BRIEF COMMUNICATION: *In vitro* fermentation characteristics of ryegrass-white clover sward containing different proportions of chicory

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Introduction

Chicory (*Cichorum intybus*) is a deep-rooted grazeable forage used to overcome production and nutritive value shortfalls of perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) swards during summer drought periods in New Zealand (Waugh et al. 1998; Chapman et al. 2012). However, there is little and contrasting data on the proportion of chicory to be used in mixed swards to influence livestock production. For example, Golding et al. (2011) showed that feeding chicory in a mixed sward at 18–40% of total dry matter, increased lamb post-weaning liveweight gain (LWG) compared with a traditional ryegrass sward. On the other hand, Cheng et al. (2017) reported similar daily LWG for heifers offered a mixed sward of 50% chicory and 50% ryegrass-white clover or 100% perennial ryegrass-white clover. These contrasting results justify more research on the chicory proportion in mixed swards and its effect on animal performance.

Ammonia (NH_3) and volatile fatty acids (VFA) are the major products of rumen fermentation. Ammonia represents the main source of nitrogen (N) for rumen microbes, and VFA provides 70% of the energy for the host animal. Changes in rumen fermentation affect production and composition of the milk. Therefore, studying the effect of the proportion of chicory in perennial ryegrass-white clover sward on *in vitro* fermentation characteristics can help understand the effect of feeding chicory on nutritive value of the mix sward. The objective of this study was to evaluate the *in vitro* fermentation pattern of ryegrass-white clover-based sward containing different proportions of chicory.

Materials and methods

Herbage harvesting

Perennial ryegrass, white clover and chicory herbage were harvested from the Lincoln University Research Dairy Farm (LURDF), Lincoln, New Zealand (43º 38'S, 172º 27'E). Vegetative herbage was cut at approximately 5 cm above ground level of each sward, frozen at -20ºC, freeze-dried, and then ground through a 1 mm sieve (ZM200, Retsch).

Rumen fluid collection, preparation and in-vitro fermentations

Rumen fluid (approximately 4000 mL) was collected from four fistulated, lactating Friesian × Jersey-cross dairy cows that previously been grazing ryegrass-white clover sward at the LURDF. Collected rumen fluid was put into two preheated 2000 mL thermos bottles and rapidly transferred to the laboratory where it was blended and then filtered through four layers of cheesecloth into a pre-heated (39.5ºC) flask. The flask was continuously purged with CO_2.

Four fermentation jars from a commercial anaerobic incubator (DAISY II-200/220, ANKOM Technology Co. Ltd. NY, USA) were prepared containing 1596 mL of buffer solution. The method was conducted according to the operating instructions supplied by ANKOM (2013). The buffer was made by mixing two parts buffer solution; part A and part B in a 5:1 ratio to obtain a final solution of pH 6.8 at 39.5ºC. Buffer solution A contained KH_2PO_4 (10 g/L), $MgSO_4 \cdot 7H_2O$ (0.5 g/L), NaCl (0.5 g/L), $CaCl_2 \cdot 2H_2O$ (0.1 g/L), and urea (0.5 g/L); and buffer solution B contained Na_2CO_3 (15.0 g/L) and $Na_2S \cdot 9H_2O$ (1.0 g/L).

To prepare the artificial rumen liquor (buffer + rumen fluid) for the fermentations, 400 mL of the blended and strained rumen fluid was added to 1596 mL of the buffer in each fermentation jar. Over two runs, four freeze-dried substrate treatments (12 g each; 11.4 g DM) containing either 100% ryegrass-white clover (Ch0%); 25% chicory + 75% ryegrass-white clover (Ch25%); 50% chicory + 50% ryegrass-white clover (Ch50%); and 100% chicory (Ch100%) were randomly assigned to the four fermentation jars. Proportion of white clover was 10% of the total amount of ryegrass-white clover mixture in all treatments. The fermentations were then carried out at 39.5ºC in the DAISY II-200/220, using an approach described by Martinez et al. (2006).

Measurements and analysis

Herbages were sub-sampled (100 g) and analysed for chemical composition using wet chemistry. The degradability of acid detergent fibre (ADF) and neutral detergent fibre (NDF) of each herbage were determined following the method of Van Soest et al. (1991). Nitrogen content of herbage was determined using an Elementar (Variomax CN Analyser, Elementar Analysensysteme, Germany), and digestibility was determined *in vitro,* using the pepsin cellulase method (Clarke et al. 1982). Metabolisable energy of herbages was estimated based on the equation [ME (MJ/kg DM] = digestible OM content ×

0.016 (g/kg of DM)] (AFRC 1993).

