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Fermentation in the rumen of sheep fed *Atriplex nummularia* cv. De Kock supplemented with incremental levels of barley and maize grain

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Abstract

The aim of this study was to investigate the effect of two carbohydrate sources, barley and maize, on the ruminal fermentation of *Atriplex nummularia* cv. De Kock fed to sheep. Barley was used as a source of rapid fermentable carbohydrates while maize was used as a moderately fermentable source. Ten rumen cannulated Merino wethers were fed different increments of ground maize and barley supplements (0%, 15%, 30%, and 45% of the ration dry matter to a basal diet of *A. nummularia* over four different periods. Supplementation of *A. nummularia* with energy tended to increase ruminal fermentation. Rumen ammonia nitrogen (NH_3-N) decreased at the 30% level of supplementation, though an increase in rumen NH_3-N concentration was observed for maize at the 45% level. This decrease might have been due to an improvement in microbial protein synthesis. Total rumen volatile fatty acids increased with increasing levels of both barley and maize supplementation. Evidence of the increase in volatile fatty acid production was supported by a decrease in ruminal pH values, with the barley at 45% level of supplementation yielding the lowest rumen pH values. Supplementing sheep with barely and maize at 30% level increased fermentation in the rumen of sheep fed on *A. nummularia* and enhanced utilisation of degradable protein available in the rumen.

Keywords: *Atriplex*, carbohydrates, fermentability, foliage, leaves
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Introduction

Atriplex nummularia has shown promise for reclamation of degraded rangelands. This species has proven to be particularly well adapted to areas that receive low annual rainfalls (Van Niekerk *et al*., 2004). In terms of nutritive value, *A. nummularia* leaves have an energy concentration of *ca*. 6.1 MJ ME/kg dry matter (DM) and a crude protein (CP) concentration of 176 - 234 g/kg DM (Van Niekerk *et al*., 2004). The high CP content of *A. nummularia* could be misleading since its CP is extensively degraded to ammonia in the rumen (Weston *et al*., 1970). Consequently, the available energy from *A. nummularia* is insufficient for the efficient utilization of the ammonia by rumen microorganisms. Increasing nutrient availability for higher levels of microbial growth, digestion and subsequently ruminant production can be achieved by one or more of the following strategies: Increasing the quantity of dry matter intake, improving the ratio of rumen fermentation end-products or supplementing by-pass nutrients for subsequent absorption in the small intestine (McCarthy *et al.*, 1989). Generally carbohydrates and proteins are the major nutrients supporting microbial growth, and the possible energy deficiency in *A. nummularia* would likely be manifested in terms of inefficient rumen fermentation and subsequent poor animal production (Hoover & Stokes, 1991). Supplementing *Atriplex* material with carbohydrate sources may rectify this. However, digestion of fibre decreases when the amount and proportion of readily fermentable carbohydrate sources in the rumen increase beyond an optimal level (Hoover, 1986). The aim of this study was to quantify the influence of source (high and medium fermentability) and level of carbohydrate supplementation on ruminal fermentation of sheep fed *A. nummularia* cv. De Kock diets.

Materials and Methods

Ten adult Merino wethers (43 ± 14.3 kg body weight) fitted with ruminal cannulae were randomly allocated to two groups of five animals. Each group received a diet consisting of sun-dried *A. nummularia* supplemented with one of the two carbohydrate sources, maize (moderate fermentability) or barley (high fermentability), each at four levels (0%, 15%, 30% and 45%/kg DM of the diet) of supplementation. The carbohydrate sources were finely ground through a 1 mm sieve and fed with *A. nummularia* as a total mixed ration on an *ad libitum* basis. The sheep were housed in individual metabolism cages. The experimental animals were adapted to their diets for 14 days prior to a 10 day collection period. The experiment was carried out in four sequential periods. Diets were allocated randomly to each of the four experimental periods. Within each period five animals received the same diet in the following order of supplementation: 0%, 30%, 15% and 45%. Between two consecutive experimental periods animals were fed a lucerne diet for a period of 15 days to avoid residual effects from the preceding diet.

