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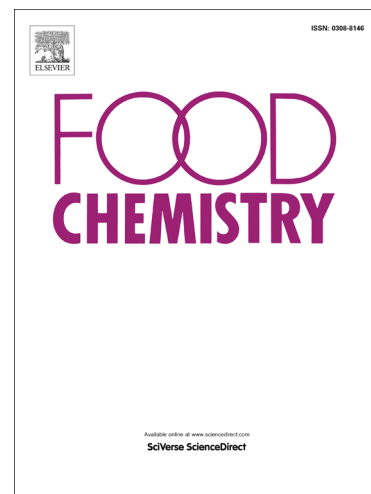
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Impact of US Brown Swiss genetics on milk quality from low-input herds in Switzerland:

interactions with season

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ABSTRACT

This study investigated the effect of, and interactions between, US Brown Swiss (BS) genetics and season on milk yield, basic composition and fatty acid profiles, from cows on low-input farms in Switzerland. Milk samples (n=1,976) were collected from 1,220 crossbreed cows with differing proportions of BS, Braunvieh and Original Braunvieh genetics on 40 farms during winter-housing and summer-grazing. Cows with more BS genetics produced more milk in winter but not in summer, possibly because of underfeeding potentially high-yielding cows on low-input pasture-based diets. Cows with more Original Braunvieh genetics produced milk with more (i) nutritionally desirable eicosapentaenoic and docosapentaenoic acids, throughout the year, and (ii) vaccenic and α-linolenic acids, total omega-3 fatty acid concentrations and a higher omega-3/omega-6 ratio only during summer-grazing. This suggests that overall milk quality could be improved by re-focussing breeding strategies on cows' ability to respond to local dietary environments and seasonal dietary changes.

Keywords: milk, fatty acid, low-input, season, Brown Swiss, Original Braunvieh

1. Introduction

Crossbreeding is the mating of animals of different breeds and has been widely used in commercial livestock production, especially for beef cattle, pig and poultry (Sorensen, Norberg, Pedersen, & Christensen, 2008). In contrast, crossbreeding has not been used extensively in dairy cattle, except for low-input and pasture-based production systems (Ferris, 2007; Stergiadis, et al., 2015b). However, there is increasing interest in developing crossbreeding strategies for European dairy systems (Sorensen, et al., 2008; Weigel & Barlass, 2003) because crossbreeding has been shown to (1) improve fertility, robustness, health and survival rates and reduce depression due to inbreeding in intensively managed herds (Sorensen, et al., 2008; Weigel, et al., 2003) and (2) increase milk yield in low-input grazing herds (BRAUNVIEH, 2016; Maxa, Neuditschko, Russ, Forster, & Medugorac, 2012). Overall, crossbreed herds may also be more profitable, especially when pricing systems based on milk solids, rather than volume, are introduced (Weigel, et al., 2003).

In Switzerland, the Original Braunvieh (OB) population consists of traditional pure-bred animals and has maintained substantial genetic diversity, due to the use of a relatively high number of natural service sires and relatively weak genetic selection for milk yield (Hagger, 2005; Maxa, et al., 2012). Between 1869 and 1910, OB animals were exported to the U.S.A., and strong genetic selection for yield within this population resulted in the generation of a divergent US Brown Swiss breed (BS) (Hagger, 2005). Since the 1960s, when artificial insemination allowed a global trade in semen, BS genetics were widely imported into Europe to cross OB, resulting in a large population of Braunvieh (BV) cattle, which are crosses between BS and OB and are nowadays also considered a separate breed (Hagger, 2005; Maxa, et al., 2012). All 3 breeds (BS, BV and OB) are currently used widely in Alpine areas of Austria, Germany, Italy and Switzerland (Maxa, et al., 2012).

Previous studies reported that, in addition to feeding regimes and season, breed choice may affect milk composition, and in particular fatty acid (FA) profiles (Carroll, DePeters, Taylor, Rosenberg, Perez-Monti, & Capps, 2006; Croissant, Washburn, Dean, & Drake, 2007; Stergiadis, et al., 2015a;

Stergiadis, Seal, Leifert, Eyre, Larsen, & Butler, 2013). Significant differences in milk fat composition among Holstein, Brown Swiss and Jersey cows have been reported (Carroll, et al., 2006; Stergiadis, et al., 2013) and, more recently, crossing Holstein Friesian cows has been shown to affect milk FA profiles (Stergiadis, et al., 2015b; Stergiadis, et al., 2012). Production season (e.g. summer-grazing vs winter-indoor) is also known to have a substantial effect on milk composition and FA profiles (Butler, et al., 2008; Kliem, Shingfield, Livingstone, & Givens, 2013; Stergiadis, et al., 2012). Summer milk fat had more monounsaturated FA (MUFA), polyunsaturated FA (PUFA), omega-3 PUFA (n-3), oleic acid (OA, c9 C18:1), vaccenic acid (VA, t11 C18:1), α-linolenic acid (ALA, c9c12c15 C18:1), *cis*-9, *trans*-11 conjugated linoleic acid (CLA9, c9t11 C18:2), and a higher omega-3/omega-6 ratio (n-3/n-6), but less saturated FA (SFA), palmitic acid (C16:0) and linoleic acid (LA, c9c12 C18:2) than had winter milk (Butler, et al., 2008; Kliem, et al., 2013; Stergiadis, et al., 2012). This is thought to be due to higher PUFA consumption from fresh grass and lower intakes of conserved forage and concentrate feeds during the grazing period (Butler, Stergiadis, Seal, Eyre, & Leifert, 2011; Dewhurst, Shingfield, Lee, & Scollan, 2006; Kliem, et al., 2013; Stergiadis, et al., 2012). However, only very few studies have investigated the potential interactions between dairy genetics and production season or feeding regimes/fresh forage intake (Croissant, et al., 2007; White, Bertrand, Wade, Washburn, Green Jr, & Jenkins, 2001) and most of these have focussed on Holstein-Friesian and Jersey genetics. A previous study, with grazing cows, reported concentrations of nutritionally beneficial FA were higher in milk from cows with increased OB contribution to their genetics but only when pasture intake was > 75% DMI (Stergiadis, et al., 2015a). Whether this applies in winter, when cow eat conserved forage and concentrates has yet to been investigated.

This study aims, for the first time, to (1) quantify the effects of, and the interactions between, dairy cow genotypes (proportion of BS genetics in crossbreed cows) and season (summer-grazing versus winter-indoor period) on milk yield and FA profiles, and (2) investigate associations between cow genotype (proportion of OB, BS and BV genetics), cow characteristics (days in milk (DIM), parity)

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and dietary drivers (type and amounts of pasture, conserved forage and concentrates) with milk yield and composition (basic composition, FA profile), using redundancy analysis (RDA).

This study reports specific milk FAs, thought to be relevant for human health, including: (1) total saturated FA (SFA), and the individual SFAs, lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), (all linked to an increased risk of cardiovascular diseases (European Food Safety Authority, 2010; Givens, 2010)), (2) total MUFA, and individual MUFAs, VA and OA (associated with reduction of plasma cholesterol, LDL-cholesterol and triglycerides, improvement of immune function and protection against atherosclerosis in mice, and, potentially, anti-cancer properties (Field, Blewett, Proctor, & Vine, 2009; Givens, 2010; Haug, Hostmark, & Harstad, 2007)), and (3) total PUFA, CLA9, n-3, and individual n-3, ALA, eicosapentaenoic acid (EPA, c5c8c11c14c17 C20:5), docosapentaenoic acid (DPA, c7c10c13c16c19 C22:5) and docosahexaenoic acid (DHA, c4c7c10c13c16c19 C22:6), (linked to reduced risk of cardiovascular disease, cancer and/or obesity, improved body composition, bone density, foetal development, and enhanced immune, neurological and cognitive functions and/or anti-inflammatory function (Swanson, Block, & Mousa, 2012)).

2. Materials and methods

All animal-related procedures were in compliance with the Swiss animal welfare act and the animal welfare ordinance, as well as the animal experimentation ordinance, and these procedures were approved by the responsible authority (Cantonal Veterinary Office, Aargau, Switzerland).

2.1. Experiment/survey design

The current study presents results from 1,976 milk samples collected from 1,220 cows registered in the Swiss Brown cattle herd book and under regular milk recording by Braunvieh Schweiz (Zug, Switzerland) on 40 low-input farms in the north east of Switzerland. Thirty eight farms were certified organic according to Swiss organic farming standards, and two, not certified, used very similar production methods. Detailed breeding value records were assessed by Qualitas AG (Zug,

Switzerland). Milk was collected once when cows were housed during winter (between January and March 2010, n = 1040) and once during summer-grazing (between June and September 2010, n = 937). Corresponding animal data (parity, DIM), management and feeding practices (type/amounts of conserved forage, other feeds, supplements offered) were recorded on each date using questionnaires completed by an interviewer, in collaboration with producers. Live weights were estimated based on average breed live weight, as shown in Appendix, Table A1. Estimated total dry matter intake (DMI) and pasture intake (by difference) were calculated as described by Butler et al. (2008), based on average breed live weight and milk yield records.

