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The effects of dietary iodine content, milking system, and farming practices on milk iodine concentration and quality traits

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ABSTRACT

Various management practices can influence milk quality traits in dairy cattle. As an example, an increasing investment in automatic milking system to substitute milking parlors has been observed in the last 2 decades in dairy farms which could have affected certain bulk milk quality traits. What is more, milking practices can also affect certain milk parameters; as an example, teat disinfectants containing I are used in commercial farms where pre- or postdipping is performed, leading to presence of some I in the bulk milk. However, this trace mineral is also supplied in cows' diet to fulfill their nutritional requirements, partly contributing to the milk I final concentration. Therefore, the aim of this study was to evaluate the sources of variation of milk I along with other traditional milk quality traits. A total of 91 dairy farms in northeastern Italy were enrolled in the study. In each farm, diet and bulk milk samples were collected on the same day for chemical analysis. Concentration of I, in particular, was determined in both milk and feed with gold standard. Pearson correlations were calculated among the traits available for milk and diet, and a general linear model was used to test significance of fixed effects (feeding system, milking system, farming system, herd size, herd stage of lactation, and sampling month) on milk quality traits including the I concentration. In the case of milk I, diet I and presence of I-based predipping and postdipping teat disinfect application were also tested as fixed effects. Results showed a positive linear correlation between milk and diet I content (correlation coefficient [r] = 0.78). Although milk I was also positively correlated with lactose content (r = 0.25), dietary I was not correlated with other milk traits. Milk I content was significantly affected by dietary I, I-based predipping teat disinfectant application, and herd composition. Compared with conventional farms, organic farms showed lower protein content and greater somatic cell score (SCS) but similar milk I. Milking system significantly affected only lactose content and SCS of milk. Sampling month was only significant for milk urea nitrogen and herd composition, feeding system, herd size, and herd average days in milk did not modify milk gross composition and SCS. In conclusion, dietary supply of I is the main factor affecting milk I concentration and findings suggest that I level in milk can be naturally improved in dairy cows by modulating the I content in the diet administered. However, further research is needed to evaluate the effect of I-based sanitizers on milk I.

Key words: cow milk, human health, organic, robotic milking

INTRODUCTION

Iodine is a trace element necessary for the biosynthesis of thyroid hormones in humans and animals (Flachowsky et al., 2014; Niero et al., 2023). Although most of the countries where I deficiency occurs have implemented a national salt (NaCl) iodization programs the salt intake is recommended to be reduced by 2025 (WHO et al., 2007; Censi et al., 2020; Santos et al., 2021). In this context, alternative I sources for humans such as milk and dairy products become important.

In dairy cattle, I dietary fortification has indirectly increased milk I, as dietary I which is not assimilated is excreted via urine and milk (Miller and Lansing, 1991) because of the high carry-over of I from feed to food (Franke et al., 2009). Moreover, using I teat sanitizers has also been identified to increase milk I (Miller and Lansing, 1991; Van Der Reijden et al., 2018). In fact, milk I long-term monitoring in Germany, Norway, and Switzerland revealed an increase up to 128% due to changes in feed supplementation (Walther et al., 2018).

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A recent study in northern Italy observed that milk I concentration decreases with parity, increases with lactation stage, and is greater in winter than spring suggesting that climatic conditions, diet, and management are the most relevant factors of milk I variability (Costa et al., 2021).

In northern eastern Italy, retail cow milk presented on average 269 ± 73 μg/L of milk I (Girelli et al., 2004; Watutantrige Fernando et al., 2013). Most children in that region consume at least 200 mL of milk per day, which means between 50 to 100 μg of daily I intake (Girelli et al., 2004). Both WHO et al. (2007) and the EFSA NDA Panel (2014) recommend consumption of at least 150 μg/d of I for nonpregnant, nonlactating adults (Bath et al., 2022). This means that in the Italian region, a cup of milk (200 mL) consumption provides between 33% and 66% of WHO/EFSA recommendations for daily I intake (Girelli et al., 2004; WHO et al., 2007; EFSA NDA Panel, 2014). Therefore, apart from iodized salt, milk is one of the most important I sources along with sea fish and shellfish upon average daily food consumption (Haldimann et al., 2005; Flachowsky et al., 2014; Censi et al., 2020). Despite a recent report published by the national observatory for the monitoring of I prophylaxis in Italy revealed that children have achieved I sufficiency (Olivieri and De Angelis, 2021), adults still presented median values below the EFSA and WHO adequate intake recommendations (EFSA NDA Panel, 2014; Iacone et al., 2021; WHO et al., 2007) placing Italy as a country with mild I deficiency status (Gärtner, 2016). Moreover, I dietary intake seems to be inversely associated with age (Iacone et al., 2021), which might be due to a decrease in milk consumption.

