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Detailed comparison between organic and conventional milk from Holstein-Friesian dairy herds in Italy

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ABSTRACT

Several studies have reported gross composition differences between organic and conventional milk; however, most studies have not considered other factors such as breed and diet ingredients, which are known to influence milk composition. Thus, this study aimed to provide a detailed characterization of Holstein-Friesian cow milk from organic (ORG) and conventional (CONV) herds with similar diet ingredients and in the same geographic area. Bulk milk samples (n = 225) of 12 ORG and 12 CONV farms were collected from September 2019 to August 2020. Farms were located in Northern Italy, included corn (meal, silage, or both) in the lactating diets, and had similar management conditions, but ORG herds spent a period on pasture. Factors affecting milk composition were tested using a linear mixed model, which included calendar month, farming system (ORG and CONV), and their interactions as fixed effects, and farm nested within farming system as random effect. Results showed that total fat, lactose, vitamin E, and AA did not significantly differ between farming systems. Total protein and casein contents were significantly lower in ORG than CONV herds, and somatic cell score (SCS) was greater in ORG than CONV. Among minerals, differences were observed for Fe, K, Mg, and S in some months, being lower in ORG than CONV for K, Mg, and S and greater or lower for Fe depending on the month. Among fatty acid (FA) groups, index, and ratios, only polyunsaturated FA and n-3 FA tended to be greater in ORG than CONV, and *cis*-FA were greater in ORG than CONV during October. Among the most abundant individual FA, only C16:1n-9 differed, being lower in ORG than CONV. The calendar month (and hence seasonal feed ration) was significant for milk gross composition, SCS, vitamin E, mineral profile (except for Mo, Sr, and Zn), AA profile, FA groups (except for medium-chain FA), FA index and ratios, and individual FA (except C16:0). We conclude that the overall milk composition was quite similar between the 2 farming systems. This could be related to the similarity of the selected farms, the Holstein-Friesian breed, and generally high level of intensity in both farming systems.
Key words: amino acids, fatty acid, mineral, organic milk, farm intensity

INTRODUCTION

Organic cow milk production has doubled since 2008, accounting in 2018 for 3.40% of dairy cows' production in the European Union (Willer et al., 2020). Consumers of both organic (**ORG**) and conventional (**CONV**) foods assume that ORG food is healthier and of better quality than CONV food (Rodríguez-Bermúdez et al., 2020). Along with the increase of ORG production and consumers' demands, the research on ORG production has also increased (Manuelian et al., 2020). In Europe, information on health, robustness, and phenotypic traits of native breeds for dairy production is scarce, and neither the rules for ORG production nor the literature clarify which breeds should be considered (Padel, 2019). In fact, it is commonly believed that breeds with a high milk production, such as Holstein-Friesian (**HF**), are not suitable for ORG farming or in agreement with ORG principles, but empirical evidence comparing breeds remains limited (Padel, 2019). Holstein-Friesians have been selected under different conditions, including grazing systems, and genetic variability exists within the breed that farmers have been selecting to better fit their production system (Padel, 2019). Thus, in some areas, HF are raised under ORG conditions because they are well adapted to the area characteristics and needs.

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Several studies have reported compositional differences between ORG and CONV milk. A review on the topic revealed that ORG and CONV milk had similar concentrations of SFA and MUFA, but ORG milk had higher total PUFA, n-3 PUFA, and α-tocopherol content (Średnicka-Tober et al., 2016). Nevertheless, milk fatty acid (**FA**) compositions in ORG and CONV pasture-based retail milk purchased in supermarkets differ in most of the individual and groups of FA (Liu et al., 2018). In addition, lower concentrations of Co, Cr, Cu, I, Se, and Zn and higher As have been reported in ORG than in CONV milk (Rodríguez-Bermúdez et al., 2018).

However, to be able to correctly attribute those differences to the farming systems (ORG or CONV), all the other factors that influence milk composition must be similar (Schwendel et al., 2015). The main factors influencing milk yield, fat, protein, and lactose concentrations are breed and diet, and many authors do not provide that information (Schwendel et al., 2015). For example, higher reported fat concentration in milk from ORG systems could be due to a preference for breeds other than HF in ORG herds (Schwendel et al., 2015) and a generally lower farming intensity. In other words, those studies (Średnicka-Tober et al., 2016) compared ORG and CONV food where differences such as breed, pasture-feed, and intensity interplayed. Here, we compared the same herd breed composition and similar levels of intensity. This could be described as input-substitution ORG, where mainly the feed (ORG feed), stocking density, and veterinary treatments change but all other inputs and management remain unchanged.

Therefore, this study provides a detailed characterization (mineral, AA, and FA profile) of bulk milk produced organically and conventionally in HF dairy farms located in the same area and under similar managerial conditions.

MATERIALS AND METHODS

Farms Recruited

Animal care and use approval was deemed unnecessary because we worked with bulk milk collected on a commercial farm as part of routine milk testing.

A total of 12 ORG and 12 CONV farms were enrolled in the study (Tables 1 and 2) from September 2019 to August 2020. Farms were selected after a personal interview with the farmers before enrollment in the study, and their responses were recorded through a questionnaire. Farms were recruited in Northern Italy, which has a high density of dairy industry, and presented similar management conditions and annual average milk production per cow and day, but spending a period of time on pasture when ORG. Twenty-one herds had only HF cows and 3 had ≥50% HF cows. The diet of lactating animals included corn (meal, silage, or both). Samples of the TMR administered to lactating cows were collected and analyzed at the Feed Analysis Laboratory of the Department of Veterinary Science of the University of Parma (Parma, Italy), and were found to show no differences between the farming systems, as reported in Manuelian et al. (2021b).