The pH of the fermenting substrate was recorded using a benchtop pH meter (Orion 2-Star, Thermo Scientific, Beverly, USA). Fermentations were sampled (1.5 mL) after 4, 8, 12, 24, and 48 h of incubation for VFA and NH_3 analysis. Fermentation jars were flushed with CO_2 and re-sealed after each sampling. Samples for NH_3 determination were immediately acidified, and centrifuged at 3000g for 20 min at 4°C. The supernatants were then transferred into new tubes and frozen at -20°C until analysis. NH_3 concentration was determined by an enzymatic UV method, using a Randox ammonia kit and the Randox Rx Daytona analyser (United Kingdom) as described by Neeley and Phillipson (1988). For VFA analysis, 100 µl of the rumen supernatant was placed into a 2 ml Eppendorf tube and 20 µl of the internal standard and 40 µl of metaphosphoric acid were added. Samples were then diluted ten times with 50:50 acetone/water diluent. Concentrations of acetate, propionate and butyrate were then determined as described by Chen and Lifschitz (1989) using a Gas Chromatograph (GC: Shimadzu GC-2010, Japan) fitted with a SGE BP21 30 m × 530 µm × 1.0 µm wide-bore capillary column.

Statistical Analysis

GenStat 16 (Lawes Agricultural Trust, Rothamsted, UK) was used for analysis. All measurements were analysed using repeated-measure ANOVA with the chicory proportion as treatment, run as replicate and sampling time as time effect. Results were declared significant at P<0. 05.

Results and discussion

Chemical composition of herbage is presented in Table 1. Herbage quality was typical of high-quality herbage in New Zealand (Burke et al. 2000; Litherland & Lambert 2007). Increases in herbage nutritive value are associated with greater DM intake and animal production. In this study, perennial ryegrass-white clover sward with increasing proportions of chicory were fermented *in vitro* in order to determine potential changes in the rumen fermentation pattern *in vivo*. There was no effect of chicory treatment on *in vitro* pH value, NH_3 or VFA concentration (Table 2). This result suggests a potential similarity in the nutrients supplied by swards containing increasing proportions of chicory as compared to ryegrass-white clover. Cheng et al. (2017) reported similar LWG of heifers offered either a mixed sward of 50% chicory and 50% ryegrass-white clover or 100% perennial ryegrass-white clover. Similarily, Muir et al. (2014) reported similar milk production of cows fed either a mixed sward of 50% chicory and 50% ryegrass-white clover or ryegrass-white clover. In contrast, Kusmartono et al. (1996) reported greater LWG by deer eating chicory than those eating ryegrass-white clover sward. However, the difference in LWG in the latter study was a result of greater DM intake for deer grazing chicory than those grazing ryegrass-white clover sward.

Burke et al. (2006) reported greater VFA yield from chicory compared to ryegrass after 24 hours *in vitro* incubation. Despite the lack of a significant effect in our study, concentration of total VFA (mmol/l) was 27% greater for Ch100% than Ch0%. The low number of replicates (two replicates) in this study may have contributed to the non-significant effect of chicory treatment. In addition, a high quality sward of ryegrass-white clover (ME = 12.5 MJ/kg DM and CP = 24% DM) was used in the current study compared to New Zealand herbage (ME range from 10-12 MJ/kg DM and CP range from 17-27% DM; Litherland & Lambert 2007), which may have limited the advantages of incorporating chicory in such a sward. These advantages could be more significant if a base-sward of lower nutritive quality was used. Our preliminary results suggest that the fermentation pattern of herbage containing increasing proportions of chicory is similar to 'conventional' ryegrass-white clover, thus including chicory in conventional swards might not alter the nutritive value of herbage.

Table 1 Chemical composition (g/kg DM) and metabolisable energy (MJ/kg DM) of ryegrass-white clover and chicory herbage

Item	Ryegrass-white clover	Chicory
Dry organic matter	89.4	93.2
Dry matter digestibility	83.3	85.4
Acid detergent fibre	23.7	16.6
Neutral detergent fibre	41.0	19.1
Crude protein	24.0	23.9
Metabolisable energy	12.5	12.2

Table 2 The *in vitro* fermentation characteristics of ryegrass-white clover herbage containing different proportions of chicory

Item	Substrates[1]				SEM	P-value		
	Ch0%	Ch25%	Ch50%	Ch100%		Trt.	Time	Trt. x Time
pH	6.5	6.5	6.5	6.5	0.02	0.69	0.01	0.89
NH_3 (mmol/l)	15.1	17.7	20.4	23.3	1.98	0.14	0.07	0.08
Total VFA (mmol/l)	24.5	27.2	29.8	33.4	5.14	0.68	0.45	0.82
Propionate (mmol/l)	4.4	5.0	5.6	6.2	1.12	0.73	0.33	0.69
Acetate (mmol/l)	17.4	19.0	20.8	23.5	3.37	0.65	0.52	0.83
Butyrate (mmol/l)	2.7	3.1	3.5	3.8	0.66	0.72	0.36	0.72
Acetate:propionate ratio	4.0	3.8	3.7	3.8	0.20	0.81	0.53	0.11

[1]Substrates: Ch0 = 100% ryegrass-white clover; Ch25% = 25% chicory + 75% ryegrass-white clover; Ch50% = 50% chicory + 50% ryegrass-white clover; Ch100% = 100% chicory.

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