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Intake and digestibility in cattle grazing temperate grass associated with legume and/or energetic supplementation

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Abstract: The intercrop between grasses and legumes is an alternative to maintain and increase the animal production. The study was conducted to evaluate the effect of grass-legume mixtures with or without supplementations on rumen fermentation, nutrient intake, and microbial protein synthesis. Six Holstein steers fitted with ruminal cannula were kept in a double 3 × 3 Latin square design. The treatments were: 1) oat, annual ryegrass, and supplement (GS), 2) oat, annual ryegrass, and vetch (GL), 3) oat, annual ryegrass, vetch, and supplement (GLS). Supplementation of ground maize was given daily at 11 h at 1% of body weight. Total digestible nutrient intake was higher in steers fed GS and GLS. Animals on GL ingested higher concentrations of nitrogen (N) compared to animals on GS and GLS diets. Ruminal pH and ammonia concentration were higher in GL. Grass-legume mixtures and supplements showed higher concentrations of sugar, α-amino acids, and peptides. The ruminal fermentative parameters, ruminal pH, ammonia, and sugars ranged cubically across the day. Microbial protein synthesis was similar amongst the treatments. Animals exclusively consuming temperate grass produce higher ruminal pH and ammonia concentrations. Therefore, using temperate legumes in pasture systems can be included in the cattle diet in lieu of utilizing energy supplements.

Keywords: ammonia concentration; *Avena strigosa*; dry matter intake; nitrogen balance; microbial protein synthesis; *Vicia sativa*

Forages are the main component of bovine diets in Brazil; however, the seasonal productivity of grasses can limit animal performance. Currently, the mixture of grasses and legumes is a viable alternative to maintain and increase animal production (Hirai et al. 2015). Oats and ryegrass are among the main winter annual forages grown in the southern region of Brazil. They can be intercropped with legumes (e.g. vetch), which have the capacity to fix atmospheric nitrogen in the soil (through symbiosis with bacteria in rhizomes) enhancing the nutritional quality of the forage supplied (Mangaravite et al. 2014; Hirai et al. 2015). In addition, legumes rich in condensed tannins can reduce methane production (Ramirez-Restrepo and Barry 2005) suggesting that this strategy may be a sustainable alternative for livestock systems.

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The intake of animals on pasture is one factor that determines animal performance, and it is typically obtained in an indirect way. Currently there is extensive information on animal consumption within grass pastures (Costa et al. 2011; Da Silva et al. 2013); however, information regarding the grass-legume consortium is more limited.

Temperate grasses have a greater digestibility and higher total and soluble nitrogen, providing higher concentrations of ammonia to ruminal bacteria, and amino acids in the small intestine (Amaral et al. 2011). In this situation, energy supplementation of rapidly fermentable carbohydrates can boost animal performance by improving the forage nitrogen use. This generates an increase in microbial populations, thereby enhancing microbial protein production (Kaur et al. 2008). Energy supplementation is widely used in bovine production systems; however, it is rarely used in intercropped grass-legume systems.

The hypothesis of the present study was that energy supplementation associated with temperate grass and legume pasture can improve rumen fermentation with increased consumption and synthesis of ruminal microbial protein. The objective of this work was to evaluate the effects of three diets: 1) oats and annual ryegrass pasture plus supplement, 2) oats and annual ryegrass pasture plus vetch, and 3) oats and annual ryegrass pasture plus vetch and supplement on nutrient intake, ruminal parameters and microbial protein synthesis in cattle.

MATERIAL AND METHODS

Locality

This study was carried out in Dois Vizinhos, Parana State, Brazil (25°44'01"S and 53°03'26"W). According to the Köppen classification, the local climate is a subtropical humid mesotherm (*Cfa*) (Alvares et al. 2013).

Animals, diets, experimental design and management

The experiment was conducted in accordance with the approval number 2015/15 of the Ethics Committee on Animal Use (CEUA). The used experimental design was a double Latin square 3 × 3 (three diets × three periods), using six steers cannulated in the rumen according to the CEUA (approval number 2013/003). Steer average live weight (LW) was 350 kg. They were kept in cultivated pastures of oat (*Avena strigosa* Schreb.) and annual ryegrass (*Lolium multiflorum* Lam.) which were used in three different treatments: 1) oats-ryegrass pasture plus vetch (GL); 2) oats-ryegrass pasture plus ground maize offered at 1% of live weight (dry matter basis) (GS); and 3) oats-ryegrass pasture plus vetch and ground maize at 1% of live weight (GLS). At the end of each experimental period, the animals were weighed to adjust the amount of the supplement.

