Effects of high potassium on *in vitro* fermentation pattern and C18 fatty acid metabolism of pasture grass

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ABSTRACT

The effect of high potassium concentration on rumen fermentation and fatty acid biohydrogenation (BH) were investigated in a 6 hour *in vitro* incubation. A pasture grass sample with 26.3 g/kg dry matter (DM) of potassium was used as control. Supplemental levels of 10, 20 and 30 g/kg DM of potassium were added as potassium chloride. Results showed that high potassium concentration had no significant effects ($P > 0.05$) on pH, molar concentrations of total or individual volatile fatty acids (VFA), concentrations of $C_{18:0}$, $C_{18:1\ trans\ 11}$, $C_{18:1\ cis\ 9}$, $C_{18:2n-6}$, or BH of $C_{18:2n-6}$ after incubation. However, concentration and BH of $C_{18:3\ n-3}$ were significantly ($P < 0.01$) affected by potassium supplementation. Both the 20 and 30 g/kg DM supplementation significantly ($P < 0.05$ and $P < 0.01$, respectively) decreased the concentration but increased BH of $C_{18:3n-3}$. This preliminary study indicates that high concentrations of potassium alter rumen fatty acid metabolism. Further studies are needed to confirm this and investigate the possible causal mechanisms.

K**eywords:** potassium; rumen fermentation; volatile fatty acids; C18 fatty acids.

INTRODUCTION

It is well known that pasture in areas with intensive livestock production is generally rich in potassium due to frequent fertilization (Fisher *et al.*, 1994; Schonewille *et al.*, 1997). Concentrations of potassium commonly exceed 35 g/kg dry matter (DM) in fresh grass (Jittakhot *et al*., 2004), which is approximately 2.5 fold higher than in total mixed ration (TMR) diets (Kolver & Muller, 1998). Other research has demonstrated that elevated dietary potassium increased rumen pH (Jittakhot *et al*., 2004; Khorasni & Armstrong, 1990) and altered rumen fermentation patterns (Spears & Harvey, 1987). Potassium levels can also alter the activity and survival of some strains of rumen microorganisms under some circumstances. For example, Dawson and Boling (1984) showed that the minimum concentration of the selective growth inhibitor lasalocid required to inhibit growth of different strains of *Bacteriodes ruminicola* was 8 to 64 times greater when the bacteria were grown in a high-potassium medium compared with a low potassium medium. Rumpler *et al*. (1986) reported that increasing dietary potassium to 25 g/kg DM increased methanogenesis by 8.8% in steers fed lasalocid.

Ruminal pH was also reported to be lower than 6.2 for cows fed pasture-based diets and might be as low as 5.8 for grazing cows (Kolver & de Veth, 2002). Decreasing rumen pH has been related to reduced milk fat percentage (Allen, 1997). However, only half the reduction of milk fat percentage was observed in pasture based dairy cows compared to TMR based cows when rumen pH decreased over the same range (from 6.5 to 5.8; Kolver & de Veth, 2002). Therefore, Kay *et al*. (2005) proposed that some factors additional to ruminal pH and dietary fatty acid content may influence milk fat synthesis in pasture based cows. The rumen factors that result in the specific milk fatty acid composition in pasture based cows are as yet undefined, and there is no existing research on the effect of high potassium concentrations on rumen lipid metabolism. This study was designed to investigate the effects of high potassium concentration on the ruminal *in vitro* fermentation pattern and fatty acid composition.

MATERIALS AND METHODS

Experimental design

An *in vitro* batch culture experiment was conducted to examine the effect of high potassium on fermentation pattern, $C_{18:2\ n-6}$ and $C_{18:3\ n-3}$ biohydrogenation (BH) and C18 fatty acid intermediates accumulation. A ryegrass and clover pasture was obtained from the Lincoln University Dairy Farm, Canterbury. The sample used (Table 1) was of mid-range crude protein (CP) in order to maximise the effect of potassium supplementation, as potassium content was hypothesised to be positively related to CP. This sample was freeze dried and supplemented with either 0 (control), 10, 20 or 30 g/kg DM of potassium by the addition of potassium chloride, and triplicates were used for each supplemental level.

TABLE 1: Chemical and fatty acid composition of the pasture sample. DM = Dry matter.

