Journal homepage: http://www.sasas.co.za/journals

South African Journal of Animal Science 2019, 49 (No. 3)

Effect of alternating total mixed ration and pasture feeding on the fatty acid content and health indices of Jersey and Fleckvieh x Jersey milk

S. Abel[1#], C.J.C. Muller[2,3] & B. Sasanti[1]

[1] Chemoprevention Research Group, Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, PO Box 1906, Bellville 7535, South Africa

[2] Western Cape Department of Agriculture, Directorate: Animal Sciences, Private Bag X1, Elsenburg 7607, South Africa

[3] Faculty of Agri-Sciences, Department of Animal Science, University of Stellenbosch, Matieland 7602, South Africa

(Received 20 February 2018; Accepted 12 January 2019; First published online 15 May 2019)

Abstract

Certain fatty acids (FAs), such as omega-3 and conjugated linoleic acid (CLA) are considered essential FAs with beneficial health effects for humans. Milk is considered a relatively inexpensive and readily available source of these FAs and is part of a recommended healthy daily diet. The aim of the study was to determine the effect of diet changes on the FA composition of Jersey (J) and Fleckvieh x Jersey (FxJ) milk. Cows were alternately put on kikuyu-ryegrass pasture, followed by a feedlot system, then returned to kikuyu-ryegrass pasture for four weeks each. The same concentrate mixture was fed to cows regardless of feeding system. The feedlot system consisted of a partial total mixed ration (pTMR). Milk samples were collected two and four weeks after diet changes and stored at −20 °C until laboratory analysis by gas chromatography. The FA concentration of milk was not affected by breed, although it was affected significantly by diet changes. Most notably, the total omega-3 and -6 FAs decreased when cows were fed pTMR while increasing when the diet was changed back to pasture feeding. The CLA content of milk was similarly affected. That is, concentrations decreased significantly when the cows were on the pTMR diet and increased when cows were put back on the pasture-based diet. The results suggested that the health benefits of milk fat are affected negatively when cows are fed pTMR compared with being fed in a pasture-based system. The health benefits of milk are reduced owing to decreased levels of the CLA content of milk fat. Therefore, feeding additional hay in a pasture-based production system should be reconsidered when aiming to produce milk that provides health beneficial qualities.

Keywords: breed, diet, n-3 fatty acid, n-6 fatty acid, pasture, Fleckvieh
[#] Corresponding author: AbelS@cput.ac.za

Introduction

Until recently, fat in milk, specifically saturated fatty acids (SFAs), was regarded as an unhealthy food source as it was linked to obesity, heart disease and some forms of cancer (Salter, 2005). This resulted in fat-free and low-fat milk (0% or 2% fat, respectively) becoming popular choices among consumers. However, more recent results indicate the beneficial effects of milk fat for humans as it is not composed solely of SFAs.

Milk contains a number of types of FAs, of which several appear to have beneficial effects on the health of human beings (Muller & Delahoy, 2016). Conjugated linoleic acid (CLA) in particular seems to have beneficial effects on human health because of cancer-preventive properties (Parodi, 2001; Banni*et et al*., 2003). Various isomers of CLA have been identified, of which *cis*-9,*trans*-11 (*c*-9,*t*-11) ('rumenic' acid) is the most abundant natural form (Muller & Delahoy, 2016). Therefore, reducing the fat content in milk to produce skimmed and low-fat milk decreases or removes beneficial FAs from milk, thereby in effect reducing the health status of low-fat milk compared with full-cream milk.

Breeding and selection in dairy herds has always been aimed at increasing the fat and protein content of milk. However, there is growing interest in increasing the value of milk by changing the FA content through feeding or breeding. According to Soyeurt *et al*. (2007), heritability estimates for FAs in milk and fat range from 5% to 38%. The effect of genetic correlations among FAs indicated an association with the metabolic

URL: http://www.sasas.co.za
ISSN 0375-1589 (print), ISSN 2221-4062 (online)
Publisher: South African Society for Animal Science
http://dx.doi.org/10.4314/sajas.v49i3.4

origin of several groups of FA types. It was also shown that an increased milk fat content did not correlate directly with undesirable milk fat composition, particularly with regard to SFAs. While the effect of breeding can be observed only in the long term, changing the diet has a short-term effect on the composition and FA content of milk (Chilliard *et al*., 2001). Another strategy may include changing breeds for the production of milk and FA content. Grega *et al*. (2005) and Hennessy *et al*. (2007) found that Simmental-derived breeds such as the Fleckvieh (F) from Germany have higher milk CLA levels than other breeds on similar feeding programmes. Sasanti *et al*. (2015) also showed breed differences between Jersey (J) and FxJ cows with regard to milk yield, milk composition and the CLA content of the milk fat of cows. The Fleckvieh breed has dual-purpose characteristics, producing beef and high solids milk, and has crossbreeding potential to improve the lifetime performance of dairy cows. Goni (2014) and Goni *et al*. (2015a; 2015b; 2016) found higher beef production, fat and protein yields and fertility in FxJ cows than in J cows.

