Milk production and milk fatty acid composition of grazing dairy cows supplemented with fodder beet

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Abstract

A study was conducted to evaluate the effect of supplementing a perennial ryegrass-based diet with fodder beet on milk production and milk fatty acid (FA) composition, of dairy cows in early lactation. Sixty Friesian × Jersey cows, were blocked into six groups of 10 cows, and groups randomly allocated to three replicates fed either 18 kg DM/day of ryegrass herbage (H), or 14.4 kg DM/day of ryegrass herbage + 4 kg DM of harvested FB bulbs (FBB). Dry matter intake (DMI) was similar between H and FBB (15.0±0.77 and 14.2±0.48 kg DM/day respectively). Although milk yield tended to be greater for H than FBB (20.0 and 18.9 kg/day respectively; P=0.09); milk solids production was not affected by treatment (P=0.89). Supplementation with FBB increased the saturated (80.6 versus 73.2±0.39 g/100g FA; P<0.001) and medium chain milk FA (66.7 versus 56.2±0.783; P<0.001) content, compared with H. Under the conditions of the present study, our results suggest that, supplementing grazing dairy cows with FBB in early lactation, may not improve milk production and increases the saturated FA content of milk.

Keywords: fodder beet; milk; fatty acid; pasture

Introduction

The large crop yields achieved by fodder beet, *Beta vulgaris* M., (FB) have led to its extensive use as a winter forage crop in New Zealand dairy systems. Compared with an alternative forage such as kale, FB can produce >20 t DM/ha (Chakwizira et al. 2013), which can be grazed or harvested and fed elsewhere or stored if necessary. This versatility is attractive for many farmers, as FB may be harvested to return the land to pasture and the FB fed to supplement the early lactation herbage supply. Although FB bulbs are high in metabolisable energy [ME: 11.8 MJ ME/kg DM (Clark et al. 1987)], studies undertaken internationally report minimal improvement of milk yield when FB is fed alongside various levels of protein (Fisher et al. 1994), or concentrates (Ferris et al. 2003). However, the milk response to supplementing a grazed herbage diet with FB has had little study.

Human food production from agriculture has traditionally focused on quantity. However, consumers are becoming increasingly aware of quality and associated health risks or rewards. For example, the concentration of polyunsaturated fatty acids (PUFA) such as conjugated linoleic acid (CLA: C18:2 cis-9, cis-12) or α-linolenic acid (C18:3 cis-9, cis-12, cis-15), have anti-carcinogenic properties and are associated with a range of benefits related to human health (Chilliard et al. 2000). Conversely, saturated FAs (SFA), have been linked with increased plasma concentrations of low density lipoprotein cholesterol, which may be a risk factor for cardiovascular disease (Shingfield et al. 2013). While herbage contains high concentrations of C18:3, and to a lesser extent CLA, ruminal bio-hydrogenation reduces their abundance in milk (Chilliard et al. 2000). Supplementation with starch has the potential to increase the PUFA content of milk as a result of reduced bio-hydrogenation at lower rumen pH (Kolver & De Veth 2002). While FB contains little starch, it is rich in soluble sugars, principally sucrose (Clark et al. 1987), which may also lower rumen pH and increase the PUFA content of milk. Consequently, the objective of this research was to determine the effect of substituting the herbage of grazing dairy cows with FB on milk production and milk FA composition.

Methods

Experimental site and design

All animal treatments and measurements in this experiment were approved by the Lincoln University Animal Ethics Committee (#2016-30).

The experiment was conducted between the 10th and 25th of November 2016 at Lincoln University's Ashley Dene Research and Development Station in Canterbury (-43.65 º North, 172.33 º East), New Zealand. Sixty Friesian x Jersey (F9 J6) dairy cows were blocked into three replicate groups according to live weight (438± 3.1), age (3.6 ± 0.12 years) days in milk (DIM: 85 ± 4.8 days), and milk solids (MS: 2.02 ± 0.18 kg/day) and allocated to two treatments in a completely randomised design. Treatments were: perennial ryegrass (RG: *Lolium perenne* L.*)* and white clover (WC: *Trifolium repense*) sward, offered as an herbage only diet (H); or herbage + 4 kg DM/day of harvested FB bulb (FBB).