At the end of a collection period individual rumen fermentation parameters were determined on eight rumen fluid samples collected over a 24 h period at 5:00, 7:00, 10:00, 14:00, 16:00, 22:00, 2:00 and 5:00 the following day. Rumen fluid samples were collected as described by De Visser *et al*. (1992). Immediately after collection the pH was measured, using an electronic pH meter. Samples for ammonia nitrogen (NH_3-N) analysis (10 mL rumen fluid) were preserved with 2 mL of a 0.5 M H_2SO_4 solution and stored at -10°C. Samples for volatile fatty acid (VFA) analysis (10 mL rumen fluid) were preserved with a 1 mL 10% NaOH solution and stored at –10 °C. The NH_3-N concentration of rumen fluid was determined using a Technicon autoanalyser, according to the AOAC (2000). A modified derivatization technique of Puttman *et al*. (1993) for high performance liquid chromatography (HPLC) and a UV detector were used for the analysis of VFA in the fluid.

All parameters measured were analysed using one-way analysis of variance by the PROC GLM of SAS (2001). It is assumed that there will be no difference between the experimental periods (except treatment effect) due to simultaneous harvesting and drying of the *A. nummularia* used in the experimental diet. Where treatment effect was significant, differences between means were determined by using Bonferrani’s test (Samuels, 1989).

Results and Discussion

The supplementation of *A. nummularia* with maize appeared to have increased the DM intake compared to the control, but the difference was statistically significant ($P < 0.05$) only at the 45% supplementation level (Table 1). In the barley supplemented groups, intakes did not differ significantly from that of the control. The effect of carbohydrate source (barley *vs*. maize) on rumen pH value was not significant ($P > 0.05$), while with both sources rumen pH values decreased with increasing levels of supplementation above the 15% level (Table 1). This agrees with Overton *et al*. (1995) who reported a decrease in pH as the amount of readily fermentable carbohydrate in the diet increased. According to Hoover & Stokes (1991) pH greatly modifies microbial growth efficiency and nutrient digestion. Overton *et al*. (1995) reported a decrease in fibre degradation because of a decrease in pH. Similarly, Du Toit *et al*. (2004) reported a lower level of neutral detergent fibre digestibility of *A. nummularia* with an increasing level of supplementation of maize and barley due to negative associative effects. It is likely that the low ruminal fluid pH decreased cellulolytic activity of the bacteria and depressed ruminal fibre degradation of the diets (Ben-Ghedalia *et al*., 1989). However, the extent to which rumen cellulolysis is inhibited varies with the type and level of carbohydrate and nitrogen sources (Hoover & Stokes, 1991). Furthermore, the inhibition of fibre degradation could be partially alleviated if rumen pH was maintained at a level of 6.7, normally associated with the fermentation of an all roughage diet (Mould *et al*., 1983). In the present study the pH decreased from 6.98 to 6.10 for the maize and from 7.05 to 5.94 for the barley supplement. Except for 45% barley supplemented treatment, the pH values recorded were higher than the pH optima for protozoan proteases (3 to 4.5), but it was within the range of pH optima for bacteria (6.0 and 7.0) (Kopecny & Wallace, 1982; Ben-Ghedalia *et al*., 1989). The lack of significant differences between barley and maize, in terms of rumen pH values, contradicts with De Visser *et al*. (1992), who reported higher rates of degradability (%/h) of barley starch compared to that of maize starch (25.8 *vs*. 8.7%).

