Animal Feed Science and Technology 231 (2017) 107–110

Contents lists available at ScienceDirect

Animal Feed Science and Technology

journal homepage: www.elsevier.com/locate/anifeedsci

Short communication

Associative effects between red clover and kikuyu grass silage: Proteolysis reduction and synergy during *in vitro* organic matter degradation

Gabriela Cristina Guzatti[a], Paulo Gonçalves Duchini[a], Gilberto Vilmar Kozloski[b], Vincent Niderkorn[c], Henrique Mendonça Nunes Ribeiro-Filho[a,*]

[a] *Departamento de Produção Animal e Alimentos, Universidade do Estado de Santa Catarina, Av. Luiz de Camões, 2090, Lages, SC, 88520-000, Brazil*
[b] *Departamento de Zootecnia, Universidade Federal de Santa Maria, Campus Camobi, Santa Maria 97105-900, RS, Brazil*
[c] *Université Clermont Auvergne, INRA, VetAgro Sup, UMR Herbivores, F-63122 Saint-Genès Champanelle, France*

ARTICLE INFO

Keywords:
Associative effects
In vitro rumen fermentation
Legume–grass mixture
Polyphenol oxidase

ABSTRACT

The aim of this study was to evaluate the effects of the association between red clover (RC; *Trifolium pratense*) and a tropical grass (kikuyu grass) on the proteolysis of ensiled material and the *in vitro* degradation of protein and organic matter. Red clover and kikuyu grass were ensiled in the following proportions: 0:1000, 250:750, 500:500, 750:250, and 1000:0 g/kg of dry matter (DM). The fraction of rapidly degradable protein, the ammonia nitrogen (NH_3-N) content of the silo, the *in vitro* protein degradation, and the degradation rate decreased linearly ($P < 0.001$) as the RC content in the ensiled material increased. Cumulative gas production after 24 h incubation showed a positive quadratic effect when RC was increased to 500 g/kg ($P < 0.001$). The silages with the highest RC content reduced proteolysis more effectively during ensiling and ruminal fermentation. Inter-species synergistic effects positively affected *in vitro* gas production, which was optimal when RC and kikuyu grass were ensiled in the same proportions as that of total DM.

1. Introduction

Ensiling usually reduces the quality of the plant material because true protein is transformed into non-protein nitrogen (NPN) (Repetto et al., 2005). This can be ameliorated by using certain legumes that contain bioactive compounds capable of decreasing proteolysis in the silo (Jones et al., 1995; Jones et al., 1995; Sulivan and Hatfield, 2006) and during the ruminal degradation of forage protein.

The legume red clover (RC; *Trifolium pratense*), particularly as silage, could considerably improve the efficiency of nitrogen (N) metabolism in ruminants because it contains polyphenol oxidase (PPO). In the presence of oxygen, this enzyme can oxidize the phenolic compounds released from plant vacuoles into quinones, which are, in turn, capable of forming complexes with proteins and reducing proteolysis in both the silo (Jones et al., 1995; Lee et al., 2008) and the rumen (Broderick et al., 2004; Merry et al., 2006). A recent study demonstrated that mixing RC and temperate-climate grass silages decreases urinary N excretion, and increases the digestible organic matter (OM) intake and N retention in sheep (Niderkorn et al., 2015). However, tropical grasses have higher fibre content and lower digestibility than that of temperate grasses, which may result in a different synergistic effect. Therefore, the effects of ensiling RC with a tropical grass, such as kikuyu (*Pennisetum clandestinum*), deserves investigation because of the potential for

* Corresponding author.
E-mail address: henrique.ribeiro@udesc.br (H.M.N. Ribeiro-Filho).

http://dx.doi.org/10.1016/j.anifeedsci.2017.07.008
Received 17 March 2017; Received in revised form 12 July 2017; Accepted 13 July 2017

broader geographical coverage.

This study tested the following hypotheses: i) that an increase in the proportion of RC in the silage mixture synergistically reduces proteolysis during ensiling and *in vitro* rumen fermentation; and ii) that mixing RC and kikuyu grass leads to better *in vitro* OM degradation than fermenting the same species individually.