Animals were kept in an area of 4.7 ha, divided into paddocks of 0.78 ha each. The pasture implantation was performed using the no-tillage system on maize crop residues between April and May 2014. Per hectare: 80 kg of oat seed, 40 kg of ryegrass seed, and 30 kg of vetch seed were used, with 300 kg of fertilizer containing nitrogen, phosphorus, and potassium (NPK) at a ratio of 10 : 18 : 20 distributed along the line.

During the experiment, 100 kg/ha of urea was split into three applications and used for cover fertilization. Further information related to weather conditions and pasture management was described in detail in Lazzarotto et al. (2019).

The experiment was conducted in three 18-day periods, in which the first 14 days were used to habituate the animals to the new routine and diet. All samples were collected on the last four days of the experimental periods, during the full vegetative stage of the forages. The pasture was managed in a continuous stocking grazing system. Daily concentrate supplementation was administered once at 14:00; apart from this, the animals remained on pasture. In all paddocks the animals had access to water and minerals.

Analytical procedures

Faecal production was estimated using chromium oxide as an external marker. Ten grams of Cr_3O_2 were used per animal, placed directly in the rumen via a rumen cannula for 12 days. During the last five days of chromium oxide supply, faecal collections occurred and were performed directly from the rectum, twice a day (7:00 and 11:00) (Kozloski

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et al. 2006). Faecal samples were stored and frozen at −10 °C. For the analysis, faecal samples were dried in a forced air ventilation oven at 55 °C for 72 h and milled (1 mm sieve). Atomic absorption spectrophotometry was used after nitric/perchloric digestion to determine chromium in faecal samples according to the methodology described by Chen and Gomes (1992).

The pasture samples were collected at each experimental period by the pasture simulation method, weighed and then dried in a ventilated oven at 55 °C for 72 h and milled with a 1 mm sieve. The chemical composition of each diet is shown in Table 1.

The dry matter (DM) content was obtained by oven drying at 105 °C for at least 8 h; mineral matter was determined by combustion at 600 °C for 4 h and organic matter (OM) by mass difference.

Table 1. Chemical composition and *in vitro* digestibility of the experimental diets

Component	Forage			Concentrate maize
	GL	GS	GLS	
DM (g/kg)	227.22	231.88	232.38	886.45
Composition (g/kg of DM)				
OM	837.31	853.26	843.86	849.97
NDF	496.53	481.66	482.53	228.95
ADF	270.03	286.05	283.79	37.81
ADL	27.87	24.20	27.44	5.75
EE	21.49	22.86	18.38	20.16
NFC	297.45	326.87	315.31	656.07
TDN	646.58	664.21	639.64	809.47
CP	182.28	149.35	174.60	77.10
Composition (g/kg of CP)				
NDIP	25.20	14.31	22.29	5.39
ADIP	10.66	5.60	16.95	5.39
IVDDM (g/kg)	830.46	818.16	812.23	859.85
IVDOM (g/kg)	734.45	735.57	722.37	827.48

ADF = acid detergent fibre; ADIP = acid detergent insoluble protein; ADL = acid detergent lignin; CP = crude protein; DM = dry matter; EE = ethereal extract; GL= oats, ryegrass and vetch; GLS = oats, ryegrass, vetch and supplement; GP= gross protein; GS = oats, ryegrass and supplement; IVDDM = *in vitro* digestible dry matter; IVDOM = *in vitro* digestible organic matter; NDF = neutral detergent fibre; NDIP= neutral detergent insoluble protein; NFC = non-fibre carbohydrates, NFC = OM – (N × 6.25) + EE + (NDF – neutral detergent insoluble N × 6.25) (Mertens 1997); OM = organic matter; TDN = total digestible nutrients (NRC 2001)

Total nitrogen (N) was obtained by the Kjeldahl method (Method 984.13; Association of Official Analytical Chemists, AOAC 1997), ethereal extract (EE) by ether extraction at 90 °C by the XT15 Ankom® fat extractor (ANKOM Technology, Macedon, NY, USA); neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the Ankom A2000 Automated Fibre Analyzer (ANKOM Technology, Macedon, NY, USA) using the NDF and Table 1.