The use of automatic milking systems (**AMS**), also known as robotic milking, has increased worldwide from ~1,250 farms at the beginning of the 2000s to over 38,000 units roughly estimated to be installed nowadays (Hogenboom et al., 2019). In Europe, where this technology is more widely used, the main countries employing AMS are Denmark with 25% of dairy farms, followed by Sweden, Iceland, and the Netherlands (Hogenboom et al., 2019). The main goal with their implementation has been to improve quality of performed labor and farmers lifestyle, and studies regarding milk composition have shown variable results. Although, some authors did not find differences in milk fat and protein content (De Marchi et al., 2017; Tse et al., 2018), other reported increased milk fat, protein, and MUN (Toušová et al., 2014). From the best of our knowledge, none of them have investigated the effect of this milking system on milk I content.

In the AMS, cows are attracted by the feedstuff supplied during the milking procedures, in the meantime the robotic arms clean, sanitize, and stimulate the udder and teats, adapting the milking conditions to the cow's identification tag. Consequently, the milking is carried out in the absence of a human operator and therefore of a visual control of the udder and milk, utilizing automated mastitis detection (Hogenboom et al., 2019). Several active compounds are available for the pre- or postdipping teat disinfection in dairy cows including chlorhexidine and I (National Mastitis Council, 2014; Fitzpatrick et al., 2021). In these products, I concentration usually range from 0.15% to 1.35% wt/wt (Fitzpatrick et al., 2021).

Due to the important role of milk as an I dietary source, the aim of this study was to evaluate the sources of variation of milk I and composition, particularly the milking system.

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Farms Recruited

A total of 91 commercial dairy farms (mean ± SD; lactating cows, 79.93 ± 55.61; DIM, 186.49 ± 48.90) in the Veneto and the Friuli-Venezia Giulia regions (northeastern Italy) were enrolled in the study between December 2020 and June 2022. Farms were selected to be representative of the intensive and semi-intensive production systems of the area based on a questionnaire designed to gather information on: (1) characteristics of the herd; (2) management; (3) general herd health; (4) milking; and (5) diet of lactating cows. Each farm was only sampled once during the study, and milk and feed were sampled on the same day. The sampling day was selected to maximize milk and feed I content variability. The breeds present were Holstein-Friesian, Simmental, Brown Swiss, Burlina, and Rendena (Table 1). Of the 91 farms involved, 60 were single-breed and 31 multibreed (Table 1).

Feeding Sampling and Chemical Analysis

A representative sample (in the amount of 4 kg as fed) of the TMR administered to the lactating cows was collected for chemical analysis before milk sampling in each farm. Dietary formulas and the average amount of each ingredient included in more than one diet are reported in Table 2. One aliquot of the diet was dried, grinded, and analyzed to determine its chemical and nutritional composition using the DS2500

Table 1. Overview of farms' characteristics, including farming system, herd composition, herd size, barn type, bedding material or surface, feed, milking system, and procedure

Item	n	Relative frequency, %	95% CI, %
Farming system			
Conventional	83	91.2	85.4–97.0
Organic	8	8.8	3.0–14.6
Herd composition			
Single-breed	60	65.9	56.2–75.7
Holstein-Friesian	53	58.8	48.1–68.4
Simmental	3	3.3	0–7.0
Brown Swiss	2	2.2	0–5.2
Burlina	1	1.1	0–3.2
Rendena	1	1.1	0–3.2
Multibreed	31	34.1	24.3–43.8
Including Holstein-Friesian	28	30.8	21.3–40.3
Herd size			
<55 cows	30	33.0	23.3–42.6
55 ≤ cows <90	29	31.9	22.-41.4
≥90 cows	32	35.2	25.4–45.0
Barn			
Freestall barn	67	73.6	64.6–100
Bedded-pack barn	10	11.1	4.6–17.4
Freestall barn + bedded-pack barn	9	9.9	3.8–16.0
Tiestall (not free to move)	5	5.5	0.8–10.2
Bedding materials or surface			
Straw	55	60.4	50.4–70.5
Wood shavings/sawdust	10	11.0	4.6–17.4
Mattress for animals	6	6.6	1.5–11.7
Sand	1	1.1	0–3.2
Other	19	20.9	12.5–29.2
Feed			
TMR	82	90.1	84.0–96.2
Hay and concentrate fed separately	9	9.9	3.8–16.0
Milking system			
Milking parlor	45	49.5	39.2–59.7
Automatic milking system	28	30.8	21.3–40.3
Pipeline milking system	10	11.0	4.6–17.4
Other	8	8.8	0.03–14.6
Milking parlor			
Fish bone	27	60.0	45.7–74.3
Parallel	10	22.2	10.1–34.4
Tandem	6	13.3	3.4–23.3
Rotatory	2	4.4	0–10.5
Milking			
2 times/d	61	67.0	57.4–76.6
3 times/d	2	3.0	0–5.2
Free access[1]	28	9.5	21.3–40.3
Predipping			
Yes	75	82.4	74.6–90.2
No	16	17.6	9.8–25.4
Use of I-based products in predipping			
Yes	4	5.5	0.3–10.7
No	69	94.5	89.3–99.7
Postdipping			
Yes	84	92.3	86.8–97.8
No	7	7.7	2.2–13.2
Use of I-based products in postdipping			
Yes	28	34.1	23.9–44.4
No	54	65.9	55.6–76.1
Udder cleaning			
Disposable paper	56	88.9	81.1–96.6
Cloth towels	5	7.9	1.3–14.6
Disposable paper and cloth towels	1	1.6	0–4.7
Sponge	1	1.6	0–4.7
Forestripping[2]			
Yes	58	92.1	85.4–98.7
No	5	7.9	1.3–14.6