2.2. *Milk analysis*

Milk samples were immediately frozen after collection and stored at -20° C prior to analysis. Basic composition (fat, protein, lactose and urea contents) and somatic cell count (SCC) analyses of milk were performed by Braunvieh Schweiz (Zug, Switzerland), using Fourier transform infrared spectrophotometry (The MilkoScan™ FT+; FOSS, Hilleroed, Denmark) and flow cytometry (Fossomatic™ FC; FOSS, Hilleroed, Denmark) respectively. Analysis of milk FA profile was based on the method of Chilliard et al. (2009), using previously described modifications and peak identification techniques (Stergiadis, et al., 2015a).

2.3. *Statistical analysis*

The 1,220 cows used in the present study were categorized in four groups depending on the proportion of BS genetics (BS1, 75-99 %, n = 940; BS2, 50-74 %, n = 147; BS3, 25-49 %, n = 54; BS4, 0-24 %, n = 79) and represented by 77.0 %, 12.0 %, 4.4 % and 6.5 % of cows respectively. Cows were crosses between BS, BV and OB genotypes, except for (i) 27 purebred OB cows, and (ii) 21 cows whose pedigree was made up (98-100%) of one (purebred) or more (crossbred) of other breeds (Holstein, Red Holstein, Simmental, Swiss Fleckvieh, Rhaetian Grey, Limousin, Normande) at 98-100%. These exceptions were therefore included in the BS4 group which represented 0-24 % BS in cows' genetics.

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All analyses of variance (ANOVA), derived from linear mixed-effects models (Pinheiro & Bates, 2000), were performed in R statistical environment (R Development Core team, 2009). Variables expressed as proportions (breed parameters, dietary ingredients, individual FA and groups of FA as proportions of total FA) were arcsine transformed, SCC values were log transformed and all other variables were used untransformed. Residual normality was assessed in the transformed variables, where applicable, using the qqnorm function (Crawley, 2007), with no data showing deviation from normality. The ANOVA considered BS contribution in the cows' genetics (4 levels: BS1, 75-99 %; BS2, 50-74 %; BS3, 25-49 %; BS4, 0-24 %) and season (2 levels: summer, winter) as fixed factors and cow ID as random factor. Cows from BS1, BS2, BS3 and BS4 groups were distributed on 39, 33, 14 and 12 farms respectively. Each "crossbreed x season" sub-group used in the present study was represented by at least 54 cows spread over 12 different farms. Pairwise comparisons of means (P < 0.05) were performed using Tukey's honestly significant difference test, by also applying general linear hypothesis test ('glht' function) and multcomp package in R. Homoscedasticity, normality, and/or balanced group sizes are not assumed in this test and we were therefore allowed to perform multiple comparisons in unbalanced models with arbitrary error distribution and hence arbitrary data distribution and variance structure (Bretz, Horthorn, & Westfall, 2011). Δ9-desaturase activity index was calculated according to Kay et al. (Kay, Mackle, Auldist, Thomson, & Bauman, 2004).

Redundancy analysis was used to investigate the influence of cow's genetics and individual diet components on milk basic composition and FA profile, using the CANOCO package (Ter Braak & Smilauer, 1998), with automatic forward selection of variables and significances calculated using Monte Carlo permutation tests. In the RDA biplots, arrow length and direction indicate the relative effects of driver variables (genetics and diet) relative to the response variables. Drivers related to genetics were proportions of each breed (BS, BV, OB) in animal genotype. Drivers related to animal details were parity and DIM. Drivers related to nutrition were dietary proportions of estimated grazing, zero-grazing (fresh-cut grass provided within 1-2 days of harvest), grass silage, grass/clover

silage, maize silage, other silage, hay/straw, whole crop (ensiled whole wheat plants, harvested approximately 1 month before grain maturity), cereals, concentrate feed and other feeds, as well as oil supplements, minerals and vitamins. Milk yield and basic composition parameters (contents of fat, protein, lactose, SCC and urea), individual FA (C12:0, C14:0, C16:0, stearic acid (C18:0), VA, OA, LA, ALA, CLA9, EPA, DPA, DHA), FA groups (SFA, MUFA, PUFA, n-3, omega-6 PUFA (n-6)) and n-3/n-6 ratio and were the response variables.

3. Results

The data available allowed milk quality, to be compared by 2-factor ANOVA for: (1) four genotype groups with different proportions of BS genetics (BS1, 75-99 %; BS2, 50-74 %; BS3, 25-49 %; BS4, 0-24 %) and two seasons (summer-grazing and the winter-indoor). Records of animal crossbreeding and dietary management and measurements of milk yield, basic composition and FA profile also allowed RDA using cow genetics and dietary components as drivers and milk composition parameters as response variables.

3.1. Animal data, crossbreeding composition and diets

Details on estimated animal liveweight, parity, DIM, genetic composition and diet composition, including the types and amounts of conserved forages, concentrate feeds and feed supplements are shown in Table 1. As expected, the proportion of genetics from OB and other breeds increased with decreasing BS proportion (BS1 < BS2 < BS3 < BS4), although differences between BS1 and BS2 were not significant. Differences in DIM between the different crossbreed groups were also not significant, however, cows in the BS2 group were on average two years older than cows in the other crossbreed groups.

Although differences in diet including intakes of total DM, fresh forage, grazing (estimated), fresh-cut grass, grass silage, grass/clover silage, maize silage, wholecrop, and concentrates were statistically significant, on the whole differences were numerically small. For example, estimated

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DMI increased with BS proportion (BS1 > BS2 > BS3 > BS4), although the differences between BS3 and other groups were not significant, and the maximum mean difference between BS1 and BS4 was only 1 kg/cow/day or approximately 5% DMI. Estimated pasture intakes for the four crossbreed groups ranged between 24.9 % and 30.6 % of DMI for BS1 and BS4, respectively, with intermediated intakes for BS2 and BS3. Fresh-cut grass was offered at much lower levels (0.2-1.8 kg DM/cow/day) - highest for BS3, and lowest for BS4 cows, with moderate amounts in BS1 and BS2 cows. This resulted in similar intakes of total fresh forage (grazed plus fresh-cut forage) across all groups (although significant, differences were < 1 kg DM/cow/day). Cows in BS1 and BS2 groups were offered 1.7-2.9 times more grass silage and slightly more maize silage than BS3 and BS4 cows although, again, not all differences were statistically significant and again, amounted to less than 1 kg DM/cow/day. BS4 cows were offered 2.6 to 3.2 times more grass/clover silage than cows in other groups. A similar, and substantial, amount of hay was provided to all groups (5.9 – 6.9 kg DM/cow/day). Other silages, wholecrop, cereals, other feeds, oil supplements and minerals/vitamins averaged less than 0.4 kg DM/cow/day across all groups, with the exception of BS3 group, offered 1.1 kg DM 'other silage'/cow/day. Although BS1 and BS4 cows received more concentrates than BS2 cows, low levels of concentrates (< 1 kg DM/cow/day) were fed to all groups.

Production season also significantly influenced yield and composition (Table 1). The genetic make-up of the cattle populations sampled during winter and summer were very similar, although significant but numerically small differences for BS and BV were detected. As expected, winter and summer feeding strategies differed considerably with (1) nearly 65 % of DMI from grazing/fresh-cut grass in summer but none in winter and (2) more conserved forage (total and individual forages) and concentrate feed offered when cows were housed, with the exception of wholecrop which was offered at slightly higher amounts during summer. Mineral supplementation was similar in summer and winter, while oil supplements were used on some farms but only during the winter.

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3.1. Milk yield and basic composition

Analysis of variance detected significant effects of crossbreed group on milk yield and concentrations of milk lactose and urea, but not milk fat, protein or SCC (Table 2). Milk yield increased with increasing proportion of BS genetics, although differences were only significant when crossbreed cows with the highest and lowest proportion of BS genetics were compared (7 % higher in cows with the highest proportion of BS genetics). Lactose and urea concentrations were highest in cows with the lowest proportion of BS genetics.

Significant effects of season were also detected (Table 2). Milk yield and concentrations of fat, protein and lactose were higher in winter but summer milk had higher SCC and urea concentrations. Significant crossbreed group x season interactions were identified for milk yield and lactose content (Fig. 1). Differences in milk yield per cow between crossbreed groups were significant in winter, with yield falling with decreasing proportions of BS genetics (BS1 > BS2 > BS3 > BS4) but did not differ when cows were under pasture-based diets in summer. Crossbreed groups with more of the BS genetics (BS1 and BS2) produced milk with slightly less lactose than did groups with a lower proportions of BS genetics (BS3 and BS4), but differences were more pronounced during the winter season.