Component	Composition (g/kg DM)
Organic matter	900
Crude protein	169
Neutral detergent fibre	364
Acid detergent fibre	190
Water soluble carbohydrate	291
Potassium	26.3
Fatty acids:	
$C_{12:0}$	0.1
$C_{14:0}$	0.73
$C_{16:0}$	6.35
$C_{16:1\ cis-9}$	0.08
$C_{18:0}$	0.44
$C_{18:1\ cis-9}$	0.78
$C_{18:2\ n-6}$	8.44
$C_{18:3\ n-3}$	25.3

In vitro incubation and sampling

One gram of grass sample with, and without, potassium was incubated with 60 mL incubation media (buffer solution and rumen fluid, 2:1, v/v) at 39°C for 6 hours. The buffer solution was made up according to the method of Lee *et al*. (2007). Rumen fluid was obtained from a ruminally cannulated cow grazing pasture at 07:30 h. One litre of rumen fluid was hand-squeezed into thermos flask pre-warmed to 39°C and transferred immediately to the laboratory, mixed in a pre-warmed blender at high speed for 30 seconds and then strained through four layers of cheesecloth into a pre-warmed beaker, and mixed with pre-warmed buffer solution by a magnetic stirrer at 39°C under anaerobic conditions. Zero hour incubations were freeze-dried for fatty acid analysis and BH calculation.

After 6 hours incubation, pH was measured immediately and fermentation was stopped using iced water. The incubation bottles were shaken and mixed thoroughly, 10 mL of contents were removed for volatile fatty acid (VFA) analysis and the bottles were then frozen. Samples for VFA were centrifuged at 13,000 rpm for 15 min, 5 mL of supernatant was mixed with 1 mL of deproteinizing solution (25 % metaphosphoric acid) and stored at -20°C until analysis. The remaining pellets were frozen, freeze-dried together with the contents in the bottles, weighed, and kept at -20°C until fatty acid analysis.

Chemical analysis

Composition of grass samples were analysed by near infrared reflectance spectroscopy (Foss Systems 3000-1, Hillerød, Denmark) and potassium was measured by flame atomic absorption spectrophotometry (GBC Avanta, Australia) (Table 1). VFA concentrations were determined by gas chromatography (GC-HP 6890, USA; column: 25 m x 530 µm x 0.5 µm, SGE BP21, wide-bore capillary column).

Fatty acid in substrates and residues of incubation were methylated with 4 ml of 0.5 mol/L sodium methoxide for 15 min at 50°C followed by an 4 mL of 5% HCl / methanol for one hour at 50°C as described by Kramer *et al*. (2001). Separation and quantification of the fatty acid methyl esters (FAME) were achieved by gas chromatograph (GC-HP6890,USA) with a fused silica capillary column (100 m, 0.25 mm×0.25 µm). Helium was used as carrier gas. The temperature programme used as described by Ribeiro *et al*. (2007), which was reported to be optimized to separate most of the $C_{18:1}$ fatty acids in the first isothermal range. Detector and injector temperatures were 260°C, and the split ratio was 80:1. Oven

TABLE 2: The pH and molar concentration (mM) of volatile fatty acids (VFA) after incubation of grass with or without potassium supplementation. DM = Dry matter, SEM = Standard error of mean.

Component	Potassium supplemental level (g/kg DM)				SEM	Significance
	Control (0)	10	20	30		
pH	6.73	6.72	6.75	6.79	0.01	NS
Total VFA	52.0	52.4	52.1	51.8	0.5	NS
Individual VFA						
Acetate	33.2	33.5	33.4	32.8	0.3	NS
Propionate	13.2	13.2	13.0	13.4	0.2	NS
Butyrate	3.99	3.96	3.94	3.98	0.05	NS
iso-Butyrate	0.41	0.44	0.42	0.43	0.02	NS
Valerate	0.16	0.15	0.16	0.16	0.01	NS
iso-Valerate	0.28	0.29	0.29	0.28	0.01	NS
Succinate	0.61	0.62	0.69	0.46	0.05	NS
Hexanoate	0.18	0.20	0.19	0.19	0.01	NS
Acetate:propionate	2.51	2.55	2.56	2.44	0.03	NS

temperature was 166°C for 39 min, increased by 10.0°C/min to 240°C, held for 10 min, increased by 3.0°C/min to 245°C, and held for 10 min. Heneicosanoic acid methyl ester ($C_{21:0}$) was used as internal standards and ME 61 and $C_{18:1trans\ 11}$ and CLA methyl esters were used as external standard to identify the fatty acids.

Statistical analyses

Estimation of fatty acid BH was based on the disappearance of the unsaturated fatty acid, calculated as the proportional loss of fatty acid from the initial time point to the incubation end point. Total weights of residues after incubation were estimated by summing up the freeze-dried weights of contents in the incubation bottles and those pellets from VFA samples. One-way analysis of variance procedure of SPSS 15.0 was used for all statistical analyses.

RESULTS

pH and VFA concentration

The pH after incubation was 6.73 in the control, and increased slightly with 20 and 30 g/kg DM potassium supplementation, but no statistical difference ($P > 0.05$) was observed (Table 2). Neither molar concentration of total, individual VFA, nor acetate to propionate ratio was altered ($P > 0.05$) by potassium supplementation (Table 2).