[1]Under automatic milking system.

[2]Forestripping is not considered in the automatic milking system farms, thus this parameter was only available for farms classified for the milking system as milking parlor (n = 45), pipeline milking system (n = 10), and other (n = 8).

Table 2. Feeds included in the diets, and their amount of inclusion (kg of DM)

Ingredient[1]	n	Mean	SD	Minimum	Maximum
Straw	28	1.03	0.32	0.18	1.76
Mixed hay	56	3.58	2.24	0.44	10.44
Wheat hay	1	3.92	0.00	3.92	3.92
Mixed graminaceous silage	10	2.39	1.06	0.80	4.00
Pea forage hay	2	2.61	1.23	1.74	3.48
Ryegrass haylage	6	2.52	2.31	1.26	7.20
Wheat haylage	12	2.29	1.13	0.16	4.48
Sorghum silage	12	5.40	2.16	0.79	7.04
Oat haylage	1	1.31	0.00	1.31	1.31
Barley silage	2	3.45	1.91	2.10	4.80
Alfalfa haylage	18	1.56	0.91	0.24	3.36
Alfalfa hay	53	3.15	1.42	0.79	7.04
Corn silage	62	6.22	1.59	1.92	9.92
Corn earlage	24	3.99	1.25	1.97	8.41
High moisture ear corn	4	3.71	1.02	2.72	4.96
Whole soybean meal[2]	3	1.33	0.17	0.90	1.86
Soybean meal[2]	44	2.34	0.76	0.89	4.72
Sunflower meal	3	1.52	0.14	1.37	1.64
Rapeseed meal[2]	4	1.60	0.84	0.91	2.82
Barley meal	5	2.49	1.53	1.32	5.46
Corn meal	60	3.39	1.65	0.44	8.36
Corn flakes	8	2.05	1.42	0.71	5.34
Wheat meal	2	2.58	0.00	0.71	4.45
Lineseeds[2]	18	0.27	0.16	0.02	0.74
Fat	16	0.20	0.10	0.01	0.40
Cottonseeds	4	1.27	0.23	0.92	1.38
Commercial feedstuff[2]	80	3.54	2.00	0.26	10.01
Cane molasses	22	0.69	0.24	0.29	1.17
Energetic liquid supplement	2	0.27	0.00	0.24	0.29
Corn distillers	3	1.61	0.36	1.18	1.82
Wheat distillers	1	2.73	0.00	2.73	2.73
Wheat bran	10	2.24	2.07	0.35	6.96
Beet pulp[2]	7	1.85	1.42	0.45	4.86
Brewers' grains	1	0.18	0.00	0.18	0.18
Sodium bicarbonate	9	0.16	0.12	0.02	0.40
Calcium bicarbonate	4	0.24	0.03	0.20	0.25
Mineral-vitamin premix[3]	23	0.50	0.36	0.05	2.97

[1]Water was included in 13 diet formulas at an average amount of 5.97 ± 4.44 kg. Only ingredients included in more than one farm were listed here.

[2]Ingredients described as goitrogens in the literature (Borucki Castro et al., 2011; Erickson and Kalscheur, 2020; Niero et al., 2023).

[3]Because this field study included a number of different farms that used around 70 different mineral-vitamin premixes, a detailed breakdown of the premix composition is not given here.

(Foss Electric A/S, Hillerød, Denmark). The equations used to predict TMR composition were developed in house and validated with 240 samples through a 15-fold cross-validation. Coefficients of determination of cross-validation (SE) were 0.58 (0.94), 0.63 (0.81), 0.87 (1.92), 0.35 (0.81), 0.85 (0.54), and 0.80 (2.04) for DM, CP, NDF assayed with a heat-stable amylase and expressed with residual ash included (**aNDF**), lignin determined by solubilization of cellulose with sulfuric acid and expressed without residual ash (**lignin(sa)**), and starch, respectively. The second aliquot was sent to a commercial laboratory (Eurolab, Bassano; Italy) for I content determination after being preprocessed as described below following the procedure described by Niero et al. (2019).

