# Multi-breed herd approach to detect breed differences in composition and fatty acid profile of cow milk

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Abstract: The objective of the present study was to estimate the effect of breed on milk fatty acid (FA) composition of dairy (Brown Swiss, Holstein-Friesian, and Jersey) and dual-purpose cows (Simmental and Alpine Grey) in multi-breed herds. Information on individual milk samples was collected during routine cow milk testing between 2011 and 2014, and consisted of 285 606 observations from 17 445 cows in 617 herds. Fixed effects included in the mixed model were breed, parity, stage of lactation and the interaction between parity and stage of lactation, and random effects were cow, herd-test-date and residual. Contrast estimates for the studied traits were used to compare specific sets of breeds. Holstein-Friesian produced more milk than the other cattle breeds, with the greatest *trans* FA and C18:1 and the lowest C18:0 content. Comparison between the specialised dairy vs the dual-purpose breeds highlighted significant differences for all traits except for polyunsaturated FA and *trans* FA content. Specialised dairy breeds had greater milk saturated FA, short-chain FA, medium-chain FA, C14:0 and C16:0 content, and dual-purpose breeds produced milk with greater content of monounsaturated FA, long-chain FA, C18:0 and C18:1. Results demonstrated that, although specialised dairy produced more milk than dual-purpose breeds, milk FA profile of the latter was more favourable from a human nutrition point of view.

Keywords: dairy specialised breed; dual-purpose breed; mid-infrared spectroscopy; phenotyping

Milk chemical composition and fatty acid (FA) profile are key points for the dairy industry that seeks for a more efficient processing of milk to offer products following new consumers' trends. Although diet is the major source of variation influencing milk FA profile, there are other important factors affecting milk FA composition such as breed, individual animal, stage of lactation and parity (Ferlay et al. 2011; Niero et al. 2016; Gottardo et al. 2017). The effect of breed on milk composition, FA profile and coagulation properties has highlighted some inconsistent results (Kelsey et al. 2003; Ferlay et al. 2011; Penasa et al. 2014; Gottardo et al. 2017; Stocco et al. 2017), which partially arise from some limitation of the studies.

The small sample size is usually related to the cost of the gas chromatographic analysis (Arnould and Soyeurt 2009), which is the traditional laboratory method for FA determination. This limitation can be overcome with the application of specific midinfrared spectroscopy (MIRS) prediction models (Hein et al. 2016; Gottardo et al. 2017) in a cheap and fast way, thus allowing the routine milk recording of data at population level (De Marchi et al. 2014). Differences in milk composition between breeds can be helpful for breeding purposes to enhance milk composition and technological ability. Therefore, the present study aimed to estimate the breed effect on cow milk yield, chemical composition, somatic cell score (SCS) and FA profile in multi-breed dairy herds.

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#### MATERIAL AND METHODS

Multi-breed herds and data editing. Information on individual milk samples from multi-breed herds collected during routine cow milk testing in January 2011–December 2014 was obtained from the South Tyrolean Dairy Association (Bolzano, Italy) and the Breeders Association of Bolzano province (Bolzano, Italy). The Bolzano province is located in a mountainous area of the Italian Alps (North Italy) and it is characterised by small herds (on average, 15 cows/herd) with feeding mainly based on forage or hay and concentrates. Cows are usually moved to highland pastures during summer season (Visentin et al. 2018). Cows were from three specialised dairy (BS, Brown Swiss; HF, Holstein-Friesian; and JE, Jersey) and two dualpurpose cattle breeds (SI, Simmental; and AG, Alpine Grey). Each cow was checked every 4 weeks for milk production and composition. Milk fat, protein, casein, lactose and FA were analysed with MilkoScan FT6000 (FOSS, Denmark) using MIRS prediction models developed and commercialised by FOSS. The FA considered were those included in the FOSS Application Note 64. Briefly, FA content was expressed as relative amount (g/100 g total FA) and determined as 8 groups, namely saturated FA (SFA), unsaturated FA (UFA), monounsaturated FA (MUFA), polyunsaturated FA (PUFA), trans FA, short-chain FA (SCFA), medium-chain FA (MCFA) and long-chain FA (LCFA), and 4 individual FA, namely C14:0, C16:0, C18:0 and C18:1 (Hein et al. 2016; Gottardo et al. 2017). Goodness-of-fit statistics of MIRS prediction models for individual FA and groups of FA can be retrieved from Gottardo et al. (2017) who reported that the coefficient of determination in external validation for FA groups ranged from 0.72 to 0.98, and for individual FA from 0.55 to 0.81. Somatic cell count (SCC) was assessed by Cell Fossomatic 250 (FOSS) and logarithmic transformation was adopted to convert SCC to SCS using the equation  $SCS = 3 + \log_2(SCC/100)$  where SCC is somatic cell count in cells/µl (Wiggans and Shook 1987).