Factors that affect CLA concentration in milk include the forage content and the maturity of the forage in the diet of dairy cows. Vanhalato *et al*. (2007) showed that early cut red clover pasture enhanced milk fat monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) content compared with grass silage. Increases in CLA in milk were first noticed more than 60 years ago when cows were put on pasture in the spring (Muller & Delahoy, 2016). Kelley *et al*. (1998) found a two-fold increase in CLA in milk when cows were put on pasture as the sole forage compared with cows on a total mixed ration (TMR). Khanal *et al*. (2007) found cows grazing perennial ryegrass pasture produced 300% - 350% more *c*-9,*t*-11 CLA compared with cows on a TMR, with no further increases resulting from the supplementation of linoleic acid through full-fat extruded soybeans or soy oil. Liu *et al*. (2016) found that diets with different forage qualities affect milk FAs and milk production levels. Pasture-based dairy farmers in South Africa provide additional feeds such as hay or silage and sometimes a partial TMR (pTMR) to cows to increase the milk output. However, feeding such conserved feeds may affect the FA, and in particular the CLA content of milk.

After research that showed the beneficial effects of specific FAs, various health indices were suggested as indicators of the health benefit of products such as milk fat. These indices indicate the relationship between FAs in food and their contribution to the prevention of coronary disease (Turan *et al*., 2007). They include the atherogenicity (AI), thrombogenicity (TI) and the hypocholesterolemic/hyper-cholesterolemic ratio (HH) indices. The AI is a criterion for the level and interrelationship of some milk FAs that are pro-atherogenic as opposed to those that are not. A high AI is assumed to be more detrimental to health. In particular, the FAs C14:0 and C16:0 should be controlled in the diet as they are considered hyperlipidemic, being responsible for an increase in plasma cholesterol. MUFAs and PUFAs are used in the calculation as attenuating modulators of atherogenesis (Ulbricht & Southgate, 1991). The TI is an indicator of the tendency to form clots in blood vessels, and is defined as the relationship between the pro-thrombogenetic FAs (C14:0, C16:0, C18:0) and the anti-thrombogenetic FAs (MUFA, n-6 and n-3 PUFA). The HH index is associated with the cholesterol metabolism, in which the ratio of hypocholesterolemic FAs (MUFAs and PUFAs) to hypercholesterolemic FAs (C14:0 and C16:0) is relevant. A higher index value indicates better nutritional quality with potential for lowering plasma cholesterol (Santos-Silva *et al*., 2002; Veira *et al*., 2015).

The aim of the study was therefore to determine the effect of changing diets from a pasture-based to a pTMR-based feeding system on the milk FA composition, in particular the CLA content and the health indices of the milk fat of J and FxJ cows.

Methods and Materials

The study was conducted as part of a crossbreeding study using F sires on J cows at Elsenburg Research Farm, Western Cape, South Africa (Goni, 2014). Starting from 10 days after calving, milk was sampled from individual cows for milk recording, with the evening milk collection being pooled with the following morning's milk sample, according to standard milk recording procedures. For the study, milk samples for FA analysis (n = 97 for J, n = 98 for FxJ) were collected from J (n = 17) and FxJ (n = 18) cows twice after diet changes. Cows were initially put on kikuyu-ryegrass pasture for two milk collection events (at two and four weeks), after which they were transferred to a feedlot system and fed a pTMR consisting of 48% oat hay, 43% lucerne hay and 9% soybean meal (on an 'as is' basis). Milk samples were collected as described above. After the second milk collection, cows were put onto kikuyu-ryegrass pasture and milk collection was repeated. The amount of pasture available before grazing was judged to ensure a daily pasture consumption of approximately 10 to 12 kg DM per day, while the pTMR was fed at 12 kg per cow per day. Regardless of the pasture- and pTMR-based feeding system, all cows of both breeds were supplemented twice a day after each milking session with the same pelleted concentrate mixture at 3.5 kg in the morning and 3.5 kg in the afternoon.

Milk samples collected for the study were divided into two subsamples, one for FA analysis (500 mL) and another subsample (40 mL) being preserved with bronopo-B_2 preservative pills. The milk samples that

were preserved with bronopo-B_2 were analysed for fat, protein, lactose, milk urea nitrogen and somatic cell count with a Milk-O Scan 133B (Foss, Denmark) at the Dairy Laboratory of the Animal Production Institute of the Agricultural Research Council at Elsenburg according to standard milk recording analyses. Only the data that were recorded for fat were used in this study.

Milk samples were kept at -20 °C until laboratory analyses. Milk FA composition and CLA content of milk samples were analysed by gas chromatography at the Institute of Biomedical and Microbial Biotechnology, Cape Peninsula University of Technology, Bellville, Western Cape, South Africa.

Separation of milk fat was based on the method described by Chouinard *et al*. (1999). Briefly, milk (500 mL) stored at -20 °C was thawed at 36 - 38 °C and homogenized by polytron. An aliquot of homogenized milk (40 mL) was centrifuged at 4000 g for 30 minutes at 8 °C and the fat layer (fat cake) was removed. The lipid from the isolated fat cake was extracted based on the method of Hara & Radin (1978) with hexane/isopropanol (3 : 2, v/v) containing butylated hydroxytoluene (0.01%) as antioxidant. A C19:0 triglyceride was added (12 mg) as internal standard to the fat cake prior to lipid extraction. Saturated sodium sulphate solution (3.5 mL) was added and the top hexane-rich layer of the biphase solution, which contained lipid extract, was removed. Fatty acid methyl esters (FAME) were prepared from the lipid extract according to Christie (1982) with modifications by Chouinard *et al*. (1999). Briefly, 0.5 M sodium methoxide (40 µL) was added to an aliquot of dried lipid extract (40 mg), re-dissolved in hexane (2 mL), allowed to react for 15 minutes at 50 °C, cooled to room temperature and centrifuged at 1000 rpm for 12 minutes. A 1 µL aliquot of FAME in hexane was removed and injected into a gas chromatograph for analyses.