Milk Sampling and Chemical Analysis

In each farm, 2 aliquots of bulk milk (50 mL each) were collected in plastic tubes. In one aliquot, 200 μL of preservative (bronopol, 2-bromo-2-nitropropan-1,3-diol; D&F Inc., Dublin, CA) were added to avoid bacterial growth and proliferation and was immediately sent to the milk laboratory of Veneto Breeders' Association (Vicenza, Italy) for the determination of gross composition (fat, protein, casein, and lactose percentages), MUN (mg/dL), pH, and SCC via the Combifoss benchtop instrument (FOSS Electric A/S, Hillerød, Denmark). The SCC values (cells/μL) were transformed into SCS through the formula $SCS = 3 + \log_2(SCC/100)$, where SCC was expressed as cells/μL (Wiggans and Shook,

1987). The second aliquot, without preservative, underwent a preliminary pretreatment and was then used to determine the I content at Eurolab (Bassano, Italy). In this case, samples were stored at −20°C until analysis performed within 6 h after collection following the same procedure previously described for feed I content determination.

For I determination, milk samples were homogenized by warmed to temperate for 1 h, gently inverted 20 times, and subsequently diluted (1:24) in 0.6% ammonia solution in disposable 50-mL plastic tubes (Niero et al., 2019). The mixture was incubated in a water bath at 90°C for 1 h to promote I extraction. After cooling at room temperature, samples were filtered using a 0.45-µm syringe filter. Finally, 5 mL of the filtered solution was diluted (1:1) in 0.6% ammonia, to a final volume of 10 mL. The resulting solution was 50-fold diluted compared with the starting milk to keep expected sample salinity below 0.2%, as recommended for ICP-MS trials (Beauchemin, 1999). The ration underwent the same extraction technique, with the only variation being the initial homogenization of the material. Subsequently, it was dried at 65°C for 96 h, with regular turning every 24 h. Finally, it was finely ground using a cutting mill (Retsch, Germany) and passed through a sieve to achieve a final product with a fineness of 0.5 mm. Quantification of the extracted I (µg/L) was achieved by optical emission spectrometry as reported in detail by Niero et al. (2019). Null values and those below the limit of detection were removed. Results were then re-calculated for the dilution used.

Statistical Analysis

Before starting the sampling, a power analysis was conducted to determine the number of samples needed using the G*Power software ver. 3.1.9.6 (Heinrich-Heine-Universität Düsseldorf, Germany; Faul et al., 2007, 2009). The sample size calculated was based on F test, ANOVA, numerator degrees of freedom for herd composition variable (1), Cohen's medium effect size (0.30), power analysis (0.80), and α-error probability (0.05).

The SAS software v. 9.4. (SAS Institute Inc., Cary, NC) was used for the data preparation, editing, and analysis. Descriptive characteristics of the farms are shown as median with its 95% confidence interval ($\mathbf{CI_{95}}$) calculated using the UNIVARIATE procedure for quantitative variables, and relative frequency with CI_{95} calculated as $p \pm z \times \sqrt{\frac{p(1-p)}{n}}$, where p is the proportion, z is z-score 1.96 for the CI_{95}, and n is the total number of responses.

Inconsistent information on SCC, and values outside the range mean ± 3 standard deviations were treated as missing values. In addition, normality was assessed with the UNIVARIATE procedure. The phenotypic variability of the traits was determined by the coefficient of variation (**CV**, %) calculated as the ratio of the standard deviation to the raw mean. Pearson correlations among traits were calculated using the CORR procedure.

After testing several models, sources of variation were investigated using the GLM procedure, according to the following linear model for milk I ($\mathbf{IOD_M}$):

$$\begin{aligned} y_{ijklmnopqrs} &= \beta(IOD_{Ri}) + feeding_j + milking_k \\ &+ herdcompo_l + farmsys_m + predip_n + postdip_o \\ &+ herdsize_p + month_q + herdlact_r + \varepsilon_{ijklmnopqrs}, \end{aligned}$$

where $y_{ijklmnopqrs}$ is the vector of the phenotypic observation of IOD_M; IOD_{Ri} is the fixed effect of the ration I content included as a covariate with the coefficient of regression β; $feeding_j$ is the fixed effect of the jth feeding (j = TMR or hay plus concentrate); $milking_k$ is the fixed effect of the kth type of milking system (k = milking parlor, AMS, pipeline milking system, other); $herdcompo_l$ is the fixed effect of the lth herd composition (l = single breed or multibreed); $farmsys_m$ is the fixed effect of the mth system farming (m = conventional or organic); $predip_n$ is the fixed effect of the nth predipping teat I-based disinfection (n = yes or no); $postdip_o$ is the fixed effect of the oth postdipping teat I-based disinfection (o = yes or no); $herdsize_p$ is the fixed effect of the pth herd size (P = class 1, cows <55; class 2, 55 ≤ cows <90; class 3, ≥ 90 cows); $month_q$ is the fixed effect of the qth month (q = October, December, March, May, or June); $herdlact_r$ is the fixed effect of the rth herd average DIM represented by the average DIM of the herd (r = class 1, <170 DIM; class 2, 170 ≤ DIM < 195; class 3, ≥195 DIM); and $\varepsilon_{ijklmnoprs}$ is the random residual $\sim N\left(0;\delta_e^2\right)$, where δ_e^2 is the residual variance.

