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Phenotypic variation of milk fatty acid composition of Pinzgauer cattle breed

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ABSTRACT

This paper investigated sources of variation of milk fatty acid (FA, g/100 g total FA) profile routinely predicted by mid-infrared spectroscopy in Pinzgauer dual-purpose cattle breed. A total of 19,578 individual milk samples collected from 1232 cows and 77 herds between 2011 and 2014 were available for phenotypic characterisation. Data were analysed using a linear mixed model which included stage of lactation, parity and their interaction as fixed effects, and cow and herd-test-day as random effects. Milk yield averaged 20.87 kg/d, and means for fat, protein and casein were 4.01, 3.44 and 2.72%, respectively. Saturated FA (SFA), monounsaturated FA (MUFA) and polyunsaturated FA (PUFA) averaged 69.20, 25.43 and 3.19 g/100 g total FA, respectively. About half of the SFA were provided by C16:0 (31.24 g/100 g total FA), and almost all MUFA by C18:1 (22.18 g/100 g total FA). Fixed effects were significant in explaining the variation of the studied traits. Milk of first-parity cows had the lowest percentage of SFA, medium-chain FA and C14:0, and the greatest percentage of MUFA, PUFA, *trans* FA, short- and long-chain FA, and C18:1. Saturated FA, C14:0 and C16:0 increased from calving until 120 days in milk, whereas unsaturated FA, MUFA, PUFA, long-chain FA, C18:0 and C18:1 decreased. Although both parity and stage of lactation affected milk FA, greater variation was observed during lactation than among parities.

Introduction

Pinzgauer (PI) is a dual-purpose (dairy and beef) local cattle breed of the alpine areas. In Europe, PI herds are mainly located in Austria (Salzburg, Tyrol, Carinthia, Styria), Italy (South Tyrol) and Germany (Bavaria). South Tyrol (Bolzano province) accounts for approximately 80% of the Italian PI population (2104 heads in 2016), where the breed is the fifth in terms of population size after the most popular Brown Swiss, Simmental, Holstein–Friesian and Alpine Grey (BDN 2017). PI cows are very appreciated because of their hardiness, resilience and longevity, and high adaptability to highland pasture (APR 2017). Milk yield per lactation after the third calving averages 6000 kg with 4% and 3.5% of fat and protein, respectively (APR 2017).

The characterisation of milk fatty acid (FA) profile can contribute to the valorisation of local breeds, such as PI, which produce less milk compared to the most cosmopolitan dairy cattle populations but are crucial for the maintenance of a close link between farmers, environment (e.g. mountain areas) and social and traditional aspects. To our knowledge, FA profile of PI breed has not been studied yet, mainly because of the high cost of reference analysis. Mid-infrared spectroscopy (MIRS), which is routinely used in milk-recording system, has become an essential tool to collect milk traits, including FA composition, at population level in a rapid and cost-effective way (De Marchi et al. 2014). Therefore, the present study aimed to assess the phenotypic variation of major milk FA groups and individual FA predicted by MIRS in PI cattle breed.

Information on individual milk samples from purebred PI cows collected during routine cow milk testing from January 2011 to December 2014 was obtained from the database of the South Tyrol Dairy Association (Bolzano, Italy) and the Breeders Association of Bolzano province (Bolzano, Italy). Milk fat, protein, casein, lactose and FA were determined by MilkoScan FT6000 (FOSS, Hillerød, Denmark) using MIRS prediction models developed and commercialised by the

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same company. Content of FA were expressed as relative amount (g/100 g total FA) and determined for eight groups and four individual FA: saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), trans FA, short-chain FA (SCFA), medium-chain FA (MCFA), long-chain FA (LCFA), C14:0, C16:0, C18:0 and C18:1 (Hein et al. 2016; Gottardo et al. 2017). Individual FA included in the aforementioned groups are detailed in Hein et al. (2016), and fitness statistics of MIRS prediction models for groups of FA and individual FA can be retrieved from Gottardo et al. (2017). Somatic cell count (SCC) was assessed by Cell Fossomatic 250 (FOSS Electric A/S; Hillerød, Denmark) and transformed to somatic cell score (SCS) as $SCS = 3 + \log_2(SCC/100{,}000)$ (Wiggans and Shook 1987).

The original dataset ($n = 30{,}477$) was edited to retain records of cows between 6 and 450 days in milk (DIM) and from parity 1 to 9. Age at calving was checked to ensure the consistency within parity, and animals whose age at calving deviated more than three standard deviations (SD) from the respective parity mean were discarded. Inconsistent measures for milk yield and SCC, as well as values of milk composition and FA profile greater than 3 SD from their respective mean, were treated as missing data. Contemporary groups were defined as cows tested in the same herd and date (HTD), and HTD with less than three animals were removed from the database. Moreover, cows with less than three observations within lactation were removed. The final edited dataset consisted of 19,578 observations from 1232 cows in 77 herds.