The RDA examined associations between drivers linked to breed, animals and feeding regime with milk yield and basic composition; all three main breeds (but mainly OB and BS), many of the animal data (parity, DIM) and feed components (grazed grass, grass silage, maize silage, hay/straw, wholecrop, cereals, concentrates, vitamins, oil and other feeds) were identified as significant drivers of yield and composition (Fig. 2). Intakes of fresh-cut grass, grass/clover silage, other silage and minerals/vitamins were not significant. Approximately 41% of variation in milk composition and quality, including FA profiles, was explained by selected drivers (25.0 % by axis 1 and a further 16.4 % by axis 2). RDA indicated milk yield, and to a minimal extent, milk lactose concentrations, were strongly positively associated with cow parity, and dietary concentrate, cereals and oil (along the

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negative axis 1) and BS genetics, other feeds maize silage, grass silage and hay/straw in the diet (along the negative axis 2). In contrast, milk protein and to a lesser extent milk fat and SCC concentrations, were positively associated with some conserved forages (grass silage, hay/straw) in the diet, and to a greater extent DIM (mainly along the positive axis 1), but negatively associated with cow parity and dietary concentrates and cereals (along the negative axis 2). OB genetics, grazed grass, and dietary grass/clover silage and wholecrop (which all aligned with the positive axis 1) were positively correlated to milk urea concentrations.

3.2. Milk fatty acid profile

The crossbreed groups significantly influenced concentrations and ratios of a range of nutritionally relevant FA and FA groups, including C12:0, C14:0, VA, ALA, EPA, DPA, PUFA and n-3, and the n-3/n-6, C14:1/C14:0 and CLA9/VA ratios (Table 2). C12:0 concentrations were 6.4 % higher in milk from the BS2 group when compared with the BS1 or BS4 groups, respectively. Milk from BS1 and BS2 cows was slightly (about 3.8 %), but significantly, lower in C14:0 than was milk from BS3 and BS4 cows, but difference between the BS1 and BS3 groups was not significant. Cows from the BS1 group had less VA (-10.5 %) than had milk from BS3 cows) and PUFA (-5.9 % than BS4 milk) and a slightly, but significantly, lower n-3/n-6 ratio compared to the BS3 and BS4 groups. However, milk from cows with low proportions of BS genetics (BS3 and BS4 groups) had significantly more n-3, ALA, EPA and DPA than had milk from BS1 and BS2 cows. Concentrations of total PUFA, n-3, EPA and DPA were 6.3 %, 16.3 %, 32.6 % and 26.7 % higher in milk from BS4 cows than in that from BS1 cows. Ratios of C14:1/C14:0 and CLA9/VA were higher in BS1 and BS2 cows than in BS3 and BS4 cows, but the difference between BS2 and BS3 was not significant for the C14:1/C14:0 ratio.

The season of production had a significant effect on all reported FA, except for n-3 and n-6 and ratios of n-3/n-6 or OA/C18:0 (Table 2). When compared with summer, winter milk had higher concentrations of C12:0 (+10 %), C14:0 (+5 %), C16:0 (+8 %), LA (2 %), ALA (+9 %) and total

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SFA (+6 %), but lower concentrations of C18:0 (-13 %), VA (-38 %), OA (-14 %), CLA9 (-36 %), EPA (-17), DPA (-20 %), DHA (-40 %), total MUFA (-13 %) and PUFA (-11 %). Also, the Δ^9-desaturase index and ratio of C16:1/C16:0 were lower in winter, when ratios of C14:1/C14:0 and CLA9/VA were higher, compared to summer milk.

Interactions between the crossbreed groups and season were significant for the concentrations of many fatty acids; C14:0, VA, ALA, EPA, DPA, DHA, SFA, PUFA, n-3 and n-6, and ratios of n-3/n-6 (Table 2; Fig. 3 and Fig. 4). Milk contained less SFA in summer than in winter, for all crossbreed groups; however, summer milk from BS1 cows had significantly more SFA than that from BS2 cows, while the opposite was found in winter (Fig. 4). The interaction for C14:0 concentrations was very similar, except that the difference between BS1 and BS2 in summer (Fig. 3) was not significant. Total PUFA and VA in milk increased with decreasing proportions of BS (BS1 < BS2 ≤ BS3 ≤ BS4) in summer, whereas differences between crossbreed groups were not significant in winter (Fig. 3 and Fig. 4). For LA, differences between summer and winter milk were only significant for BS1, and differences between crossbreed groups were not significant in either season (Fig. 3). Significantly higher concentrations of ALA were detected for BS3 cows in winter but, for BS4 cows, in summer, while there was no significant difference in ALA concentrations between summer and winter milk for BS2 and BS3 cows (Fig. 3). Total ALA concentrations in milk increased with decreasing proportions of BS (BS1 < BS2 < BS3 ≤ BS4) in summer but significant differences were not detected in winter. A similar pattern was found for milk n-3 concentrations, which were higher during summer for BS2, BS3 and BS4 cows, while there was no difference in n-3 concentrations between summer and winter milk for BS4 cows (Fig. 4). Total n-6 concentrations were higher during the summer for BS2 cows only, while there was no significant difference between summer and winter milk for the other three crossbreed groups (Fig. 4). The n-3/n-6 ratio increased with decreasing proportion of BS genetics in summer (BS1 ≤ BS2 < BS3 ≤ BS4), while the n-3/n-6 ratio in BS4 cows was numerically lower than that of BS3 cows in winter (Fig. 4). Cows from BS1, BS2

and BS3 groups produced milk with more EPA in summer than in winter, but the effect of season on milk EPA concentrations was not significant for the BS1 group (Fig. 3). Concentrations of EPA were highest in milk from BS4 cows in winter and BS3 cows in summer, but the difference between these groups was only significant during winter. DPA concentrations increased with decreasing proportion of BS genetics (BS1 ≤ BS2 ≤ BS3 ≤ BS4) but the relative differences between crossbreed groups were smaller in winter than in summer milk (Fig. 3). For DHA, concentrations in milk were significantly higher only for the BS1 group and in summer (Fig. 3).

The RDA also identified significant associations between breed, animal and feeding regime drivers with milk FA profile; again, all three main breeds (but mainly OB and BS) and the animal data (parity, DIM) and feed components (grazed grass, grass silage, maize silage, hay/straw, wholecrop, cereals, concentrates, vitamins, oil and other feeds) were significant (Fig. 2). Whereas intakes of fresh-cut grass, grass/clover silage, other silage and minerals/vitamins were not significant. The RDA drivers explained approximately 41.1 % of the variation (25.0 % by axis 1 and a further 16.4 % by axis 2) in the variables assessed, which also included milk basic composition parameters. The RDA showed milk concentrations of many beneficial FA; VA, ALA, CLA9, EPA, DPA, PUFA and n-3, the ratio of n-3/n-6 were positively associated with OB genetics, and intakes of grazed grass and wholecrop (along the positive axis 1) and DIM, as lactation progressed (along the positive axis 2). The same FA were negatively associated with BS genetics, intakes of maize silage, grass silage, hay/straw (along the axis 2), concentrates, cereals, oils and cow parity (along the negative axis 1). Results for OA and MUFA were similar to these individual and total PUFA although the link with DIM was negative (along the axis 1). In contrast, concentrations of LA and n-6 were strongly positively associated with cow parity and dietary concentrates and cereals (along the negative axis 1). The main individual SFA (C12:0, C14:0, C16:0) and total SFA were linked positively with BS genetics and intakes of maize silage, grass silage, hay/straw and other feeds (along the negative axis

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2) and negatively with OB genetics and intakes of grazed grass, grass/clover silage and wholecrop (along axis 2).

4. Discussion

During the grazing season, BS genetics has been found to affect milk FA profiles but the extent of this effect was dependent on pasture intake (Stergiadis, et al., 2015a); for example, contents of VA, EPA, n-3 and the ratio of n-3/n-6 in milk were higher in BS3 and BS4 cows, than in BS1 and BS2 cows, but only when pasture intake was > 75% of DMI. Given the strong interaction between pasture intake and BS genetics it is therefore undefined whether these differences would appear in winter, when pasture is unavailable. The current study reports, for the first time, the interactions between genetics and production season on milk yield and fat composition in crossbreed cows with varying BS genetic proportion on low-input systems in Switzerland.

4.1. Milk yield and basic composition

Redundancy analysis showed the main drivers affecting milk yield were dietary, especially the supply of concentrates and cereals, although cows' genetics also had an effect. Previous reports show that crossbreed cows with high proportion of OB genetics produced 14 % less milk than did crossbreed cows with more BS genetics (BRAUNVIEH, 2016), a finding which has been confirmed in this study. For example, BS4 cows produced 1.5 kg less milk per day than did BS1 cows, despite the slightly higher concentrate and cereals intake of the former, possibly because cows with more BS genetics have a higher maximum yield potential (Maxa, et al., 2012). However, yield differences between crossbreed groups were only significant during winter. This may be explained by BS genetics relying on relatively high concentrate feed intakes to reach their maximum yield potential, since concentrate supplementation was very low (less than 1.5 % of DMI) in summer, while in winter (when BS1 cows had higher milk yield than had BS4 cows) cereals and other concentrates accounted for 7.5 % of DMI. This would be consistent with other studies reporting that pasture-based

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diets did not allow high-yielding cows to reach their yield potential (Ferris, 2007), without high levels of concentrate supplementation. This implies that, when high-yielding BS genetics are used in crossbreeding in low-input systems, producers could possibly ensure that feeding practices are appropriate, although levels of supplementation will depend on economics and producers' attitude to intensification (Hoffstetter, Frey, Gazzarin, Wyss, & Kunz, 2014), as well as to compliance with Swiss organic standards which only allow for up to 10 % of concentrates to be included in cows' diets. In the current study there was no genetic influence on milk fat and protein concentrations, consistent with recent reports in Switzerland (BRAUNVIEH, 2016), and differences in lactose and urea concentrations although significant, were numerically very small.