FAME were analysed on a dual-channel Varian 3300, equipped with dual flame-ionization detectors and hydrogen as carrier gas. Capillary columns were BPX-70 120 m (ID 0.25 mm, film 0.25 µm)(SGE, Austin, Texas, USA) with injector and detector temperatures maintained at 240 °C and 280 °C, respectively, and a split ratio of 80 : 1. The initial oven temperature of 80 °C was held for 8 min followed by two temperature ramps (9 °C/min) to 175 °C and 225 °C, respectively, and separated by a 52 min holding time. Unknown chromatogram peaks were identified by a comparison of the retention times with pure standards of FAME and CLA (Nu-Check Prep, Elysian, MN, USA). The concentration of each FA was calculated according to the internal standard (C19:0) and expressed as g FA/100g fat.

The health indices for milk were calculated using specific FAs in milk fat:

- Atherogenicity index: [C12:0+(4xC14:0)+C16:0]/[MUFA+PUFA] (Ulbricht & Southgate, 1991)
- Thrombogenicity index: [C14:0+C16:0+C18:0]/[(0.5xMUFA)+(0.5xn-6 PUFA)+(3xn-3 PUFA)+(n-3 PUFA/n-6 PUFA)] (Ulbricht & Southgate, 1991)
- Hypocholesterolemic/hypercholerolemic index: (C18:1n-9+PUFA)/(C14:0+C16:0) (Santos-Silva *et al*., 2002)

All milk samples collected for FA analysis were used to compare breeds and diets for their milk fat and FA content. Data were subjected to a two-way repeated measure of analysis of variance (ANOVA) using the mixed procedure model of SAS Enterprise Guide 5.1. The model included the effect of breed, diet, lactation and number (stage), and interaction effect of breed and diet as fixed effects, whereas animal influence within treatments was specified as a random effect. Significance was declared at P <0.05. Where necessary, the Tukey-Kramer multiple comparison test was used to detect pair-wise significant differences among groups of more than two means.

Results and Discussion

The effects of diet and breed on FA content are shown in Table 1. In contrast with an earlier study which showed that breed and lactation stage affected the content in milk fat and some FAs (Sasanti *et al*., 2015), the present study showed that the FA content of the milk of cows was not affected by breed (P >0.05) except for C8:0 (P <0.008) and C12:0 (P <0.026). There was no (P >0.05) lactation stage effect, although a linear trend was observed for most FAs, however, with no significant difference between means. The overall mean of the lactation number for both breeds was 3.12 ± 1.52. There was no significant difference between the breeds for days in milk (DIM), with overall means for the DIM of both breeds at the three time points being 44 ± 18, 107 ± 20, and 166 ± 33, respectively, for Pasture 1, pTMR and Pasture 2. Therefore, the data (Table 1) represent the combined overall means (ANOVA) of J and FxJ breeds, because feed had a major effect, whereas breed did not. The lack of lactation stage effect is probably related to specific cows in the study as the CLA content of milk varied between cows, indicating the possibility of genetic variation among individuals. Kelsey *et al*. (2003) also found a threefold variation in the CLA content of milk fat among individual Holstein cows with the range being 2.3 to 7.2 mg/g of FA. Other studies have shown similar variations among cows (Jiang *et al*., 1996; Kelly *et al*., 1998). Pilarczyk *et al*. (2015) showed that the FA profiles of the milk from Simmental cows differed (P<0.05) from those of Holstein-Friesian cows in an organic

farm, and described other studies that indicated breed differences when on the same feeding system. Although winter and summer feeding systems differed in their study, Pilarczyk *et al.* (2015) did not describe the effect of the differing seasonal feed on the milk FA content between the breeds.

Table 1 Overall geometrical means ± SE fatty acid content in the milk fat of Jersey and Fleckvieh x Jersey cows as affected by diet