ADF solutions were prepared according to the methodology proposed by Van Soest et al. (1991); lignin was determined according to Robertson and Van Soest (1981); neutral detergent insoluble N (NDIN) and acid detergent insoluble N (ADIN) according to Licitra et al. (1996). In vitro digestible DM (IVDDM) of the pasture samples was determined using a method adapted from Tilley and Terry (1963).

The total dry matter intake (DMI) was estimated using the equation: DMI = faecal production/(1 – IVDDM); and faecal production, in kg/DM day, using the following formula: FP = chromium oxide administered (g/day)/faecal chromium oxide (g/kg of DM) (Pond et al. 1989). Forage dry matter intake was obtained by discounting the supplement intake from the total DMI.

Ruminal fluid samples were collected during the last four days of the experimental period at eight-hour intervals, advancing 2 h each day, culminating in a set of samples representing every two-hour interval of a 24-hour period. After each collection, the pH of the ruminal fluid was measured and two 10 ml aliquots were acidified, one with 1.0 ml of 20% sulphuric acid and the other with 1.0 ml of 50% trichloroacetic acid, centrifuged (1 000 × *g*) for 20 min, and frozen until further analysis. Through this, the levels of ammonia N (Weatherburn 1967), soluble sugars (Dubois et al. 1956), of amino acids and peptides (Palmer and Peters 1969) were determined.

Spot urine samples were collected once a day at 11:00 over the last four days of the experimental period (Chizzotti et al. 2008). Urine samples (10 ml) were subsequently acidified with 40 ml of sulphuric acid (0.036 N) in a 50 ml volumetric flask, identified and frozen until further analysis.

Creatinine (CRE) concentrations in the urine samples were colorimetrically determined us-

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ing commercial kits to obtain daily urine output (UO). Absorbed purines (AP) were calculated from the excretion of purine derivatives in the urine (sum of total allantoin and total uric acid) by means of the equation: AP = 0.85 DP + 0.385 PV where 0.85 is the recovery of absorbed purines as urinary derivatives of purines and 0.385 PV is the endogenous contribution to purine excretion (Verbic et al. 1990). The levels of allantoin (ALA) in the urine were obtained through the technique proposed by Fujihara et al. (1987), described by Chen and Gomes (1992), and uric acid was determined using a commercial kit (LABTEST, Lagoa Santa, MG, Brazil). Rumen microbial synthesis (g N microbe/day) was calculated from absorbed purines (Chen and Gomes 1992).

Nitrogen balance was obtained from the difference between the total amount of nitrogen ingested and the total amount of nitrogen excreted in faeces and urine. Estimates of daily urinary excretion of N-urea were calculated from the product of urine urea concentration divided by the estimated urinary volume and multiplied by 0.466 (corresponding to the N content in urea). Estimates of daily excretion of N in faeces were determined by the concentration of N in total faecal production. True N digestibility was calculated by discounting neutral detergent insoluble N (NIDN) present in faeces.

Statistical analysis

Data were analyzed using the MIXED procedure of SAS/STAT® software (2002, SAS Institute, Inc., Cary, NC, USA) at a 5% level of significance including: the effects of animals, periods, treatments, time and time × treatment interaction according to the statistical model:

$$Y_{ijkl} = \mu + A_i + P_j + D_k + A(P \times D)_{ijk} + T_l + (D \times T)_{kl} + e_{ijkl} \quad (1)$$

where:

$Y_{ij(k)}$ – dependent variables;
μ – mean of the observations;
A_i – effect of animals;
P_j – effect of periods;
D_k – effect of treatments;
$A(P \times D)_{ijk}$ – random effect between experimental units;
$(D \times T)_{kl}$ – effect of the treatment by time interaction;
e_{ijkl} – residual error.

Consumption means, N balance, microbial synthesis and ruminal parameters were compared using Tukey's test and orthogonal contrasts when the treatments were evaluated. In addition, regression analysis was used for the means of the ruminal parameters in relation to the sample collection time.

RESULTS

Intake and digestibility

The dry matter intake DMI as well as the forage dry matter intake (FI) were similar among the tested diets ($P > 0.05$) (Table 2).

The animals on the GS and GLS diets exhibited higher non-fibrous carbohydrate intake compared to animals on the GL diet ($P < 0.05$). The highest total digestible nutrient intake occurred in the GS diet and the lowest in the GL diet, and the GLS diet did not significantly differ from the other two diets.