To evaluate the factors affecting variability of fat, protein, casein, and lactose content, SCS, MUN, and pH, the same model was applied without including the effect of the feeding iodine ($\mathbf{IOD_R}$) and the application of pre- and postdipping. Finally, least squares means (**LSM**) multiple comparisons were adjusted with Tukey-Kramer. Overall, the significance reported in the present study was established at $P < 0.05$, unless otherwise indicated.

RESULTS

Farm Descriptions

Farms included in the study had between 6 and 356 lactating cows, ranging from an average of 85 to 360

DIM, and declared an incidence of up to 12 mastitis/mo (median, 1; minimum, 0). As reported in Table 1, farms were mostly under conventional farming systems, had single-breed herds (60 vs. 31), and raised Holstein-Friesian cows.

Cows were housed mainly in freestall with straw as bedding material. Around 90% of the farms—particularly those that implemented AMS or had a milking parlor—fed TMR to their animals. Half of the farms used a milking parlor (mainly fishbone design), and almost one-third of the farms had AMS implemented. Most farms cleaned the udder with disposable paper and applied forestripping. Most of the not-AMS farms milked twice a day, whereas AMS farms offered the cows permanent free access to the milking apparatus. Most of the farms included in the study applied predipping and postdipping using non-I-based products. The main forage sources included in the diets analyzed were corn silage (n = 62), and alfalfa hay (n = 53), additionally mixed hay was included in more than a half of the diets (Table 2). Soybean and corn meal were the most used concentrates (n, 47 and 60, respectively), together with commercial feedstuffs (n = 80; Table 2). Rapeseed meal was only included in a few diets (n = 4) with a maximum inclusion of 2.85 kg/DM. The IOD_R content expressed as mg/kg of DM never exceeded 2.78 and was on average 0.62 mg/kg of DM (Table 2).

Descriptive Statistics and Correlations

The descriptive statistics of the complete data set is reported in Table 3. A total of 19 farms showed a SCC >400,000 cells and most of them were farms using AMS or the milking parlor. In particular, this high level of SCC was found in 7 out of 28 (25%) farms for AMS, and 6 out of 45 (13%) for the milking parlor. The 16.4% and 12.1% of milk and diet samples, respectively, had an I content below the limit of detection. Moreover, IOD_M and IOD_R revealed the greatest CV (~87% and ~94%, respectively). Among milk composition traits, fat showed the greatest CV (~15%) and lactose the lowest one (~2%). The SCS and MUN presented a CV of ~19% and ~23%, respectively. The lowest CV was observed for pH (~1%). Among feed chemical composition, DM variability was quite low (~3%) and the greatest CV was observed for ash content. The CV for CP, aNDF, and lignin(sa) ranged from 11% to 17%.

Out of the 91 farms included in the study, 77 had information on IOD_M, because either the aliquot for I analysis was not available or the concentration of the mineral itself was below the limit of detection. For the same reasons, only 78 farms had the IOD_R available. After outliers removal (2 for IOD_R and 3 for n IOD_M), we ended up with 65 farms with both IOD_M and IOD_R. A positive linear correlation was observed between IOD_M and IOD_R content ($P < 0.001$; Figure 1). Although IOD_M was also significantly correlated with lactose content, whereas IOD_R was not correlated with any other milk traits (Table 4). Fat was negatively correlated with lactose, and positively correlated with SCS (Table 4). Protein was positively correlated with casein, and lactose was negatively correlated with SCS (Table 4).

Table 3. Descriptive statistics of milk and feed traits after editing

Trait[1]	n	Mean	SD	Minimum	Maximum
Milk					
IOD_M, µg/L	74	245.07	215	50	1,000
Fat, %	85	3.99	0.60	2.16	6.02
Protein, %	87	3.33	0.14	2.93	3.64
Casein, %	88	2.62	0.13	2.31	3.01
Lactose, %	85	4.77	0.09	4.44	4.89
SCC ($\times 10^3$/mL)	88	309	190	25.00	1,005
SCS	88	4.39	0.87	1.00	6.33
MUN, mg/dL	87	23.31	5.43	7.70	34.60
pH	87	6.60	0.04	6.49	6.71
Feed					
IOD_R, mg/kg DM	78	0.62	0.59	0.11	2.78
DM, % of predried	85	93.12	2.43	87.49	100
CP, % DM	84	14.58	2.13	9.83	19.53
aNDF, % DM	84	45.00	5.00	30.19	55.37
Lignin(sa), % DM	84	5.26	0.87	3.16	7.08
Starch, % DM	84	20.72	5.11	7.59	35.14
Ash, % DM	85	5.48	1.56	0.96	8.62

[1]IOD_M = milk iodine; IOD_R = feeding iodine; aNDF = neutral detergent fiber assayed with a heat-stable amylase and expressed inclusive of residual ash; lignin(sa) = lignin determined by solubilization of cellulose with sulfuric acid.