Fatty acid concentration and BH

Potassium supplementation did not alter ($P > 0.05$) concentration of $C_{18:0}$, $C_{18:1trans\ 11}$, $C_{18:1\ cis9}$ or $C_{18:2n-6}$ after incubation, or BH of $C_{18:2n-6}$ (Table 3). However, concentration of $C_{18:3n-3}$ decreased with potassium supplementation ($P < 0.01$). Compared with control, 20 g/kg DM potassium supplementation significantly decreased $C_{18:3n-3}$ concentration ($P < 0.05$), and a further decrease was observed by 30 g/kg DM supplementation ($P < 0.01$), which was also different ($P < 0.05$) to 10 g/kg DM supplementation. Compared with the control and 20 g/kg DM supplementation, 10 g/kg DM potassium supplementation had no effect ($P > 0.05$) on concentration of $C_{18:3n-3}$. As a result, BH of $C_{18:3n-3}$ increased significantly ($P < 0.01$) with potassium supplementation, which was affected similarly to that of $C_{18:3n-3}$ concentration.

TABLE 3: Major C18 fatty acid concentrations (FAC) (mg/incubator) and biohydrogenation (BH) (%) of $C_{18:2n-6}$ and $C_{18:3n-3}$ of grass after incubation with or without potassium supplementation. DM = Dry matter, SEM = Standard error of mean.

Component	Potassium supplemental level (g/kg DM)				SEM	Significance
	Control (0)	10	20	30		
C18 fatty acid concentration (mg/incubation)						
$C_{18:0}$	1.07	1.07	1.11	1.06	0.02	NS
$C_{18:1trans\ 11}$	0.31	0.31	0.34	0.33	0.01	NS
$C_{18:1cis9}$	0.55	0.58	0.60	0.62	0.06	NS
$C_{18:2n-6}$	3.50	3.51	3.54	3.56	0.08	NS
$C_{18:3n-3}$	5.46^{Aa}	5.36^{Aab}	5.00^{ABbc}	4.67^{Bc}	0.11	**
Biohydrogenation (%)						
C18:2n-6	60.0	60.0	59.6	59.3	0.9	NS
C18:3n-3	78.6^{Aa}	79.0^{Aab}	80.3^{ABbc}	81.7^{Bc}	0.4	**

Different letters within the same row indicate significant differences among treatments (Capital letters, $P < 0.01$; Small letters, $P < 0.05$).

DISCUSSION

This is the first study to investigate the effect of high potassium concentration on rumen fatty acid metabolism. These results showed that BH of $C_{18:3n-3}$ increased significantly ($P < 0.01$) with increasing potassium content, suggesting some influence on rumen fat metabolism. However, neither BH of $C_{18:2n-6}$ or total concentrations of C_{18} fatty acids were affected.

Several studies have investigated the effects of increasing dietary potassium on *in vivo* rumen pH and fermentation. Jittakhot *et al.* (2004) showed that rumen pH was positively correlated with rumen potassium concentrations, and Khorasni and Armstrong (1990) reported that both rumen contents pH and total ruminal VFA concentration were significantly ($P < 0.05$) increased by increasing dietary potassium from 6.5 g/kg DM to 35 g/kg DM when sheep were fed a semi-purified diet. Grings and Males (1987) observed that ruminal molar proportions of acetate increased linearly with increasing dietary potassium level, while molar proportions of butyrate ($P < 0.01$) and valerate decreased linearly. However, in the present study, increasing potassium concentration of pasture grass from 26 g/kg DM to 56 g/kg DM only increased pH, and did not influence either total or individual VFA concentrations.

The potassium salt used in this study was potassium chloride, which has a lower buffering capacity than potassium bicarbonate used by Jittakhot *et al.* (2004) or Khorasni and Armstrong (1990), which may have had an influence on the pH recorded in

this study. Furthermore, the potassium chloride buffering capacity in this study could have been influenced by the relatively high pH after incubation of 6.7, which is toward the upper limit reported *in vivo* in pasture fed cows (Kolver & de Veth, 2002), with the result being a reduced effect on *in vitro* fermentation.

Potassium content in pasture sample used was 26.3 g/kg DM, which is lower than the mean level (37.4 g/kg DM) of pasture reported by Roche *et al.* (2005). This difference may be related to the lower content of CP in our sample (169 g/kg DM, Table 1) compared to 286 g/kg DM in the study of Roche *et al*. (2005).

In conclusion, this preliminary study demonstrated that high potassium concentration did influence fatty acid BH, however, further research is required to determine the specific processes altered by increased dietary potassium, the effect at a range of pH and the possible mechanisms of action.

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