Sources of variation of milk FA traits were investigated through a generalised linear model using the MIXED procedure of SAS (SAS 2013). The model was:

$$y_{ijklm} = \mu + S_i + Parity_j + (S \times Parity)_{ij} + HTD_k + Cow_l + \varepsilon_{ijklm},$$

where y_{ijklm} is the dependent variable, μ is the overall intercept of the model, S_i is the fixed effect of the ith class of stage of lactation ($i = 1-14$, the first being a class from 6 to 30 DIM, followed by 12 classes of 30 DIM each, and the last being a class from 391 to 450 DIM), $Parity_j$ is the fixed effect of the jth class of parity of the cow ($j = 1-6$, with the last including parities 6–9), $(S \times Parity)_{ij}$ is the fixed interaction effect between stage of lactation and parity, HTD_k is the random effect of the kth herd-test-date ($k = 1-2353$) $\sim N(0, \sigma^2_{HTD})$, Cow_l is the random effect of the lth cow ($l = 1-1232$) $\sim N(0, \sigma^2_{COW})$, and ε_{ijklm} is the random residual $\sim N(0, \sigma^2_e)$. Multiple comparisons of means

Table 1. Descriptive statistics of milk yield, composition, somatic cell score (SCS) and fatty acid (FA) profile in Pinzgauer cattle breed after editing.

Trait	n	Mean	SD	Minimum	Maximum
Milk yield, kg/d	19,492	20.87	7.36	1.80	43.40
Milk composition, %					
Fat	19,379	4.01	0.70	1.72	6.32
Protein	19,444	3.44	0.40	2.22	4.77
Casein	19,451	2.72	0.30	1.81	3.71
Lactose	19,466	4.82	0.20	3.99	5.45
SCS, units	19,539	2.80	1.80	−2.64	9.60
Groups of FA, g/100 g total FA					
SFA	13,673	69.20	3.88	56.72	80.61
UFA	13,640	30.37	4.29	20.12	44.42
MUFA	13,646	25.43	3.68	13.96	37.58
PUFA	13,658	3.19	0.58	1.37	5.06
trans FA	13,673	2.12	0.64	0.14	4.12
SCFA	13,651	10.63	1.19	6.63	14.40
MCFA	13,691	43.13	7.03	20.72	65.32
LCFA	13,633	32.09	4.98	16.55	48.61
Individual FA, g/100 g total FA					
C14:0	13,620	11.93	1.37	7.25	16.35
C16:0	13,702	31.24	3.13	21.21	40.94
C18:0	13,670	10.55	1.68	5.36	15.98
C18:1	13,633	22.18	3.74	10.53	34.62

SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SCFA: short-chain fatty acids; MCFA: medium-chain fatty acids; LCFA: long-chain fatty acids.

were performed for the fixed effects using Bonferroni's test ($p < .05$).

Results and discussion

The coefficient of variation of the studied traits ranged from 4% (lactose) to 64% (SCS; Table 1), which partially agreed with Gottardo et al. (2017) who reported greater variation for SCS (76%) considering Brown Swiss, Holstein–Friesian, Simmental and Alpine Grey cows reared in the same province of the current study. Regarding other local dual-purpose cows reared in mountain area, Niero et al. (2016) reported greater variation for lactose (5.4%) and lower for SCS (56%) in milk of Burlina cattle breed compared with the present study. PI produced more milk, with greater fat and protein percentage than Aosta Red Pied breed (Renna et al. 2014). Moreover, PI cows yielded milk with greater fat, protein, and lactose percentage, and lower SCS than Burlina (Niero et al. 2016).

Milk FA derive mainly from four pathways (directly from the diet, from the mammary gland by *de novo* synthesis, from the rumen by biohydrogenation, and from body fat mobilisation), and any modification on these pathways may change milk FA composition (Stoop et al. 2009). In particular, the diet is an important factor affecting milk FA composition. Even if we did not have direct information on the diet used to fed cows, the inclusion of HTD as random effect in the

statistical analysis allowed us to account, at least in part, for the variation due to farm management, including feeding, and to calculate the incidence of managerial conditions on milk FA composition by means of the ratio between the HTD variance and the total variance of each FA. The proportion of total variance explained by HTD was around 40%, except for C18:0 (30%), *trans* FA (15%), and SCFA and MCFA (12%). Comparison of milk FA composition among studies is difficult due to the different analytical methods and units of expression adopted. Milk of Burlina cows showed lower SFA and PUFA percentage, and greater UFA, MUFA, C14:0, C16:0 and C18:0 percentage (Niero et al. 2016) than milk of PI cows from our study. In cows grazed on alpine pastures, Falchero et al. (2010) detected twice PUFA and greater LCFA percentage compared with the present study, and Roda et al. (2015) reported greater C18:0, and lower C14:0 and C16:0 percentage than our findings. All mentioned studies used gas chromatography as analytical method for milk FA determination.