Bulk Milk Sampling and Gross Composition, SCS, and Vitamin E Determination

A total of 225 bulk milk samples (100 mL) were collected monthly in the farms, and 0.1 mL of azidiol preservative prepared at the central laboratory of Granarolo S.p.A (Bologna, Italy) was added (Benedet et al., 2018). Samples were transported to the central laboratory of Granarolo S.p.A. and then sent to the corresponding laboratory for analysis. Due to COVID-19 access restrictions in 2020, we were not able to collect samples in March and April.

Table 1. Descriptions of the organic and conventional farms that participated in the study, expressed as median and 95% CI

	Organic farms			Conventional farms		
Item	n	Median	95% CI	n	Median	95% CI
Certified organic, yr	12	6.5	2–19	—	—	—
Herd size, no.	12	545	160–1,020	12	340	130–780
Cows in lactation, no.	12	244.5	84–460	12	240	60–340
Daily average milk production, L/cow	12	28.4	21–33	12	31.6	26–35
Dry cows, no.	12	34	16–70	12	40	15–65
DIM, d	12	198	180–320	12	181.5	160–240
Cows' space if permanent litter, m^2/head	7	9	6–20	3	6	5–15
Cows/box if permanent litter, no.	7	75	40–180	3	10	6–70
Cubicles/cows in lactation, no.	7	315	33–405	10	292.5	63–396
Places/milking parlor, no.	11	16	8–30	12	19	8–28
Mastitis/month, no.	12	2	1–10	12	2.5	1–5

Table 2. Descriptions of the organic (n = 12) and conventional (n = 12) farms that participated in the study expressed as relative frequency (RF, %) and 95% CI

	Organic farms		Conventional farms	
Item	RF	95% CI	RF	95% CI
Breed				
At least 50% animals Holstein-Friesian	16.7	0–40.3	8.3	0–25.9
All Holstein-Friesian	83.3	59.7–100	91.7	74.1–100
Housing				
Cubicles	41.7	10.3–73.0	66.7	36.7–96.6
With permanent litter	41.7	10.3–73.0	16.7	0–40.3
With permanent litter and cubicles	16.7	0–40.3	16.7	0–40.3
Bedding material in permanent litter[1]				
Straw	87.5	59.9–100	100	—
Other	12.5	0–40.1	—	—
Bedding material in cubicles[2]				
Straw	100	—	80.0	51.4–100
Other	—	—	20.0	0–48.6
Includes external paddock				
Yes	100	—	50	18.2–81.8
No	—	—	50	18.2–81.8
Period on pasture during lactation				
Yes	58.3	27.0–89.7	—	—
No	41.7	10.3–73.0	100	—
Reposition with period on pasture				
Yes	66.7	36.7–96.6	—	—
No	33.3	3.4–63.3	100	—
Dry cows with period on pasture				
Yes	83.3	59.7–100	—	—
No	16.7	0–40.3	100	—
Refrigeration/ventilation system				
Nebulizers and fans	50	18.2–81.8	50	18.2–81.8
Fans	50	18.2–81.8	50	18.2–81.8
Offspring separation from their dams				
<12 h	66.7	36.7–96.6	91.7	74.1–100
12–24 h	16.7	0–40.3	8.3	0–25.9
Other	16.7	0–40.3	—	—
Milking parlor				
Automatic milking system	16.7	0–40.3	—	—
Parallel	8.3	0–25.9	16.7	0–40.3
Herringbone	75.0	47.5–100	83.3	59.7–100
Milking				
2 times/d	66.7	36.7–96.6	91.7	74.1–100
3 times/d	16.7	0–40.3	8.3	0–25.9
Free access	16.7	0–40.3	—	—
Pre-dipping				
No	25.0	0–52.5	25.0	0–52.5
Yes	75.0	47.5–100	75.0	47.5–100
Post-dipping				
No	16.7	0–403	8.3	0–25.9
Yes	83.3	59.7–100	91.7	74.1–100
Use of iodine in post-dipping[3]				
No	30.0	0–62.8	25.0	0–61.2
Yes	70.0	37.2–100	75.0	38.8–100
Udder cleaning				
Water	8.3	0–25.9	—	—
Paper towel	91.7	74.1–100	100	—
Voluntary vaccination				
IBR and BVD[4]	75.0	47.5–100	25.0	0–52.50
None	25.0	0–52.50	75.0	47.5–100
Main use of antibiotic				
During dry period	33.3	3.4–63.3	58.3	27.0–89.7
During lactation	58.3	27.0–89.7	33.3	3.4–63.3
Do not use	8.3	0–25.9	8.3	0–25.9
Use of phytotherapy				
No	50.0	18.2–81.8	50.0	18.2–81.8
Yes	50.0	18.2–81.8	50.0	18.2–81.8

[1]Organic = 8 farms; conventional = 4 farms.
[2]Organic = 7 farms; conventional = 10 farms.
[3]Organic = 10 farms; conventional = 8 farms.
[4]IBR = infectious bovine rhinotracheitis; BVD = bovine viral diarrhea.

Bulk milk composition (fat, protein, casein, and lactose percentages) was determined through mid-infrared spectroscopy prediction models available on the MilkoScan FT6000 (Foss Electric A/S) in the laboratory of the Breeders Association of Veneto Region (Padova, Italy), within 4 d from milk sampling. Milk quality traits were processed according to the standard methods used in official milk-recording schemes for fat, protein, casein, and lactose. Moreover, SCC was determined by Fossomatic flow cytometers (Foss Electric A/S), and values of SCC were transformed to SCS through the formula $SCS = 3 + \log_2(SCC/100)$, where SCC was expressed as cells per microliter (Wiggans and Shook, 1987).

