Supplemental fumarate has little effect on the detailed composition of bovine milk during early lactation

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ABSTRACT

Future farming practices need to be assessed by the dairy industry for any impacts they may have on the detailed composition and processability of milk. These impacts may change the milk price a farmer receives and the milk processability, product range and product quality. The use of sodium fumarate (an organic acid) to reduce methane production from dairy cows has previously been reported. This experiment investigated the effect of intra-ruminal infusions of sodium fumarate on the detailed composition of milk from dairy cows. Fumarate (5% of daily dry matter intake) was infused into 8 lactating cows while 8 cows received infusions of water as a control. Milk samples were collected during a covariate period, then again following a 16-day treatment period, of which cows were housed indoors for the last 9 days. Gross composition of milk and other production parameters have been presented previously. Milk yield, fat and protein were not affected but lactose concentrations were significantly higher in the milk of cows receiving the fumarate. In the current study, none of the individual casein proteins measured showed an effect of treatment. Of the whey proteins examined, concentrations of α-lactalbumin (α-La) were higher (1.07 *vs*. 0.98 mg/mL; sed 0.038; $P < 0.05$) and bovine serum albumin (BSA) concentrations were lower (214.6 *vs*. 251.0 mg/L; sed 15.1; $P < 0.05$) in the milk of cows receiving fumarate. None of the individual fatty acids or minerals examined showed a treatment effect. The addition of sodium fumarate to the diet of dairy cows in early lactation does not have a major influence on the composition of milk, suggesting that the product could be used with minimal impact on milk composition and product quality at this early stage of lactation.

Keywords: fumarate; bovine milk; milk composition; milk quality

INTRODUCTION

Dairy farm management practices, including nutritional modification, can result in changes in both the volume and composition of milk (Thomson, 1999; Kay *et al.*, 2002; Turner *et al.*, 2005). Thus an understanding should be developed of the effect new management practices have on milk characteristics. This information can be used by farmers who are interested in the impact of the new technology on the milk payout, and by milk processors to assess the effect these technologies have on the quantity and quality of manufactured products. Any changes in the concentrations of minor component bioactives, which are currently sought after by nutriceutical markets, could also be determined, providing an avenue for the future production of higher concentrations.

A nutritional modification that has received recent study has been dicarboxylic organic acids. Malate and fumarate, which naturally occur in grasses and legumes, have been proposed as a method of methane mitigation (Callaway & Martin, 1996). Mode of action is principally through altered ruminal fermentation, by providing an alternative electron sink for hydrogen following the reduction of fumarate to succinate in the succinate-propionate pathway of ruminal bacteria (Martin, 1998). As well as a reduction in methane, ruminal concentrations of propionate have been shown to be significantly elevated when diets based on grass (Kolver *et al.*, 2004) and grain (Callaway & Martin, 1996) have been fermented with fumarate *in vitro*. These changes in ruminal metabolism would be expected to have subsequent effects on milk composition. An increased supply of propionate has been reported to increase the yield, content, and composition of milk protein (Raggio *et al.*, 2006). The fatty acid profile of milk could also be expected to change as a result of elevated propionate supply, with the synthesis of linear odd-chain fatty acids (C15:0 and C17:0) being elevated (Vlaeminck *et al.*, 2006).

This study tested whether the addition of fumarate to the diet of dairy cows fed pasture increased milk protein content, and modified the composition of milk protein and fatty acids.

MATERIALS AND METHODS

Experimental design and animal measurements

This study was conducted as part of a larger experiment investigating whether supplementing dairy cows with sodium fumarate reduced methane emissions and increased milk production (Kolver & Aspin, 2006). Briefly, sixteen ruminally

fistulated Friesian or Friesian cross dairy cows (100 ± 20.7 days in milk; DIM) were offered *ad libitum* a diet of fresh pasture with either no supplemental fumarate (control, n=8) or supplemental sodium fumarate at 5% of DM intake (n=8, 98.7% sodium fumarate dibasic anhydrous $C_4H_2O_4Na_2$; Sulkem Company Ltd., Auckland, New Zealand; 931 ± 126 g/cow/day). The full study included 3 sequential periods: Days 1-4 during which cows grazed as one herd and received no treatment (covariate period); days 5-11 during which cows grazed pasture at a high pasture allowance (>50 kg DM/cow/day) as one herd and received treatment (adjustment period); and days 12-20 in which cows received treatment and were housed in metabolism stalls (experimental period). Fumarate was administered in solution (90 g/L) with a total of 10 L/cow/day administered three times daily (3.33 L/cow/dose) at 0730, 1200, and 1630 h via the rumen fistula during the adjustment period, or continuously infused into the rumen (10.3 ± 1.18L/cow/day as a solution to provide 931 ± 126g sodium fumarate/cow/day) during the experimental period. Control cows were infused with an equivalent volume of water during the adjustment and experimental periods.