The original dataset (n = 1491347) was edited to retain herds with 2 or 3 breeds, and cows between 6 and 450 days in milk (DIM) and from parity 1 to 9. Cows whose age at calving deviated more than 3 standard deviations from the respective parity mean were discarded from the dataset, as well as cows with less than 5 observations within lactation,

herds with less than 3 cows per breed and breed combinations spread in less than 5 herds. Also, inconsistent records for the studied traits were treated as missing values. Contemporary groups were defined as cows tested in the same herd and date (herd-test-date, HTD), and HTD with less than 5 animals were removed from the database. After editing, the dataset consisted of 285 606 observations from 17 445 cows and 617 multibreed herds. The frequency for each breed was: BS, 434 herds and 6733 cows; HF, 420 herds and 5675 cows; JE, 42 herds and 217 cows; SI, 306 herds and 3732 cows; and AG, 94 herds and 1088 cows. Twelve breed combinations were available in the dataset: BS + HF (219 herds), BS + SI (120 herds), HF + SI (100 herds), BS + HF + SI (42 herds), SI + AG (37 herds), HF + AG (27 herds), BS + JE (22 herds), BS + AG (18 herds), HF + JE (12 herds), BS + HF + JE (8 herds), HF + SI + AG (7 herds), and BS + HF + AG (5 herds).

Statistical analysis. Milk yield, composition, SCS and FA traits were analysed through a linear mixed model in ASReml (Gilmour et al. 2015) including the fixed effects of breed (BS, HF, JE, SI, AG), parity (6 classes, with the last including parities 6 to 9), stage of lactation (14 classes, 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), and the interaction between parity and stage of lactation. Random effects were cow, HTD and residual. Contrast estimates between Least Squares Means for milk yield, SCS, composition and FA traits were used to compare specific sets of breeds. Significance was set at P < 0.05 unless otherwise stated.

### **RESULTS AND DISCUSSION**

Descriptive statistics. BS and HF accounted for 38.6% and 32.5% of total cows in the dataset, respectively, and JE was the least represented breed with 1.2% of cows (Table 1). HF produced by 3.6–8.7 kg/day more milk than other breeds, with the lowest protein, casein, SCFA and C18:0 contents, and the greatest trans FA and C18:1 percentage. Compared to the Canadian Holsteins (Fleming et al. 2018), HF cows of the present study produced less milk, with greater SCS, and fat and protein percentages. JE cows yielded milk with greater SCS and fat, protein, casein, SFA, SCFA,

MCFA, C14:0 and C16:0 content, and lower lactose, UFA, MUFA, PUFA, trans FA, LCFA and C18:1 content compared to the other breeds (Table 1). For traditional traits, the lowest coefficient of variation was observed for lactose percentage (4% for all breeds), and the greatest for SCS which ranged from 52% for JE to 72% for SI. For FA groups, the lowest variability was observed for SFA (5% for all breeds), and the greatest for trans FA which ranged from 32% for SI and HF to 43% for JE (Table 1).

All fixed effects considered in the statistical analysis were significant in explaining the variation of milk yield, composition, SCS and FA profile (P < 0.001; Table 1). The proportion of total variance explained by HTD (between 3% and 10%) was lower than for cow effect (between 34% and 50%)

for milk yield, composition and SCS (Table 1), which agreed with Stocco et al. (2017). Moreover, the lower proportion of variance explained by HTD for trans FA (15%), MCFA (13%) and SCFA (10%), and the greater for LCFA (30%) compared to the cow effect (trans FA, 42%; MCFA, 49%; SCFA, 53%; and LCFA, 22%) supported the hypothesis that diet affected more LCFA than MCFA, SCFA and trans FA (Stoop et al. 2008; Bilal et al. 2014). The pattern of variation of FA content in different parities and across lactation (data not shown) resembled those reported by several authors (Stoop et al. 2009; Ferlay et al. 2011; Gross et al. 2011; Bilal et al. 2014; Niero et al. 2016; Gottardo et al. 2017) who have reported greater UFA and LCFA, and lower MCFA content in milk from primiparous