Vitamin E (**VitE**) content of milk was analyzed in the Feed Analysis Laboratory in the Department of Veterinary Science of the University of Parma. In brief, frozen milk samples were thawed in a water bath at 50°C for 30 min, and they were turned after 15 min of incubation. Thereafter, they were shaken for 10 s and left resting for 1 h. Then, they were shaken again, and 0.5 mL of sample was aspirated with a syringe and poured into the tube with the solvent of the iCheck Vitamin E Kit (BioAnalyt GmbH). The VitE concentration was determined using the iCheck fluorimeter/spectrophotometer and expressed as milligrams per liter.

Minerals, AA, and FA composition were determined at the Department of Agronomy, Food, Natural resources, Animals and Environment of the University of Padova (Legnaro, Italy), as described in De Marchi et al. (2021), after samples were thawed during 24 h at 4°C.

Bulk Milk Mineral Profile

Briefly, minerals were quantified after mineralization of the sample (2 mL) with 7 mL of 67% nitric acid (HNO_3) and 2 mL of 30% hydrogen peroxide in closed vessels by a microwave system (200°C for 15–18 min, cooled to 35°C, and made up to volume with distilled water; Ethos 1600, Milestone S.r.l.) using inductively coupled plasma optical emission spectrometry (Arcos EOP, Spectro Analytical Instruments GmbH) according to AOAC International (2016) method no. 2011.14. The complete list of minerals and wavelengths used is shown in De Marchi et al. (2021). Instrument operating parameters were optimized for acid solution, and calibration standards were matched with 5% HNO_3 (vol/vol) solution using 65% HNO_3 Suprapur (100441, Merck). Operating conditions of inductively coupled plasma optical emission spectrometry were 2 mL/min of sample aspiration rate, plasma power 1,350 W, coolant flow 12 L/min, auxiliary flow 0.80 L/min, nebulizer flow 0.90 L/min, and integration time of 28 s. The calibration solutions for each mineral were prepared from single-element solutions (Inorganic Ventures) in a concentration range between 0 and 100 mg/L. Minerals were expressed in milligrams per kilogram.

Bulk Milk AA Profile

Briefly, Ala, Arg, Asp, Cys, Gly, Glu, Ile, His, Leu, Lys, Met, Phe, Pro, Ser, Tyr, Thr, and Val determination, a laboratory internal method was used. Samples were prepared by acid hydrolysis with acid chloride 6 *M* and heated at 105°C for 24 h. After hydrolysis, the samples were neutralized with sodium hydroxide 8 *N*, adjusted to volume and filtered at 0.45 μm. Then, the derivatization step was conducted according to the manufacturer's instructions (AccQTag Ultra Derivatization Kit, Waters Corp.). Separation and quantification of AA were performed using an Agilent 1260 Infinity HPLC (Agilent Technologies) equipped with a reversed-phase C18 column (Cortecs C18 Column, 90 Å, 2.7 μm, 250 mm × 2.1 mm; Waters Corp.) kept at 45°C and a diode array detector (Agilent 1260 Series, DAD VL+, G1315C). Mobile phases consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). Flow rate was 0.4 mL/min and volume injection 5 μL. Determination of Trp followed the methodology described by Yust et al. (2004). Briefly, 7.5 mL of 4 *N* sodium hydroxide was added to the sample and incubated in an oven at 100°C for 16 h. After hydrolysis, the samples were neutralized with HCl 6 *N*, adjusted to volume, and filtered at 0.45 μm. Separation and quantification of Trp were performed using an Agilent 1260 Infinity HPLC (Agilent Technologies) equipped with an AdvanceBio AAA column kept at 18°C and a diode array detector (Agilent 1260 Series, DAD VL+, G1315C). Injection volume was 20 μL. An isocratic elution system consisting of 25 m*M* sodium acetate/acetonitrile (91:9) was delivered at 0.9 mL/min. Amino acids results were expressed in milligrams per 100 g.

Bulk Milk Fatty Acid Profile

For FA profiling, total lipids were extracted by an accelerated solvent extraction method using a Dionex ASE 350 system (Thermo Scientific) with petroleum ether/isopropanol (2:1) as solvent as indicated by the manufacturer (application note 345). Total fat content was determined after solvent evaporation under nitrogen flow at 30°C and expressed as a percentage. Fatty acid methyl esters of total lipids were prepared with an

internal method adapted from Christie (1982). Briefly, for 40 mg of extracted fat, 200 μL of sodium methylate 1 *M*, and 2 mL of hexane were added. The solution was placed in a stirrer for 15 min and 300 μL of oxalic acid and 4 mL of sodium sulfate were added. At the end of methylation, FAME solutions were centrifuged for 10 min at 693 × *g* at 10°C, and 1.6 mL of supernatant was collected in a 2-mL vial.

Separation and quantification of FAME were performed using an Agilent 7820A GC System equipped with an automatic sampler G4567A (Agilent Technologies) and a flame ionization detector. The capillary column (length 30 m, inner diameter 0.25 mm, film thickness 0.25 μm) comprised an Omegawax capillary GC column (24136 Supelco, Sigma-Aldrich). The carrier gas was hydrogen at flow rate of 1.4184 mL/min with an average speed of 39.5 cm/s. The injector and temperature detector were both set at 250°C. The oven temperature was initially 50°C for 2 min and then increased at 4°C/min until reaching 220°C, at which point this temperature was held for 18 min. The individual FA were identified by comparing their retention times with those of a standard fatty acid (Supelco FAME mixC4-C24 #18919-1AMP, Sigma-Aldrich). Peak areas were calculated using OpenLAB CDS ChemStation Edition C.01 XX software (Agilent Technologies) and expressed as percentage of total FA.

The following FA groups were obtained by summing up individual FA as described in De Marchi et al. (2021): SFA, MUFA, PUFA, CLA, n-3 FA, n-6 FA, short-chain FA, medium-chain FA, long-chain FA, all *cis*-stereoisomers of FA (***cis*-FA**), and all *trans*-stereoisomers of FA except in CLA.