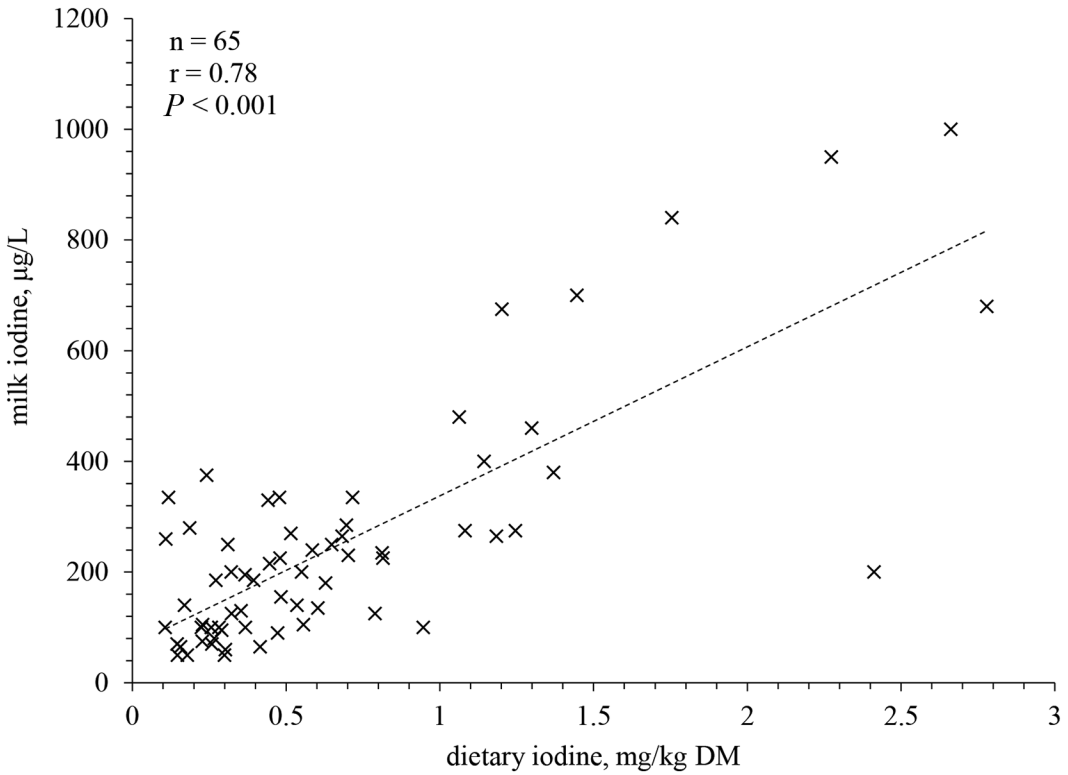

Figure 1. Plot of milk I versus dietary I. Pearson correlation coefficient (r) and its significance (P) are reported, along with the number of samples (n) available.

Sources of Variation of Milk I and Composition

Results for IOD_M are displayed in Table 5. The model presented a coefficient of determination of 0.75, with the following significant fixed effects: IOD_R ($P < 0.001$), herd composition ($P = 0.044$), and I-based disinfectant predipping ($P = 0.014$). When increasing or decreasing IOD_R by 1 mg/kg DM, IOD_M increased or decreased by 228 µg/L. Multibreed herds showed greater IOD_M than single-breed herds (250 vs. 129 µg/L, respectively). Farms using I-based disinfectant in predipping achieved greater IOD_M concentration than those applying other predipping treatments (365 vs. 13 µg/L, respectively), however the groups were hardly imbalanced for this variable (Table 1). In contrast, milking system ($P = 0.948$), feed ($P = 0.880$), farming system ($P = 0.163$), I-based disinfectant postdipping ($P = 0.825$), month ($P = 0.190$), herd size ($P = 0.938$), and herd average DIM ($P = 0.842$) were far from the significance threshold and apparently did not influence the bulk milk IOD_M.

