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SOLUBILITY AND DEGRADATION OF WHITE CLOVER IN THE LACTATING COW

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SUMMARY

The *in sacco* technique was used in 2 experiments to identify differences in the patterns of dry matter (DM) and crude protein (CP) degradation in fresh white clover samples, compared with freeze-dried and oven-dried (at 45 and 60°C) preparations. Preparation methods and basal diets fed were found to have a marked effect on the immediately soluble fraction, and the rate and potential degradation. All 3 dried preparations failed to describe the degradation pattern of the fresh treatment adequately enough to be interchangeable. Fresh samples consistently had a lower immediate solubility, and a higher potential degradation, with the rate of degradation generally being higher. For all the fresh samples, CP degraded more rapidly and had a greater potential degradation than DM. Two additional experiments were conducted to determine the immediately soluble DM and CP fractions released on mastication by the lactating cow, and these were compared with *in sacco* estimates. Mastication was found to release between 24-29% of the DM and 28-32% of CP. The estimates obtained from the *in sacco* technique were lower; 18% for DM and 15% for CP. These lower values are suggested to be a result of the greater cuticle damage through mastication as compared with fresh herbage cut to 1 cm lengths.

Keywords: in sacco, white clover, protein solubility, lactating cows.

INTRODUCTION

White clover can provide high quality feed for milk production and liveweight gain, especially in the northern Victorian irrigation zone where pasture growth can be maintained through summer and autumn. For a high producing dairy cow, an adequate supply of metabolisable protein can only be assured if enough nitrogen (N) is made available for microbial protein synthesis in addition to feed bypassing the rumen (Lindberg 1981). The former is usually more than sufficient in fresh legume herbages (Beever *et al.* 1985). For accurate predictions of the intestinal supply, information is needed on the relative contributions of feed protein and microbial protein in the lactating cow.

The *in sacco* technique as described by Mehrez and Orskov (1977) is often used to evaluate the ruminal degradation patterns of feed components, but relatively little work has been done with fresh legume forages. Sample preparation method may influence protein degradability estimates. However, more consistency in the preparation of samples can be achieved when using freeze-dried or oven-dried samples, and provided that these treatments accurately describe the patterns observed with fresh herbage, they may prove preferable for future degradability studies. Two nylon bag experiments were conducted using rumen fistulated lactating cows to measure the degradation of white clover from fresh, freeze-dried and oven-dried (at 45 and 60°C) preparations.

When fresh herbage is eaten by the dairy cow, some cell solubles are rapidly released for immediate fermentation in the rumen. The release of this immediately soluble fraction is of interest, as mastication of feed prior to entering the rumen could affect subsequent microbial degradation, and hence utilisation by the cow. This fraction was initially estimated using the in *sacco* technique. In 2 additional experiments, the effect of mastication on the extent of dry matter (DM) and crude protein (CP) solubilisation of fresh white clover was examined. The *in sacco* and *in vivo* methods were compared.

MATERIALS AND METHODS

Experiments 1 and 2

Two experiments were conducted in late winter-early spring 1993, using Holstein-Friesian cows fitted with 100 mm rumen cannulae. All cows were in early lactation. In experiment 1, 4 cows were pen-fed a mixed ration *ad libitum*, twice daily. The diet consisted of 45% maize silage, 34% clover hay, 8% whole cotton seed and 13% whole barley grain, supplemented with calcium and phosphorus. Intakes averaged 15.7 ± 2.7 kg DM/day and cows produced 30.3 ± 4 L of milk/day. In experiment 2, 6 cows grazed to appetite on white clover dominant pastures and produced 27.4 ± 1.5 L/day. For both experiments, the adaptation period on the diets was 2 weeks.

All samples were harvested from clover dominant pastures (> 70% clover content). Fresh samples were cut to 1 cm lengths, while freeze-dried (-42°C for 72 hours) and oven-dried (45 and 60°C for 24 hours) preparations were milled through a 1 mm sized screen before weighing. Five grams (DM) of sample was placed into each bag. All treatments were included in each experiment and each cow. The *in sacco* technique employed was that suggested by the AFRC (1992) publication, unless stated otherwise.