As in previous studies into low-input dairying systems (Stergiadis, et al., 2015b), milk yield and concentrations of fat and protein were higher in winter, likely due to the greater reliance on both conserved forages and concentrates during the winter. For example, in this study cows had an approximately 4-times higher intake of hay and concentrates during winter which will provide an adequate balance of fibre (for milk fat synthesis), and energy (to support milk yield and production of milk protein). The RDA also confirmed this strong association between hay/straw intake with fat and protein contents of milk, and between concentrates intake and milk yield.

4.2. Milk fatty acid profile

Although the effect of season on milk FA profile has been extensively investigated (Butler, et al., 2011; Kliem, et al., 2013; Lock & Garnsworthy, 2003; Stergiadis, et al., 2015b), the fat composition of milk produced by commonly used crossbreed cows in Swiss low-input dairying systems (crosses between BS, BV and OB) has only previously been reported under summer grazing conditions (Stergiadis, et al., 2015a). This study recorded milk FA profiles from these crossbreed cows during both winter and summer, when animals were fed conserved forages and concentrates and pasture-based diets, respectively. Cows with less BS genetics produced milk with higher concentrations of nutritionally desirable n-3; ALA, EPA and DPA although differences were not all consistent between

summer and winter. As previously reported (Stergiadis, et al., 2015a), the current study found OB genetics were positively associated with FAs linked to beneficial effects in human health, such as VA, ALA, EPA, DPA, n-3, and PUFA, while BV and BS genetics showed very little effect on these FA.

However, the study reported here shows, for the first time, that during the summer, traditional OB genetics improved the nutritional quality of FA profiles without compromising productivity (Stergiadis, et al., 2015a). Also, this study shows that differences in fat composition between breeds were much smaller in winter, when cows with high levels of BS genetics produced more milk. These results suggest that differences in beneficial FA (and especially n-3 PUFA) concentrations in milk between crossbreed cows only become apparent when PUFA intakes from pasture-based diets are high. Transition from winter (conserved forages and concentrates) to summer diets (grazing) is likely to (i) alter the supply of FA, transferred directly to milk or acting as substrate for conversion to other milk FAs, and (ii) exert metabolic changes in the rumen and/or the cow, possibly altering activity of nutritionally-sensitive enzymes responsible for *de novo* synthesis of short and medium chain SFA or desaturation of FA in the mammary gland (Lock, et al., 2003). Breed influence on the responses to dietary/seasonal changes has been reported for retail milk (Stergiadis, et al., 2013), but has not been confirmed in other studies (Croissant, et al., 2007; Palladino, Buckley, Prendiville, Murphy, Callan, & Kenny, 2010; White, et al., 2001), which compared Holstein and Jersey genotypes.

Milk from cows with low contribution of BS genetics has higher concentrations of MUFAs, such as VA (the main *trans* FAs found in milk) during summer. This may be of nutritional relevance since there has been concern about potential health risks from the consumption of *trans* FA (European Food Safety Authority, 2010). However, VA does not appear to adversely affect human health, and some studies have linked increased VA consumption to improvements in dislipidemia, atherosclerosis, immune function and anti-cancer action (Field, et al., 2009). Previous work has shown that the ability of cows with high OB genetics to supply milk with more VA concentrations

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depends on high intakes of fresh forage (> 75 % DMI as forage) (Stergiadis, et al., 2015a); suggesting that a combination of appropriate genetics and substrates from grazing is required to optimise VA content in milk.

Much of the CLA9 in milk is produced from VA in the mammary gland under the actions of Δ^9-desaturase (Griinari, Corl, Lacy, Chouinard, Nurmela, & Bauman, 2000) and results here suggest that cows with a high proportion of BS genetics have greater Δ^9-desaturase activity, especially by its best indicator of C14:1/C14:0 (Griinari, et al., 2000). Possible differences on Δ^9-desaturase activity between breeds have been reported, although a consistent effect of breed has not been found (Croissant, et al., 2007; Palladino, et al., 2010; Soyeurt, Dardenne, Dehareng, Bastin, & Gengler, 2008; White, et al., 2001). Inconsistencies between studies may be explained by differences in diets, influencing enzyme activity and substrate supply, as well as wide within-breed variation overriding differences between breeds (Lock, et al., 2003; Palladino, et al., 2010). Overall CLA9 concentrations in milk were similar for all crossbreed categories in this study, suggesting little or no scope to alter milk CLA9 content in these low-input systems by changing crossbreeding strategies. However, in summer, milk from cows with lower proportions of BS and more OB genetics could well, indirectly, provide more CLA9 to consumers as a result of its higher VA concentrations; 19-25% of VA is converted to CLA9, significantly contributing to supply, given that its concentration in milk is generally 2-3 times higher than CLA9 (Field, et al., 2009; van Wijlen & Colombani, 2010).

The main factors influencing EPA and DPA concentrations in milk are dietary intake of EPA, DPA and ALA, the extent of their biohydrogenation in rumen (RBH), the efficiency of absorption, transport and uptake from the mammary gland, and the rate of ALA conversion to EPA and DPA (Rymer & Givens, 2003). Potential genetic differences in the efficiency of these mechanisms cannot therefore be excluded. For example, the finding that cows with high OB genetics had higher milk EPA and DPA concentrations both under indoor/conserved forage and concentrate and outdoor/grazing diets, may imply that there are certain genetic mechanisms which favour higher

rumen turnover or escape RBH and/or higher uptake of EPA and DPA from the mammary gland. Support for the latter mechanism also comes from the positive association between OB genetics and EPA and DPA in milk found in the RDA of both the present and other work (Stergiadis, et al., 2015a). These mechanisms may also interact with levels of dietary PUFA intake through cows' diet since the response to switching from winter to summer diets varied according to the crossbreed group. This hypothesis could be supported by previous work showing that cows with high OB genetics produce milk with higher concentrations of EPA but, as for other beneficial traits, only when pasture intake was high (>75 % DMI) (Stergiadis, et al., 2015a). In humans, EPA and DPA are produced from ALA under the effect of various enzymes, such as elongase, Δ^5-desaturase and Δ^6-desaturase (Rymer, et al., 2003); whether a similar mechanisms exists in ruminants is not clear, but a similar process could also be affected by genetic and nutritional influences, as has already been proven for other enzymes (e.g. synthase, Δ^6-desaturase) (Lock, et al., 2003; Palladino, et al., 2010; Rymer, et al., 2003).

The seasonal variation observed in the present study for milk yield, production and FA profile was expected; dairy management in low-input pasture-based systems is heavily influenced by season, especially the availability and consumption of fresh grass. This is in contrast to more intensive housed production systems where feeding system and other management practices tend to be uniform throughout the year (Hoffstetter, et al., 2014; Stergiadis, et al., 2012). Lower concentrations of nutritionally undesirable SFA and higher concentrations of desirable PUFA have been extensively reported for milk produced in summer, when compared with winter milk (Butler, et al., 2011; Kliem, et al., 2013; Lock, et al., 2003). Lower concentrations of C12:0 and C14:0, produced by *de novo* synthesis in the mammary gland, in summer milk have been widely reported (Butler, et al., 2011; Kliem, et al., 2013; Lock, et al., 2003). Although the mechanism through which season affects the production of C12:0 and C14:0 is still unclear, it is suggested there may be changes in metabolism when cows eat fresh grass, affecting the activity of synthase, the main enzyme involved in producing

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SFA in mammary gland (Lock, et al., 2003). Fresh grass is a relatively rich source of PUFA, and particularly ALA (Dewhurst, et al., 2006). While some dietary PUFA escape RBH and are transferred into milk, the majority undergo RBH by rumen microorganisms (Rymer, et al., 2003). As a result, intermediate (e.g. VA) and finite (C18:0) products of this process are produced (Rymer, et al., 2003); a proportion of these FAs can then be transformed in the udder by Δ^9-desaturase to CLA9 and OA, respectively, and secreted into milk (Griinari, et al., 2000; Haug, et al., 2007). Summer pasture-based diets provide more PUFA (than most conserved forage and concentrate diets), and these will either be transferred into milk or breakdown to substrates for subsequent change to other beneficial FAs, such as VA, CLA9 and OA (Butler, et al., 2011; Kliem, et al., 2013; Stergiadis, et al., 2012). Interestingly, ALA concentrations in milk were slightly higher in winter than in summer, despite the expectation that ALA intake by cows would be higher when fresh grass was fed (Dewhurst, et al., 2006). This is in contrast to other studies (Butler, et al., 2011; Kliem, et al., 2013), although there is evidence that RBH of dietary ALA (to VA) can be higher in grazing animals than in those fed grass silage-based diets (Mohammed, et al., 2009). This could partly explain the similar ALA contents in summer and winter milk in conjunction with nearly 60 % higher milk VA and CLA9 concentrations in summer. The nature of the swards may also affect milk FA profile (Stergiadis, et al., 2015a), although the botanical composition of the natural swards on these farms was not assessed.