Parameters	Overall mean	Feed			*P*-values	
		Pasture 1	pTMR	Pasture 2	Breed	Feed
Milk fat %	4.14±0.16	3.94^{a}±0.17	4.28^{b}±0.16	4.21^{ab}±0.16	NS	0.0092
Fatty acid (g FA/100 g fat)						
C4:0	1.66±0.08	1.77^{a}±0.08	1.58^{b}±0.08	1.64^{ab}±0.08	NS	0.008
C6:0	0.69±0.11	0.97^{a}±0.12	0.72^{b}±0.11	0.44^{c}±0.11	NS	0.000
C8:0	0.87±0.05	0.85±0.05	0.86±0.05	0.90±0.05	0.008	NS
C12:0	2.20±0.12	1.99^{a}±0.12	2.24^{ab}±0.11	2.31^{b}±0.11	0.026	0.001
C14:0	8.02±0.35	7.62^{a}±0.37	8.18^{b}±0.34	8.16^{b}±0.34	NS	0.032
C16:0	22.26±0.90	21.22^{a}±0.95	23.12^{b}±0.88	22.34^{ab}±0.87	NS	0.008
C18:0	8.32±0.44	8.96^{a}±0.46	7.70^{b}±0.43	8.44^{a}±0.42	NS	0.000
Total SFA	47.16±1.53	46.29±1.61	47.61±1.50	47.49±1.48	NS	NS
C18:1n-7	0.31±0.03	0.33^{a}±0.03	0.27^{b}±0.03	0.32^{a}±0.03	NS	0.000
C18:1n-9	13.97±0.65	14.60±0.69	13.39±0.64	14.05±0.63	NS	NS
Total MUFA	16.23±0.72	16.76±0.76	15.69±0.70	16.35±0.69	NS	NS
C18:2n-6 (LA)	1.31±0.08	1.37^{a}±0.08	1.12^{b}±0.08	1.45^{a}±0.08	NS	0.000
C20:3n-6	0.07±0.01	0.06^{a}±0.01	0.06^{ab}±0.01	0.07^{b}±0.01	NS	0.010
C20:4n-6	0.08±0.01	0.09±0.01	0.08±0.01	0.08±0.01	NS	NS
Total n-6	1.49±0.09	1.55^{a}±0.09	1.31^{b}±0.09	1.64^{a}±0.09	NS	0.000
C18:3n-3 (ALA)	0.33±0.04	0.29^{a}±0.04	0.26^{a}±0.04	0.42^{b}±0.04	NS	0.000
C20:5n-3	0.03±0.01	0.02^{a}±0.01	0.02^{a}±0.01	0.03^{b}±0.01	NS	0.000
C22:5n-3	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01	NS	NS
Total n-3	0.39±0.04	0.36^{a}±0.04	0.32^{a}±0.04	0.49^{b}±0.04	NS	0.000
Total PUFA	1.89±0.11	1.91^{a}±0.12	1.63^{b}±0.11	2.13^{a}±0.11	NS	0.000
CLA *c*9,*t*11	0.53±0.05	0.53^{a}±0.06	0.37^{b}±0.05	0.68^{c}±0.05	NS	0.000
C18:1 *t*11	0.42±0.04	0.43^{a}±0.04	0.35^{b}±0.04	0.47^{a}±0.04	NS	0.000
LA/ALA	4.78±0.43	5.35^{a}±0.45	4.80^{ab}±0.42	4.31^{b}±0.42	NS	0.002
n-6/n-3	4.37±0.35	4.86^{a}±0.36	4.36^{ab}±0.34	3.99^{b}±0.34	NS	0.004
P/S	0.04±0.01	0.04^{a}±0.01	0.03^{b}±0.01	0.04^{a}±0.01	NS	0.000

Data presented as geometrical means ± standard error from the combined overall means of both breeds together and transformed from logarithmic (Ln) data.
Means within rows with different superscripts differ at P <0.05.
FA: fatty acid(s); SFAs: saturated fatty acids; MUFAs: mono-unsaturated fatty acids; PUFAs: polyunsaturated fatty acids; LA: linoleic acid (18:2n-6); ALA: α-linolenic acid (18:3n-3); CLA: conjugated linoleic acid (*cis*-9,*trans*-11 isomer); n-6/n-3: total n-6/total n-3 fatty acid ratio; P/S: total polyunsaturated/total saturated fatty acid ratio
pTMR: partial total mixed ration

Kelsey *et al*. (2003) speculated that although the physiological and genetic basis for the individual variations had still to be identified, it may be related to rumen output of *trans*-11 18:1 (C18:1 *t*11), and to a lesser extent, *c*9,*t*11 CLA. Although Nogalski *et al*. (2012) showed that a high mobilization of body fat reserves could lead to a change in FA composition, changes in body reserves for cows in the present study would have been the same as those of cows at the same lactation stage. The fat content in milk for both breeds

increased (P <0.05) when the feed was changed from a pasture-based feeding system to pTMR. Kay *et al*. (2005) and Schroeder *et al*. (2005) also showed increases in milk fat percentages when cows were moved from pasture to a TMR feeding system. However, in the present study when cows were transferred back to pasture feeding, milk fat percentage was not affected (P >0.05). Similar to the FAs, a linear trend was observed for lactation stage, but with no significant difference between means.

Various factors have been suggested to change milk fat percentage, such as alterations in PUFA dietary intake, which can affect rumen fibre digestion and consequently change short chain FA synthesis, and *de novo* FA synthesis and suppression of FA synthesis by higher levels of *trans* 18:1 isomer (Brown *et al*., 2008). It appears that breeds with a lower milk fat percentage are associated with a healthier milk fat profile with respect to FA composition, that is, lower levels of SFAs and higher PUFAs than breeds with higher milk fat. In particular, dual-purpose and crossbreds show better FA composition and therefore a more advantageous fat composition than purebreds such as Jersey and Holstein cows (Samkova *et al*., 2012).

Diet has a major effect on the CLA content of milk fat (Kelsey *et al*., 2003). In the present study, diet also affected (P <0.05) the FA content of the milk with some FAs, that is, the C18 and C18 isomers, decreasing when the diet was changed from a pasture- to a pTMR-based feeding system (Table 1). These FAs increased (P <0.05) again when cows were transferred back to a pasture-based diet. Studies that monitored the effect of feed on milk FA composition found that some FAs are lower for cows on TMR compared with a pasture-based feeding system (Kay *et al*., 2005). In the present study, the saturated FAs C4:0, C6:0 and C18:0 decreased (P <0.05) when cows were on a pTMR, while C14:0 and C16:0 increased (P <0.05). The C8:0 was not affected, whereas C12:0 showed a tendency to increase. Transferring the cows back to pasture feeding (Pasture 2) led to an increase only in C18:0 (P <0.05), restoring it to the Pasture 1 level, while C12:0, C14:0 and C16:0 remained elevated. The C6:0 did not recover after cows were put back on pasture, but decreased further (P <0.05). In general, short- and medium-chain SFAs in milk such as C4:0 to C18:0 are lower in pasture feeding systems, while PUFA, CLA and *trans* C18:1 are higher compared with TMR feeding systems (Chilliard *et al*., 2001; Pilarczyk *et al*., 2015; Mierlita, 2016).