The significance of the fixed effects for milk composition, SCS, and pH are reported in Table 6, whereas LSM are shown in Table 7. Farming system significantly affected protein and SCS, with a greater protein content and a lower SCS in conventional than

Table 4. Pearson correlation among traits[1]

Item	IOD_M, µg/L	IOD_R	Fat	Protein	Casein	Lactose	SCS	MUN
Fat, %	0.116	0.136						
Protein, %	0.124	0.032	−0.012					
Casein, %	0.158	0.080	0.103	0.958***				
Lactose, %	0.247*	0.140	−0.332**	0.160	0.046			
SCS	−0.124	−0.135	0.375***	−0.222*	−0.173	−0.468***		
MUN, mg/dL	−0.123	−0.003	0.178	−0.011	−0.060	−0.048	0.140	
pH	−0.010	0.013	−0.121	0.040	−0.042	0.197	−0.182	0.050

[1]IOD_R = feeding iodine; IOD_M = milk iodine.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 5. The LSM (±SE) of milk I for each fixed effect

Fixed effect	n	Milk I, µg/L
Farming system		
Conventional	70	419.47 ± 120.06
Organic	4	0.00 ± 240.79
Herd composition		
Single breed	50	128.60 ± 110.16[b]
Multibreed	24	249.87 ± 100.47[a]
Feed		
TMR	65	207.51 ± 142.11
Hay and concentrate fed separately	9	170.96 ± 171.46
Milking system		
Milking parlor	32	198.13 ± 129.13
Automatic milking system	26	194.28 ± 144.82
Pipeline	9	231.19 ± 145.80
Other	7	133.34 ± 126.13
Use of I-based products in predipping		
Yes	3	365.27 ± 155.62[a]
No	56	13.20 ± 127.45[b]
Use of I-based products in postdipping		
Yes	23	194.96 ± 109.54
No	43	183.52 ± 99.46
Herd size		
<55 cows	27	178.66 ± 103.99
55 ≤ cows <90	20	202.93 ± 112.13
≥90 cows	27	186.12 ± 107.87
Month		
March	14	126.97 ± 141.51
May	19	273.98 ± 148.21
June	12	91.70 ± 141.43
October	6	210.70 ± 113.99
December	23	242.83 ± 125.87
Herd average DIM		
<170 DIM	25	169.22 ± 114.89
170 ≤ DIM <195	23	189.01 ± 106.87
≥195 DIM	26	209.22 ± 102.54

[a,b]Estimates within a fixed effect with different superscript letters are significantly different ($P < 0.05$).

organic farms. Milking system significantly affected lactose content and SCS. Lactose content was lower ($P = 0.029$) using a pipeline than "other" milking system (not including milking parlor and AMS), whereas SCS was greater ($P = 0.041$) when using a pipeline than when using a milking parlor. Values for the AMS did not differ from those obtained with the milking parlor. A monthly effect was only significant for MUN, with greater values in May than December ($P = 0.040$). Herd composition, feeding (TMR or hay and concentrate fed separately), herd size, and herd average DIM did not modify neither gross composition, SCS, MUN, nor pH.

DISCUSSION

Data Variability

The diversity of farms and herd management practices included in the present study was properly maximized to achieve a great variability in the analyzed traits, including IOD_M. Many feed ingredients contain

Table 6. Significance (P-value) of the fixed effects on milk composition,[1] SCS, and pH

Fixed effect	Fat	Protein	Casein	Lactose	SCS	MUN	pH
Farming system	0.115	0.019	0.150	0.319	0.033	0.268	0.752
Herd composition	0.211	0.263	0.144	0.523	0.878	0.919	0.461
Feeding	0.578	0.997	0.966	0.077	0.334	0.409	0.793
Milking system	0.777	0.307	0.163	0.049	0.034	0.880	0.324
Herd size	0.744	0.575	0.755	0.294	0.649	0.501	0.050
Month	0.411	0.427	0.182	0.573	0.290	0.041	0.441
Herd average DIM	0.770	0.065	0.092	0.265	0.508	0.789	0.188

[1]Milk I is not included in this table as the model was slightly different for this trait.