4.3. Potential confounding effect of animal and diet on milk FA profile

The RDA was conducted to separate and identify the relative contribution of genetics, animal and feeding details to milk yield and composition. The stage of lactation (as DIM) was found to have a stronger effect on milk FA profile than breeding and diet, however, this is unlikely to mask the effect of these main factors (crossbreeding group and season) since the crossbreed groups (i) were balanced for stage of lactation (averaging 156-182 DIM), and (ii) had very similar proportions of cows in the early lactation (BS1 29 %, BS2 27 %, BS3 30 %, BS4 27 % of total cows; winter 19 %, summer 19

% of total cows). Other studies report the effect of stage of lactation mostly associated with a negative energy balance soon after calving (approximately 1-100 DIM); beyond 100 days the effect has been found to be minimal (Bilal, Cue, Mustafa, & Hayes, 2014; Kelsey, Corl, Collier, & Bauman, 2003; Kgwatalala, Ibeagha-Awemu, Mustafa, & Zhao, 2009).

The age of the cows did differ slightly between groups with more cows in BS 2 surviving into older age. Cows in BS2 group were 31 % in parities 1-3, 43 % in 4-7 and 25 % in 8-14, whereas other groups had 60-65 %, 30-34 % and 4-9 % of cows in corresponding parity ranges. However, previous studies report contradictory effects of parity on milk FA profile (including significant but also non-significant effect) and, when significant differences are reported, this is mainly between primiparous and multiparous cows (Bilal, et al., 2014; Kelsey, et al., 2003; Kgwatalala, et al., 2009). Therefore, a potential confounding effect of parity, which had similar strength to that of OB and BV in the RDA, may not be excluded in comparing BS2 cows with other groups although whether differences between 3rd (average in BS1, BS2 and BS4 groups) and 5th parity (average in BS2 group) strongly influences milk FA profile is perhaps questionable.

Although dietary differences between breeding groups proved to be significant, they were numerically small. As expected, crossing with high-yielding BS genetics was associated with slightly more intensive feeding regimes, on some Swiss farms. For example, cows with greater BS genetics received slightly more maize silage instead of grass/clover silage relative to cows with less BS genetics. Hence a confounding effect of the different diet cannot be excluded since, as revealed by the RDA, feeding has a stronger influence on milk FA profiles than the breed. However, farms selection and study design allowed these differences to be minimised. For example, fresh forage intake (summary of estimated grazing and fresh-cut grass), and forage:concentrate ratio, both major drivers of FA profile in milk (Dewhurst, et al., 2006; Stergiadis, et al., 2015b; Stergiadis, et al., 2012), were very similar across the crossbreed groups, ranging only between 30 % to 36 % of DMI and 76:34 to 87:13, respectively. The largest difference observed between groups for individual

forage intakes was 3.7 kg DM/d (BS3 vs BS4, for grass/clover silage) and RDA indicated grass/clover silage was not linked to variation on milk basic composition or FA profiles. Maximum differences in the intake of other forages, between the crossbreeding groups were < 1.8 kg/d, with differences for most ingredients being less than 350 g DM/d (less than 2% of DMI) and therefore considered rather small to exert a major impact on the observed results.

5. Conclusions

The present study provides evidence that using OB genetics in crossbreeding schemes in Swiss low-input production systems can improve nutritional composition of milk during summer, with higher concentrations of nutritionally desirable VA, ALA, EPA, DPA and lower concentrations of undesirable SFAs. Although there were fewer composition differences during the winter indoor feeding period, milk from OB continued to have higher concentrations of the nutritionally desirable very long chain omega-3 FAs EPA and DPA. However, cows with more OB genetics produced less milk in winter, despite the higher intakes of cereals and concentrates, which tend to support greater yields. The strong interaction between season and crossbreed type identified in the present study implies that a combination of dietary and crossbreeding strategies may be necessary to effectively manipulate milk FA profile. The similarity in milk yields among all crossbreed types in summer may indicate underfeeding of high-merit animals, with more BS genetics, under low-input management. Crossbreeding with more BS genetics could give the potential to increase productivity, but may require better quality grazing swards or higher supplementation of energy-rich concentrates in pasture-based diets. However, the latter approach may impose a risk for low-input systems under the current volatility of feed and milk prices, given that current recommendations to improve profitability in Swiss dairy production are to reduce feeding costs, while compliance with organic regulations requires a less than 10 % contribution of concentrates in cows' diets.

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Figure keys

Fig. 1. Interaction means ± SE for the effects of crossbreed group (% US Brown Swiss genetics; BS1, 75-99%; BS2, 50-74%; BS3, 25-49%; BS4; 0-24%) and season (winter, summer) on milk yield from individual cows from 40 low-input dairy farms in Switzerland during the year. P represents the ANOVA P-value for the interaction. Bars labelled with different lower case letter are significantly different within the same season; bars labelled with different upper case letter are significantly different within the same crossbreed group (Tukey's honestly significant difference test, P < 0.05). No lower or upper case letters indicate that there are no differences between individual season or crossbreed groups, respectively.

Fig. 2. Biplot derived from the redundancy analysis showing the relationship between animal variables (par = parity, days in milk = dim); production system variables (breeding variables expressing proportion of each breed in cows' genetics (bs = US Brown Swiss genetics, bv = Braunvieh, ob = Original Braunvieh); production system variables expressing proportion of feed in cows' diet (cer = cereals, con = concentrates, gra = estimated grazing, gs = grass silage, hs = hay/straw, ms = maize silage, oil = oil supplements, os = other silage, oth = other feeds, vit = minerals/vitamins, wc = wholecrop), and (a) milk yield (shown as dot; yie = yield), basic composition (shown as dots; concentrations of fat, pro = protein, lac = lactose, urea and scc = somatic cell count in milk), and fatty acid (FA) composition (shown as dots; c12 = lauric acid, c14 = myristic acid, c16 = palmitic acid, c18 = stearic acid, va = vaccenic acid, oa = oleic acid, la = linoleic acid, ala = α-linolenic acid, cla9 = c9t11 conjugated linoleic acid, epa = eicosapentaenoic acid, dpa = docosapentaenoic acid, dha = docosahexaenoic acid, sfa = saturated fatty acids, mufa = monounsaturated fatty acids, pufa = polyunsaturated fatty acids, n3 = omega-3 fatty acids, n6 = omega-6 fatty acids, n3n6 = omega-3/omega-6 ratio). Continuous variables (shown as arrows) were bs (P = 0.002), bv (P = 0.002), cer (P = 0.002), con (P = 0.002), dim (P = 0.002), gra (P = 0.002), gs (P = 0.002), hs (P = 0.002), ms (P =

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0.002), ob (P = 0.002), oth (P = 0.002), par (P = 0.002), vit (P = 0.002), oil (P = 0.042); Axis 1 explained 25.0 % of the variation and axis 2 a further 16.4 %.

Fig. 3. Interaction means ± SE for the effects of crossbreed group (% US Brown Swiss genetics; BS1, 75-99%; BS2, 50-74%; BS3, 25-49%; BS4; 0-24%) and season (winter, summer) on the concentrations of myristic acid (C14:0), palmitic acid (C16:0), vaccenic acid (VA), linoleic acid (LA), α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA) of milk collected from individual cows from 40 low-input dairy farms in Switzerland during the year. P represents the ANOVA P-value for the interaction. Bars labelled with different lower case letters are significantly different within the same season; bars labelled with different upper case letter are significantly different within the same crossbreed group (Tukey's honestly significant difference test, P < 0.05). No lower or upper case letters indicate that there are no differences between individual season or crossbreed groups, respectively.

