilised species, ryegrass stands out for its exceptional resistance, palatability, high yield, and digestibility (Meinerz et al., 2012).

In more intensive production systems, where animal nutritional requirements are high, better quality roughage should be considered to reduce costs by decreasing the concentrate fraction of the diet. Including forage legumes in the roughage fraction of the diet is important to increase its nutritional value. Among legumes, alfalfa is distinguished by its valuable characteristics, such as DM productivity ranging from 14 to 24 t per hectare per year, crude protein contents between 22 and 25%, and acceptable palatability (Costa et al., 2003).

Mixing grasses and legumes improves diet quality and increases yield and forage supply (Tambara et al., 2017). In addition, legumes can symbiotically fix atmospheric nitrogen, providing more nitrogen to the soilplant system and contributing to the enhanced development and productivity of plants in a mixed system. Lorensetti et al. (2021) observed that cattle fed on pastures in combination with ryegrass and vetch, consumed and retained more N, as well as showed greater efficiency in N utilisation. Therefore, more studies evaluating the inclusion of legumes in animal diets are necessary to verify whether these positive effects can be consistently maintained. Therefore, the hypothesis of the current study posited that high levels of alfalfa hay in the diet would promote increased nutrient intake, improved ruminal metabolism, and enhanced microbial protein synthesis in sheep. Accordingly, the present study aimed to investigate the effects of partially replacing ryegrass silage (*Lolium multiflorum* L.) with alfalfa hay (*Medicago sativa* L.) up to 45% in the diet to evaluate nutrient intake, N utilisation efficiency, rumen parameters, and microbial protein synthesis in sheep.

Material and methods

Location

The study was conducted at Universidade Tecnológica Federal do Paraná (UTFPR), Dois Vizinhos campus, PR, Brazil. The experiment was approved by the Institutional Ethics Committee on the Use of Animals (CEUA), approval number 2018/014.

Animals, experimental design, and diets

The experimental design was a 4×4 Latin square, with four treatments and four replicates. Diets were formulated to replace ryegrass silage with alfalfa hay at 0, 15, 30, or 45%. Ryegrass was ensiled at the pre-flowering stage, and the material was sun-dried for 4 h after harvesting. Subsequently, the material was stored in an above-ground silo. Four wethers, without a defined breed, with an average live weight of 50 kg and rumen cannulation, were used following the protocol approved by CEUA (protocol No. 2016-011).

The animals were housed individually in cages with a useful area of 0.96 m^2, equipped with feeders and drinkers. The experiment was conducted in four periods of 20 days each, 15 days for adaptation, and 5 days for samplings. The basal diet was formulated to be isoprotein and isoenergetic. It consisted of a roughage fraction, i.e., ryegrass silage and alfalfa hay, and a concentrate fraction, consisting of soybean meal (2%), ground maize (94%), calcitic limestone (2%), and dicalcium phosphate (2%), in a 60:40 ratio, respectively. The diet was designed to meet the requirements of 12.5% CP and 66% total digestible nutrients (TDN) for an average daily gain (ADG) of 0.250 kg (NRC, 2007) (Tables 1 and 2).

Table 1. Chemical composition of concentrate and experimental forages

Item	Concentrate	Alfalfa hay	Ryegrass silage
Dry matter (DM), g kg^{-1}	892.0	952.6	188.9
Composition, g kg^{-1} DM			
organic matter	940.7	916.0	909.4
ether extract	39.5	21.0	28.2
neutral detergent fibre	398.7	594.8	669.5
acid detergent fibre	80.7	486.2	554.1
lignin	23.4	159.9	95.6
N	18.2	24.9	19.3
neutral detergent insoluble nitrogen	1.1	1.1	4.8
acid detergent insoluble nitrogen	-	74.1	51.6
total digestible nutrients	792.7	624.7	629.4

Feeding was divided into two daily meals, provided at 8:00 and 16:00. The amount offered was adjusted daily to allow for 8% orts, ensuring *ad libitum* feeding. Orts were collected and weighed each day during the experimental period, before the morning meal, to determine daily intake.