Table 1. Within-breed mean (standard deviation in parentheses) of milk yield, somatic cell score (SCS), composition, and fatty acid traits, and proportion of phenotypic variance explained by herd-test-date (HTD) and cow effects

Trait	Breeds					Random effects (% of total variance)	
	BS	HF	JE	SI	AG	HTD	cow
Cows n	6 733	5 675	217	3 732	1 088		
Records n	109 728	92 161	3461	62 146	18 110		
Milk yield (kg/day)	23.20 (6.82)	26.75 (7.43)	19.53 (5.44)	23.19 (6.99)	18.09 (5.91)	10.34	50.25
SCS	2.94 (1.77)	3.04 (1.83)	3.47 (1.82)	2.54 (1.82)	2.69 (1.79)	2.96	43.33
Milk composition (	%)						
Fat	4.26 (0.68)	4.07 (0.71)	5.26 (0.74)	4.09 (0.68)	3.81 (0.62)	10.10	34.00
Protein	3.67 (0.41)	3.38 (0.40)	4.02 (0.42)	3.52 (0.39)	3.48 (0.40)	9.84	48.21
Casein	2.88 (0.31)	2.65 (0.30)	3.16 (0.32)	2.77 (0.30)	2.74 (0.30)	10.20	49.40
Lactose	4.79 (0.19)	4.75 (0.18)	4.69 (0.18)	4.76 (0.19)	4.82 (0.20)	6.34	47.22
<b>Groups of FA</b> (g/100	0 g total FA)						
SFA	70.42 (3.38)	70.27 (3.40)	73.47 (3.35)	70.25 (3.46)	68.88 (3.62)	33.29	25.84
UFA	28.97 (3.71)	29.19 (3.74)	25.92 (3.68)	29.23 (3.78)	30.67 (4.00)	28.57	27.07
MUFA	24.24 (3.26)	25.17 (3.23)	22.24 (3.03)	25.16 (3.31)	25.70 (3.55)	26.47	28.35
PUFA	3.02 (0.54)	2.92 (0.55)	2.66 (0.55)	2.92 (0.55)	3.26 (0.59)	38.22	29.21
Trans FA	1.64 (0.58)	2.02 (0.64)	1.22 (0.52)	1.84 (0.59)	1.92 (0.66)	15.18	41.70
SCFA	11.22 (1.09)	10.21 (1.14)	11.64 (0.99)	10.50 (1.13)	10.76 (1.19)	10.26	52.75
MCFA	45.44 (6.88)	43.28 (6.77)	50.17 (6.57)	46.55 (6.77)	41.56 (7.17)	13.17	48.85
LCFA	29.93 (4.35)	31.03 (4.22)	27.31 (4.22)	31.17 (4.36)	31.79 (4.75)	30.25	22.40
Individual FA (g/10	0 g total FA)						
C14:0	12.23 (1.17)	12.22 (1.20)	12.61 (1.11)	12.01 (1.22)	11.95 (1.33)	26.73	23.88
C16:0	31.86 (2.89)	32.68 (3.00)	34.62 (2.85)	32.46 (2.99)	31.04 (3.14)	34.86	26.70
C18:0	9.85 (1.54)	9.37 (1.58)	9.76 (1.38)	10.06 (1.62)	10.26 (1.64)	22.96	33.38
C18:1	20.59 (3.33)	22.23 (3.21)	19.49 (2.97)	21.60 (3.37)	21.81 (3.65)	26.85	29.10

FA = fatty acid, 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, BS = Brown Swiss, HF = Holstein-Friesian, JE = Jersey, SI = Simmental, AG = Alpine Grey

than multiparous cows, and greater UFA, *trans* FA and LCFA at the beginning of lactation.