In addition, the desaturation index of C16:0 was calculated as (C16:1)/(C16:0 + C16:1) × 100; desaturation index of C18:0 as (C18:1)/(C18:0 + C18:1) × 100; and elongation index as (C8:0 + C10:0 + C12:0 + C14:0)/(C4:0 + C6:0). The atherogenic index (AI) and the thrombogenic index (TI) were calculated by applying the formula proposed by Ulbricht and Southgate (1991):

$$\text{AI} = [\text{C12:0} + (4 \times \text{C14:0}) + \text{C16:0}]/(\text{MUFA+PUFA});$$

$$\text{TI} = (\text{C14:0} + \text{C16:0} + \text{all C18:0})/[(0.5 \times \text{MUFA}) + (0.5 \times \text{n-6}) + (3 \times \text{n-3}) + (\text{n-3/n-6})].$$

The nutritional value (NV) was calculated by applying the formula proposed by Estévez et al. (2004):

$$\text{NV} = (\text{C12:0} + \text{C14:0} + \text{C16:0})/(\text{C18:1} + \text{C18:2n-6}).$$

The hypocholesterolemic/hypercholesterolemic ratio (h/H) was calculated by applying the formula of Fernández et al. (2007):

$$\text{h/H} = (\text{C18:1} + \text{C18:2n-6} + \text{C18:3n-3} + \text{C18:3n-6} + \text{C18:4n-3} + \text{C20:1n-9} + \text{C20:2n-6} + \text{C20:3n-6} + \text{C20:3n-3} + \text{C20:4n-6} + \text{C20:5n-3} + \text{C22:1n-9} + \text{C22:2n-6} + \text{C22:6n-3} + \text{C24:1n-9})/(\text{C14:0} + \text{C16:0}).$$

Statistical Analysis

Descriptive characteristics of the farms are shown as median with its 95% confidence interval (95% CI), calculated using the UNIVARIATE procedure of SAS version 9.4 (SAS Institute Inc.) for quantitative variables, and relative frequency with 95% CI, calculated as $p \pm t(n-1) \times \sqrt{p(1-p)n}$, where p is the proportion, t is the *t*-value, and n is the number of responses for categorical variables.

Bulk milk chemical composition (fat, protein, casein, lactose), SCS, VitE, and mineral, AA, and FA profiles were investigated through the MIXED procedure of SAS version 9.4, using a model that included farming system, calendar month of sampling, and their interaction as fixed effects, and farm nested within farming system as random effect. Results are reported as least squares means (LSM), and multiple comparisons of LSM were performed for the main effects of farming system, calendar month of sampling, and their interaction, using Bonferroni adjustment when necessary. The LSM for the calendar month of sampling are not shown in detail nor discussed, despite being significant, because they follow the same pattern already described in the literature. An LSM for the interaction is shown only when significant between both farming systems for the same calendar month of sampling. Significance was declared at $P < 0.05$ unless otherwise stated.

RESULTS

Description of Farms

All farms selected included corn (meal, silage, or both) as an ingredient in the lactating diets, and the annual average milk production per cow was similar. Table 1 displays the farms information from the continuous quantitative variables. Half of the ORG farms had been certified for 6.5 yr or less; however, the 95% CI was quite wide (17 yr). Herd size seemed to be larger and with a wider range in ORG (median, 545 cows) than CONV farms (median, 340 cows). Despite the

medians of animals in lactation, dry animals and DIM were similar, but the range for the number of lactating cows and DIM was wider in ORG (376 cows and 140 DIM) than CONV herds (280 cows and 80 DIM). Although very few CONV farms used permanent litter, ORG cows had more space (+3 m^2) and included more cows per box. The ORG farms showed more variability in the number of cubicles per cows in lactation, with a difference (of the median) of 22 cubicles. In spite of similar variability of the number of places in the milking parlor, a difference (of the median) of 3 places was observed between ORG and CONV farms. However, even if the median number of mastitis cases per month (2 in ORG and 2.5 in CONV) was similar, a wider range was observed in ORG than CONV herds.

Table 2 displays the farms' information in relation to the discrete variables. Farms included in the study reared HF. However, 1 of the CONV herds and 2 of the ORG herds also had other breeds. The use of cubicles was more frequent in CONV than ORG herds. In both farming systems, the most frequent bedding material indicated was straw. Other bedding materials were also indicated by some ORG farmers. Separation of the offspring within the first 12 h after calving was more frequent in CONV than in ORG herds; however, farms that kept dams and calves together for a longer period were included in both farming systems. Although herringbone milking parlors and milking twice a day were the most common structure and milking routine, few ORG farms (n = 2) also stated that they use an automatic milking system. Pre- and post-dipping and use of paper towels to clean the teats were the most common practices during the milking routine in both farming systems. The majority of farms (ORG = 7 out of 10; CONV = 6 out of 8) in both groups use products including iodine during post-dipping. Farms in both farming systems were equal (50%) in relation to the type of ventilation system and the application of phytotherapy. Nevertheless, we did not include questions related to the amount of phytotherapy used (or medication bills as proxy for amount of phytotherapy used) because this was beyond the scope of the present work.