Bags were made from woven nylon cloth with a mean pore size of 25 μm (Scrynal NY25HD x 115 cm). Ten bags/preparation were suspended in the ventral sac of the rumen at time t = 0 (1100 hours) and 1 bag/treatment.cow was sequentially withdrawn at t = 1, 2, 4, 6, 9, 16, 24, 30, 48 and 72 hours. Bags were washed in a washing machine for 45 minutes, followed by 5 minutes on low spin (500 rpm), frozen and freeze-dried. On removal from the freeze-drier, samples were weighed for DM loss determination and then ground to 1 mm to ensure sample homogeneity for N analysis. The N contents were determined by combustion (Leco Corporation model 160FP478) using a white clover standard. Losses of DM and CP (CP = N x 6.25) were then plotted against time. Exponential curves (Orskov and McDonald 1979) were fitted to all points using a statistical program (Genstat 5.0). The curves were then used to derive the immediately soluble portion, and the rate and extent of degradation of the various white clover preparations.

Experiments 3 and 4

Two separate experiments were undertaken in the spring of 1992 and 1993 using 4 rumen fistulated lactating cows. Cows were grazing clover pastures *ad libitum* prior to both trials. Samples of herbage were harvested from swards of clover dominant pastures (10-15 cm in height). Cows were emptied of rumen contents and fed 7 samples of approximately 500 g of fresh white clover in experiment 3, and 15 in experiment 4. Food boluses were collected at the cardia by hand. Each bolus collected was weighed and a subsample retained, while the remainder was squeezed in a gauze cloth and washed of the soluble component. All samples were analysed for DM and N contents, corrected for 100% DM recovery, and solubilities of DM and CP due to the effect of mastication were determined.

RESULTS

The degradability data obtained for DM and CP for each feed preparation were fitted to the first order model of Orskov and McDonald (1979);

$$P = A + B\,(1 - e^{-CT}) \qquad \text{(equation 1)}$$

where, P is the cumulative amount degraded at time T, A is the readily soluble fraction (%), B is the potentially degradable fraction in the rumen (%), C is the fractional degradation rate of B (%/hour), and T is time (hours).

The degradability coefficients (ie. A, B and C) for experiments 1 and 2 are shown in Table 1. These results highlight the differences that exist between sample preparation methods, and provide a comparison of these values for the 2 diets fed. All dried preparations failed to describe the fresh situation accurately. The immediate solubility and potential degradation for both the DM and CP situations were significantly different ($P < 0.05$) between treatments. Although the rates for the dried treatments were similar to the fresh for the DM situation in experiment 2, this response was not duplicated in experiment 1 for the CP situation.

Table 1. Degradability coefficients[A] of white clover dry matter and crude protein for experiments 1 and 2

Sample preparation	Dry matter			Crude protein		
	A	B	C	A	B	C
Experiment 1						
Fresh clover (1 cm)	18.9^{b}	72.9^{a}	7.2^{a}	14.8^{c}	79.4^{a}	8.4^{a}
Freeze-dried	28.6^{a}	61.6^{b}	5.9^{b}	20.4^{b}	72.7^{b}	5.2^{b}
Oven-dried(45°C)	29.0^{a}	54.4^{c}	6.0^{b}	35.9^{a}	50.2^{c}	6.4^{b}
Oven-dried (60°C)	27.7^{a}	64.2^{b}	4.9^{b}	18.7^{b}	77.0^{ab}	4.1^{c}
S.E.D	2.03	2.71	0.65	2.04	2.57	0.64
Significance	< 0.001	< 0.001	0.03	< 0.001	< 0.001	< 0.001
Experiment 2						
Fresh clover (1 cm)	17.9^{c}	68.1^{a}	9.1^{a}	14.5^{c}	72.9^{a}	13.8^{a}
Freeze-dried	34.7^{a}	51.7^{c}	9.6^{a}	42.8^{a}	45.2^{c}	12.3^{ab}
Oven-dried (45°C)	28.4^{b}	58.5^{b}	8.7^{a}	31.4^{b}	60.4^{b}	8.9^{b}
Oven-dried (60°C)	24.6^{b}	60.7^{b}	8.6^{a}	25.9^{b}	66.4^{ab}	6.3^{b}
S.E.D	2.80	3.60	1.62	4.40	4.26	2.55
Significance	< 0.001	0.003	0.91	< 0.001	< 0.001	0.038

