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

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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

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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 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...
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 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/hypercholesterolemic 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 prothrombogenic FAs (C14:0, C16:0, C18:0) and the anti-thrombogenic 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 PUFA s) 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-B2 preservative pills. The milk samples that
were preserved with bronopo-B2 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 biphasic 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**: \[ \frac{[C12:0+(4xC14:0)+C16:0]}{[0.5xMUFA]+(0.5xn-6 PUFA)+(3xn-3 PUFA)+(n-3 PUFA/n-6 PUFA)] \] (Ulbricht & Southgate, 1991)
- **Thrombogenicity index**: \[ \frac{[C14:0+C16:0+C18:0]}{(0.5xMUFA)+(3xn-3 PUFA)+(n-3 PUFA/n-6 PUFA)} \] (Ulbricht & Southgate, 1991)
- **Hypocholesterolemic/hypercholesterolemic index**: \[ \frac{(C18:1n-9+PUFA)}{(C14:0+C16:0)} \] (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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Overall mean</th>
<th>Feed</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pasture 1</td>
<td>pTMR</td>
</tr>
<tr>
<td>Milk fat %</td>
<td>4.14±0.16</td>
<td>3.94±0.17</td>
<td>4.28±0.16</td>
</tr>
<tr>
<td>Fatty acid (g FA/100 g fat)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C4:0</td>
<td>1.66±0.08</td>
<td>1.77±0.08</td>
<td>1.58±0.08</td>
</tr>
<tr>
<td>C6:0</td>
<td>0.69±0.11</td>
<td>0.97±0.12</td>
<td>0.72±0.11</td>
</tr>
<tr>
<td>C8:0</td>
<td>0.87±0.05</td>
<td>0.85±0.05</td>
<td>0.86±0.05</td>
</tr>
<tr>
<td>C12:0</td>
<td>2.20±0.12</td>
<td>1.99±0.12</td>
<td>2.24±0.11</td>
</tr>
<tr>
<td>C14:0</td>
<td>8.02±0.35</td>
<td>7.62±0.37</td>
<td>8.18±0.34</td>
</tr>
<tr>
<td>C16:0</td>
<td>22.26±0.90</td>
<td>21.22±0.95</td>
<td>23.12±0.88</td>
</tr>
<tr>
<td>C18:0</td>
<td>8.32±0.44</td>
<td>8.96±0.46</td>
<td>7.70±0.43</td>
</tr>
<tr>
<td>Total SFA</td>
<td>47.16±1.53</td>
<td>46.29±1.61</td>
<td>47.61±1.50</td>
</tr>
<tr>
<td>C18:1n-7</td>
<td>0.31±0.03</td>
<td>0.33±0.03</td>
<td>0.27±0.03</td>
</tr>
<tr>
<td>C18:1n-9</td>
<td>13.97±0.65</td>
<td>14.60±0.69</td>
<td>13.39±0.64</td>
</tr>
<tr>
<td>Total MUFA</td>
<td>16.23±0.72</td>
<td>16.76±0.76</td>
<td>15.69±0.70</td>
</tr>
<tr>
<td>C18:2n-6 (LA)</td>
<td>1.31±0.08</td>
<td>1.37±0.08</td>
<td>1.12±0.08</td>
</tr>
<tr>
<td>C20:3n-6</td>
<td>0.07±0.01</td>
<td>0.06±0.01</td>
<td>0.06±0.01</td>
</tr>
<tr>
<td>C20:4n-6</td>
<td>0.08±0.01</td>
<td>0.09±0.01</td>
<td>0.08±0.01</td>
</tr>
<tr>
<td>Total n-6</td>
<td>1.49±0.09</td>
<td>1.55±0.09</td>
<td>1.31±0.09</td>
</tr>
<tr>
<td>C18:3n-3 (ALA)</td>
<td>0.33±0.04</td>
<td>0.29±0.04</td>
<td>0.26±0.04</td>
</tr>
<tr>
<td>C20:5n-3</td>
<td>0.03±0.01</td>
<td>0.02±0.01</td>
<td>0.02±0.01</td>
</tr>
<tr>
<td>C22:5n-3</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>Total n-3</td>
<td>0.39±0.04</td>
<td>0.36±0.04</td>
<td>0.32±0.04</td>
</tr>
<tr>
<td>Total PUFA</td>
<td>1.89±0.11</td>
<td>1.91±0.12</td>
<td>1.63±0.11</td>
</tr>
<tr>
<td>CLA c9,t11</td>
<td>0.53±0.05</td>
<td>0.53±0.06</td>
<td>0.37±0.05</td>
</tr>
<tr>
<td>C18:1 t11</td>
<td>0.42±0.04</td>
<td>0.43±0.04</td>
<td>0.35±0.04</td>
</tr>
<tr>
<td>LA/ALA</td>
<td>4.78±0.43</td>
<td>5.35±0.45</td>
<td>4.80±0.42</td>
</tr>
<tr>
<td>n-6/ALA</td>
<td>4.37±0.35</td>
<td>4.86±0.36</td>
<td>4.36±0.34</td>
</tr>
<tr>
<td>P/S</td>
<td>0.04±0.01</td>
<td>0.04±0.01</td>
<td>0.03±0.01</td>
</tr>
</tbody>
</table>

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 t11), and to a lesser extent, c9,t11 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 crossesbreds 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 TMR-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. (2003) 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 CLAc-9,11 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 C18:1f11, 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Overall mean</th>
<th>Pasture1</th>
<th>pTMR</th>
<th>Pasture2</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Breed</td>
</tr>
<tr>
<td>AI index</td>
<td>3.20 ± 0.14</td>
<td>2.93&lt;sup&gt;a&lt;/sup&gt; ± 0.15</td>
<td>3.45&lt;sup&gt;b&lt;/sup&gt; ± 0.14</td>
<td>3.17&lt;sup&gt;ab&lt;/sup&gt; ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td>TI index</td>
<td>3.25 ± 0.13</td>
<td>3.12&lt;sup&gt;a&lt;/sup&gt; ± 0.13</td>
<td>3.49&lt;sup&gt;b&lt;/sup&gt; ± 0.12</td>
<td>3.13&lt;sup&gt;a&lt;/sup&gt; ± 0.12</td>
<td>NS</td>
</tr>
<tr>
<td>HH Index</td>
<td>0.53 ± 0.02</td>
<td>0.58&lt;sup&gt;a&lt;/sup&gt; ± 0.03</td>
<td>0.48&lt;sup&gt;a&lt;/sup&gt; ± 0.02</td>
<td>0.54&lt;sup&gt;a&lt;/sup&gt; ± 0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

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/hypercholesterolemic 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 (Figueroedo 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, Ayshire 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 t11 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-curors 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 t11 and the CLA c9,t11 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.

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**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**