Linoleic acid (C18:2n-6 (LA)) decreased (P <0.05) when the feed of cows changed from pasture to a pTMR-based feeding system. However, it increased (P <0.05) when cows were put back onto pasture. The level of C18:3n-3 (α-linolenic acid (ALA)) also increased (P <0.05) when changed from pTMR back to pasture. The decrease in PUFA content when on the pTMR diet was due mainly to the decrease in the C18:2n-6 level, while the change-over back to the pasture diet increased both C18:2n-6 and C18:3n-3, thereby increasing the PUFA level. Interestingly, C18:3n-3 increased more than C18:2n-6. The higher total PUFA content or cows on pasture in this study agrees with findings in other studies. Parodi (1999) noted higher levels of PUFAs with cows on pastures, with a similar tendency shown by Grega *et al*. (2005) and Kraft *et al*. (2003). Zapletal *et al*. (2009) found similar results, although their study focused on comparing beef breeds (Montbéliarde and Czech Fleckvieh). Of particular interest for CLA*c*-9,*t*-11 was that the same trend was observed for C18:2n-6, that is, decreasing (P <0.05) when cows received the pTMR-feed and increasing (P <0.05) when cows were transferred to pasture again. The same pattern was observed for C18:1*t*11, a precursor of CLA in tissues. Interestingly, the increase in CLA and C18:3n-3, when cows were put back on pasture (Pasture 2) was higher (P <0.05) than the initial levels on pasture (Pasture 1). This higher content is probably related to lactation stage, although was not significant between means, similar to a study by Sasanti *et al*. (2015) which showed that while CLA levels differed between breeds, higher levels were shown towards the end of the lactation period.

The n-6/n-3 ratio (Table 1) is also an important determinant of the potential health benefits of a food source. The ratio indicated a declining trend, which was significantly lower at Pasture 2 compared with Pasture 1, probably indicating a lactation effect. According to the World Health Organization and the United Nations Food and Agriculture Organization (FAO/WHO, 1994), a healthy ratio ranges from 5 : 1 to 10 : 1, while a ratio below 4 : 1 is recommended for decreasing the risk of certain non-communicable diseases of lifestyle (Simopoulos, 2002). A balanced ratio is important because n-6 FAs are indicative of promoting pro-inflammatory effects, enhancing the production of cytokines resulting in vasoconstriction which promotes platelet aggregation. These events are related to increased incidence of cardiovascular and inflammatory diseases. In contrast, the n-3 FAs are associated with the prevention of cardiovascular and inflammatory diseases by promoting vasodilation and preventing platelet aggregation. These factors are associated with the prevention of hypertension, atherosclerosis, hypercholesterolemia, arthritis, inflammatory diseases and various cancers. Because the FA composition in milk is increasingly being regarded as a health benefit, it is important to determine the effect of a diet change on the quality of the milk being produced. The type of dietary fat that is consumed has been associated with cardiovascular disease (CVD) with some FAs having a greater role in atherogenesis, and others in thrombogenesis.

The effects of breed and feed on health indices of milk are presented in Table 2.

Table 2 Effects of breed and feed on specific health indices for atherogenicity, thrombogenicity and cholesterol that are attributed to milk

Parameter	Overall mean	Pasture1	pTMR	Pasture2	*P*-values	
					Breed	Feed
AI index	3.20 ± 0.14	2.93^{a} ± 0.15	3.45^{b} ± 0.14	3.17^{ab} ± 0.14	NS	0.000
TI Index	3.25 ± 0.13	3.12^{a} ± 0.13	3.49^{b} ± 0.12	3.13^{a} ± 0.12	NS	0.000
HH Index	0.53 ± 0.02	0.58^{a} ± 0.03	0.48^{b} ± 0.02	0.54^{a} ± 0.02	NS	0.000

Data presented as geometrical means ± standard error from the combined overall means of the breeds and transformed from logarithmic (Ln) data
Row means within rows with different superscripts differ significantly at P <0.05
AI: atherogenicity index [C12:0+(4xC14:0)+C16:0]/[MUFA+PUFA]
TI: thrombogenicity index [C14:0+C16:0+C18:0]/[(0.5xMUFA)+(0.5 x n-6PUFA)+(3xn-3PUFA)+(n-3PUFA/n-6PUFA)]; HH: hypocholesterolemic/hypercholerolemic index (C18:1n-9+PUFA)/(C14:0+C16:0)
pTMR: partial total mixed ration; NS: not significant