[A]Degradability coefficients as described in equation 1, where A = readily soluble fraction (%), B = potentially degradable fraction (%) and C = fractional rate of degradation of B (%/hour).
Different superscripts denote significant differences within columns ($P < 0.05$).

Mastication released between 24-29% of the DM. Protein solubility of white clover was greater, in the order of 28-32% (Table 2).

Table 2. Mean solubility (%) of dry matter (DM) and crude protein (CP) of fresh white clover as released on mastication

Experiment	Date	No. of samples	Leaf (%)	CP offered (%)	DM solubility (%)	CP solubility (%)
3	Spring 92	7	44.0	22.8	23.8	27.6
4	Spring 93	15	58.8	24.9	29.0	32.1

DISCUSSION

All of the dried preparations used failed to accurately describe the DM and CP degradation patterns observed for the fresh white clover preparation. The immediate solubility and potential degradation for the dried preparations significantly varied to the fresh, with the immediate solubility for the fresh being consistently lower. The rate constant for the fresh was generally higher.

The fresh white clover preparation results indicate that the potential degradability and rate of degradation of white clover was higher for CP than for the DM for all experiments. These higher values are possibly due to the fact that herbage CP is generally degraded more rapidly than the fibre component. The CP had a lower immediate solubility to the DM possibly due to a delay in the release of some proteins.

The basal diet fed was found to have a large influence on the rates of degradation of white clover. Hence, comparisons between *in sacco* experimental results can only be made for similar diets, as suggested by AFRC (1992). These differences are possibly due to the pH effect on the rumen environment, and the protein solubility which may influence the microbial activity and degradative capabilities. The diet in experiment 2 was altered to that in experiment 1, as it gives a better indication of what happens in grazing cows. The feed level suggested by the AFRC (1992) technique was modified in all experiments and cows were fed to appetite *(ad libitum)* to maintain milk production.

Immediate solubilities were higher *in vivo* compared to estimates derived using the *in sacco* technique. This is possibly due to the higher cuticle damage experienced during mastication. The protein solubility of the plant appears to be a combined effect of the protein content of the plant, the proportion of soluble proteins to the more slowly degraded proteins, the non-protein N content and the degree of cuticle damage predisposing the cells to microbial colonisation and attack. It is important to stress that not all of the solubilised protein will necessarily be degraded. Fermentative degradation is not immediate; so that some of the soluble protein may conceivably escape rumen degradation (bypass), and be digested in the small intestine. This is particularly so in lactating cows fed clover where the rumen outflow rates exceed 200 L/day (Wanjaiya 1993) and the fractional outflow rates are in excess of 0.12%/hour. However, the fermentative degradation of the rapidly released protein must contribute significantly to the total amount of rumen fluid ammonia produced, and a high proportion of ammonia may result in the inefficient utilisation of dietary proteins.

CONCLUSION

Fresh samples of white clover had lower immediate solubilities, higher potential degradabilities and generally degraded at a faster rate than any of the dried samples. To accurately describe the degradation pattern of white clover, the immediate solubility, potential degradability and the rate at which the DM and CP are degraded must be considered. In this case, the fresh treatment was the only suitable method. Diets need to be identical if comparisons between experiments are to be made. Experiments 1 and 2 also indicate that with the fresh samples, protein degradation is faster for CP than for DM, since protein is more readily solubilised than the fibre. The immediate solubilities estimated *in sacco* for fresh samples were lower than those observed *in vivo*. This indicates that mastication is more effective than cutting to 1 cm lengths, in increasing initial solubility. Further, a high level of solubilisation is not necessarily accompanied by a high degradation in the rumen.

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