Although most farms in both farming systems used antibiotic treatments, ORG mostly administered antibiotics during lactation (58%), whereas CONV mostly applied them during the dry period (58%; Table 2). Only one farm in each group declared not using antibiotics. Voluntary vaccination against infectious bovine rhinotracheitis and bovine viral diarrhea seems to be more frequent in ORG (75%) than CONV (25%) farms (Table 2). However, the main difference between both farming systems is related to the presence of external paddocks and the use of pastures (Table 2). The external paddock is a free-access outdoor area in the facilities where the cows are kept. Use of pastures refers to moving the cows to the pasture where they can graze. Although all ORG farms had an external paddock for the animals, only half of the CONV farms did. In addition, animals in ORG farms spent at least 1 of the 3 periods (lactation, the dry period, and the heifer stage) on pasture, especially during the dry period, whereas none of the animals in CONV farms spent a period in the pasture. However, it should be noted that only 7 out of the 12 ORG farms reported moving animals to the pasture during lactation.

Bulk Milk Gross Composition, SCS, and VitE

Milk composition, SCS, and VitE content are reported in Table 3. Gross milk composition revealed significantly lower contents of protein ($P = 0.037$) and casein ($P = 0.047$) in ORG (3.25% and 2.53%, respectively) than CONV (3.37% and 2.64%, respectively) milk samples. In addition, SCS was greater ($P = 0.009$) in ORG than CONV farms (4.30 and 3.90, respectively; Table 4). By contrast, VitE did not differ between farming systems. The calendar month of sampling was significant for all the traits, whereas the interaction was not.

Mineral, AA, and Fatty Acid Profiles

Among all the minerals (Table 4), only Fe, K, Mg, and S were affected by the farming system during some months. In particular, in those months, K (July), Mg (October, July, and August), and S (October, July, and August) content in milk from ORG farms was lower than in milk from CONV farms ($P < 0.05$). In contrast, Fe content in milk from ORG farms was greater in November and December, and lower in May than in milk from CONV farms ($P < 0.05$). The calendar month of sampling was always significant except for Mo, Sr, and Zn. Heavy metals As, Cd, Pb, Ni, Tl, Sn, and Hg were below the limit of detection, as were Be, Co, Li, Sb, Se, and V.

The AA profile did not differ between farming systems (Table 5) and was not affected by the interaction. However, calendar month of sampling was highly significant ($P < 0.001$) for all the AA analyzed.

Fatty acid groups, index, and ratios are shown in Table 6. Moreover, Table 7 reports individual FA present at least at 1% of total FA. Among all of them (Table 6 and Table 7), we found significant differences only for palmitoleic acid (C16:1n-9; Table 7), which was greater ($P = 0.049$) in CONV (1.68%) than ORG (1.58%) milk, and also a greater ($P < 0.001$) *cis*-FA content in October in ORG (1.14%) than CONV (0.69%) milk. In

Table 3. Cow bulk milk gross composition and vitamin E content (LSM ± SE) during the study from the organic (n = 12) and conventional (n = 12) farms

	Organic milk		Conventional milk		
Trait	n	LSM ± SE	n	LSM ± SE	*P*-value
Milk composition, %					
Fat	109	3.75 ± 0.08	113	3.91 ± 0.08	0.170
Protein	109	3.25 ± 0.04	114	3.37 ± 0.04	0.037
Casein	109	2.53 ± 0.03	114	2.64 ± 0.03	0.047
Lactose	107	4.81 ± 0.02	114	4.83 ± 0.02	0.539
SCS	109	4.30 ± 0.10	114	3.90 ± 0.10	0.009
Vitamin E, mg/L	102	1.88 ± 0.08	107	1.82 ± 0.08	0.591

addition, PUFA (Table 6), n-3 (Table 6), C6:0 (Table 7), and α-linoleic acid (C18:3n-3; ORG, 0.59 ± 0.03%; CONV, 0.50 ± 0.03%) tended ($P < 0.10$) to be greater in ORG than CONV milk. By contrast, eicosapentaenoic acid (C20:5n-3; ORG, 0.018 ± 0.002%; CONV, 0.017 ± 0.002%) and docosahexaenoic acid (C22:6n-3; 0.0137 ± 0.010%; CONV, 0.0124 ± 0.0009%) did not differ between groups. The calendar month of sampling was always significant except for medium-chain FA and palmitic acid (C16:0). In addition, tendencies were detected for n-6 ($P = 0.074$), hypocholesterolemic/hypercholesterolemic ratio ($P = 0.060$), and linoleic acid (C18:2n-6; $P = 0.088$).

DISCUSSION

Differences Between Farms in Each System

As already discussed in Manuelian et al. (2021b), the main difficulty in comparing these results with the literature is the lack of control of most published studies in the variables that affect milk composition (e.g.,

Table 4. Cow bulk mineral composition (LSM ± SE, mg/kg) during the study from organic (n = 12) and conventional (n = 12) farms; results for the interaction (type of production × calendar month of sampling) are shown when significant