2. Materials and methods

The forages were ensiled in 3.8-L plastic buckets using the following ratios of RC and kikuyu grass (based on DM): 0:1000, 250:750, 500:500, 750:250, and 1000:0 g/kg (n = 15 silos, 3 field repetitions × 5 treatments). Details about the forage and silage preparation methods are described in the supplementary content. After 100 days of ensiling, the silos were opened and two representative samples of the ensiled material were collected per silo. One sample (~400 g) was compacted using a hydraulic press to collect the fluid. The fluid was filtered using paper (20 µm, quick filtration), the pH was immediately measured, and the fluid was frozen prior to analysing the ammonia nitrogen (NH_3-N). Another sample (~200 g) was freeze-dried, ground through a 1 mm porosity sieve, and stored until required for chemical analysis and *in vitro* rumen fermentation.

Three *in vitro* runs were carried out, which generated a total of 90 observations (3 field repetitions × 5 treatments × 2 replicates × 3 runs). For each treatment, the values were averaged per replicate and field repetition within each run, which was considered the experimental unit. There were 15 remaining observations (3 per treatment). Details about *in vitro* rumen fermentation and the chemical analyses are described in the supplementary content.

The curves for gas production throughout the incubation time were adjusted using the unicompartmental logistic model (Schofield et al., 1994) so that the gas production rate (kd) could be estimated. The *in vitro* N degradation (IVND) at each incubation time-point was calculated using the equation: IVND = (([NH_3-N] − [NH_3-N Br]) × volume (mL))/incubated N (mg), where [NH_3-N] = concentration (mg/mL) of N-ammonia measured in the bottle containing the sample; and [NH_3-N Br] = concentration of N-ammonia in the sample collected from blanks at time 0. The fractional rate of protein degradation was estimated as the regression coefficient between the natural logarithm values of the non-degradable fraction (Ln (1 – IVND), y) *vs* the incubation time (×) according to Broderick (1987).

The quality parameters of the ensiled material were subjected to analysis of variance using the SAS PROC GLM programme (SAS Institute, Cary, NC, USA). The treatment was considered a fixed effect. The *in vitro* incubation parameter data were analysed using the SAS PROC MIXED programme using a model that included the fixed effect of the treatment and the random effect of the run. The effect of increased RC proportion over all evaluated parameters was tested by an orthogonal polynomial contrast that considered the linear and quadratic effects. The differences were significant at $P < 0.05$.

3. Results

The acid detergent fibre (ADF) and non-fibre carbohydrate (NFC) content increased and the neutral detergent fibre (aNDF) content decreased linearly ($P \leq 0.001$, Table 1) as the RC proportion rose. Organic matter (OM), crude protein (CP) content and protein fraction B were similar in all treatments. However, protein fraction A decreased and protein fraction C increased linearly as the RC proportion rose in the silage ($P < 0.001$). The pH values decreased as the RC proportion in the ensiled material increased, and both the linear and quadratic effects were significant ($P < 0.001$). The NH_3-N in the fluid extracted from the silage decreased linearly as the RC proportion increased ($P < 0.001$).

Protein degradation at the different incubation times and the protein degradation rate both decreased linearly ($P < 0.001$) as the

Table 1
Chemical composition (g/kg DM), pH, and NH_3-N content (g/kg of total N) of the ensiled materials containing different proportions of red clover and kikuyu grass.

	Red clover (g/kg of total DM)					RSD	P-value	Contrasts	
	0	250	500	750	1000			L	Q
Composition (g/kg DM):									
DM	349	367	395	417	459	28.6	0.006	< 0.001	0.492
OM	903	897	893	898	896	4.9	0.268	0.205	0.151
aNDF	585	545	511	477	435	13.7	< 0.001	< 0.001	0.869
ADF	278	288	293	305	316	11.7	0.019	0.001	0.768
NFC	131	159	179	215	252	16.5	< 0.001	< 0.001	0.354
CP	200	200	202	201	200	6.4	0.995	0.932	0.768
Protein fractions (g/kg CP)									
A	520	508	483	410	348	31.9	< 0.001	< 0.001	0.057
B	428	423	422	462	486	38.1	0.229	0.049	0.256
C	52	69	95	128	166	13.7	< 0.001	< 0.001	0.122
Silage parameters									
pH	5.48	5.28	5.01	5.00	4.90	0.021	< 0.001	< 0.001	< 0.001
NH_3-N	103	90.5	76.3	70.5	47.6	5.93	< 0.001	< 0.001	0.358

(DM) dry matter; (OM) organic matter; (aNDF) neutral detergent fibre; (ADF) acid detergent fibre; (NCF) non-fibre carbohydrate; (CP) crude protein. RSD = residual standard deviation. (L) linear and (Q) quadratic contrasts.