Low values of AI and TI indices of milk are considered more beneficial to health, as higher levels of the MUFA and PUFA are associated with preventing the emergence of CVD, whereas a higher ratio for the HH index indicates a better potential of the food to lower cholesterol (Turan *et al.*, 2007). Pasture grazing improved the health potential of the milk (P <0.05), as indicated by the lower AI and TI indices and higher HH index. The C12:0, C14:0 and C16:0 increased with pTMR feeding, while PUFAs decreased, due mostly to lower C18:2n-6 content. Although the shift to pTMR influenced the indexes detrimentally, this was attenuated by Pasture 2, mostly by increasing the n-6 and n-3 PUFA levels. This indicates the potential benefit of feeding pasture as opposed to pTMR in influencing the FA content of milk towards a healthier profile. Saturated FAs with a chain length of 12, 14 or 16 C atoms have a cholesterol-raising effect and are therefore considered atherogenic, while those with a chain length of 14, 16 or 18 C atoms are possibly thrombogenic (Ohlsson, 2010). In contrast, the MUFAs, and n-6 and n-3 PUFAs are considered protective. MUFAs and n-6 PUFAs reduce plasma total cholesterol and low-density-lipoprotein cholesterol concentrations, while the n-3 PUFAs have only a small effect on plasma cholesterol level, but decrease plasma-triacylglycerols, thromboxane B, and platelet activity and prolong bleeding time and heparin-thrombin clotting time (Figueredo *et al*., 2017, Wang & Hu, 2017).

Although this study did not show breed differences, other researchers observed differences. Maurice-Van Eijnhoven *et al*. (2011) compared four cattle breeds in the Netherlands with breed differences. However, results were confounded with breed-herd effects, as only one breed per farm was sampled. Samkova *et al.* (2012) also found that the level of 18:1n-9 differed in levels among dairy breeds, that is, Holstein, Jersey, Brown Swiss, Ayrshire and Montbéliarde. However, a study by Zapletal *et al*. (2009), which compared Montbéliarde and Czech Fleckvieh breeds, showed that total MUFA level did not differ, possibly because of the similarity of the breeds, both being Simmental derived.

The present study showed that the possible health benefit of milk fat is affected negatively when cows are fed a pTMR compared with a pasture-based diet, owing to lower levels of the PUFA and CLA content of milk fat. This could be because the pTMR has a lower content of PUFAs, particularly C18:3n-3, than pasture (Collomb *et al*., 2006) and therefore the dietary source of PUFAs is lower. Kay *et al*. (2005) and Rego *et al*. (2004) also demonstrated that pasture feeding can increase the CLA and PUFA content of cow milk. This is partially due to C18:3n-3 being considered an additional source of CLA as it is converted to C18:1 *t*11 in the rumen and later enzymatically to CLA in the tissues (Shingfield *et al*., 2012). The lower CLA in cows fed pTMR can be ascribed to the process of drying grass to make hay, which in effect decreases the content of the 'parent' fatty acids of CLA, because the pre-cursors of CLA are oxidized in changing the green plant material to the conserved feed products. Muller & Delahoy (2016) found that the CLA content in the milk of cows fed TMR that contained conserved forages was lower than that of cows fed fresh pasture plus concentrate, that is, 6 mg/g fat versus 18, respectively. Cows fed TMR in addition to pasture had CLA levels closer to the TMR only diet. This means that while a pasture-based feeding system may increase the CLA content of milk, feeding additional roughages or a TMR to a pasture-based diet may reduce the level of CLA in milk, resulting in a negative effect on milk quality with regard to healthy FAs. This would be important for processors that use the CLA content of milk as a marketing tool. Therefore, feeding additional hay in a

pasture-based production system should be reconsidered when aiming to produce milk that provides health beneficial qualities.

Conclusion

In the current study, no breed differences in milk fat or FA content was observed when J and FxJ cows were fed the same diet and shared a management system. Changing the diet of J and FxJ cows from a pasture- to a pTMR-feeding system reduced the CLA content of milk by 30% (calculated from CLA level of pasture as baseline), while changing the diet back to pasture increased the CLA content by 84% (calculated from pTMR CLA level as baseline). This suggests that feeding preserved forages, as part of pTMR to dairy cows, compared with a pasture-based system, negatively affects the FA content of milk fat thereby reducing the health benefits of milk owing to lower CLA and PUFA contents. The calculated health indices AI, TI, and HH were also negatively affected by the pTMR feed, indicating a lowering of potential health benefits. The nutritional value of milk, with regard to FA content, can be manipulated by feed as was evidenced by the higher levels of MUFA (C18:1n-9), PUFA (C18:3n-3), and CLA-related FAs such as C18:1 *t*11 and the CLA *c*9,*t*11 isomer. For this reason, the common practice in South Africa of including additional hay or pTMR in pasture-based production systems to increase the carrying capacity of the milking platform should be reconsidered when the aim is to produce milk of high health quality. Changing the feeding system could affect the marketability of milk, possibly helping to create a market for healthier alternatives and products derived from this milk. This could be of benefit to the general public, increasing the potential for a healthier lifestyle.

Acknowledgements

This study was part of a breed comparison study conducted at the Elsenburg Research Farm towards an MTech degree for B Sasanti. The authors express gratitude to Western Cape Department of Agriculture for permission to conduct the study and Western Cape Agricultural Research Trust and Bayern-Genetik (Germany) for funding this study. J.A. Botha, research technician, and personnel at the Elsenburg dairy are also thanked for feeding and milking cows, and for collecting milk samples.

Authors' Contributions

CJCM conceptualized the study and planning at Elsenburg, the laboratory sample preparation and analyses was carried out by BS. SA interpreted the data and wrote the manuscript in collaboration with CJCM.

Conflict of Interest Declaration

The authors declare that there is no conflict of interest.