Fig. 4. Interaction means ± SE for the effects of crossbreed group (% US Brown Swiss genetics; BS1, 75-99%; BS2, 50-74%; BS3, 25-49%; BS4; 0-24%) and season (winter, summer) on the concentrations of total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), total omega-3 fatty acids (n-3), total omega-6 fatty acids (n-6) and the omega-3/omega-6 ratio of milk collected from individual cows from 40 low-input dairy farms in Switzerland during the year. P represents the ANOVA P-value for the interaction. Bars labelled with different lower case letters are significantly different within the same season; bars labelled with different upper case letter are significantly different within the same crossbreed group (Tukey's honestly significant difference test, P < 0.05). No lower or upper case letters indicate that there are no differences between individual season or crossbreed groups respectively.
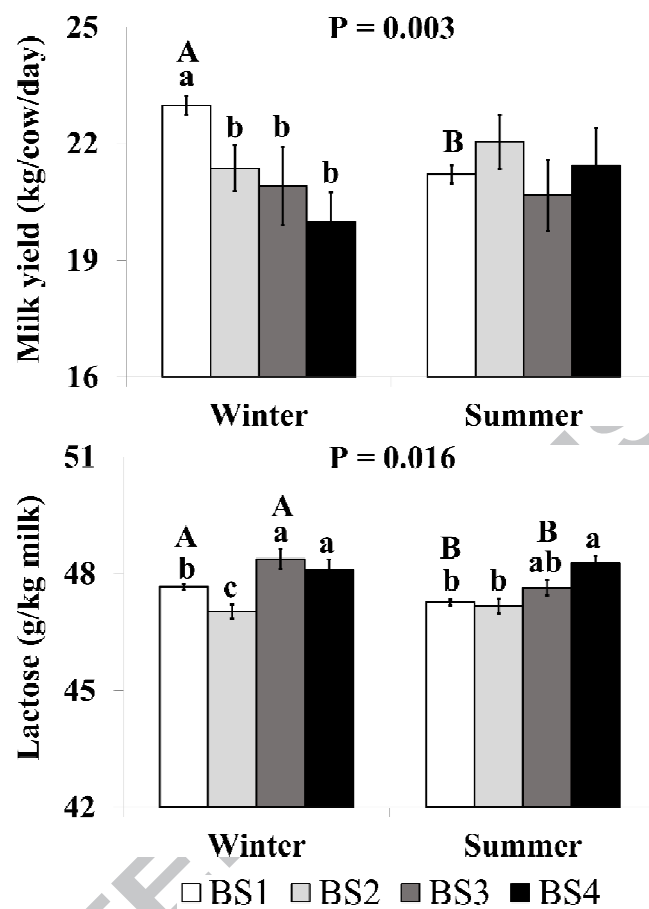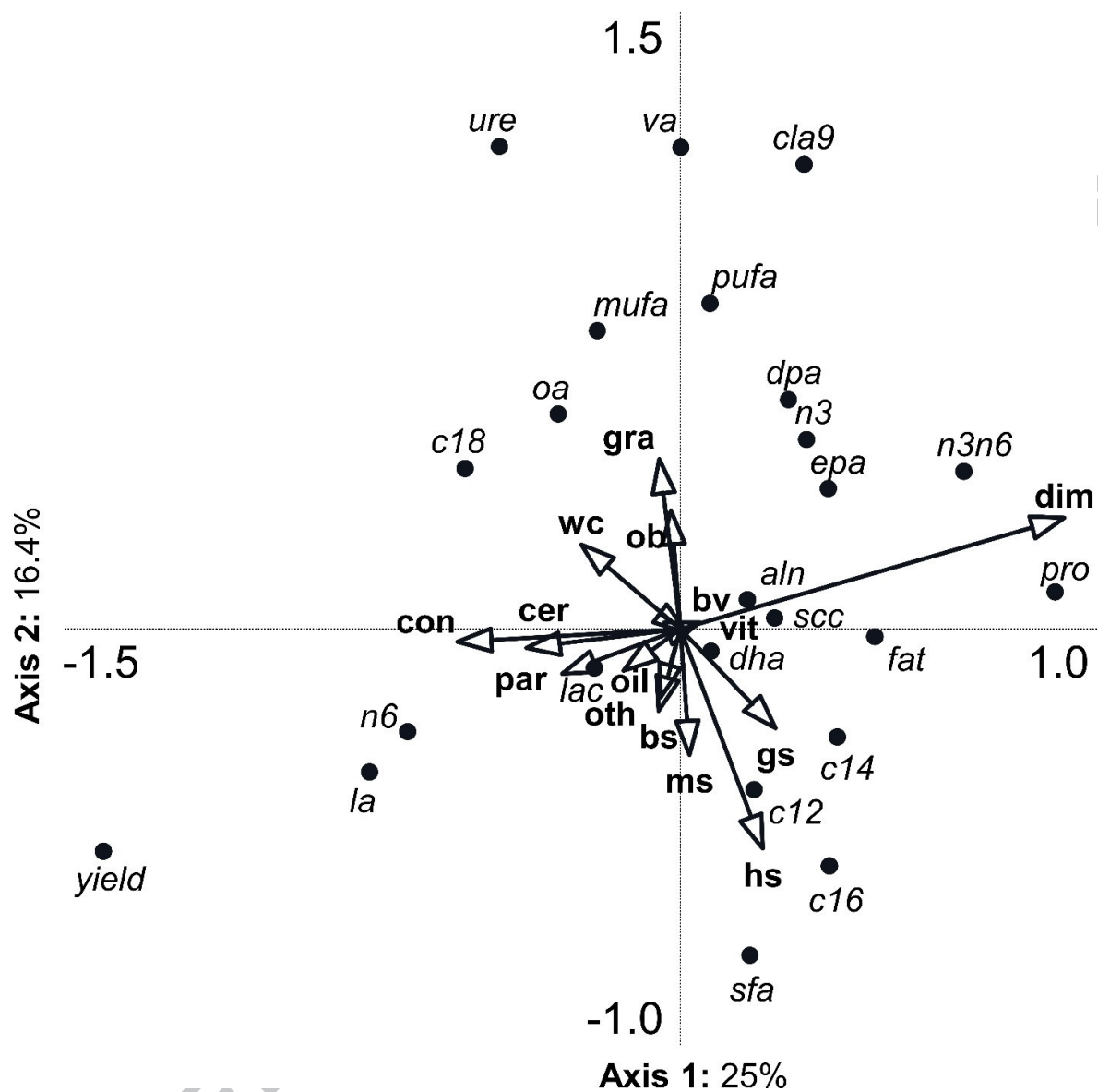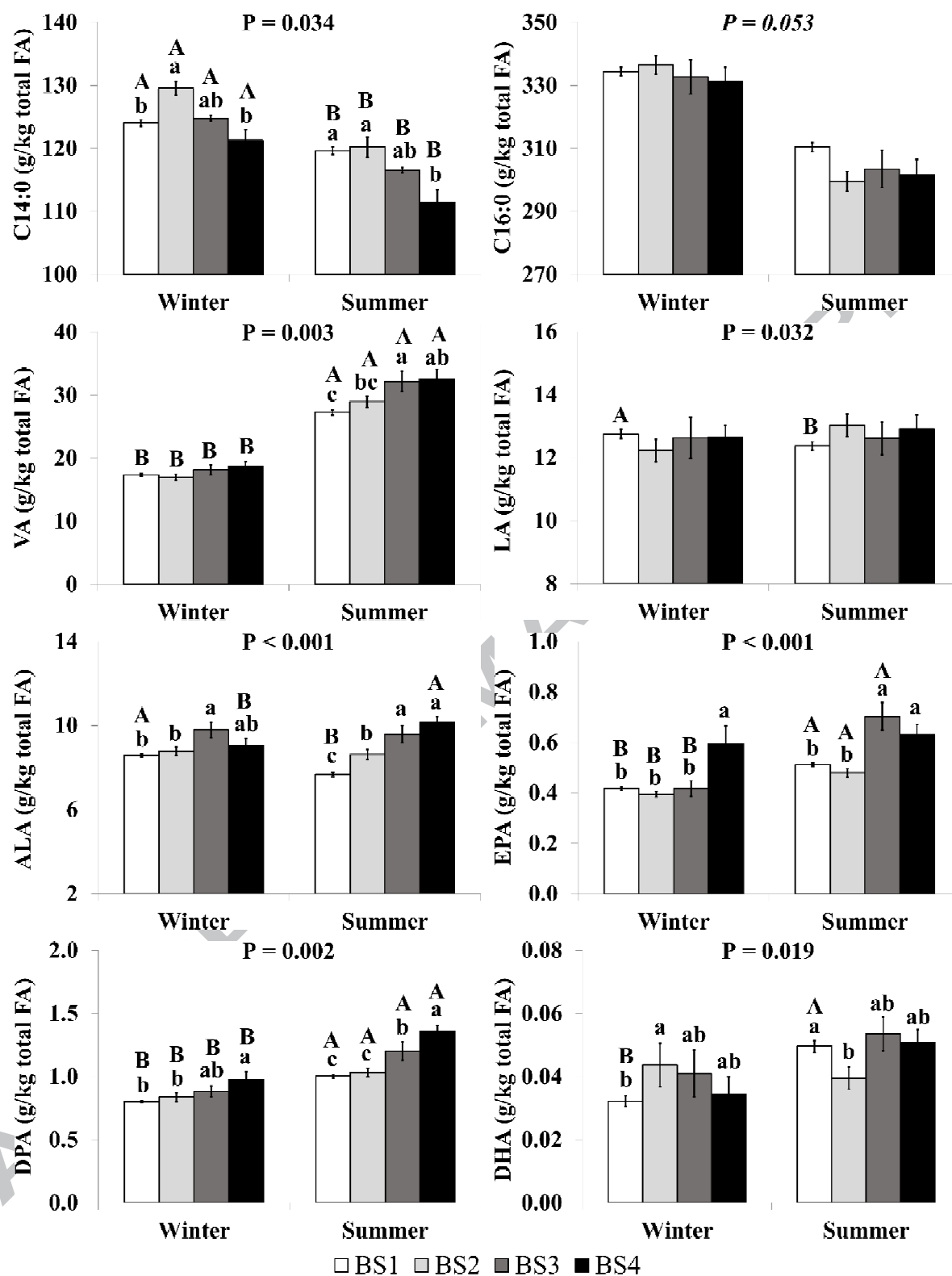
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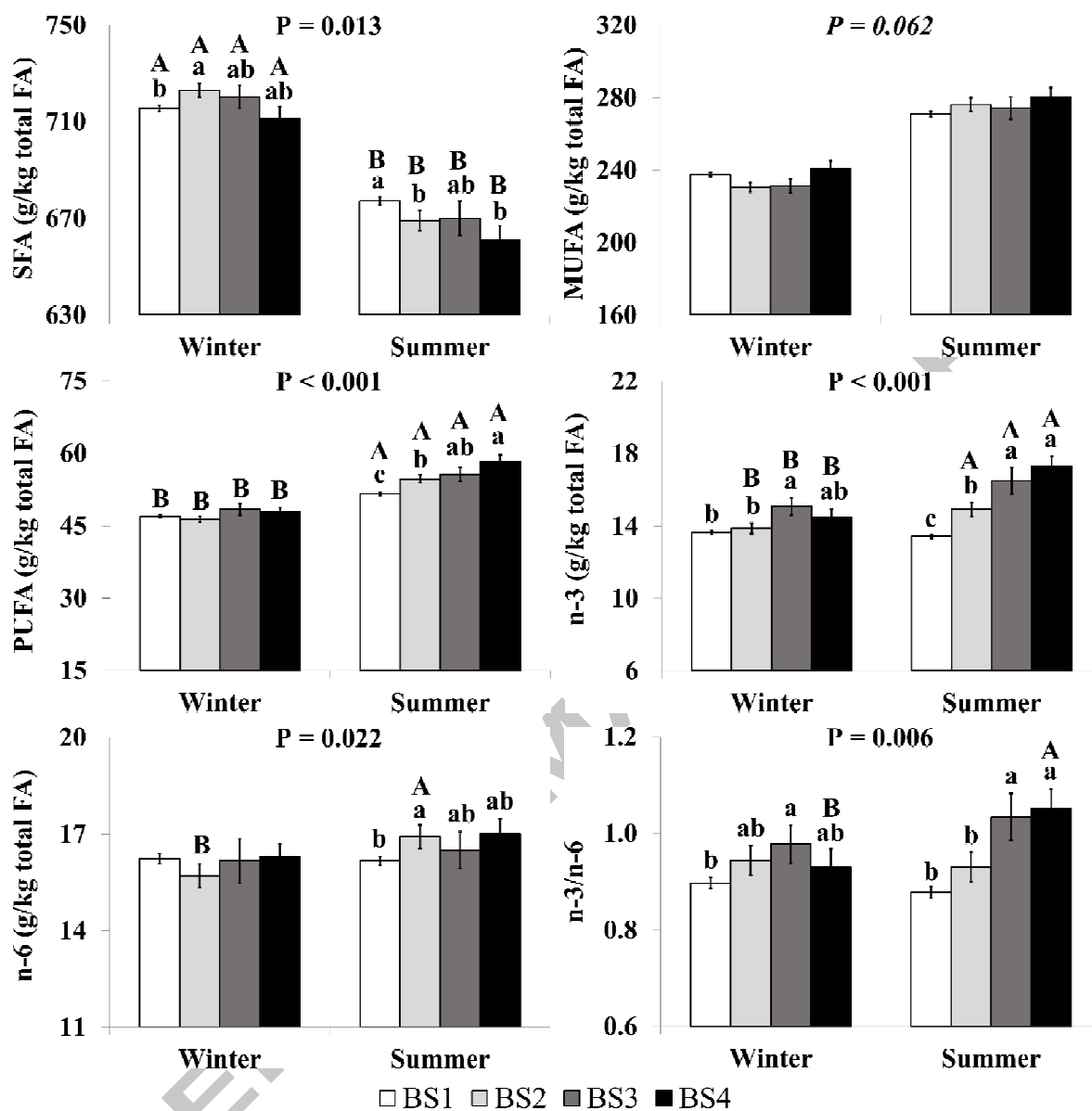

