Exploiting cow genotype to increase milk value through production of minor milk components

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ABSTRACT

Milk value could be increased on-farm by supplying milk with enhanced concentrations of minor compounds that have bioactive properties. Compounds of interest in this study were the whey proteins lactoferrin (Lf) and lactoperioxidase (LP), and milk fatty acids; particularly conjugated linoleic acid (CLA) and omega-3 fatty acids. Mixed genotype cows (20 Friesian, 20 Jersey and 20 crossbred) were sampled to determine the effect of cow genotype and age on Lf concentration, Lf yield and LP activity at 4 times during the season. In milk fat, concentration of CLA and omega-3 fatty acids were determined twice during the season. Cow genotype had no effect on Lf concentration but age did ($P < 0.002$), with 4-year and older cows producing greater concentrations in milk than 3 year old cows. For harvesting Lf, Friesian cows would be the most profitable, with the greatest Lf yields, particularly in mid-late lactation. The highest LP activity levels throughout lactation were demonstrated in Jersey milk. In milk fat, Friesian cows produced milk of a putatively healthier profile, with greater concentrations of unsaturated fatty acids, CLA and omega-3 fatty acids.

Key words: milk value; cow genotype; lactoferrin; lactoperoxidase; conjugated linoleic acid; omega-3 fatty acids.

INTRODUCTION

The nutriceutical industry is continuing to grow and along with this there is an increasing demand for naturally occurring compounds with bioactive properties. As a result, there is the potential for milk value to be increased on-farm by supplying colostrum or milk containing enhanced concentrations of bioactive compounds for specialty or niche products. To meet this demand information is required to determine the type of cow needed to produce such specialised milk. Two minor whey proteins currently being harvested in New Zealand for value-added products are lactoferrin (Lf) and lactoperoxidase (LP). There is also interest in bioactive fatty acids (FA) such as conjugated linoleic acid and omega-3 fatty acids.

Both Lf and LP are glyco-proteins that bind iron (Nuijens *et al.,* 1996; Kussendrager & van Hooijdonk, 2000) and have anti-bacterial and anti-viral activity making them part of the non-specific defense mechanism of the mammary gland. Lactoferrin is part of a protective immune response after infection (reviewed by van Hooijdonk *et al.,* 2000). As a result of this, Lf is sold as a health supplement in tablet form and is included in a diverse range of products, such as infant formulae, human health products and animal feeds. Lactoperoxidase is one of the major enzymes found in milk, with a wide anti-microbial activity (reviewed by Kussendrager & van Hooijdonk 2000), thereby preserving milk. Commercial applications have been developed using LP as a natural biopreservative in food, for example in yoghurt where it suppresses acidity and increases shelf life, speciality feeds and in cosmetics.

The term conjugated linoleic acid is used to refer to positional and geometric isomers of linoleic acid ($C_{18:2}$), two of which are known to have biological activity (Pariza *et al.,* 2001). Numerous physiological effects are attributed to conjugated linoleic acid, such as anti-cancer (Ip *et al.,* 1999) and weight loss, and these effects vary depending on the isomer (Pariza *et al.*, 2001). For the purpose of this paper, only the *cis*-9, *trans*-11 $C_{18:2}$ isomer (CLA) will be discussed. Omega-3 FA are long chain FA that are synthesized in small amounts by the human body and are largely derived from the diet. They consist of a precursor, α-linolenic acid ($C_{18:3}$), that can be converted by the liver into 2 physiologically active FA, eicosapentaenoic (EPA, $C_{20:5}$) and docosahexaenoic (DHA, $C_{22:6}$). In humans the conversion is limited, and it is estimated that only 5-15% of α-linolenic acid is converted to DHA (Holub, 2002). While EPA regulates cardiac physiology, blood clotting and inflammation, and may have an important role in the management of mental disorders such as depression (Holub, 2002), the major benefits of DHA are seen in the fetus and newborn due to its effects on the growth and development of the brain and retina (Innis, 2004). As a result, products containing EPA are formulated for ageing adults, whereas DHA is considered an important ingredient in infant formulae.

There is little information about these minor proteins or FA in colostrum or milk of New Zealand Friesian or Jersey cows (the main parent breeds) and no

information on cross-bred cows. The aim of this study is to examine whether there is a difference in the amounts of these minor components in colostrum or milk produced by the three main genotypes of New Zealand dairy cows grazed in a pastoral system.