Grazing management

Fodder beet (cv. Rivage) was sown in October 2015, harvested commercially and stored five weeks prior to the study. Perennial ryegrass and white clover swards were grazed 4±1 weeks prior to the experiment and fertilised with 46 kg N/ha as urea. Prior to the experiment, all cows grazed a PRG WC sward supplemented with 3.5 kg DM/d

of harvested FBB. An eight-day transition period prior to the experiment enabled animals in H to adapt back to an herbage only diet, and the FBB cows to reach FB allocation.

Over the measurement period, herbage DM allocation for cows offered either H or FBB were 18 and 14.4 kg DM/cow per day, respectively above a residual herbage compressed height (as determined by rising plate meter, RPM) of 3.5 cm or 1500 kg DM/ha. Access to water was *ad libitum*. Cows were offered a fresh allocation of herbage each morning behind a temporary electric fence with a back fence to prevent grazing of residual regrowth. Allocation area was calculated from herbage mass estimated by RPM (Jenquip Ltd, New Zealand); using a standard equation for PRG WC swards (kg DM/ha = 140 x RPM reading + 500). Replicate groups of FBB grazed a single paddock split into three breaks, while two groups in H shared a paddock and the remaining group grazed alone. Prior to the experiment the DM (20.3%) of FBB was determined by random selection of bulbs in the stack, and oven drying (60°C for 48 hours). Daily DM allocations of FBB were weighed and fed out by mixer wagon onto a feed pad, and fed to cows after the morning milking. Upon meal completion, cows were returned to pasture. Animals were milked at 0700 and 1600 h daily.

Feed measurements and analyses

Herbage was sampled on four occasions, before and after grazing, by plucking ten random hand grab samples to grazing height in each allocation. Herbage was bulked and sub-sampled to assess DM (oven-dried at 60° C for 48 hours), botanical and chemical composition. Samples of FBB were also collected, minced and stored at -20ºC. Supplement refusals were collected daily and weighed to determine apparent intake. Sub-samples of pasture were freeze-dried then ground (ZM200 Retsch) for chemical analysis (FA, crude protein: CP, acid detergent fibre: ADF, neutral detergent fibre: NDF, organic matter: OM, and water soluble carbohydrates: WSC) using near infrared spectrophotometry (NIRS. Model: FOSS NIRS Systems 5000, Maryland USA). Ground samples of FBB were assessed for N: Elementar (Variomax CN Analyser, Elementar Analysensysteme, Germany), ADF, NDF (Van Soest et al. 1991), ash and WSC (Pollock & Jones 1979).

Animal measurements and analyses

Milk yield (kg/d) and live weight were recorded automatically at each milking (Waikato milking systems, Hamilton, New Zealand). Bulk milk from individual cows was sub-sampled from two consecutive milkings on six occasions to determine milk fat, protein and lactose, using Milkoscan (Foss Electric, Hillerod, Denmark, courtesy of Livestock Improvement Corporation, Christchurch). A skimmed sample of milk was used to determine MUN, using a Randox RX Daytona analyser (United Kingdom). Fatty acid methyl esters of milk, pasture and FB were prepared by transmethylation and analysed by gas chromatography (AOAC method 2012.13) (Shimadzu GC-2010, Japan with AOC-20i auto-sampler) using a Varian CP742 silica capillary column (0.25 x 100m x 0.2 μm).

Statistical Analysis

All individual animal variables were combined and averaged over sampling days and analysed by ANOVA using GenStat (v.18 VSN International LTD, 2015) with two treatments and three replicates equating to six experimental units. Treatment effect was deemed significant if $P \leq 0.05$.

Results

Feeds

The pre-grazing herbage mass and ME intake were similar between H and FBB groups. However, post-grazing herbage mass ADF, NDF, and reproductive pasture were greater, and CP lower in herbage fed to FBB compared to that of H (Table 1). Fodder beet contained more WSC and less CP, ADF and NDF than herbage (Table 1). While there was no variation in the FA content of herbage between treatments, FBB contained less C18:3 (0.17 verses 8.9 mg FA/g DM, $P<0.001$) and CLA (1.29 verses 2.25 mg FA/g DM, $P<0.001$) than herbage (Table 1).