For the purposes of the current study, individual milk samples were collected using in-line milk meters from a consecutive PM and AM milking during the covariate period (d 2 PM + d 3 AM), and again during the experimental period (d 18 PM + d 19 AM). Milk samples were combined to give one 'daily' sample per cow at each sampling occasion. In comparison, the data presented by Kolver & Aspin, (2006) are from measurements made throughout the entire experimental period (d12-20).

Milk sample analyses

All milk samples were analysed for gross composition (fat, crude and true protein, casein, lactose and total solids) using an infrared milk analyser (FT120; Foss Electric, Hillerød, Denmark), with additional milk samples analysed by reference procedures as described by Turner *et al.* (2006). Milk pH was measured using a CyberScan 510 pH meter (Eutech Instruments Pte Ltd, Singapore).

Lactoferrin (Lf) concentrations were measured using a bovine Lf ELISA quantification kit (Bethyl Laboratories. Inc, Montgomery, TX, USA) as described by Turner *et al.* (2003).

Lactoperoxidase activity of the milk samples was assayed as described by Turner *et al.* (2005).

Urea concentrations in the milk were measured using a kinetic UV assay (Alpha Scientific, Hamilton, New Zealand) and citrate concentrations by UV spectrophotometry (Dagley, 1974).

Bovine serum albumin (BSA) and immunoglobulin-G (IgG) concentrations were determined using radial immunodiffusion kits Albumin 'NL' and IgG 'NL', respectively (The Binding Site Ltd, Birmingham, UK) as described by Turner *et al.* (2005)

Concentrations of individual casein (CN; α, β and κ-CN) and whey (α-lactalbumin; α-La, β-lactoglobulin; β-LG) proteins were determined by HPLC analyses at AgResearch Grasslands (Palmerston North, New Zealand) as follows: Liquid milk samples (200 µL) were added to 600 µL 6M guanidine-HCL, 0.1 M bis-Tris, 5.37 mM trisodium citrate and 1.95 mM dithiothreitol. After incubation at room temperature for 60 minutes, 500 µL were added to 490 µL 4.5 M guanidine-HCL and 10 µL 2-mercaptoethanol and mixed. They were then filtered through 0.2 um cellulose acetate filters into HPLC autosampler vials. Injection volume of standards and samples was 50 µL. Bovine standards for caseins and whey proteins were obtained from the Sigma Chemical Company (St Louis, MO, USA). A composite casein standard containing α, β and κ-CN was made up in 4.5 M guanidine-HCL to a concentration of 2 mg/ mL for each of the caseins. A composite standard for the whey proteins containing α-La (0.5 mg/mL, β-LG A (1 mg/mL) and β-LG B (1 mg/mL) was also made up in 4.5 M guanidine-HCL. Proteins were separated on a Bio-Rad High Pore RP318 reverse phase column (4.6 x 250 mm; Bio-Rad Laboratories, 2000 Alfred Nobel Drive, Hercules, CA, USA) at 40°C with a gradient of 0.2% trifluoroacetic acid in deionised water and 0.1% trifluoroacetic acid in acetonitrile/water (90:10). The flow rate was 1 mL/min and the eluted peaks were detected by UV-absorption at 280 nm in a Shimadzu LC10A HPLC system. Milk concentrations of proteins were calculated by comparing the standard peak area to the corresponding unknown peak area times the concentration of the standard, times the sample dilution factor, to give a result as mg protein/ mL milk sample.

Milk mineral (magnesium [Mg]; sodium [Na]; potassium [K]; calcium [Ca]) concentrations were measured using the nitric-perchloric mixed acids wet ashing procedure by e-Lab, Hamilton, New Zealand as described by Turner *et al.* (2005).

Milk fatty acid (FA) profiles were analysed in fat extracted from milk using the Röse-Gottlieb fat extraction procedure (IDF, 1987). Fatty acids were esterified and quantified by gas chromatography as described by Turner *et al.* (2005).

Statistical analyses

Data were analysed using ANOVA in GenStat (2002) using the pretreatment data as a covariate, and treatment as a fixed effect. Both SCC and concentrations of Lf were analysed following $\log_{10}$ transformation of the data to stabilise the variance. Both $\log_{10}$ and back-transformed means for Lf concentration are presented.