Breed effect. Contrast estimates for the studied traits are reported in Table 2. Comparison between the specialised dairy (BS, HF and JE) and dualpurpose breeds (SI and AG) showed significant differences for all traits, except for PUFA and trans FA content. Specialised dairy cows produced more milk and with greater fat, protein, casein, SCS, SFA, SCFA, MCFA, C14:0 and C16:0, and lower lactose, UFA, MUFA, LCFA, C18:0 and C18:1 contents than milk from dual-purpose cows (P < 0.05). Contrary to our results, Stocco et al. (2017) did not detect differences between dairy and dual-purpose cattle breeds for milk yield and SCS, which could be explained by the inclusion of Rendena breed along with SI and AG in dual-purpose breeds group, the considerable lower number of cows and herds, and the lower number of herds for each breed combination. Compared to other breeds, HF cows showed significant differences for all milk traits (P < 0.001), with greater milk yield, SCS, UFA, MUFA, trans FA, LCFA, C14:0, C16:0 and C18:1. These results are consistent with findings of Hein et al. (2018) who reported breed differences between Danish Holstein and Danish Jersey for the same individual FA and FA groups included in the current study. The higher LCFA in HF compared to other breeds could be related to a higher body fat mobilisation due to a possible greater and longer negative energy balance originating from the high milk production (Bauman and Griinari 2001). Moreover, some authors have also pointed out that C18:1 was greater in HF than in other breeds (Palmquist et al. 1993; Ferlay et al. 2011; Hein et al. 2018). In addition, the greater MUFA

Table 2. Contrast estimates between Least Squares Means of milk yield, somatic cell score (SCS), composition and fatty acid traits for different sets of breeds

Trait	BS + HF + JE vs SI + AG	HF vs others	BS + HF vs JE	SI vs AG
Milk yield (kg/day)	3.17***	6.57***	6.02***	5.70***
SCS	0.49***	0.19***	-0.36***	-0.09***
Milk composition (%)				
Fat	0.54***	-0.35***	-1.17***	0.26***
Protein	0.15*	-0.34***	-0.54***	0.01*
Casein	0.12*	-0.27***	-0.42***	0.00 <sup>ns</sup>
Lactose	-0.07*	-0.02***	0.06***	-0.07***
Groups of FA (g/100 g	total FA)			
SFA	1.74***	-0.57***	-3.07***	1.31***
UFA	-1.90***	0.56***	3.11***	-1.46***
MUFA	-1.56***	0.84***	2.41 <sup>ns</sup>	$-0.64^{\rm ns}$
PUFA	$-0.21^{\text{ns}}$	-0.03***	0.30***	-0.36***
Trans FA	$-0.21^{\text{ns}}$	0.37***	0.60***	-0.10***
SCFA	0.36***	-0.87***	$-0.94^{ns}$	-0.32***
MCFA	1.82***	-2.90***	-5.70***	4.98***
LCFA	-2.00***	1.05***	3.15***	-0.57***
Individual FA (g/100 g	g total FA)			
C14:0	0.40***	0.05***	-0.36***	0.07***
C16:0	1.21**	0.13***	-2.22***	1.36***
C18:0	-0.46***	-0.59***	-0.14***	-0.16***
C18:1	-0.91***	1.42***	1.93***	$-0.23^{ns}$

BS = Brown Swiss, HF = Holstein-Friesian, JE = Jersey, SI = Simmental, AG = Alpine Grey, FA = fatty acid, 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, ns = not significant, \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05

and lower MCFA content in HF than in other breeds agreed with the greater delta-9 desaturase activity reported for HF compared to BS and JE (Arnould and Soyeurt 2009), which is the enzyme responsible to convert MCFA into MUFA. Within specialised dairy breeds, BS + HF differed from JE breed for all traits (P < 0.001), except for MUFA and SCFA. In particular, JE cows produced less milk with greater SCS and fat, protein, casein, SFA, MCFA, C14:0, C16:0 and C18:0 than other breeds, which agreed with Palmquist et al. (1993), Arnould and Soyeurt (2009) and Ferlay et al. (2011). Hein et al. (2018) have also reported greater SFA, SCFA, MCFA, C16:0, C14:0 and lower C18:0 in Danish Jersey than in Danish Holstein. On the other hand, Stocco et al. (2017) did not detect differences for SCS between BS + HF and JE. Regarding dual-purpose breeds, SI differed from AG for all traits (P < 0.05), except for casein, MUFA and C18:1, being SI the one that produced more milk with greater fat, protein, SFA, MCFA, C14:0 and C16:0. However, Gottardo et al. (2017) have reported the same milk production and SCS in SI and AG, probably due to the greater number of records included in our study.

### **CONCLUSION**

Parity, stage of lactation and breed were important sources of variation for milk traits, including FA composition. Our results demonstrated that although dairy breeds yielded more milk than dual-purpose breeds, milk from the latter exhibited better FA profile for human health. This study also supported the utility of technologies such as MIRS to collect phenotypic information at population level. Further research is needed to deepen the effects of farm characteristics, especially feeding, on the variation of milk FA traits to identify strategies for addressing FA content towards a more desirable profile.

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