Table 1 Pre- and post-graze herbage mass and chemical composition of herbage offered to cows grazing either an herbage only (H) or an herbage and fodder beet diet (FBB herbage). The chemical composition of fodder beet bulbs (FB bulbs) is also presented.

	H	FBB herbage	FB Bulb	SEM	P Value
Pre-graze mass (kg DM/ha)	3642[a]	3581[a]		170	0.804
Post-graze mass	1663[a]	2003[b]		57.4	<0.001
Reproductive grass (% DM)	32.2[a]	57.1[b]		3.65	<0.001
Vegetative grass	40.6[a]	32.8[a]		5.03	0.111
Clover	10.5[a]	0.98[b]		1.81	0.001
Dead	5.67[a]	6.20[a]		1.26	0.745
Weeds	11.1[a]	5.16[a]		2.45	0.237
Herbs	0[a]	6.20[a]		0.24	0.329
Dry Matter (%)	22.4[a]	26.5[b]	20.3[c]	1.73	0.033
N	2.44[a]	1.89[b]	1.30[c]	0.99	<0.001
WSC	24.1[a]	26.8[b]	54.9[c]	0.41	<0.001
ADF	25.2[a]	28.8[b]	6.7[c]	1.03	0.021
NDF	41.6[a]	45.7[b]	14.2[c]	0.93	0.005
CP	15.3[a]	11.8[b]	8.5[c]	0.45	<0.001
OM	80.8[a]	79.5[a]	94.7[b]	0.10	<0.001
ME (MJ ME/kg DM)	11.7[a]	11.5[a]		0.12	0.132
Fatty Acid Content					
C16:0 (mg/g DM)	3.01[a]	2.95[a]	0.84[b]	0.1	<0.001
C18:0	0.27[a]	0.24[a]	0.07[a]	0.1	0.109
C18:1	0.45[a]	0.47[a]	0.68[a]	0.22	0.733
C18:2	2.3[a]	2.2[a]	1.29[b]	0.04	<0.001
C18:3	9.0[a]	8.80[a]	0.17[b]	0.71	0.001
Σ Total FA	16.2[a]	16.1[a]	3.32[b]	0.62	<0.001

[a-c] Means of the same variable in the same row with different subscripts differ

Animal

Animals did not achieve target herbage residuals (1500 kg DM/ha) resulting in average apparent herbage DM intakes of 14.9 and 10.2 ± 0.625 kg DM/day for H and FBB respectively during the measurement period. When supplement is accounted for, there was no difference in total apparent DMI between FBB and H (14.2 vs. 15.0 ± 0.63 kg DM/d; P=0.4) respectively. While live weight declined in both treatments over the experimental period, this was more pronounced in FBB groups than H (-9.93 vs. -4.95 kg). Animals offered FBB tended (P=0.09) to produce less milk, but the MS yield was not different from those measured in the H groups (Table 2). Lactose yield (0.98 versus 1.04 ± 0.03 kg/day, P=0.022) and MUN (3.48 vs 6.58 mg/dl P<0.001) were lower and percentage fat greater for cows fed FBB; however, milk fat yield was not significantly different. Concentrations of saturated (P<0.001), short chain (<C8, P<0.001), medium chain FAs (C8-C16, P<0.001) were higher and PUFA lower, in milk produced from FBB rather than H. There was no treatment effect on C14:1/C14:0 (P=0.99) or C16:1/C16:0 ratios (P=0.21); however, cows fed FBB had lower C18:1 to C18:0 (P<0.001) and CLA C18:2 *cis9*, *trans11* to C18:1 *trans11* (P<0.001) ratios than those fed H (Table 2).

Table 2 Change in live weight, yield of milk and milk constituents and milk fatty acid (FA) composition of cows fed either herbage only (H) or herbage and 4 kg DM of harvested fodder beet (FBB).