Nitrogen intake (NI), urinary nitrogen, nitrogen retention and true digestibility of N were higher ($P < 0.05$) for the GL diet, with the GS and GLS diets

Table 2. Estimate intake of nutrients of steers in the tested experimental diets.

Variables	Diets			SEM	*P*-value
	GL	GS	GLS		
DMI (kg/day)	11.01	12.00	11.77	0.84	0.698 4
FI (kg/day)	11.01	8.98	8.78	0.82	0.169 4
RR	–	0.1	0.1	0.05	0.670 7
OMI (kg/day)	9.22	10.22	9.97	0.71	0.608 7
GPI (kg/day)	2.01	1.54	1.70	0.14	0.050 4
NDFI (kg/day)	5.45	4.89	4.80	0.44	0.533 9
NFCI (kg/day)	3.29^{b}	5.07^{a}	4.90^{a}	0.29	0.004 8
TDNI (kg/day)	6.35^{b}	7.40^{a}	6.91^{ab}	0.27	0.035 1

DMI = dry matter intake; FI = forage dry matter intake; GL = oats, ryegrass and vetch; GLS = oats, ryegrass, vetch and supplement; GS = oats, ryegrass and supplement; GPI = gross protein intake; NDFI = neutral detergent fibre intake; NFCI = non-fibrous carbohydrate intake; OMI = organic matter intake; RR = replacement level of dry matter (DM) supplement (kg DM forage/kg DM supplement) by DM forage; TDNI = real total digestible nutrient intake of feed subtracted from total digestible nutrients lost in faeces

^{a,b}Means with different letters on the same row differ statistically by Tukey's test ($P < 0.05$)

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Table 3. Characteristics of use of nitrogen compounds in cattle fed the experimental diets

Variables	Diets			SEM	*P*-value
	GL	GS	GLS		
NI (g/day)	321.03[a]	247.14[b]	272.31[b]	12.5	0.001 1
UN (g/day)	41.79[a]	330.36[b]	32.51[b]	2.61	0.010 9
FN (g/day)	46.88[b]	58.68[a]	60.93[a]	2.97	0.005 3
NR (g/day)	232.36[a]	158.10[b]	178.87[b]	9.81	0.000 1
N total tract digestibility (%)					
Apparent	69.75[a]	60.74[b]	64.06[ab]	1.98	0.011 9
True	91.68[a]	83.97[b]	84.13[b]	0.82	0.000 1
NUE (g N retained/ g N consumed)	0.70[a]	0.61[b]	0.64[ab]	0.02	0.011 9

FN = faecal N; GL = oats, ryegrass and vetch; GLS = oats, ryegrass, vetch and supplement; GS = oats, ryegrass and supplement; NI = nitrogen intake; NR = nitrogen retention; NUE = N total tract digestibility, and nitrogen use efficiency; UN = urinary N

[a,b]Means with different letters on the same row differ statistically by Tukey's test ($P < 0.05$)

Table 4. Ruminal parameters of cattle fed the experimental diets

Variables (mg/dl)	Diets			SEM	*P*-value
	GL	GS	GLS		
pH	6.67[a]	6.43[b]	6.31[b]	0.04	0.000 1
Ammonia	16.77[a]	9.05[b]	8.21[b]	0.65	0.000 1
Total sugars	100.83[b]	100.67[b]	147.44[a]	5.70	0.000 1
Peptides	59.41[b]	65.36[ab]	74.57[a]	3.11	0.001 3
Amino acids	6.07[b]	6.36[b]	6.99[a]	0.12	0.000 1

GL = oats, ryegrass and vetch; GLS = oats, ryegrass, vetch and supplement; GS = oats, ryegrass and supplement

[a,b]Means with different letters on the same row differ statistically by Tukey's test ($P < 0.05$)

being similar to each other (Table 3). N apparent digestibility and nitrogen use efficiency (NUE) were higher ($P < 0.05$) for GL, lowest in GS, and intermediate in the GLS diet. Faecal nitrogen was higher ($P < 0.05$) for the GS and GLS diets, with the GL diet being lowest (Table 3).

Rumen fermentation

Ruminal pH and ammonia were higher ($P < 0.05$) for animals fed the GL diet. Total concentration of sugars and amino acids in the rumen fluid varied significantly ($P < 0.05$) across the experimental diets, with the GLS diet having the highest values compared to GL and GS.

Concentration of peptides was highest ($P < 0.05$) in GLS, lowest in GL, with GS being intermediate (Table 4).