	Organic milk		Conventional milk		
Mineral	n	LSM ± SE	n	LSM ± SE	*P*-value
Al[1]	107	0.76 ± 0.15	111	0.63 ± 0.11	0.711
B	108	0.36 ± 0.02	111	0.38 ± 0.02	0.460
Ba	108	0.057 ± 0.004	109	0.061 ± 0.004	0.473
Ca	108	1,161 ± 11.6	110	1,170 ± 11.6	0.579
Cr	107	0.0228 ± 0.0006	109	0.0226 ± 0.0005	0.751
Cu	96	0.041 ± 0.002	104	0.043 ± 0.002	0.398
Fe	106	0.264 ± 0.005	110	0.261 ± 0.005	0.661
November	10	0.372 ± 0.017	11	0.319 ± 0.016	0.025
December	12	0.306 ± 0.015	12	0.254 ± 0.015	0.019
May	11	0.223 ± 0.016	11	0.286 ± 0.016	0.006
K	107	1,509 ± 7.76	107	1,514 ± 7.75	0.690
July	9	1,561 ± 14.70	8	1,609 ± 15.44	0.026
Mg	108	112.7 ± 0.95	110	114.8 ± 0.95	0.133
October	11	108.6 ± 1.31	12	112.2 ± 1.28	0.028
July	9	113.2 ± 1.39	9	118.3 ± 1.39	0.010
August	11	112.7 ± 1.31	10	116.9 ± 1.35	0.028
Mn	107	0.0212 ± 0.0006	111	0.0225 ± 0.0006	0.158
Mo	102	0.0493 ± 0.0037	108	0.0449 ± 0.0037	0.400
Na	100	413.1 ± 5.92	111	410.0 ± 5.78	0.703
P	108	921.3 ± 8.20	106	928.6 ± 8.24	0.537
S	108	306.7 ± 2.75	110	316.9 ± 2.75	0.015
October	11	307.4 ± 3.71	12	319.9 ± 3.62	0.016
July	9	296.9 ± 3.93	9	320.3 ± 3.93	<0.001
August	11	295.9 ± 3.71	10	315.5 ± 3.81	<0.001
Si	108	69.61 ± 0.72	110	69.96 ± 0.72	0.737
Sr	108	0.498 ± 0.036	111	0.484 ± 0.036	0.791
Ti	98	0.0109 ± 0.00002	100	0.0111 ± 0.00002	0.515
Zn	108	3.89 ± 0.05	109	3.96 ± 0.05	0.338

[1]Al results presented as raw means instead of LSM of the inverse transformation for its interpretation.

Table 5. Cow bulk protein profile (LSM ± SE, mg/100 g) during the study from the organic (n = 12) and conventional (n = 12) farms

	Organic milk		Conventional milk		
AA	n	LSM ± SE	n	LSM ± SE	*P*-value
His	105	103.0 ± 1.34	109	105.1 ± 1.30	0.259
Arg	106	71.7 ± 1.25	110	73.9 ± 1.23	0.216
Ser	106	178.1 ± 2.21	110	180.8 ± 2.18	0.391
Gly	106	54.3 ± 0.70	110	55.1 ± 0.69	0.431
Asp	106	259.1 ± 3.75	110	263.2 ± 3.71	0.439
Gln	106	846.1 ± 10.64	110	858.4 ± 10.53	0.419
Thre	106	126.6 ± 1.85	110	129.8 ± 1.84	0.232
Ala	106	95.6 ± 1.46	110	96.9 ± 1.44	0.555
Pro	106	311.5 ± 4.08	110	317.9 ± 4.04	0.278
Lys	106	289.2 ± 3.84	110	294.4 ± 3.79	0.341
Tyr	105	136.2 ± 1.52	110	138.6 ± 1.50	0.264
Met	106	55.8 ± 1.59	110	56.5 ± 1.58	0.744
Val	106	143.3 ± 2.16	110	145.2 ± 2.13	0.521
Ile	105	115.9 ± 1.77	110	118.3 ± 1.74	0.351
Leu	105	296.6 ± 3.62	110	301.4 ± 3.57	0.359
Phe	105	150.9 ± 1.70	110	153.9 ± 1.68	0.232
Trp	106	50.5 ± 0.79	110	51.8 ± 0.79	0.281
Cys	106	26.2 ± 0.34	110	26.7 ± 0.33	0.294

breed, diet formula and ingredients, pasture-based systems). It is almost impossible to find ORG and CONV farms with the same management conditions, to allow control of factors that influence milk quality. We tried to minimize those differences by including the same breed, which is known to affect FA (Manuelian et al., 2019) and mineral profiles (Manuelian et al., 2018), in both farming systems (Table 2). We also selected farms with a similar daily average milk production per cow (Table 1) to avoid the dilution effect on milk components. In addition, not all ORG farms had animals on pasture during the lactation period, when milk samples were collected, which means that diets of some ORG farms relied on TMR, the same as CONV farms. In particular, only 5 ORG farms did not have the herd on pasture during the lactation period, which we expect to reduce differences between ORG and CONV milk, as none of the CONV farms had cows on pasture. Moreover, all farms were selected to ensure that the TMR ingredients included corn, which is a C4 plant commonly used in CONV concentrate feed. Its use in ORG farms makes it impossible to differentiate between the systems when using the carbon stable isotope ratio (^{13}C) in milk method (Schwendel et al., 2015; Inácio and Chalk, 2017), despite ^{13}C being the most promising isotopic marker to differentiate ORG and CONV milk (Inácio and Chalk, 2017).

The differences observed between farming systems were in agreement with the Italian ORG dairy sector and EU ORG legislation (European Union, 2009, 2018), with longer lactations, more space per animal, access to external paddock, and a period of time on pasture. Although antibiotics are the last resource for ORG farmers, their application is allowed under the prescription of a veterinarian (European Union, 2009, 2018). Therefore, the use of antibiotics in ORG conditions was expected, as those treatments are still the preferred ones by ORG farmers for most health issues (Manuelian et al., 2021a), in particular to treat mastitis (Orjales et al., 2016a), which is one of the main problems in ORG dairy farming (Hovi et al., 2003; Sutherland et al., 2013; Brock et al., 2021). It is interesting to note that more ORG than CONV farmers declared using antibiotics during the lactation period, considering that the withdrawal period for allopathic treatments established by ORG legislation must be twice as long in ORG than in CONV farms (European Union, 2009, 2018).