Fixed effects included in the statistical model, including the interaction, were significant ($p < .001$) in explaining the variation of all the studied traits (results not shown) and least squares means of milk yield, composition, SCS and FA profile in different parities and stages of lactation are displayed in Table 2. Milk of first-parity cows had lower percentage of SFA, MCFA and C14:0, and greater percentage of MUFA, PUFA, *trans* FA, SCFA, LCFA and C18:1 than milk of multiparous animals

($p < .05$). Moreover, milk of primiparous had greater UFA percentage than second- and third-parity cows, greater C18:0 than second-parity cows, and lower C16:0 than second-, third- and fourth-parity cows ($p < .05$; Table 2). These results partially agreed with findings of Gottardo et al. (2017) who reported lower percentage of SFA, C14:0 and C16:0, and greater percentage of UFA, LCFA, C18:0 and C18:1 in milk of primiparous than multiparous cows. Also, lower SFA and greater UFA in first- than later-parity Burlina cows were observed by Niero et al. (2016). Statistical differences for FA profile between multiparous cows were rarely detected in the present study (Table 2). The greatest unsaturated fat content in milk of first- than later-lactation cows agreed with findings of Bilal et al. (2014), Niero et al. (2016) and Gottardo et al. (2017), who reported that the differences could be related to a less metabolically active mammary gland in first-parity cows and thus to a lower synthesis of *de novo* FA (C4:0–C14:0 and 50% of C16:0); lower dry matter intake and higher passage rate in first lactation which favour the acidogenic ruminal fermentation and thus the increment of UFA content in milk; and the higher proportion of concentrates in the diet of multiparous cows which favour the synthesis of *de novo* FA.

Saturated FA, C14:0 and C16:0 increased from calving until 120 DIM and decreased thereafter; UFA, MUFA and PUFA showed an opposite pattern with minimum values at around 120 DIM; and SCFA and *trans* FA continuously decreased through lactation ($p < .05$; Table 2). The LCFA and C18:1 decreased until

Table 2. Least squares means of milk fatty acid (FA) profile (g/100 g total FA) in different parities and stages of lactation.