Ruminal pH varied cubically over the course of the hours (Figure 1) for all diets, showing that a significant decrease in pH occurs in the first 4 h after feeding, stabilizing between the 6th and 10th hour, and increasing after 10 h from feeding.

The relationship between the ruminal parameters and the temporal space (24 h) is presented in Figure 2. Since there were no interactions ($P > 0.05$) between time and treatment for any of the variables, the mean 24-hour variation of all treatments is presented together.

Concentrations of ammonia and sugars varied cubically, while concentrations of peptides and amino acids varied linearly over time ($P < 0.05$).

Rumen microbial protein synthesis

Daily urine output, creatinine, allantoin, uric acid, absorbed purines, N fixed, microbial protein (MP) synthesis and N intake that was recovered by the rumen microbes and incorporated in MP (N-fixed-NI) did not change significantly for any of the tested diets ($P > 0.05$; Table 5).

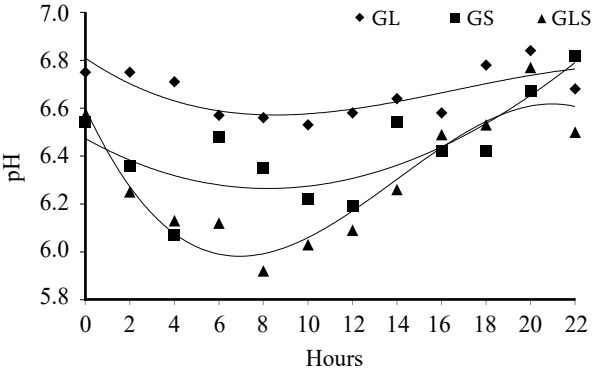

Figure 1. Variation of ruminal pH over a 24-hour period in cattle fed temperate pastures associated with legume or energy supplementation

Ruminal pH varied cubically during the 24-hour period for all diets

GL = oats, ryegrass and vetch; GLS = oats, ryegrass, vetch and supplement; GS = oats, ryegrass and supplement

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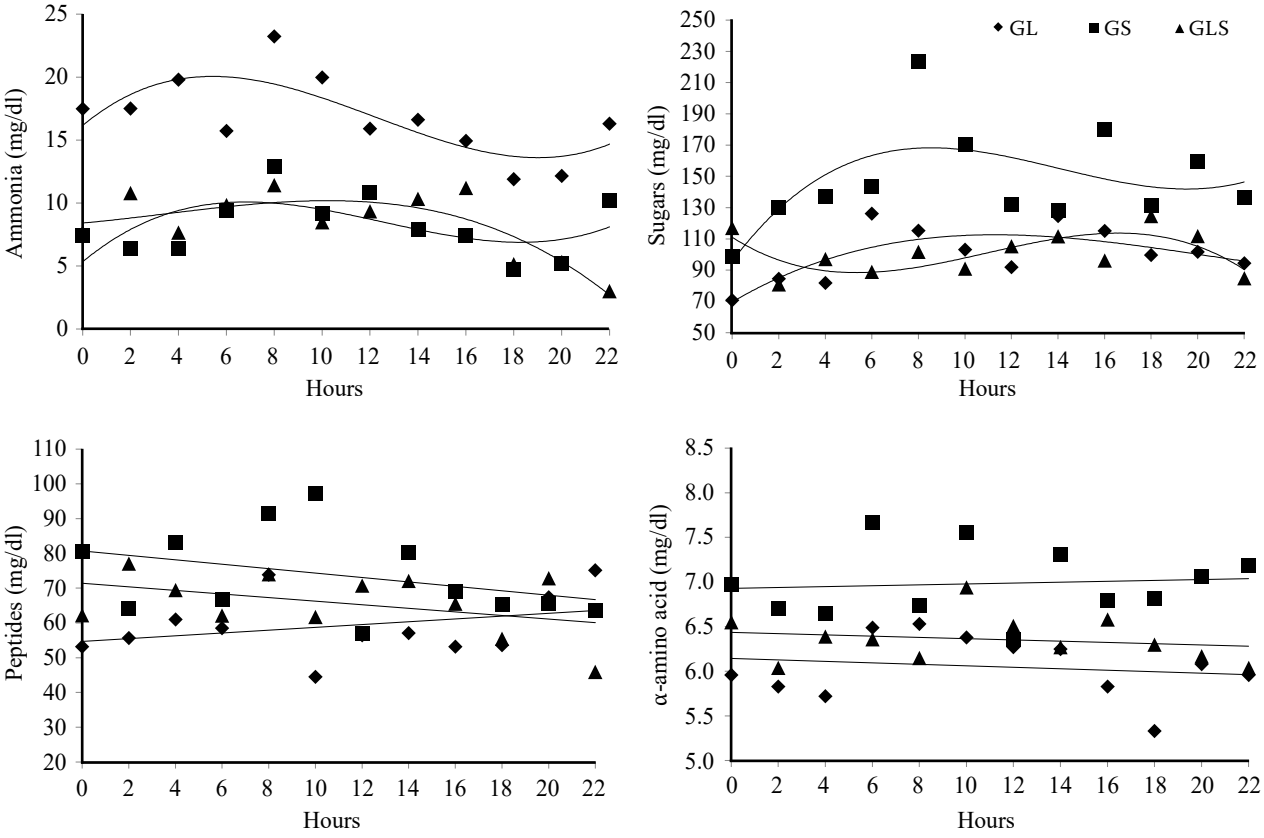