METHODS AND MATERIALS

The experiment was carried out with the approval of the Ruakura Animal Ethics Committee. A herd of mixed genotype dairy cows (20 Friesian, 20 Jersey and 20 crossbred cows) were calved at pasture in July-September 2003. Cows selected were: Friesian (F:J 16/0, BW 105), Jersey (F:J 0/16, BW 96), crossbred (F:J 10/6, BW 111). The cows were grazed on 2 farmlets in an all-pasture system, with 10 cows of each genotype per farmlet at a stocking rate of 3.3 cows/ha.

Effect of cow genotype on Lf and LP was determined at 4 times during the season. Samples for analysis were taken at the first milkings after calving (July-September), October (peak lactation), February (mid lactation) and April-May (drying-off). In milk fat, effect of cow genotype was measured twice during the season (October and February). Samples for all analyses were taken from a composite sample of morning and afternoon milk. Colostrum and milk yield (litres/milking) was measured using in-line milk meters (Westfalia Surge Metatron P21).

Concentration of Lf in colostrum and milk was measured using a bovine Lf ELISA quantification kit (Bethyl Laboratories. Inc, Montgomery, Texas, United States of America) with modifications as described by Turner *et al.* (2003b). LP activity of the colostrum and milk samples was assayed as follows: 3 ml 0.1 M citrate buffer (pH 5.5), 200 µl 7.5 mM ABTS (2,2' azino-di-[3-ethylbenzthiazoline-6-sulphonic acid]) and 100 µl of milk were mixed together. H_2O_2 solution (10.5 mM; 50 µl), was added, mixed and the absorbance measured at 413 nm at 120 and 240 seconds following the addition of the H_2O_2. One unit of activity is defined as the amount of enzyme resulting in the oxidation of 1 mM ABTS/minute under the standard assay conditions.

Milk FA profiles were analysed as described by Thomson *et al.* (2003). Briefly, fat was extracted from the milk using the Röse-Gottlieb fat extraction procedure (IDF, 1987) and fatty acid methyl esters were quantified by gas chromatography after methylation using a Gas Chromatogram (Shimadzu Corporation, Kyoto, Japan GC 17A-FID) with a 120-m BPX-70 column.

Data were analysed using residual maximum likelihood (REML) in the GenStat statistical package (GenStat 2002). Breed and age group (3 year old cows vs cows 4 years and older) were fitted as fixed effects. Data were analysed at each time individually. Lf concentration and yield were analysed following $\log_{10}$ transformation, which was used to stabilise cow variation. For ease of understanding and to allow comparison with published values, both the $\log_{10}$ and back-transformed means are presented in Table 1.

RESULTS

Cow genotype had no effect on Lf concentration (Table 1) in colostrum or milk. However, age ($P < 0.002$; data not presented) did. Based on the total season's production (colostrum and milk), 4-year and older cows produced higher Lf concentrations in their milk (409 mg/l) than 3 year old cows (268 g/l). There was a significant effect of cow genotype on Lf yield in February and at drying-off (Table 1) with Friesian and crossbred cows producing higher Lf yields than Jersey cows. Older cows also produced greater ($P < 0.001$; data not presented) Lf yields, with cows 4 years and older producing on average 4.31 g/day whereas 3 year old cows produced on average 2.75 g/day.

Colostrum and milk of Friesian cows had lower LP activity levels compared with milk of crossbred and Jersey cows (Table 1). In all genotypes LP activity was less in colostrum compared with milk, Jersey colostrum being the lowest having 57% of milk activity compared with 67% in Friesian cows and 71% in crossbred cows. The highest LP activity was observed in the milk of Jersey cows, with milk of crossbred cows having approximately 84%, and Friesian milk having approximately 67% of the LP activity in Jersey milk. In contrast to Lf, there was no effect of age on LP activity.

Cow genotype affected the concentration of saturated and unsaturated FA (Table 2). Milk fat from Jersey cows had a greater concentration of total saturated FA compared with Friesian and crossbred cows' milk fat in both October and February. The concentration of total saturated FA in milk fat differed between Friesian and crossbred cows in February only, with crossbred cows having the greater saturated FA concentration. At both sampling times, milk fat from Jersey cows also had the lowest concentration of total unsaturated FA than Friesian and crossbred cows' milk fat. Again, there was a difference between Friesian and crossbred cows in February only, with Friesian cows producing milk fat with the greater unsaturated FA concentration.