References

Banni, S., Heys, C.S.D. & Wahle, K.W.J., 2003. Conjugated linoleic acid as anticancer nutrients: Studies in vivo and cellular mechanisms. In: J. Sebedio, W.W. Christie & R. Adolf (eds). Advances in Conjugated Linoleic Acid Research. Volume 2. AOCS Press, Champaign, IL. pp. 267-281.

Brown, W., Abu Ghazaleh, A.A. & Ibrahim, S.A., 2008. Milk conjugated linoleic acid response to fish oil and linseed oil supplementation of grazing dairy cows. Asian-Austral. J. Anim. Sci. 21, 663-670.

Chilliard, Y., Ferlay, A. & Doreau, M., 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest. Prod. Sc. 70, 31-48.

Chouinard, P.Y., Corneau, L., Saebo, A. & Bauman, D.E., 1999. Milk yield and composition during abomasal infusion of CLA in dairy cows. J. Dairy Sci. 82, 2737-2745.

Christie, W.W., 1982. A simple procedure for rapid transmethylation of glycerolipids and cholesterol esters. J. Lipid Res. 23, 1072-1075.

Collomb, M., Schmid. A., Sieber. R., Wechsler, D. & Ryhänen, E.L., 2006. Conjugated linoleic acid in milk fat variation and physiological effects. Int. Dairy J. 16, 1347-1361.

FAO/WHO, 1994. Lipids in early development. Fats and oils in human nutrition. Report of a Joint FAO/WHO Expert Consultation. 57, 49-55.

Figueiredo, P.S., Inada, A.C., Marcelino, G., Cardozo. C.M.L., De Cássia Freitas, K., De Cássia Avellaneda Guimarães, R., De Castro, A.P., Do Nascimento, V.A. & Hiane, P.A., 2017. Fatty acids consumption: The role metabolic aspects involved in obesity and its associated disorders. Nutrients 9, 1158.Doi: 10.3390/nu9101158.

Goni, S., 2014. Production and reproduction performance of Jersey and Fleckvieh x Jersey cows in a pasture-based system. MSc(Agric) thesis, University of Stellenbosch, Stellenbosch, South Africa.

Goni, S., Muller, C.J.C., Dube, B. & Dzama, K., 2015a. Milk production of Jersey and Fleckvieh x Jersey cows in a pasture-based feeding system. Trop. Anim. Health Prod. 47, 139-144.

Goni, S., Muller, C.J.C., Dube, B. & Dzama, K., 2015b. Reproductive performance of Jersey and Fleckvieh x Jersey heifers and cows in a pasture-based feeding system. S. Afr. J. Anim. Sci. 45, 379-385.

Goni, S., Muller, C.J.C., Dube, B. & Dzama, K., 2016. Effect of crossbreeding on beef production of Jersey herd using Fleckvieh sires maintained on a pasture-based feeding system. Open J. Anim. Sci. 6, 163-168. http://dx.doi.org/10.4236/ojas.2016.63021

Grega, T., Sady, M., Najgebauer, D., Domagala, J., Pustkowiak, H. & Faber, B., 2005.Factors affecting the level of conjugated linoleic acid (CLA) in milk from different cow breeds. Bio. Anim. Husb. 21, 241-244.

Hara, A. & Radin, N.S., 1978. Lipid extraction of tissues with low-toxicity solvent. Anal. Biochem. 90, 420-426.

Hennessy, A.A., Ross, R.P., Stanton, C., Devery, R. & Murphy, J.J., 2007. Development of dairy based functional foods enriched in conjugated linoleic acid with special reference to rumenic acid. In: M. Saarela (ed.). Functional Dairy Products. Volume 2. Woodhead Publishing Limited, Cambridge, England. pp. 443-520. https://doi.org/10.1533/9781845693107.3.443

Jiang, J.L., Bjoerck, L., Fonden, R. & Emanuelson, M., 1996. Occurrence of conjugated *cis*-9, *trans*-11-octadecadienoic acid in bovine milk: Effects of feed and dietary regimen. J. Dairy Sci. 79, 438-445.

Kay, J.K., Roche, J.R., Kolver, E.S., Thomson, N.A. & Baumgard, L.H., 2005. A comparison between feeding systems (pasture and TMR) and the effect of vitamin E supplementation on plasma and milk fatty acid profiles in dairy cows. J. Dairy Sci. 72, 322-332.

Kelly, M.L, Berry, J.R., Dwyer, D.A., Griinari, J.M., Chouinard, P.Y., Van Amburgh, M.E. & Bauman, D.E., 1998a. Dietary fatty acid sources affect conjugated linoleic acid concentrations in milk from lactating dairy cows. J. Nutr. 128, 881-885.

Kelsey, J.A., Cori, B.A., Collier, R.J. & Bauman, D.E., 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. J. Dairy Sci. 86, 2588-2597.

Khanal, R.C., Dhiman, T.R., Boman, R.L. & McMahon, D.J., 2007. Influence of supplementing dairy cows grazing on pasture with feeds rich in linoleic acid on milk fat conjugated linoleic acid (CLA) content. Asian-Austral. J. Anim. Sci. 20, 1374-1388.

Kraft, J., Collomb, M., Mockel, P., Sieber, R. & Jahreis, G., 2003. Differences in CLA isomers distribution of cows' milk lipids. Lipids 38, 657-664.

Liu, S., Zhang, R., Kang, R., Meng, J. & Ao, C., 2016. Milk fatty acid profiles and milk production from dairy cows fed different forage quality diets. Anim. Nutr. 2, 329-333.