Effect	N	FA groups								Individual FA			
		SFA	UFA	MUFA	PUFA	*trans* FA	SCFA	MCFA	LCFA	C14:0	C16:0	C18:0	C18:1
Parity													
First	5444	68.09[a]	31.39[b]	26.97[c]	3.37[b]	2.26[d]	10.85[e]	42.96[a]	33.64[b]	11.43[a]	30.82[a]	10.66[b]	23.54[b]
Second	4735	68.70[b]	30.94[a]	25.76[b]	3.20[a]	1.96[c]	10.58[d]	43.74[b]	32.42[a]	11.78[b]	31.19[b]	10.52[a]	22.39[a]
Third	3447	68.85[b]	30.86[a]	25.49[a]	3.17[a]	1.88[b]	10.42[c]	44.07[bcd]	32.11[a]	11.87[bc]	31.21[b]	10.55[ab]	22.17[a]
Fourth	2563	68.83[b]	30.96[ab]	25.39[ab]	3.18[a]	1.82[a]	10.30[b]	44.02[bc]	32.07[a]	11.90[c]	31.22[b]	10.57[ab]	22.05[a]
Fifth	1553	68.58[b]	31.02[ab]	25.43[a]	3.20[a]	1.81[ab]	10.16[a]	44.54[bcd]	32.13[a]	11.87[bc]	31.12[ab]	10.64[ab]	22.13[a]
Six and later	1836	68.83[b]	30.86[ab]	25.22[ab]	3.16[a]	1.81[ab]	10.09[a]	45.00[d]	31.99[a]	11.95[c]	31.13[ab]	10.54[ab]	21.94[a]
Lactation stage, d													
6–30	1440	66.15[a]	34.00[g]	27.84[g]	3.35[f]	2.05[ef]	10.97[h]	40.48[b]	37.88[h]	10.63[a]	27.51[a]	13.48[f]	24.94[h]
31–60	1931	68.20[c]	31.92[ef]	26.11[de]	3.28[e]	2.50[j]	10.41[cde]	39.40[a]	34.72[g]	11.60[b]	29.70[b]	12.04[e]	23.32[g]
61–90	2001	69.63[f]	30.02[c]	24.86[b]	3.15[cd]	2.41[i]	10.40[cd]	41.28[c]	32.14[ef]	12.19[d]	31.22[c]	10.97[d]	21.88[cd]
91–120	1956	70.15[g]	29.20[ab]	24.31[a]	3.11[ac]	2.29[h]	10.49[dg]	42.65[d]	30.90[ab]	12.45[e]	31.84[dg]	10.36[c]	21.17[a]
121–150	1962	70.16[g]	29.02[a]	24.24[a]	3.08[ab]	2.20[g]	10.53[fg]	43.79[e]	30.51[a]	12.45[e]	31.98[g]	10.06[ab]	21.05[a]
151–180	1909	70.05[g]	29.16[ab]	24.41[a]	3.07[ab]	2.11[f]	10.48[efg]	44.88[fg]	30.53[a]	12.33[e]	32.08[g]	9.92[a]	21.20[ab]
181–210	1832	69.61[f]	29.57[b]	24.80[b]	3.12[bc]	2.04[e]	10.49[df]	45.47[gh]	30.98[ac]	12.18[d]	31.81[fg]	9.94[a]	21.57[bc]
211–240	1755	69.24[e]	30.23[c]	25.23[c]	3.16[cd]	1.90[d]	10.45[df]	45.13[fgh]	31.38[bcd]	12.02[c]	31.58[def]	10.02[ab]	21.91[cd]
241–270	1635	68.64[d]	30.92[d]	25.74[d]	3.24[e]	1.75[c]	10.38[bdf]	44.87[fg]	31.73[de]	11.88[c]	31.44[ce]	10.07[ab]	22.20[de]
271–300	1346	68.11[bc]	31.62[e]	26.21[ef]	3.29[e]	1.64[b]	10.29[abc]	44.73[fi]	32.30[f]	11.64[b]	31.31[ce]	10.24[c]	22.65[f]
301–330	793	67.73[b]	32.14[ef]	26.62[f]	3.30[ef]	1.58[ab]	10.17[a]	45.21[gij]	32.72[f]	11.48[b]	31.12[c]	10.29[c]	22.99[f]
331–360	484	67.56[b]	32.32[f]	26.72[f]	3.32[ef]	1.52[a]	10.18[a]	45.47[gij]	32.82[f]	11.43[b]	31.21[ce]	10.29[c]	22.94[fg]
361–390	271	67.89[bc]	31.94[ef]	26.53[df]	3.30[ef]	1.51[a]	10.23[abc]	46.23[hjk]	32.57[ef]	11.46[b]	31.26[ce]	10.22[abc]	22.79[efg]
390–450	263	67.95[bcd]	32.03[ef]	26.34[de]	3.23[de]	1.45[a]	10.14[a]	47.18[k]	32.30[df]	11.46[b]	31.52[ceg]	10.22[abc]	22.57[cdfg]

Least squares means with different superscript letters within trait and effect are significantly different ($p < .05$).
SFA: saturated fatty acids; UFA: unsaturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SCFA: short-chain fatty acids; MCFA: medium-chain fatty acids; LCFA: long-chain fatty acids.

120 DIM and slightly increased thereafter until the end of the lactation ($p < .05$). Percentage of C18:0 decreased until 150 DIM and reached a plateau thereafter ($p < .05$). Proportion of MCFA increased progressively after 60 DIM and reached a plateau at 180 DIM ($p < .05$). These results agreed with several authors (Stoop et al. 2009; Bilal et al. 2014; Niero et al. 2016; Gottardo et al. 2017) who suggested that the greater proportion of LCFA at the beginning of lactation could be explained by the mobilisation of body reserve to cope with the negative energy balance. The negative energy balance also alters the biohydrogenation capacity of the rumen, leading to a greater production of *trans* FA. Moreover, the LCFA inhibits the mammary gland lipogenic enzymes for the synthesis of *de novo* FA, which could also explain the increase of MCFA (mainly C14:0 and C16:0) during lactation due to an increase of the capacity of the mammary gland to synthesise *de novo* FA.

Conclusions

Pinzgauer breed produced more milk, with greater fat, protein and casein percentages than other local populations reported in the literature and mainly reared in mountain areas. Similarly to results from other breeds, PI cows had less saturated fat in first parity and at the beginning of the lactation. In addition, larger variation was detected during lactation than among parities.

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Disclosure statement

The authors declare that there is no conflict of interest associated with the paper. The authors alone are responsible for the content and writing of this article.

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