Rumen NH_3-N concentration of the control sheep ranged between 7.23 - 7.28 mg/100 mL (Table 1). This is relatively higher than rumen NH_3-N concentrations of 5 mg/100 mL reported by Satter & Slyter (1974) as a minimal concentration for optimum microbial protein synthesis. The relatively higher level was probably due to the high proportion of non-protein nitrogen (NPN) in *Atriplex* (470 g/kg) (Benjamin *et al*., 1995), which is highly soluble in the rumen (Weston *et al*., 1970). The high level of rumen NH_3-N concentration (8.12 and 7.90 mg/100 mL) recorded at the 15% supplementation level suggested that this level did not supply enough fermentable energy to the rumen microorganisms to make sufficient use of the available NH_3-N (McDonald *et al.,* 2002). Compared to the rumen NH_3-N concentration of at 15% supplementation level, lower levels of 4.68 and 5.24 mg/100 mL were recorded at the 30% supplementation level for maize and barley, respectively. These reduced ammonia levels could be due to a more efficient N-utilization by rumen microbes when more fermentable energy was available (Casper *et al*., 1999). These authors also reported that increased rumen liquid volumes could play a role in the reduction of NH_3-N concentrations. The significant difference in rumen NH_3-N concentration between maize and barley at the 45% level of supplementation cannot be explained and this could be due to an error.

Table 1 Mean (± s.e.) dry matter intake, rumen pH, rumen ammonia-N (NH_3-N) and total volatile fatty acids of sheep fed *Atriplex nummularia* supplemented with two carbohydrate sources at different levels

Parameter	Supplementation level (%)	Energy source	
		Maize	Barley
Intake/kg W (g/day)	0	23.46_1^{b} ±3.7	32.2_1^{ab} ±3.3
	15	23.58_1^{b} ±3.3	21.2_1^{b} ±3.7
	30	33.51_1^{ab}±3.3	37.9_1^{a} ±3.3
	45	38.63_1^{a} ±3.3	25.7_2^{b} ±3.7
pH (H_2O)	0	6.98^{a} ±0.11	7.05^{a} ±0.10
	15	6.77^{ab} ±0.10	6.75^{ab} ±011
	30	6.50^{bc} ±0.11	6.53^{b}±0.10
	45	6.10^{c} ±0.10	5.94^{c} ±0.10
NH_3-N (mg/100 mL)	0	7.28^{a} ±0.54	7.23^{a} ±0.54
	15	8.12^{a} ±0.41	7.90^{a} ±0.46
	30	4.68^{b} ±0.46	5.24^{b} ±0.41
	45	8.36_1^{a} ±0.41	5.26_2^{b} ±0.54
Total volatile fatty acids (mmol/100 mL)	0	12.89^{c} ±1.30	11.09^{c} ±1.30
	15	21.55^{b} ±1.30	22.45^{b} ±1.30
	30	25.42^{b} ±1.30	23.32^{b} ±1.30
	45	32.49^{a} ±1.30	30.71^{a} ±1.30

Means within columns (a,b,c) and rows (1,2) with different scripts differ significantly at $P < 0.05$

Significant differences were found in terms of total VFA concentrations between the non-supplemented and carbohydrate supplementation groups (Table 1). In all supplemented diets total VFA concentration increased with increasing levels of supplementation. In this study, the differences between barley and maize in terms of total VFA concentration were not significant ($P > 0.05$). This is contrary to previous findings that reported higher VFA concentrations for cows fed a barley-based diet, which correlated well with the higher ruminal starch digestibility of barley compared to cows fed a maize-based diet (McCarthy *et al*., 1989). One possible reason for this could be higher ruminal liquid volumes in the barley-fed animals that would have a diluting effect on VFA concentrations (Casper *et al*., 1999). The reason for the increase in ruminal fluid volumes when barley supplemented diets were fed is not clear, but it could possibly be related to non-structural carbohydrate solubility or the degradability of barley compared to maize. The lower VFA concentration for barley supplemented animals (Casper *et al*., 1999) could possibly be as a result of increased absorption from the rumen due to the lower ruminal pH of the barley supplemented animals (Lana *et al*., 1998).

Conclusions

Supplementing animals with barely and maize at 30% level enhanced the ruminal fermentation environment of sheep fed on *A. nummularia* without decreasing the rumen pH below the critical level that sustains optimum fibre digestion. Higher grain supplementation levels improve rumen fermentation and the utilisation of degradable protein in the rumen as indicated by the increase in total fatty acids and reduction of NH_3-N concentrations in the rumen.

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