Maurice-Van Eijnhoven, M.H.T., Hiemstra, S.J. & Calus, M.P.L., 2011. Short communication: Milk fat composition of four cattle breeds in the Netherlands. J. Dairy Sci. 94, 1021-1025.

Mierlita, D., 2016. Fatty acid profile and health lipid indices in the raw milk of ewes grazing part-time and hemp seed supplementation of lactating ewes. S. Afr. J. Anim. Sci. 46, 237-246.

Muller, L.D. & Delahoy, J.E., 2016. Conjugated linoleic acid (CLA) implications for animal production and human health. DAS 04-88. Penn State College of Agricultural Sciences.

Nogalski, Z., Wroński, M., Sobczuk-Szul, M., Mochol, M. & Pogorzelska, P., 2012. The effect of body energy reserve mobilization on the fatty acid profile of milk in high-yielding cows. Asian-Austral. J. Anim. Sci. 25, 1712-1720.

Ohlsson, L., 2010. Dairy products and plasma cholesterol levels. Food & Nutrition Research 54, 5124. Doi: 10.3402/fnr.v54i0.5124.

Parodi, P.W., 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milkfat. Symposium: A bold new look at the milk fat. J. Dairy Sci. 82, 1339-1349.

Parodi, P.W., 2001. Cow's milk components with anti-cancer potential. Aust. J. Dairy Technol. 56, 65-73.

Pilarczyk, R., Wójcik, J., Sablik, P. & Czemiak, P., 2015. Fatty acid profile and health lipid indices in the raw milk of Simmental and Holstein-Friesian cows from an organic farm. S. Afr. J. Anim. Sci. 45, 30-38.

Rego, O. A., Portugal, P.V., Sousa, M.B., Rosa, H.J.D., Vouzela, C.M., Borba, A.E.S. & Bessa, R.J.B., 2004. Effect of diet on the fatty acid pattern of milk from dairy cows. Anim. Res. 53, 213-220.

Salter, A.M., 2005.The role of diet in preventing cardiovascular disease in the 'post-statin' era. Proc. Cornell Nutr. Conf. pp. 127-137.

Samkova, E., Spicka, J., Pesek, M., Pelikanova, T. & Hanus, O., 2012. Animal factors affecting fatty acid composition of cow milk fat: A review. S. Afr. J. Anim. Sci. 42, 83-99.

Santos-Silva, J., Bessa, R.J.B. & Santos-Silva, F., 2002. Effect of genotype, feeding system and slaughter weight on the quality of light lambs. II. Fatty acid composition of meat. Livest. Prod. Sci. 77, 187-194.

Sasanti, B., Abel, S., Muller, C.J.C., Gelderblom, W.C.A. & Schmulian, A., 2015. The milk fatty acid composition and conjugated linoleic acid content of Jersey and Fleckvieh x Jersey cows in a pasture-based feeding system. S. Afr. J. Anim. Sci. 45, 411-418.

Schroeder, G.F., Couderc, J.J., Bargo, F. & Rearte, D.H., 2005. Milk production fatty acid profile of milk fat by dairy cows fed a winter oat (*Avena sativa* L.) pasture only or a total mixed ration. New Zealand J. Agr. Res. 48, 187-195.

Shingfield, K.J., Kalrenius, P., Arola, A., Paillard, D., Muetzel, S., Ahvenjarvi, S., Vanhatalo, A., Huhtanen, P., Tivonen, V., Griinari, J.M. & Wallace, R.J., 2012. Dietary fish oil supplements modify ruminal biohydrogenation, alter the flow of fatty acids at the omasum, and induce changes in the ruminal *Butyrivibrio* population in lactating cows. J. Nutr. 142, 1437-1448.

Simopoulos, A.P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. & Pharmacother. 56, 365-379.

Soyeurt, H., Gillon, A., Vandericks, S., Mayeres, P., Bertozzi, C. & Gengler, N., 2007. Estimation of heritability and genetic correlations for the major fatty acids in bovine milk. J. Dairy Sci. 90, 4435-4442.

Turan, H., Sönmez, G. & Kaya, Y., 2007. Fatty acid profile and proximate composition of the thorn backray (*Raja clavata* L. 1758) from the Sinop coast in the Black Sea. J. Fisheries Sci. 1, 97-103.

Ulbricht, T.L.V. & Southgate, D.A.T., 1991. Coronary heart disease: seven dietary factors. Lancet 338, 985-992.

Vanhalato, A., Kuoppala, K., Toivonen, V. & Shingfield, K.J., 2007. Effects of forage species and stage of maturity on bovine milk fatty acid composition. Eur. J. Lipid Sci. Techn. 109, 856-867.

Veira, C.P., Alvares, T.S., Gomes, L.S., Torres, A.G., Paschoalin, V.M.F. & Conte-Junior, C.A., 2015. Kefir grains change fatty acid profile of milk during fermentation and storage. PLoS ONE 10(10): e0139910.https://doi.org/10.1371/journal.pone.0139910.

Wang, D.D. & Hu, F.B., 2017. Dietary fat and risk of cardiovascular disease: Recent controversies and advances. Ann. Rev. Nutr. 37, 423-446.

Zapletal, D., Chladek, G. & Subrt, J., 2009. Breed variation in the chemical and fatty acid compositions of the *longissimus dorsi* muscle in Czech Fleckvieh and Montbéliarde cattle. J. Livest. Sci. 123, 28-33.