Effects on Bulk Milk Gross Composition, SCS, VitE, and AA Composition

According to Table 3, milk gross composition differed between farming systems for protein and casein contents. However, the AA profiles were similar (Table 5). This could be explained by the method of determination used. Whereas protein and casein contents were estimated using MilkoScan, AA were quantified with HPLC. The MilkoScan prediction model for protein is built on nitrogen detection, as is crude protein determination; thus, other nitrogen compounds can influence the value obtained. Therefore, our results could be in agreement with the review of Średnicka-Tober et al. (2016), who did not find differences in fat and protein contents in ORG and CONV milk. However, a study conducted in ORG and CONV retail milk showed

Table 6. Cow bulk milk fatty acid (FA) composition (LSM ± SE, %) during the study from the organic (n = 12) and conventional (n = 12) farms; results for the interaction (type of production × calendar month of sampling) are shown when significant

	Organic milk		Conventional milk		
Item[1]	n	LSM ± SE	n	LSM ± SE	*P*-value
SFA	108	69.28 ± 0.49	113	69.87 ± 0.49	0.396
MUFA	108	25.64 ± 0.35	112	25.55 ± 0.35	0.860
PUFA	107	5.11 ± 0.21	111	4.54 ± 0.21	0.071
n-3	107	0.77 ± 0.03	111	0.68 ± 0.03	0.086
n-6	107	4.31 ± 0.19	111	3.84 ± 0.19	0.103
CLA	107	0.602 ± 0.022	110	0.554 ± 0.022	0.144
cis-FA	101	0.725 ± 0.041	110	0.668 ± 0.040	0.331
October	10	1.142 ± 0.076	9	0.690 ± 0.073	<0.001
trans-FA	106	0.305 ± 0.004	110	0.299 ± 0.004	0.287
SCFA	107	8.65 ± 0.10	111	8.54 ± 0.09	0.430
MCFA	108	51.11 ± 0.72	113	52.07 ± 0.72	0.360
LCFA	108	40.23 ± 0.78	113	39.39 ± 0.78	0.454
Index and ratio					
SFA/(MUFA+PUFA) ratio	108	2.27 ± 0.05	113	2.34 ± 0.05	0.383
n-6/n-3 ratio	108	5.68 ± 0.27	113	5.71 ± 0.27	0.942
DI C16:0	108	5.23 ± 0.06	112	5.32 ± 0.06	0.331
DI C18:0	108	67.07 ± 0.39	112	67.14 ± 0.39	0.891
EI	107	4.53 ± 0.07	111	4.49 ± 0.07	0.680
AI	108	2.64 ± 0.07	113	2.70 ± 0.07	0.588
TI	107	3.49 ± 0.08	111	3.61 ± 0.08	0.250
NV	108	1.83 ± 0.06	112	1.92 ± 0.06	0.360
h/H ratio	108	0.629 ± 0.021	113	0.602 ± 0.021	0.351

[1]SCFA = short-chain fatty acids; MCFA = medium-chain fatty acids; LCFA = long-chain fatty acids; *cis*-FA = *cis*-stereoisomers of fatty acids; *trans*-FA = *trans*-stereoisomers of fatty acids, excluding CLA; DI C16:0 = desaturation index of C16:0; DI C18:0 = desaturation index of C18:0; EI = elongation index; AI = atherogenic index; TI = thrombogenic index; NV = nutritional value; h/H = hypocholesterolemic/hypercholesterolemic ratio.

greater total milk fat (g/kg of milk) in the former than in the latter (Butler et al., 2011). To the best of our knowledge, differences in the AA composition of ORG and CONV farms have not been studied. A recent study showed lower ^{13}C in Ile, Leu, Met, Phe, Pro, Ser, and Val of ORG than CONV milk (Chung et al., 2019), which could be related to the more frequent use of corn for CONV than ORG cows (Schwendel et al., 2015). It should be noted that we established in our experimental design that both groups' diets should include corn.

Few studies have evaluated differences in VitE milk content between ORG and CONV farms. Vitamin E is mainly found in pasture, and in grass and legumes, but the concentration is highly variable (Johansson et

Table 7. Most abundant individual cow bulk milk fatty acids (LSM ± SE, %) from organic (n = 12) and conventional (n = 12) farms through 1 yr

	Organic milk		Conventional milk		
Fatty acid	n	LSM ± SE	n	LSM ± SE	*P*-value
C4:0	104	2.39 ± 0.02	112	2.36 ± 0.02	0.400
C6:0	105	1.80 ± 0.02	111	1.76 ± 0.02	0.099
C8:0	105	1.20 ± 0.02	110	1.17 ± 0.02	0.334
C10:0	108	2.87 ± 0.06	113	2.83 ± 0.06	0.623
C12:0	108	3.48 ± 0.09	110	3.45 ± 0.09	0.808
C14:0	108	11.43 ± 0.14	111	11.19 ± 0.14	0.245
C14:1	105	1.088 ± 0.027	107	1.093 ± 0.027	0.905
C15:0	108	1.92 ± 0.05	113	1.90 ± 0.05	0.858
C16:0	107	30.99 ± 0.51	111	32.12 ± 0.51	0.133
C16:1n-9	108	1.58 ± 0.04	113	1.68 ± 0.04	0.049
C17:0	108	1.59 ± 0.04	111	1.56 ± 0.04	0.599
C18:0	108	10.84 ± 0.32	111	10.74 ± 0.32	0.823
C18:1n-9	108	21.00 ± 0.37	112	20.87 ± 0.37	0.806
C18:2n-6	107	3.41 ± 0.18	111	2.98 ± 0.18	0.100

al., 2014). Therefore, concentrate feeding for dairy cows reared in ORG farming systems usually incorporates a vitamin premix to ensure adequate vitamin intake levels, even if farmers are not aware of the addition of this vitamin premix (Manuelian et al., 2021a). The importance of this premix in the ORG group diet is more relevant as 5 out of the 12 ORG farms did not use pasture during the lactation period. Although Średnicka-Tober et al. (2016) indicated a significantly higher content of α-tocopherol content in ORG than CONV milk—which disagrees with our results (Table 3)—they stated no differences in contents of other vitamins (A, C, D_3) and VitE activity.