Figure 2. Variation of the concentrations of ammonia, total sugars, peptides and α-amino acid in the rumen fluid over a 24-hour period in cattle fed temperate pastures associated with legume or energy supplementation
GL = oats, ryegrass and vetch; GLS = oats, ryegrass, vetch and supplement; GS = oats, ryegrass and supplement

DISCUSSION

The quality of the consumed pasture is demonstrated by the low NDF content (487.00 g/kg of DM) and high crude protein (CP) content (168.74 g/kg of DM) of the diets, which indicates a high digestibility of the pasture (Van Soest 1994). A similar consumption of the animals across treatments is indicative of the adequate protein content of the forages, and of the dry matter digestibility (IVDDM) which was similar for all the diets.

In addition to these factors, forage mass and availability are positively related to DMI as described by Da Trindade et al. (2016). Forage mass varied among the evaluated diets (1 487.4 kg; 1 303.5 kg and 1 108.7 kg DM/ha for GS, GLS and GL, respectively), but forage availability was similar, with an average of 1.00 kg DM/kg LW (Lazzarotto et al. 2019). Not surprisingly, the supplemented animals had a higher NFC intake because ground maize had twice as much NFC as the average pasture. However, even with the higher energy consumption due to supplementation, animals did not reduce the intake of forage, as indicated by the equal replacement rate (0.1 kg DM forage for each kg DM of ground maize).

In addition to the quantity and quality of available forage, other factors that directly affect animal performance are the quantity and the nutritional characteristics of the supplement given to grazing animals (Reis et al. 2009). Keeping grazing animals on a mixture of grasses and legumes without the addition of an energetic supplement may limit energy intake, and thus reduce animal productivity, since the NFC content in the pasture is lower.

It was verified that total N, N retained, N apparent and true N digestibilities were higher for animals fed the GL diet. This was not unexpected since GL had the highest N concentrations. The true N digestibility of animals receiving ground maize was lower than that of unsupplemented animals, and this was positively correlated with the faecal excretion of N since there was a higher proportion of faecal N assessed in the GLS and GS diets

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Table 5. Creatinine concentration, urinary output, allantoin, uric acid, absorbed purines, N fixed, microbial protein (MP) synthesis and fixed-N in relation to N intake of the experimental diets

Variables	Diets			SEM	*P*-value
	GL	GS	GLS		
Creatinine (mg/dl)	531.71	722.78	608.09	90.54	0.370 2
Urinary output (l/day)	18.82	14.88	16.18	1.73	0.315 3
Allantoin (mg/kg)	53.76	57.29	46.23	8.84	0.678 0
Allantoin (mmol)	165.59	179.33	135.8	27.45	0.546 3
Uric acid (mg/kg)	1 370.4	1 762.94	1 746.85	139.1	0.139 1
Uric acid (mmol)	12.18	15.67	15.52	1.24	0.139 3
Absorbed purines (mmol)	181.96	196.42	159.0	23.9	0.560 3
N-fixed (g/day)	132.29	142.8	115.6	17.38	0.560 3
MP synthesis (g/day)	826.85	892.54	722.51	108.61	0.560 3
Fixed-N/NI (%)	45.04	58.60	47.30	11.61	0.586 6

GL = oats, ryegrass and vetch; GLS = oats, ryegrass, vetch and supplement; GS = oats, ryegrass and supplement
There was no significant difference at $P < 0.05$ or by Tukey's test at 5% probability