	H	FBB	S. E. M	P Value
LW change (kg)	-4.95[a]	-9.93[b]	0.811	<0.001
Milk (kg)	20.0[a]	18.9[a]	0.419	0.091
Fat (%)	5.32[a]	5.75[b]	0.088	<0.001
Protein	3.94[a]	4.03[a]	0.043	0.155
MS	9.26[a]	9.78[b]	0.123	0.004
Fat (kg/d)	1.05[a]	1.08[a]	0.021	0.301
Protein	0.78[a]	0.76[a]	0.014	0.217
MS	1.84[a]	1.84[a]	0.033	0.898
Lactose	1.04[a]	0.98[b]	0.022	0.036
MUN mmol/L	6.58[a]	3.48[b]	0.173	<0.001
FA (g/100g FA)				
C4:0	1.34[a]	1.41[b]	0.00	0.057
C6:0	1.48[a]	1.56[b]	0.00	0.016
C8:0	1.13[a]	1.22[b]	0.00	<0.001
C10:0	3.14[a]	3.81[b]	0.10	<0.001
C12:0	3.81[a]	5.03[b]	0.10	<0.001
C14:0	12.4[a]	14.0[b]	0.30	<0.001
C16:0	35.8[a]	41.9[b]	0.40	<0.001
C16:1 c7	0.22[a]	0.20[b]	0.00	<0.001
C16:1 c9	1.25[a]	1.28[a]	0.03	0.403
C18:0	9.28[a]	7.25[b]	0.20	<0.001
C18:1 c6	0.38[a]	0.25[b]	0.01	<0.001
C18:1 c9	15.1[a]	9.80[b]	0.23	<0.001
C18:1 t9	0.16[a]	0.11[b]	0.00	<0.001
C18:1 t11	2.28[a]	1.47[b]	0.11	<0.001
C18:1 t5 t8	0.15[a]	0.10[b]	0.00	<0.001
C18:2 c9 c12	0.90[a]	0.71[b]	0.01	<0.001
C18:2 c9 t 13	0.14[a]	0.10[b]	0.00	<0.001
C18:2 t9 c12	0.12[a]	0.07[b]	0.01	<0.001
C18:3 c9, 12, 15	0.93[a]	0.69[b]	0.02	<0.001
CLA c9 t11	0.87[a]	0.50[b]	0.03	<0.001
Σ Short chain (total FA)	7.26[a]	8.37[b]	0.12	<0.001
Σ Med chain FA	56.2[a]	66.7[b]	0.78	<0.001
Σ Long chain FA	34.8[a]	24.9[b]	0.58	<0.001
Σ SFA	73.2[a]	80.6[b]	0.39	<0.001
Σ Mono FA	22.6[a]	16.4[b]	0.51	<0.001
Σ PUFA	3.8[a]	2.86[b]	0.06	<0.001
Product: substrate ratios				
C14:1 *cis 9* to C14:0	0.06[a]	0.06[a]	0.00	0.987
C16:1 *cis 9* to C16:0	0.03[a]	0.03[a]	0.00	0.210
C18:1 *cis9* to C18:0	1.64[a]	1.36[b]	0.03	<0.001
CLA C18:2 *cis9, trans11* to C18:1 *trans 11*	0.36[a]	0.34[b]	0.01	<0.001

[a-b] Means of the same variable in the same row with different subscripts differ

Discussion

There was a tendency (P=0.09) for cows to produce less milk when fed FBB, however, due to the greater proportion of solids in milk (Table 2), MS production was similar across treatments. The higher solids in FBB milk reflects a greater percentage of milk fat (5.75 versus 5.32%), similar to previous reports (Fisher et al. 1994; Ferris et al. 2003).

The advanced phenological state of herbage (Table 1) led to a relatively low CP content of both diets, below the 18% recommended level for early lactation dairy cows (14.5 and 15 % CP, FBB and H) respectively. The animal response to this change in nutrient supply altered milk composition. For example, MUN was lower for cows fed FBB compared with those in the H treatment (3.58 and 6.58 mmol/L FBB and H) respectively.