Despite significant difference between ORG and CONV for SCS (Table 3), Średnicka-Tober et al. (2016) did not report the same in their review. However, our results are in agreement with studies conducted in Northern Spain, which showed higher SCC in ORG than CONV herds (Villar and López-Alonso, 2015; Orjales et al., 2016b, 2017), partially associated with higher prevalence of chronic subclinical mastitis in ORG farms (Villar et al., 2016). The increase in SCC has been linked to increasing parity, with the use of alternative treatments instead of antibiotics, without use of teat dipping in the milking routine, and with lower milk production (Orjales et al., 2017). This last aspect was explained by a dilution effect related to greater milk production. As discussed by Orjales et al. (2017), ORG farms are usually low-input systems that lead to lower milk production levels compared with CONV farms. However, the dilution effect does not apply to our scenario, because our study included farms with a similar annual average milk production per cow (Table 1). The greater SCS in ORG than CONV observed in our study is also in agreement with the wider range of mastitis cases per month declared by the farms (Table 1).

Milk Mineral Profiles

Few studies have evaluated differences in milk mineral profiles between ORG and CONV farms. The review of Średnicka-Tober et al. (2016) reported a significantly higher content of Fe and lower I and Se contents in ORG than CONV milk, which agrees with our results related to Fe content in November and December. In addition, van der Reijden et al. (2018) demonstrated that milk I concentration depends on the farming system (lower in ORG than CONV farms) and the use of iodine-containing teat dipping. By contrast, Średnicka-Tober et al. (2016) reported no differences in most minerals (Ca, Co, Cu, Mg, Mn, Mo, P, K, Na, and Zn) and toxic metals (Cd and Pb), which is in line with our results. However, we observed a lower content of Mg, K, S, and Fe for very few months (<3; Table 4). Nevertheless, we did not find differences in Se content, and toxic metals such as As, Cd, Pb, and Hg were below the limits of detection. However, we did not measure I content, which needs to be determined according to a different methodology (Niero et al., 2019) than the one used for the quantification of all the other minerals evaluated in the present study. It would be interesting to include this mineral determination in future studies. However, from the questionnaire compiled by the farmers, it seems that both farming systems used similar udder hygiene, which included iodine products during the post-dipping (Table 2).

A study conducted in Northern Spain, which included 39 ORG and 59 CONV dairy farms (Rodríguez-Bermúdez et al., 2018), revealed more differences between ORG and CONV farms, with significantly lower concentrations of Co, Cu, Se, Zn, I, and Cr in ORG than CONV farms, likely due to sanitary management practices (Se to treat an improve reproductive performance and I as a disinfectant after milking) and mineral supplementation (Cr, Cu, and Zn; López-Alonso et al., 2017). Moreover, López-Alonso et al. (2017) indicated that the ingestion of soil during grazing seems to have an important effect on the trace elements status in ORG cattle compared with CONV. However, the selection of farms in our study might have influenced the reduction of differences in milk mineral profiles between both farming systems. In our study, lactating cows from 7 out of 12 ORG farms grazed at pasture, and half of the CONV included an external paddock. Access to an external paddock in CONV might have contributed to soil ingestion and reduced differences of trace elements between groups.

Effects on Milk Fatty Acid Profile

Milk FA profile is one of the most studied traits in ORG milk. The review of Średnicka-Tober et al. (2016), which included 170 published studies, found no significant differences in SFA, MUFA, linoleic acid (C18:2n -6), n-6 concentrations, and n-6/n-3 ratios between ORG and CONV milk, which agreed with our results. Moreover, the greater tendencies we obtained for PUFA, n-3 (Table 6), and α-linoleic acid were in line with the findings of Średnicka-Tober et al. (2016). Nevertheless, they also reported greater contents of eicosapentaenoic acid, docosahexaenoic acid, and CLA in ORG than in CONV milk. We found significant differences only for C16:1n-9, which was greater in CONV than in ORG milk, and *cis*-FA in October, also greater in ORG than CONV milk (Table 7). In contrast, Hein et al. (2016) reported greater SFA, PUFA, short-chain FA, C16:0, and C14:0 content in ORG than CONV, whereas

MUFA, long-chain FA, C18:1, and C18:0 were greater in CONV than ORG milk. Moreover, Benbrook et al. (2013) reported 26% lower n-6 content and 62% greater n-3 content in retail ORG than CONV milk. However, those studies did not include ORG and CONV milk samples from cows reared under similar conditions.

A study conducted with retail milk (87 cartons of full-fat pasteurized milk) demonstrated that the differences observed in the FA profile between ORG and CONV were reduced when including CONV pasture-based milk along with the CONV milk (Liu et al., 2018). Therefore, the very few differences we observed could be related to the farms selected within each group; both farming systems included HF cows, and 5 out of 12 of the ORG farms did not have the lactating animals on pasture.

CONCLUSIONS

We conclude that the overall milk composition was quite similar between the 2 farming systems in Northern Italy. The only differences detected were greater SCS, lower K, Mg, and S, and lower or greater Fe content during some months, lower C16:1n-9, and greater *cis*-FA in October in ORG than in CONV milk. We also observed tendencies for greater C6:0, PUFA, and n-3 content in ORG. These few differences could be related to the similarities of the selected farms, including production level, the HF breed, and generally high levels of intensity in both farming systems. Although the present study demonstrated lack of difference in milk quality between ORG and CONV farming systems raising HF and minimizing managerial conditions, other aspects related to the production system and indicators of animal health and welfare should be considered. For instance, the expected better welfare, which includes outdoor access and lower stocking density, in ORG farming systems is often assumed by ORG consumers but is not demonstrated by its intrinsic milk quality traits. Moreover, although antibiotic residues are difficult to measure in milk and this was beyond the scope of the present work, further work could measure drug residues in milk and further compare the 2 systems.

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