RESULTS

Supplemental fumarate, ruminally infused at a rate of 5% of DM intake did not affect the milk yield of grazing dairy cows, nor the concentrations of fat, protein, casein or total solids (Table 1). Lactose concentrations were 2.5% higher in the milk of cows receiving fumarate ($P < 0.05$). No differences were apparent in milk urea, citrate, pH, SCC or concentrations of minerals (Ca, Mg, Na or K). The concentrations of individual milk caseins measured were not affected by treatment (Table 2), however, concentrations of the whey proteins α-La and BSA were 10% higher, and 14.5% lower respectively in the milk of cows supplemented with fumarate ($P < 0.05$). No significant differences were found in the concentrations of other whey proteins measured (Table 2). A range of milk fatty acids measured showed no significant effect of supplemental fumarate on the fatty acid profile (Table 3).

DISCUSSION

This paper is the first reported study examining the effect of supplemental fumarate on detailed milk composition in early lactation, and is a companion paper to that of Kolver & Aspin, (2006) which describes the effects on gross milk composition and methane production. Concentrations of the different milk components measured were comparable to those reported previously for milk from Friesian or Friesian cross dairy cows at a similar time of the season and stage of lactation (Mackle *et al.*, 1997; Auldist *et al.*, 1998; Back & Thomson, 2005).

The addition of sodium fumarate to the diet of lactating dairy cows (Kolver & Aspin, 2006) did not result in the elevated ruminal concentrations of propionate, and reduced concentrations of methane observed *in vitro* (Kolver *et al.*, 2004). While unexpected, this result was consistent with the study of McGinn *et al.* (2004) using beef cattle. Consequently there were none of the expected changes in the content of milk protein or milk fat. However, some minor differences were apparent in the detailed milk composition. Lactose is the major osmole of milk and is thus responsible for drawing water into the epithelial cells as milk is synthesised (Kuhn, 1983). While concentrations of lactose were higher in those receiving supplemental fumarate, there was no difference in milk yields between the two groups of cows. α–La plays an essential role in lactose biosynthesis where it modifies the action of galactosyltransferase to couple galactose to glucose (Kuhn, 1983). Although α–La concentrations were higher in the milk of those cows receiving fumarate, it is not known whether the fumarate supplementation resulted in up regulation of α–La synthesis within mammary epithelial cells. The higher concentration of lactose in the milk of cows receiving the fumarate is however consistent with an increased availability of α–La within the epithelial cells.

Table 1: Yield, gross composition and mineral concentrations of milk from dairy cows fed pasture (Control) or pasture supplemented with 931 g sodium fumarate (5% of DM) per cow per day (Fumarate).

	Control	Fumarate	sed	*P*
Milk yield (L/cow/day)	21.8	23.6	1.37	0.212
Fat (%)	4.46	4.32	0.115	0.241
Crude protein (%)	3.19	3.28	0.062	0.144
True protein (%)	2.98	3.08	0.061	0.122
Casein (%)	2.44	2.52	0.050	0.170
Lactose (%)	4.80	4.92	0.041	0.011
Total solids (%)	12.9	13.1	0.153	0.344
Urea (mmol/L)	5.77	5.45	0.182	0.100
Citrate (mg/mL)	1.68	1.68	0.105	0.978
pH	6.51	6.51	0.033	0.970
Log_{10} SCC	1.75	1.66	0.103	0.363
Ca (mg/100g)	119.2	123.7	5.16	0.396
Mg (mg/100g)	11.4	11.4	0.36	0.934
K (mg/100g)	161.4	152.0	5.50	0.110
Na (mg/100g)	38.6	39.3	2.07	0.747

Table 2: Concentrations of individual casein and whey proteins, and the yield of lactoferrin (Lf) in milk from dairy cows fed pasture (Control) or pasture supplemented with 931 g sodium fumarate (5% of DM) per cow per day (Fumarate).

	Control	Fumarate	sed	*P*
α-casein (mg/mL)	14.8	15.0	0.53	0.623
β-casein (mg/mL)	13.0	13.0	0.49	0.991
κ-casein (mg/mL)	3.6	3.6	0.12	0.639
α-lactalbumin (mg/mL)	1.0	1.1	0.04	0.040
β-lactoglobulin (mg/mL)	4.1	4.3	0.13	0.395
Bovine serum albumin (mg/L)	251.0	214.6	15.06	0.031
Immunoglobulin-G (mg/L)	560.6	548.7	24.26	0.632
Lactoperoxidase (U/mL)	10.8	11.4	1.98	0.790
Log_{10} lactoferrin	1.94	1.91	0.073	0.686
	(87.1)[1]	(81.3)[1]		
Lf yield (g/day)	2.27	2.24	0.378	0.947

[1]Values in parentheses are Lf concentration (mg/l) following back-transformation.