The lower lactose concentration and yield in FBB milk (Table 2), suggests a limited supply of glucogenic precursors (propionate or glucogenic amino acids), and that animals in FBB were in a state of negative energy balance (NEB). This is supported by the greater reduction of live weight of FBB cows (Table 2). However, NEB had no effect on milk FA content, as increased circulation of lipoproteins and non-esterified FAs (indicative of NEB) lead to increased proportions of C18 FA in milk (Table 2) (Chilliard et al. 2000).

Inclusion of FBB reduced the nutraceutical properties of 'pasture-based milk' by increasing the content of SFAs: C12:0, C14:0, and C16:0 by 32, 13, and 17 % respectively, compared with H. Similarly, Collomb et al. (2004) reported increased content of C16:0 when hay was supplemented with FB, compared with rapeseed or linseed (31.1, 26.2 and 24.5 g/100g FA respectively). Fatty acids less than 12 carbons in length, most C14 and about half of C16, are synthesised *de novo* in mammary tissue, from acetate and β-hydroxybutyrate derived from rumen fermentation. Alternatively, the remaining C14, C16 and all FAs longer than C18 enter the mammary gland from the arterial circulation (Chilliard et al. 2000). It is apparent that

incomplete bio-hydrogenation of unsaturated FA (UFA), reduces *de novo* synthesis in the mammary gland (Chilliard et al. 2000; Shingfield et al. 2013). Thus, in the present study, the low UFA content of FBB (Table 1), could have increased *de novo* synthesised fatty acids found in milk. Furthermore, ruminal fermentation of sucrose is reported to favour production of butyrate and may increase bio-hydrogenation of UFA (Oba, 2011), which could explain the greater milk fat percentage observed from animals fed FBB.

Unsaturated FAs are also synthesised in mammary tissue by delta 9 desaturase. Although herbage contains less cis-9, cis-11 CLA and more C18:3 FA than grain, the concentration of cis-9, trans-11 CLA in milk, is greater from cows fed herbage (Chilliard et al. 2000). In the bio-hydrogenation pathway of C18:2, cis-9, cis-12 C18:2 is isomerised to cis-9, trans-11 CLA, yielding C18:1 trans-11 and finally, C18:0. While hydrogenation of C18:3 also yields C18:1 trans-11, formation of cis-9, trans-11 CLA is not an intermediary step. This suggests a proportion of cis-9, trans-11 CLA in milk from cows fed herbage is formed through desaturation of trans-11 C18:1, by delta 9 desaturase (Griinari & Bauman 1999). In the present study, the ratios of cis-9, trans-11 CLA to trans-11 C18:1, and cis-9 C18:1 to C18:0 were reduced by FBB treatment (Table 2); indicating a reduction of endogenously synthesised UFA. This most likely reflects the lower supply of trans-11 C18:1 in FBB (Table 1), as the activity of delta 9 desaturase is dependent on substrate availability (Kay et al. 2002). Although, it is important to note that NEB can also inhibit delta 9 desaturase (Kgwatalala et al. 2009), and may also be partly responsible.

While FBB reduced the CLA cis-9, trans-11 and C18:3 content of milk (Table 2), the levels observed in H groups (0.87 CLA and 0.93 C18:3 g/100g FA) were much less than those reported elsewhere (1.81 CLA and 1.64 C18:3 g/100g) (Rugoho et al. 2014). The majority of FA synthesis occurs in chloroplasts, which contain more than half of plant protein (Rugoho et al. 2017). Therefore, the generally low CP content of herbage fed presently (<16% CP; Table 1), may have corresponded to a lower PUFA content of pasture and milk.

Our results do not support the hypothesis that rapidly fermentable carbohydrates in FBB increase the PUFA content of milk. The appearance of dietary PUFA in milk is a result of ruminal passage and escape of hydrogenation by rumen bacteria (Chilliard et al. 2000). While a low rumen pH reportedly reduces hydrogenation (Kolver & De Veth 2002), the greater proportion of fibre in FBB herbage (Table 1), may have increased rumen retention and bio-hydrogenation. The high reproductive content of herbage in FBB swards may also have contributed to lower apparent herbage intake, further limiting ruminal PUFA supply.