TABLE 1: Effect of cow genotype on lactoferrin concentration, lactoferrin yield and lactoperoxidase activity in colostrum and milk.

	Friesian	Jersey	Crossbred	SED	P value
Log_{10} Lactoferrin concentration (mg/l)[1]					
Colostrum	2.2	2.3	2.3	0.2	0.611
	(169)	(215)	(193)		
October	1.8	1.7	1.7	0.2	0.363
	(68)	(45)	(51)		
February	2.4	2.1	2.4	0.2	0.158
	(232)	(115)	(245)		
Drying-off	2.7	2.6	2.8	0.1	0.706
	(445)	(388)	(598)		
Log_{10} Lactoferrin yield (g/day)[2]					
Colostrum	0.43	0.26	0.39	0.20	0.912
	(2.66)	(1.80)	(2.43)		
October	0.20	-0.13	0.05	0.16	0.109
	(1.57)	(0.75)	(1.01)		
February	0.58^{b}	0.11^{a}	0.50^{b}	0.15	0.002
	(3.79)	(1.27)	(3.16)		
Drying-off	0.56^{b}	0.23^{a}	0.52^{b}	0.11	0.033
	(3.63)	(1.70)	(3.28)		
Lactoperoxidase activity (U/ml)					
Colostrum	4.74^{a}	6.05^{b}	6.34^{b}	0.65	0.028
October	7.35^{a}	10.82^{b}	9.26^{b}	0.90	0.001
February	6.55^{a}	10.41^{b}	7.72^{b}	0.85	0.001
Drying-off	7.47^{a}	10.73^{b}	9.85^{b}	1.14	0.013

[1] Log_{10} values from the statistical analysis are presented, with the back-transformed concentration (mg/l) presented in parentheses.
[2] Log_{10} values from the statistical analysis are presented, with the back-transformed yields (g/day) presented in parentheses.
^{a,b,c} means with different superscripts within rows are significantly different at $P < 0.05$.

The effect of cow genotype on the proportion of saturated to unsaturated FA in milk fat is demonstrated in the changes in the C_{18} FAs (Table 2). Friesian cows' milk fat had lower concentrations of $C_{18:0}$ than milk fat of Jersey and crossbred cows in both October and February, whereas Jersey cows had lower concentrations of total $C_{18:1}$ in their milk fat, and this was consistent for the CLA precursor, vaccenic acid. Jersey cows had approximately 55% and 77% of Friesian values for vaccenic acid in October and February. Concentrations of CLA were lower in Jersey cows' milk fat compared with milk fat of Friesian and crossbred cows in both February and October. Jersey cows' milk fat had concentrations of approximately 47% and 57% of Freisian cows' milk fat, whereas crossbred cows' milk fat had 68% and 73% of Friesian cows' milk fat at the two sampling times.

The observed differences between genotypes in vaccenic acid and CLA concentrations in milk fat were supported by the changes in the desaturation index. The desaturation index is the ratio between the product and substrate (the desaturated FA to its saturated FA) in milk fat caused by the Δ^9-desaturase enzyme. Cow genotype had an effect on the desaturation indices (Table 2) in both October and February, where Jersey cows' milk fat had a significantly lower ratio than occurred in milk fat from Friesian and crossbred cows. The ratio between CLA and vaccenic acid in milk fat from Jersey cows was also lower than in milk fat from Friesian and crossbred cows.

Concentration of $C_{18:3}$ was unaffected by cow genotype but there was an inconsistent effect of genotype on EPA, and DHA was not detectable. The effect of genotype on EPA concentration was detectable only in February, when Jersey cows had the lowest EPA concentration in milk fat (Table 2).

DISCUSSION

There was no effect of cow genotype on Lf concentration in colostrum or milk. At present, there are no published values for Lf concentrations in colostrum for pasture-fed cows to compare with our data. A comparison with overseas data suggests our

values are low and there are no indications as to why this might be. In milk, the Lf concentration increased during lactation and this agrees with studies in pasture-fed cows (Turner *et al.,* 2003a) and concentrate-fed cows (Hagiwara *et al.,* 2003). However, whether or not there is a genotype effect is inconclusive. In agreement with our study, Tsuji *et al*. (1990) reported there was no difference in Lf concentration between Holstein-Friesian and Jersey cows, whereas Farr *et al.* (2002) reported that in pasture-fed Friesian and Jersey cows milked once or twice a day, Friesian cows' milk had higher Lf concentrations in once-a-day samples and the morning sample of cows milked twice a day. In contrast, our study determined Lf concentrations on a composite sample of morning and afternoon milk.