Alfalfa hay was cut before feeding to sheep into 5- to 10-cm particles using a forage chopper to facilitate weighing and mixing during feeding. Samples of ryegrass silage, concentrate, and alfalfa hay were collected in each period, dried in a forced air oven at 55 °C for 72 h, and ground using a Wiley mill

Table 2. Chemical composition of the experimental diets, g kg^{-1} dry matter (DM)

Item	Experimental diets			
	ALF0	ALF15	ALF30	ALF45
organic matter	916.7	917.7	918.7	919.7
ether extract	30.4	29.3	28.3	27.2
neutral detergent fibre	552.9	541.7	530.5	519.3
acid detergent fibre	320.4	330.6	340.7	350.9
lignin	67.6	77.2	86.8	96.5
N	19.0	20.0	21.0	21.0
neutral detergent insoluble nitrogen	68.2	77.7	87.3	96.8
acid detergent insoluble nitrogen	2.4	35.4	38.7	42.1
total digestible nutrients	705.0	707.3	709.6	711.9

ALF0 – 0% alfalfa, ALF15 – 15% alfalfa, ALF30 – 30% alfalfa, ALF45 – 45% alfalfa

fitted with 1-mm sieves. Subsequently, the samples were homogenised, and a subsample was collected for chemical analysis.

Total faeces collection was performed by taking daily samples (approximately 10% of the total weight) using collector bags attached to the animal, to determine total nutrient apparent digestibility. Faecal samples were dried in a forced air oven (55 °C) for 72 h, ground in a Wiley mill fitted with 1-mm sieves and stored for further chemical analysis.

On day 20 of each experimental period, approximately 50 ml of ruminal fluid was collected manually before (0) and 1, 2, 3, 4, 6, and 9 h after the first feeding. Rumen fluid pH was immediately determined using a digital potentiometer (Technos), and two 10ml aliquots were acidified, one with 1.0 ml of 20% sulphuric acid and the other with 1.0 ml of 50% trichloroacetic acid. They were then centrifuged (3500 rpm) for 20 min and frozen for further analysis. The samples were analysed for ammonia N (Weatherburn, 1967), soluble sugars (Dubois et al., 1956), and α-amino (Palmer and Peters, 1969). Urine was collected daily during the sampling period into buckets containing 100 ml of 20% sulphuric acid (0.036 mol/l). The total volume was measured, and 10 ml was sampled, filtered, diluted four times, and stored at −20 °C until analysis (Chen and Gomes, 1992).

Allantoin analyses were performed using a colorimetric method of Fujihara et al. (1987), described by Chen and Gomes (1992). The determinations of uric acid were performed using a commercial kit (Labtest® – Uric acid liquiform, Lagoa Santa, MG, Brazil) following incubation with the enzyme xanthine oxidase, as described by Chen and Gomes (1992). Ruminal microbial synthesis was estimated based on absorbed purines, as outlined by Chen and Gomes (1992).

Chemical analysis

Dry matter content was determined for feed, orts, and faeces by drying in an oven at 105 °C for at least 8 h, while ash content was determined by calcination in a muffle furnace at 600 °C for 4 h. Total N content was determined using Kjeldahl's method (method 984.13; AOAC, 1997). Ether extract (EE) content was obtained by extraction with petroleum ether at 90 °C for 1 h using a Xt15 fat extractor (Ankom). The contents of NDF and acid detergent fibre (ADF) were determined using an ANKOM^{200} Fiber Analyzer in NDF and ADF solutions prepared according to Van Soest et al. (1991). Lignin content was determined following the method of Robertson and Van Soest (1981) and treatment in 72% sulphuric acid. Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) levels were analysed according to the method of Licitra et al. (1996).

Total digestible nutrients (TDN) were obtained using an *in vivo* method and calculated based on the ingested and excreted nutrients. True organic matter digestibility (TOMD) was calculated using the following equation:

$$\text{OMD (\%)} = (\text{\%OM ingested} - \text{OM digestibility})/\text{\%OM ingested} \times 100,$$

where: OMD – organic matter digestibility, OM – organic matter.

The following equation was used to calculate the true N digestibility:

$$\text{digestibility (\%)} = (\%_{\text{ingested}}\text{N} - \%\ \text{NDIN}_{\text{in faeces}})/\%_{\text{ingested}}\text{N} \times 100,$$

where: NDIN – neutral detergent insoluble nitrogen.

The data were subjected to the analysis of variance using the MIXED procedures implemented in SAS 9.0 (SAS Institute, Cary, NC). Additionally, regression analysis was performed for individual legume treatments and sampling times. Statistical significance was defined at $P < 0.05$, and a tendency was considered at $0.05 < P < 0.10$.

Results

Dry matter intake (DMI) and digestible organic matter intake (DOMI) (Table 3) were influenced by the levels of alfalfa hay replacing ryegrass silage, showing a linear trend ($P = 0.0970$ and $P = 0.0608$, respectively).

Substituting ryegrass silage with alfalfa hay resulted in a quadratic increase in N intake (Table 4).