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Tables

Table 1

Animal data, crossbreed composition (% BS, BV and OB genetics), and estimated dry matter intake (DMI) and dietary components (% of DMI) in cows from different crossbreed groups (BS) and season from 40 low-input dairy farms in Switzerland during the year (means ± SE)

Parameters assessed	Crossbreed group (% of US Brown Swiss genetics)				Season		ANOVA P-values[a]		
	BS1 (75-99%) n=1524	BS2 (50-74%) n=245	BS3 (25-49%) n=90	BS4 (0-24%) n=118	Winter n=1040	Summer n=937			
Animal data									
Estimated live weight[b]	640±0.1[A]	635±0.3[A]	624±1.3[B]	609±3.6[C]	637±0.5	637 0.3	***	ns	ns
Parity	3.1±0.05[B]	5.3±0.20[A]	3.6±0.25[B]	3.4±0.21[B]	3.4±0.07	3.5±0.08	***	***	*
Days in milk	173±2.7	182±7.0	156±10.2	171±9.2	162±3.2	183±3.5	ns	***	ns
Crossbreed composition (%)									
Brown Swiss (BS)	86.6±0.1[A]	67.6±0.4[B]	39.3±0.7[C]	4.1±0.6[D]	76.5±0.7	77.8±0.7	***	**	ns
Braunvieh (BV)	2.7±0.1[C]	9.2±0.6[B]	13.1±2.3[A]	5.7±1.7[B]	4.2±0.3	4.0±0.3	***	**	*
Original Braunvieh (OB)	10.8±0.1[C]	23.1±0.7[C]	46.4±2.7[B]	70.8±3.7[A]	17.4±0.6	17.7±0.7	***	ns	ns
Other breeds[c]	0.0±0.0	0.0±0.0	1.1±0.8[B]	19.5±3.7[A]	1.9±0.4	0.5±0.2	***	ns	ns
Estimated DMI (kg/cow/day)	18.8±0.0[A]	18.6±0.1[A]	18.2±0.1[AB]	17.8±0.1[B]	18.7±0.0	18.6±0.0	***	***	***
Diet components (% of DMI)									
Total fresh forage intake	30.3±0.9[C]	31.5±2.4[B]	35.8±4.3[A]	31.7±3.5[AB]	0.0±0.0	64.9±0.8	**	***	***
Estimated grazing	24.9±0.8[C]	26.9±2.1[B]	26.0±3.8[BC]	30.6±3.5[A]	0.0±0.0	53.9±0.8	**	***	***
Fresh-cut grass/"zero grazing"[d]	5.4±0.4[B]	4.6±0.8[B]	9.8±2.2[A]	1.1±0.7[C]	0.0±0.0	11.0±0.7	***	***	***
Total ensiled forage	28.8±0.7	25.7±1.7	22.3±2.8	29.1±2.7	37.8±0.8	17.4±0.7	ns	***	*
Grass silage	13.1±0.5[A]	11.3±1.2[AB]	4.5±1.3[B]	6.5±1.2[BC]	15.8±0.7	7.9±0.4	***	***	***
Grass/clover silage	5.3±0.4[B]	6.6±1.0[B]	5.6±1.4[B]	17.1±2.5[A]	10.6±0.6	1.2±0.1	***	***	***
Maize silage	10.0±0.3[A]	7.5±0.7[AB]	6.5±1.0[AB]	5.1±1.0[B]	10.3±0.4	8.1±0.4	*	***	ns
Other silage	0.5±0.1	0.3±0.2	5.8±1.6	0.4±0.4	1.2±0.2	0.2±0.0	ns	***	ns
Wholecrop[e]	1.1±0.1[B]	2.0±0.3[A]	0.4±0.4[BC]	0.2±0.1[C]	1.0±0.1	1.3±0.1	***	*	ns
Hay/Straw	33.4±0.7	35.6±2.0	36.0±3.6	32.1±2.9	52.0±0.9	13.4±0.4	ns	***	ns
Cereals	0.8±0.1	0.5±0.1	0.9±0.2	1.3±0.3	1.1±0.1	0.4±0.1	†	***	**
Other feeds/supplements	0.8±0.1	0.8±0.2	0.5±0.3	0.1±0.0	0.8±0.1	0.7±0.1	†	ns	ns
Concentrates	4.1±0.2[A]	3.2±0.3[B]	3.7±0.7[AB]	5.0±0.4[A]	6.4±0.2	1.5±0.1	*	***	*
Oil supplements	0.3±0.0	0.2±0.1	0.1±0.1	0.2±0.1	0.5±0.0	0.0±0.0	ns	***	ns

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Minerals/vitamins	0.4±0.0	0.4±0.0	0.3±0.0	0.4±0.0	0.4±0.0	0.4±0.0	***	ns	ns

[a] Significances were declared at ***, P < 0.001; **, P < 0.01; *, P < 0.05; †, 0.05 < P < 0.10 (trend); ns, P > 0.10 (non-significant). Means for crossbreed group within a row with different upper case letters are significantly different according to Tukey's honestly significant difference test (P < 0.05)

[b] Live weights were estimated based on average breed live weight as shown in Appendix, Table A1.