(60.93 g/day and 58.68 g/day of faecal N, respectively). In the present study, it was verified that the grazing system of grass/legume was more efficient in the use of the N consumed than the other two treatments, implying that a lower excretion of N to the environment occurred. Currently, many countries have requirements for producers to gradually reduce N excretion in the next few years (Gierus et al. 2012), so this effect has attributed a beneficial feature to the use of legumes in ruminant production systems. The N_2 fixation for legumes represents an important renewable source of N that can help maintain and increase soil fertility for the benefit of subsequent crops and reduce the use of nitrogen fertilizer (Barcellos et al. 2008). Besides the environmental benefits, the addition of legumes in combination with grasses promotes an increase in animal productivity and improves N balance (Castro et al. 2008). Hirai et al. (2015) verified an intermediate performance for steers kept in the intercropped pasture of white oats with vetch compared to steers kept on white oats and receiving ground maize, indicating that the grass-legume combination is a viable option for finishing cattle, especially when the prices of supplements are high.

Nitrogen intake affects the NH_3–N concentration in the ruminal fluid. The higher N intake by steers fed the GL mixture probably explains the higher concentration of NH_3–N in their ruminal fluid. Moreover, the addition of NFC from the supplement reduced the concentration of NH_3–N in the other treatments. In addition, the concentrations of ammonia and sugar over time fluctuated in opposite ways for animals receiving supplement or not, indicating that the addition of non-fibrous carbohydrates to grazing animals reduces ammonia and increases sugar concentrations.

The supply of rapidly fermenting carbohydrates may cause changes in the rumen microflora and reduce the pH (Darwin et al. 2018), as observed in this study. However, such a reduction in pH has not likely impaired the ruminal environment, since Van Soest (1994) reported that fermentative activity is impaired when pH is below 6.0, which did not occur in the present study.

The higher pH values for supplemented steers from our study compared with other studies (Kaur et al. 2008) may be due to the fact that our diets had higher levels of CP and, consequently, higher ammonia contents in the rumen, thereby contributing to the maintenance of pH even when the supplement was provided. Ammonia is a potent buffer, and in the rumen this molecule is found as ammonium ion (NH_4^+) and it flows into the blood in this way, removing protons from the system and maintaining ruminal pH homeostasis (Aschenbach et al. 2011).

The steers kept on the GLS diet presented higher levels of sugars, peptides and amino acids in the ruminal fluid, probably due to the higher consumption of NFC, which, in addition to the high CP from forages, provided the ideal conditions for microbial growth. Bailey et al. (2012) stated that for the synthesis of amino acids, carbon skeletons originated from the degradation of carbohydrates are necessary. The same is true of the synthesis of peptides, which also requires the presence of starch, pectin and sugars for more effective microbial growth, rather than fibrous carbohydrates such as cellulose and hemicellulose. However, in the present study, the microbial protein synthesis (MPS) was not affected by the diets. This lack of response in the MPS was also observed by other authors working with

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animals fed temperate forages and receiving energy supplementation (Amaral et al. 2011; Tebot et al. 2012). Dewhurst et al. (2000) reviewed several studies and confirmed a confounding difference in the estimation of MPS between the season and the physiological state of the animal. It is believed that bacteria can maintain fermentation close to normal levels even with a decrease in microorganism synthesis (Clark et al. 1992). Organic matter consumption is directly related to the supply of microbial nitrogen when ruminal N is not limiting (Hentz et al. 2012). In this study, the OM intake was similar for all diets. Furthermore, the proportions of N intake retained as MP were 45.04%, 58.60% and 47.30% for the GL, GS and GLS treatments, respectively, which were found to be similar. However, this numerical difference of 25.7% between GL and GS treatments may be relevant under other circumstances. Detmann et al. (2014) suggested that the increase in NUE improves the status of N, and this increase allows the total portion of N to be used for purposes of animal metabolism and for the overall increase in the efficiency of the use of metabolizable protein, and it seems to be a better indicator than the direct effect of increased MPS.

CONCLUSION

Temperate legumes can be included in the diet of cattle rather than using energy supplements in pasture systems. The intake of dry matter and organic matter was not affected, and N retention was higher in animals grazing temperate grass associated with the legume. Animals exclusively in temperate legume pastures obtain higher ruminal pH and ammonia concentrations.

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Conflict of interest

The authors declare no conflict of interest.

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