Table 3. Nutrient intake and digestibility in sheep fed alfalfa hay as a replacement for ryegrass silage

Item	Diets				SEM	*P*-value	
	ALF0	ALF15	ALF30	ALF45		linear	quadratic
Intake, g d^{-1}							
dry matter	655.0	885.0	1072.5	915.0	0.12	0.0970	0.1136
organic matter	605.0	777.5	992.5	842.5	0.12	0.1123	0.1981
neutral detergent fibre	350.0	447.5	562.5	480.6	0.07	0.1285	0.2091
acid detergent fibre	205.0	260.0	342.5	292.5	0.04	0.1022	0.2480
non-fibre carbohydrates	157.5	197.5	255.0	217.5	0.03	0.1005	0.2108
digestible organic matter intake	457.5	585.0	750.0	657.5	0.08	0.0608	0.2004
total digestible nutrients intake	738.1	756.6	746.1	755.3	25.4	0.7038	0.8538
Digestibility, g kg^{-1}							
dry matter	732.7	753.2	747.1	756.9	2.85	0.5849	0.8505
neutral detergent fibre	491.2	495.2	529.3	564.5	6.96	0.3943	0.8193
true organic matter digestibility	752.2	774.2	765.1	776.8	2.64	0.5669	0.8442

ALF0 – 0% alfalfa, ALF15 – 15% alfalfa, ALF30 – 30% alfalfa, ALF45 – 45% alfalfa; SEM – standard error of the mean; $P > 0.05$ indicates that data are not significantly different

Table 4. Balance of N (g day^{-1}), N digestibility in the total tract (g kg^{-1}), and microbial protein synthesis efficiency in sheep fed alfalfa hay as a replacement for ryegrass silage

Item	Diets				SEM	*P*-value	
	ALF0	ALF15	ALF30	ALF45		linear	quadratic
N intake	12.3	16.4	21.7	18.8	0.0027	0.0025	0.0125
Urinary N	2.2	2.7	3.0	2.5	0.0006	0.7108	0.4176
Faecal N	4.2	5.5	6.0	5.0	0.0013	0.6253	0.3805
N total tract digestibility							
apparent nitrogen digestibility	660.0	685.0	727.5	740.0	0.0036	0.0836	0.8616
true nitrogen digestibility	825.0	832.5	880.0	887.5	0.026	0.0474	0.9999
retained N	5.7	8.0	12.7	11.5	0.0020	0.0235	0.3803
microbial N	2.7	4.5	5.7	3.9	1.84	0.5539	0.3284
efficiency of microbial synthesis, g N kg $DOMR^{-1}$	16.8	28.3	35.5	24.5	11.51	0.5543	0.3276

ALF0 – 0% alfalfa, ALF15 – 15% alfalfa, ALF30 – 30% alfalfa, ALF45 – 45% alfalfa; DOMR – digestible organic matter in the rumen: DOMR – 0.65 × digestible organic matter (AFRC, 1993); SEM – standard error of the mean; $P < 0.05$ indicates that data are significantly different

The apparent digestibility of N showed a linear increasing trend, whereas the true digestibility of N and retained N showed an increasing linear effect with raising alfalfa hay levels in the diet.

Microbial N and the efficiency of ruminal microbial protein synthesis were not affected ($P > 0.05$) by the presence of alfalfa (Table 4).

No significant interaction ($P > 0.05$) was observed between treatments and sampling time for the rumen parameters evaluated. Additionally, there were no differences in pH and sugar values ($P > 0.05$) among individual treatments (Table 5). A quadratic response ($P < 0.05$) was observed in the concentrations of ammonia and amino acids with increasing levels of alfalfa hay in the diet.

Table 5. Rumen pH and concentrations of ammonia, sugars, and amino acids (mg dl^{-1}) of sheep fed with alfalfa hay as a replacement for ryegrass silage

Item	Diets				SEM	*P*-value	
	ALF0	ALF15	ALF30	ALF45		linear	quadratic
pH	7.1	7.1	7.00	7.1	0.05	0.7558	0.1906
Sugars	44.0	35.9	48.2	48.9	2.71	0.1921	0.3288
Ammonia	26.2	18.3	17.0	23.6	5.13	0.3707	0.0009
Amino acids	95.2	88.0	118.6	74.0	10.51	0.4103	0.0211

ALF0 – 0% alfalfa, ALF15 – 15% alfalfa, ALF30 – 30% alfalfa, ALF45 – 45% alfalfa; SEM – standard error of the mean; $P < 0.05$ indicates that data is significantly different

Discussion

The increasing levels of alfalfa hay in place of ryegrass silage led to an increase in DMI and DOMI in sheep. The higher intake observed in sheep fed higher proportion of alfalfa hay could be attributed to the lower NDF content of alfalfa hay than ryegrass silage (594.8 vs. 669.5 g kg^{-1} DM, respectively). It has been demonstrated that high NDF content in forages negatively correlates with DMI in ruminants (Cooke et al., 2008). Similarly, Hassanat et al. (2014) found a linear increase in DMI in dairy cows, as the proportion of alfalfa silage replacing timothy silage was elevated.