Conclusions

Our findings indicate that supplementing grazing dairy cows with FBB was of no advantage to yield, or nutritional value of milk, when offered in periods of high herbage supply. The extent of ruminal bio-hydrogenation and milk FA synthesis appeared to increase, while dietary supply of PUFA declined with FBB inclusion. However, we are unable to discern whether this result was independent of the variation in herbage composition across treatments thus, further study is required.

References

AOAC, Official Method 2012.13. Determination of Labeled Fatty Acids Content in Milk Products and Infant Formula. AOAC International, Gaithersburg, MD, USA 2012

Chakwizira E, Meenken ED, Maley S, George M, Hubber R, Morton J, Stafford A 2013. Effects of potassium, sodium and chloride fertiliser rates on fodder beet yield and quality in Canterbury. Proceedings of the New Zealand Grassland association 75: 261-270.

Chilliard Y, Ferlay A, Mansbridge RM, & Doreau M 2000. Ruminant milk fat plasticity: nutritional control of saturated, polyunsaturated, trans and conjugated fatty acids. Annales de Zootechnie 49(3): 181-205.

Clark P, Givens DI, Brunnen JM 1987. The chemical composition, digestibility and energy value of fodder-beet roots. Animal Feed Science and Technology 18(3): 225-231.

Collomb M, Sollberger H, Bütikofer U, Sieber R, Stoll W, & , Schaeren W 2004. Impact of a basal diet of hay and fodder beet supplemented with rapeseed, linseed and sunflowerseed on the fatty acid composition of milk fat. International Dairy Journal 14(6): 549-559.

Ferris CP, Patterson DC, Gordon FJ, Kilpatrick DJ 2003. The effect of concentrate feed level on the response of lactating dairy cows to a constant proportion of fodder beet inclusion in a grass silage-based diet. Grass and Forage Science 58(1): 17-27.

Fisher GEJ, Sabri MS, Roberts DJ 1994. Effects of feeding fodder beet and concentrates with different protein contents on dairy cows offered silage ad libitum. Grass and Forage Science 49(1): 34-41.

Griinari J, Bauman DE 1999. Biosynthesis of conjugated linoleic acid and its incorporation into meat and milk in ruminants. Advances in Conjugated Linoleic Acid Research 1: 180-200.

Kay JK, Mackle TR, Auldist MJ, Thomson NA, Bauman DE 2002. Endogenous synthesis and enhancement of conjugated linoleic acid in pasture-fed dairy cows. Proceedings of the New Zealand Society of Animal Production 62: 12-15.

Kgwatalala PM, Ibeagha-Awemu EM, Mustafa AF, Zhao X 2009. Stearoyl-CoA desaturase 1 genotype and stage of lactation influences milk fatty acid composition of Canadian Holstein cows. Animal Genetics 40(5): 609-615.

Kolver ES, De Veth MJ 2002. Prediction of ruminal pH from pasture-based diets. Jounal of Dairy Science 85(5): 1255-1266.

Oba M 2011. Effects of feeding sugars on productivity of lactating dairy cows. Canadian Journal of Animal Science 91(1): 37-46.

Pollock CJ, Jones T 1979. Seasonal patterns of fructan metabolism in forage grasses. New Phytologist 83(1): 9-15.

Rugoho I, Liu Y, Dewhurst RJ 2014. Analysis of major fatty acids in milk produced from high-quality grazed pasture. New Zealand Journal of Agricultural Research 57(3): 165-179.

Rugoho I, Cheng L, Aizimu W, Bryant RH, Edwards GR 2017. Effects of post-grazing herbage height and concentrate feeding on milk production and major milk fatty acids of dairy cows in mid-lactation. Grass and Forage Science 72(2): 211-219.

Shingfield KJ, Bonnet M, Scollan ND 2013. Recent developments in altering the fatty acid composition of ruminant-derived foods. Animal 7 (1): 132-62.

Van Soest PJ, Robertson JB, Lewis BA 1991. Symposium: carbohydrate methodology, metabolism, and nutritional implications in dairy cattle. Journal of Dairy Science 74(10): 3583-3597.