Table 3: Fatty acid concentrations (%) in milk from dairy cows fed pasture (Control) or pasture supplemented with 931 g sodium fumarate (5% of DM) per cow per day (Fumarate).

	Control	Fumarate	sed	*P*
4:0	4.49	4.53	0.143	0.768
6:0	2.66	2.72	0.102	0.548
8:0	1.54	1.54	0.053	0.969
10:0	3.68	3.56	0.108	0.280
12:0	4.05	3.92	0.132	0.354
14:0	11.32	11.45	0.364	0.722
cis-9 14:1	1.44	1.41	0.082	0.782
15:0	1.34	1.33	0.062	0.796
16:0	26.40	27.40	0.885	0.276
cis-9 16:1	1.21	1.20	0.081	0.947
17:0	0.61	0.60	0.017	0.566
18:0	10.34	10.18	0.538	0.770
trans-8 18:1	0.15	0.16	0.030	0.776
trans-9 18:1	0.15	0.14	0.017	0.703
trans-10 18:1	0.24	0.23	0.029	0.841
trans-11 18:1	3.78	3.64	0.484	0.778
cis-9 18:1	15.57	15.16	0.803	0.621
cis-11 18:1	0.72	0.70	0.063	0.760
18.2	0.56	0.58	0.044	0.656
CLA[1]	1.35	1.36	0.133	0.909
18.3	0.81	0.81	0.062	0.943
20:0	0.12	0.12	0.005	0.941
C20.4	0.05	0.05	0.007	0.561
20:5	0.10	0.08	0.020	0.398
22:5	0.12	0.12	0.014	0.799

[1]*cis-9*, *trans-11* 18:2 CLA

Concentrations of lactose in plasma, and changes in milk BSA concentrations are used as indirect measures of the integrity of the tight junctions in the mammary gland (Stelwagen *et al.*, processors, higher milk BSA concentrations can have a negative impact on cheese production (Auldist *et al.*, 1996), therefore milk with lower BSA concentrations may be of greater value. Milk BSA concentrations increase toward the end of lactation (Sheldrake *et al.*, 1983; Auldist *et al.*, 1998) due to involution. Whether supplemental 1994a; 1994b). In this study, the decreased concentrations of BSA may indicate that tight junction integrity was greater in those cows receiving the supplemental fumarate. For milk fumarate could be used, as a means of decreasing BSA concentrations in late lactation requires further study, especially given that the current study was performed in early lactation.

In spite of the fumarate infusion being a sodium salt (sodium fumarate dibasic anhydrous), resulting in a greater sodium intake (1.4% of DM; Kolver &

Aspin, 2006), milk sodium concentrations were not different between the treated and control cows. This suggests that sodium clearance from cows was not via their milk. Roche *et al.* (2005) also reported that no changes were apparent in milk sodium concentrations in cows receiving drenches containing varying amounts of sodium. That study did however show that changes in both blood and urine sodium were apparent. Changes in milk sodium concentrations in relation to milk potassium concentrations has been used previously as an indicator or tight junction permeability (Shamay *et al.*, 2003). Neither sodium nor potassium concentrations were different between the treatments suggesting that tight junction integrity was not altered. This contrasts with the changes in BSA concentrations, which suggested a possible effect on tight junctions. However, it has recently been reported that there may be some albumin synthesis by mammary epithelial cells (Shamay *et al.*, 2005). Therefore it may be possible that sodium fumarate supplementation effects mammary albumin synthesis, and not tight junction integrity.

The lack of effects of the treatment on the milk fatty acid profile indicate that supplemental fumarate does not negatively effect the health attributes of the milk fat, nor alter the FA profile in such a way to effect the processing properties of the milk.

Together these results show that supplementing cows with sodium fumarate in early lactation has no negative effects on milk composition from either the point of view of a farmer (no decreases in milk volume, or the concentrations of protein or fat), or a milk processor. Supplemental fumarate in early lactation may benefit processors due to decreases in milk BSA concentrations. The mechanism by which this might be occurring is unknown and further study is required.

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