[c] Holstein, Red Holstein, Simmental, Swiss Fleckvieh, Rhaetian Grey, Limousin, Normande

[d] Fresh cut grass provided within 1-2 days after harvest

[e] Dehydrated and pelleted whole maize plants (stem, leaves and immature grain), harvested approximately 1 month before grain maturity

Table 2
Means ± SE and ANOVA P-values for the effect of crossbreed group (BS) and season (S) on the yield, basic composition and fatty acid (FA) profile (g/kg total FA) of milk collected from individual cows from 40 low-input dairy farms in Switzerland during the year

Parameters assessed	Crossbreed group (BS; % US Brown Swiss genetics)				Season (S)		ANOVA P-values[a]		
	BS1 (75-99%) n=1524	BS2 (50-74%) n=245	BS3 (25-49%) n=90	BS4 (0-24%) n=118	Winter n=1040	Summer n=937	BS	S	BSxS
Milk yield (kg/cow/day)	22.1±0.2[A]	21.7±0.4[AB]	20.8±0.7[AB]	20.6±0.6[B]	22.5±0.2	21.3±0.2	*	***	**
Basic composition									
Fat (g/kg milk)	39.7±0.2	38.9±0.4	38.9±0.7	39.6±0.5	40.9±0.2	38.2±0.2	ns	***	ns
Protein (g/kg milk)	33.9±0.1	34.2±0.2	33.4±0.4	33.4±0.3	34.4±0.1	33.3±0.1	ns	***	ns
Lactose (g/kg milk)	47.5±0.1[B]	47.1±0.1[C]	48.0±0.2[AB]	48.2±0.2[A]	47.6±0.1	47.3±0.1	***	***	*
Urea (g/kg milk)	0.22±0.002[B]	0.23±0.005[B]	0.23±0.008[B]	0.25±0.009[A]	0.20±0.002	0.25±0.003	**	***	ns
SCC[b] (x 10³)	186±11	226±31	155±23	130±23	167±12	207±15	†	***	ns
SFA[b]									
C12:0	32.9±0.2[B]	35.0±0.4[A]	33.5±0.7[AB]	32.9±0.6[B]	34.7±0.2	31.5±0.2	***	***	ns
C14:0	122±0.4[B]	125±1.0[A]	121±1.5[BC]	117±1.4[C]	125±0.5	119±0.5	***	***	*
C16:0	323±1	319±2	319±4	319±4	334±1	308±1	ns	***	†
C18:0	99±1	96±1	101±2	101±2	92±1	106±1	ns	***	ns
MUFA[c]									
VA	22.1±0.3[B]	22.5±0.6[AB]	24.7±1.1[A]	24.6±1.0[A]	17.4±0.2	28.0±0.4	*	***	**
OA	186±1	184±2	182±3	188±3	173±1	200±1	ns	***	ns
PUFA[d]									
LA	12.6±0.1	12.6±0.3	12.6±0.4	12.8±0.3	12.7±0.1	12.5±0.1	ns	**	*
ALA	8.14±0.06[C]	8.70±0.15[B]	9.69±0.28[A]	9.51±0.23[A]	8.69±0.07	8.00±0.08	***	***	***
CLA9	10.6±0.1	10.9±0.3	10.8±0.5	10.9±0.5	8.4±0.1	13.2±0.2	ns	***	ns
EPA	0.46±0.01[C]	0.43±0.01[C]	0.55±0.03[B]	0.61±0.04[A]	0.43±0.01	0.52±0.01	***	***	***
DPA	0.90±0.01[C]	0.93±0.02[C]	1.03±0.04[B]	1.14±0.04[A]	0.82±0.01	1.03±0.01	***	***	**
DHA	0.04±0.001	0.04±0.004	0.05±0.005	0.04±0.004	0.03±0.002	0.05±0.002	ns	***	*
FA groups									
SFA	697±1	698±3	697±5	690±4	716±1	675±1	ns	***	*
MUFA	254±1	252±3	251±4	258±4	237±1	272±1	ns	***	†
PUFA	49.2±0.2[B]	50.2±0.6[AB]	51.8±1.0[A]	52.3±0.8[A]	47.0±0.2	52.6±0.3	***	***	***
n-3[e]	13.5±0.1[C]	14.4±0.2[B]	15.7±0.4[A]	15.7±0.4[A]	13.8±0.1	14.0±0.1	***	†	***
n-6[f]	16.2±0.1	16.3±0.3	16.3±0.5	16.6±0.3	16.2±0.1	16.3±0.1	ns	ns	*
n-3/n-6	0.89±0.01[B]	0.94±0.02[AB]	1.00±0.03[A]	0.98±0.03[A]	0.91±0.01	0.90±0.01	***	ns	**

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Indices

$\Delta^{9\,g}$	0.277±0.001	0.276±0.003	0.271±0.004	0.277±0.004	0.263±0.001	0.291±0.001	ns	***	†
C14:1/C14:0	0.079±0.001[A]	0.077±0.001[AB]	0.072±0.002[BC]	0.071±0.002[C]	0.080±0.001	0.076±0.001	***	***	ns
c9 C16:1/C16:0	0.046±0.0002	0.047±0.0006	0.046±0.0010	0.046±0.0008	0.045±0.0003	0.048±0.0003	ns	***	ns
OA/C18:0	1.934±0.009	1.957±0.022	1.846±0.034	1.902±0.032	1.933±0.011	1.929±0.012	ns	ns	ns
CLA9/VA	0.493±0.003[A]	0.497±0.007[A]	0.446±0.010[B]	0.452±0.009[B]	0.496±0.003	0.481±0.004	***	**	ns

[a] Significances were declared at ***, P < 0.001; **, P < 0.01; *, P < 0.05; †, 0.05 < P < 0.10 (trend); ns, P > 0.10 (non-significant). Means for crossbreed group within a row with different upper case letters are significantly different according to Tukey's honestly significance difference test (P < 0.05)

[b] SFA: C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0

[c] MUFA: c9 C14:1, c10 C15:1, c9 C16:1, t9 C16:1, c9 C17:1, t6+t7+t8 C18:1, t9 C18:1, t10 C18:1, t11 C18:1 (VA), t12+t13+t14 C18:1, c9 C18:1 (OA), t15 C18:1, c11 C18:1, c12 C18:1, c13 C18:1, c14+t16 C18:1, c15 C18:1, c8 C20:1, c13 C22:1, c15 C24:1

[d] PUFA: c9t13 C18:2, t9t12 C18:2, t8c13 C18:2, c9t12 C18:2, t9c12 C18:2, t11c15 C18:2, c9c12 C18:2 (LA), c9c15 C18:2, c12c15 C18:2, c6c9c12 C18:3, c9c12c15 C18:3 (ALA), c9t11 C18:2 (CLA9), unknown conjugated and non-conjugated C18:2 isomers, c11c14 C20:2, c8c11c14 C20:3, c11c14c17 C20:3, c5c8c11c14 C20:4, c13c16 C22:2, c13c16c19 C22:3, c7c10c13c16 C22:4 , c5c8c11c14c17 C20:5 (EPA), c7c10c13c16c19 C22:5 (DPA), c4c7c10c13c16c19 C22:6 (DHA)

[e] n-3 FA: t11c15 C18:2, c9c15 C18:2, c12c15 C18:2, c9c12c15 C18:3 (ALA), c11c14c17 C20:3, c5c8c11c14c17 C20:5 (EPA), c13c16c19 C22:3, c7c10c13c16c19 C22:5 (DPA), c4c7c10c13c16c19 C22:6 (DHA)

[f] n-6 FA: t9t12 C18:2, c9t12 C18:2, t9c12 C18:2, c9c12 C18:2 (LA), c6c9c12 C18:3, c11c14 C20:2, c8c11c14 C20:3, c5c8c11c14 C20:4, c13c16 C22:2, c7c10c13c16 C22:4. [g] Δ^9-desaturase activity index (Δ^9): (c9 C14:1+c9 C16:1+c9 C18:1+t11 C18:1)/(c9 C14:1+c9 C16:1+c9 C18:1+t11 C18:1+C14:0+C16:0+C18:0+c9t11 C18:2 conjugated), as proposed by Kay et al. (2004)

Highlights

- Breeding by season interactions in milk quality were assessed on low-input farms

- Crossbreeding with Original Braunvieh (OB) reduced milk yield during winter

- Cows with more OB genetics produced milk with improved fatty acid profile

- Desirable effect of OB genetics on milk fatty acid profile was higher in summer

- Improvement of milk quality should account for genetics by diet interaction