An important result was the effect of age on Lf concentration where milk from older cows had greater Lf concentrations. As with genotype, published data are inclusive about the effect of age. In a study by Hagiwara *et al.* (2003), the younger cows (2 & 3 years old) had higher concentrations than 5-6 year old cows,

TABLE 2: Effect of cow genotype on selected fatty acid concentrations (% of individual fatty acids in fat) in milk collected in October and February.

Fatty Acid	Month	Friesian	Jersey	Crossbred	SED	P value
Pre-formed	October	34.03^{ab}	35.08^{a}	32.41^{b}	0.94	0.022
	February	29.23	30.66	29.47	0.62	0.054
Total C_{16}	October	29.21	29.59	29.83	0.85	0.763
	February	32.78	32.59	34.05	0.77	0.135
Formed	October	35.56	34.30	36.73	1.22	0.147
	February	37.35	36.39	36.02	0.91	0.326
Total saturated	October	72.10^{a}	76.08^{b}	72.80^{a}	0.89	0.001
	February	71.21^{a}	75.08^{c}	73.51^{b}	0.64	0.001
Total	October	26.70^{a}	22.90^{b}	26.17^{a}	0.89	0.001
unsaturated	February	28.16^{c}	24.56^{a}	26.03^{b}	0.65	0.001
$C_{18:0}$	October	10.21^{a}	12.45^{b}	11.72^{b}	0.59	0.001
	February	10.43^{a}	12.83^{c}	11.02^{b}	0.50	0.001
$C_{18:1}$ total	October	20.72^{b}	18.12^{a}	20.73^{b}	0.83	0.003
	February	22.41^{b}	20.07^{a}	20.93^{a}	0.58	0.001
trans-11 $C_{18:1}$	October	2.90^{c}	1.59^{a}	2.19^{b}	0.21	0.001
	February	1.83^{b}	1.40^{a}	1.45^{a}	0.13	0.003
$C_{18:2}$ CLA[1]	October	1.16^{c}	0.54^{a}	0.79^{b}	0.08	0.001
	February	1.35^{c}	0.78^{a}	0.99^{b}	0.09	0.001
$C_{18:2}$ linoleic	October	0.97^{b}	0.87^{a}	0.96^{b}	0.04	0.044
	February	0.88^{b}	0.80^{a}	0.78^{a}	0.04	0.014
$C_{18:3}$	October	0.76	0.80	0.83	0.03	0.073
	February	0.66	0.63	0.64	0.03	0.476
$C_{20:5}$ (EPA)	October	0.10	0.10	0.09	0.01	0.549
	February	0.07^{b}	0.03^{a}	0.11^{c}	0.01	0.001
Desaturase index[2]						
$C_{14:1}/C_{14:0}$	October	0.024^{b}	0.021^{a}	0.024^{b}	0.001	0.003
	February	0.024^{b}	0.022^{a}	0.024^{b}	0.001	0.074
$C_{16:1}/C_{16:0}$	October	0.045^{c}	0.036^{a}	0.041^{b}	0.002	0.001
	February	0.051^{b}	0.038^{a}	0.043^{a}	0.003	0.001
cis-9$C_{18:1}/C_{18:0}$	October	1.53^{c}	1.19^{a}	1.43^{b}	0.066	0.001
	February	1.82^{c}	1.34^{a}	1.64^{b}	0.066	0.001
CLA/VA[3]	October	0.40^{b}	0.34^{a}	0.37^{ab}	0.020	0.025
	February	0.75^{b}	0.57^{a}	0.69^{b}	0.037	0.001

[1] $C_{18:2}$ CLA is the concentration of the *cis*-9, *trans*-11 $C_{18:2}$ isomer.
[2] Product to substrate ratio.
[3] CLA/VA is the ratio between *cis-9*, *trans-11* CLA and *trans-11* $C_{18:1}$ (vaccenic acid).
[a,b,c] means with different superscripts within rows are significantly different at $P < 0.05$.

whereas Harmon *et al.* (1975) showed no clear relationship between Lf concentration and age. At present there is no understanding of a physiological basis for an age effect. It is known that Lf concentrations are increased with inflammation of the gland (e.g mastitis; Hagiwara *et al.* 2003). The relationship between Lf concentration and somatic cell counts in this study was examined (data not presented), but no consistent relationship was detected. Our results indicate it would appear to be most profitable to harvest Lf from milk of older cows. However, given the discrepancy between our results and those previously published, further research is required to understand the effects of genotype and age.