DM intake can be affected by various factors, including fibre content, original structure of the plant, and its breakdown during digestion. Wilson and Kenedy (1996) suggested that the higher digestibility of legumes compared to grasses may be related to differences in leaf length. Grass particles typically have long and floating structures with low functional specific gravity, making them prone to tangling. Conversely, chewed legume vascular particles are shorter and thicker, and possess higher functional specific gravity, thus they more easily escape from the rumen. This mechanism could explain higher DMI values observed in sheep fed diets containing legumes.

N intake is associated with its concentration in the feed, and leguminous plants generally have higher levels compared to grasses (Van Soest, 1994; Tambara et al., 2017). In the present study, alfalfa hay contained 5.6 g kg^{-1} of N more than ryegrass silage. Additionally, the ingested N was also related to DM intake, which was notably higher for sheep receiving larger amounts of alfalfa hay. However, N intake at 45% alfalfa hay addition was reduced, possibly due to the higher proportion of ADIN in alfalfa hay compared to ryegrass silage (74.1 vs. 51.6 g kg^{-1} microbial synthesis (MS), respectively).

Increased N intake has been previously shown to correlate strongly with urinary N and to a lesser extent with faecal N excretion (Hentz et al., 2012; Schuba et al., 2017). However, in the present study, no significant effect was observed with respect to N excretion, likely due to the balanced energy-protein content of the evaluated diets. Imbalances between N and energy in the rumen can lead to increased excretion of compounds (Detmann et al., 2014; Piñeiro-Vazquez et al., 2017).

The increase in dietary alfalfa levels allowed to achieve greater apparent and true digestibility of N. According to Van Soest (1994), apparent digestibility in diets with non-fibrous components is directly proportional to consumption. True N digestibility, on the other hand, is a function of N intake and dry matter intake or faecal DM production, and typically remains constant, ranging between 85 and 95% of feed N. It should be noted that the loss of non-dietary N is inevitable during faeces production (NRC, 1985).

N retention increased with raising doses of alfalfa hay and declining ryegrass proportion. This could be attributed to the high availability of readily degradable N, a significant amount of fermentable energy, and a low concentration of ammonia N in the sheep rumen. The results of the present study corroborate the work of Detmann et al. (2014), who reported a positive relationship between N retention and digestible OM and N intake.

High microbial protein synthesis results from large amounts of fermented OM in the rumen and efficiency of microbial synthesis (EMS) during an adequate supply of N (Bach et al., 2005). Although a significant effect was anticipated for microbial N and EMS with an increase in the proportion of alfalfa hay and sufficient OM and N, no statistical difference was observed, likely due to increased variability. Nevertheless, numerically, there appeared to be a superiority for sheep fed alfalfa hay. According to Detmann et al. (2014), the average EMS obtained in tropical forages (without supplement) was 146 g microbial crude protein (MCP)/kg of digestible organic matter (DOM). In the present experiment, the average was 166 g MCP/kg DOM, ranging from 105 to 222 g MCP/kg DOM. CSIRO (2007) collated 22 experiments involving grazing cattle and found a variation from 99 to 191 g MCP/kg DOM. The higher values obtained in the present experiment can be attributed to the diet containing temperate forages and legumes, which have a higher N content and greater availability of nutrients for protein synthesis.

Ammonia concentrations for all levels of alfalfa hay inclusion exceeded the minimum value recommended, ensuring no restriction on microbial growth, i.e., 10 mg of ammonia per 100 ml of rumen fluid (Van Soest, 1994). The highest ammonia concentration was recorded in the control treatment, averaging 26.17 mg/dl. This elevation could be attributed to the low intake of digestible organic matter (457.5 g/day), indicating limited fermentable energy in sheep fed this diet. Another study has found a positive relationship between dietary DOM and CP (Detman et al. 2014).

The results of this study indicate that the inclusion of alfalfa hay in sheep's diets, as a replacement of ryegrass silage, enabled better N utilisation by the animals, while reducing N release into the environment. This decreased excretion of N through faeces and urine contributed to the reduction of greenhouse gas emissions, air pollution, terrestrial and aquatic acidification, and eutrophication caused by ammonia volatilisation (NH_3) (Tamminga, 2006).

Conclusions

The incorporation of alfalfa hay up to 30%, as a replacement for ryegrass silage, increased the efficiency of N utilisation and the consumption of digestible organic matter in sheep. Further research is needed involving other legume species to ensure positive results in animal production.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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