In the three genotypes, LP activity was higher in milk compared with colostrum. In milk, values were similar in October and at drying-off but slightly lower in February. It is not known why this fluctuation would occur as Fonteh *et al.* (2002) found that LP activity increased from the onset of lactation to peak at mid lactation and decreased thereafter. It is hard to evaluate our data as much of the work pertaining to LP has been concerned with the role of LP and the LP system (LP, hydrogen peroxide and thiocyanate) in preserving milk. While it is known that there is a large variation in LP activity between and within animals, species, feed and stage of lactation (Zapico *et al.,* 1991, Fonteh *et al.,* 2002), data available in the literature are difficult to compare because of different assays and experimental units used (Kussendrager & Hooijdonk 2000). In terms of increasing milk value through harvesting LP, there is potentially an advantage in harvesting milk from Jersey cows as higher activity in milk was maintained throughout lactation compared with milk of crossbred and Friesians cows.

The FA profile of milk fat between the three cow genotypes differed. Milk FAs are a combination of FA derived from feed, rumen biohydogenation and mammary synthesis. As all cows were grazed on similar pasture, it appears the different FAs profiles were due to a difference in ruminal biohydrogenation of the unsaturated C_{18} FAs, resulting in a different supply of precursors to the mammary gland and/or a difference in enzyme activity in the mammary gland. Milk fat Jersey cows' milk had higher $C_{18:0}$ suggesting greater ruminal biohydrogenation, resulting in lower concentrations of $C_{18:3,}$ $C_{18:2}$ and $C_{18:1}$ FA. The lower concentrations of unsaturated C_{18} FAs found in Jersey milk fat suggests a more rapid hydrogenation process or a slower rate of passage from the rumen which would allow more time for biohydrogenation to occur.

It has also been suggested that Jersey cows have lower Δ^9-desaturase enzyme activity in the mammary gland (Beaulieu & Palmquist 1995, DePeters *et al.,* 1995). The lower concentration of unsaturated FA, especially CLA, in Jersey and even crossbred milk fat supports this. That pasture-fed Jersey cows have much lower CLA concentrations in milk fat than Friesian cows has not previously been reported. In addition, there appears to be a stage of lactation effect with higher enzyme activity in February than October. The difference in the CLA:VA ratio between October and February suggests a greater proportion of vaccenic acid is converted to CLA in February in all cow genotypes. This is despite having lower vaccenic acid concentrations in February. There is limited evidence to support this, but it has been shown in sheep that the onset of lactation results in a dramatic increase in mRNA for Δ^9-desaturase in mammary tissue with a concurrent decrease in adipose tissue (Ward *et al.,* 1998). Therefore, stage of lactation and/or feed type may regulate enzyme abundance and activity in dairy cows.

To our knowledge this is the first published comparison of omega-3 FA concentrations in different cow genotypes. While concentrations of these FAs in this study are low, it is known that the elongation pathway by which omega-3 FA are produced is present in dairy cows. Feeds such as rumen protected fish oils (Kitessa *et al.,* 2004) and algae preparations (Franklin *et al.,* 1999) are being used to increase these FAs in milk fat for niche products in Europe. However, the cause of the lower concentrations of EPA in Jersey cows is unclear as little is known about the mechanisms that control synthesis of these FAs in ruminants.

Given the current trends for a more 'healthy' FA profile in foods, the greater concentration of unsaturated FA and CLA makes Friesian milk a more desirable product. Crossbred cows have a FA profile more similar to Friesians at the October sampling but more similar to the Jersey profile in February. This may mean that there are certain times of the season, i.e. early lactation, where it would be more profitable to harvest milk from crossbred cows.

The results of this study suggest there are opportunities to exploit cow genotype and age to either harvest minor components from milk or produce dairy products with enhanced concentrations. However, under the current payment system it is not possible to estimate realistic financial returns for farmers and processors. This paper also highlights the lack of understanding about the mechanisms that regulate synthesis of these minor components in milk of dairy cows and indicates that further research is required.

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