Table 7. Estimates for milk composition,[1] SCS, and pH

	Fat, %		Protein, %		Casein, %		Lactose, %		SCS		MUN, mg/dL		pH	
Fixed effect	n	LSM ± SE	n	LSM ± SE	n	LSM ± SE	n	LSM ± SE	n	LSM ± SE	n	LSM ± SE	n	LSM ± SE
Farming system														
Conventional	76	3.97 ± 0.14	81	3.32 ± 0.03^{a}	83	2.61 ± 0.03	6	4.73 ± 0.02	81	4.29 ± 0.18^{b}	80	22.30 ± 1.15	80	6.61 ± 0.01
Organic	9	4.54 ± 0.31	6	3.13 ± 0.07^{b}	8	2.51 ± 0.06	79	4.69 ± 0.04	7	5.33 ± 0.42^{a}	7	25.66 ± 2.63	7	6.61 ± 0.02
Herd composition														
Single-breed	30	4.35 ± 0.17	30	3.25 ± 0.04	30	2.58 ± 0.03	30	4.72 ± 0.02	30	4.83 ± 0.23	30	23.91 ± 1.48	30	6.61 ± 0.01
Multibreed	55	4.16 ± 0.18	57	3.21 ± 0.04	58	2.54 ± 0.04	55	4.70 ± 0.02	58	4.80 ± 0.24	57	24.05 ± 1.54	57	6.61 ± 0.01
Feed														
TMR	76	4.16 ± 0.19	78	3.23 ± 0.04	79	2.56 ± 0.04	77	4.75 ± 0.02	79	5.03 ± 0.25	78	25.13 ± 1.55	9	6.61 ± 0.01
Hay and concentrate fed separately	9	4.35 ± 0.27	9	3.23 ± 0.06	9	2.56 ± 0.05	8	4.67 ± 0.04	9	4.60 ± 0.36	9	22.83 ± 2.26	78	6.61 ± 0.02
Milking system														
Milking parlor	43	4.18 ± 0.23	43	3.29 ± 0.05	44	2.63 ± 0.04	43	4.70 ± 0.03^{ab}	44	4.40 ± 0.03^{b}	43	24.12 ± 1.90	44	6.60 ± 0.01
AMS[2]	25	4.42 ± 0.26	26	3.24 ± 0.06	26	2.58 ± 0.05	25	4.71 ± 0.04^{ab}	26	4.87 ± 0.35^{ab}	26	23.07 ± 2.19	25	6.63 ± 0.02
Pipeline	9	4.17 ± 0.28	10	3.22 ± 0.06	10	2.54 ± 0.05	10	4.65 ± 0.04^{b}	10	5.38 ± 0.36^{a}	10	25.15 ± 2.28	10	6.61 ± 0.02
Other	8	4.27 ± 0.24	8	3.17 ± 0.05	8	2.49 ± 0.05	7	4.78 ± 0.03^{a}	8	4.60 ± 0.32^{ab}	8	23.58 ± 2.03	8	6.61 ± 0.02
Herd size														
<55 cows	28	4.22 ± 0.18	29	3.20 ± 0.04	30	2.54 ± 0.03	28	4.70 ± 0.02	30	4.77 ± 0.24	30	23.37 ± 1.49	30	6.59 ± 0.01
55 ≤ cows < 90	27	4.34 ± 0.19	27	3.24 ± 0.04	27	2.56 ± 0.04	27	4.73 ± 0.03	27	4.72 ± 0.26	27	23.52 ± 1.63	26	6.62 ± 0.01
≥90 cows	30	4.21 ± 0.21	31	3.24 ± 0.05	31	2.57 ± 0.04	30	4.70 ± 0.03	31	4.94 ± 0.28	30	25.04 ± 1.75	31	6.62 ± 0.01
Month														
March	18	4.36 ± 0.23	19	3.24 ± 0.05	19	2.56 ± 0.04	18	4.71 ± 0.03	19	5.07 ± 0.30	19	22.92 ± 1.88^{ab}	19	6.60 ± 0.01
May	20	4.05 ± 0.27	21	3.20 ± 0.06	21	2.50 ± 0.05	20	4.72 ± 0.04	21	4.74 ± 0.36	21	27.98 ± 2.25^{a}	21	6.60 ± 0.02
June	13	4.52 ± 0.26	13	3.20 ± 0.06	13	2.51 ± 0.05	13	4.70 ± 0.03	13	5.07 ± 0.34	12	26.29 ± 2.22^{ab}	12	6.62 ± 0.02
October	8	4.01 ± 0.25	7	3.32 ± 0.05	8	2.67 ± 0.05	7	4.69 ± 0.03	8	4.64 ± 0.33	8	21.54 ± 2.07^{ab}	8	6.62 ± 0.02
December	26	4.36 ± 0.24	27	3.19 ± 0.05	27	2.54 ± 0.05	27	4.74 ± 0.03	27	4.55 ± 0.32	27	21.16 ± 2.03^{b}	27	6.61 ± 0.02
Herd average DIM														
<170 DIM	30	4.25 ± 0.20	31	3.28 ± 0.04	31	2.60 ± 0.04	30	4.73 ± 0.03	31	4.66 ± 0.26	30	23.47 ± 1.68	31	6.62 ± 0.01
170 ≤ DIM < 195	27	4.19 ± 0.21	28	3.19 ± 0.05	28	2.53 ± 0.04	28	4.70 ± 0.03	28	4.83 ± 0.28	28	24.50 ± 1.77	27	6.60 ± 0.01
≥195 DIM	28	4.33 ± 0.17	28	3.22 ± 0.04	29	2.55 ± 0.03	27	4.69 ± 0.03	29	4.95 ± 0.23	29	23.97 ± 1.43	29	6.61 ± 0.01

[a,b]Estimates with different superscript letters within a fixed effect are significantly different ($P < 0.05$).

[1]Milk I is not included in this table as the model was slightly different for this trait.

[2]AMS = automatic milking system.

goitrogenic substances (Borucki Castro et al., 2011; Erickson and Kalscheur, 2020; Niero et al., 2023). Examples are plants of the cruciferous family (rape, canola, and kale), but also soybean, beet pulp, millet, linseed, white clover, and sweet potato. Among the potentially goitrogenic dietary ingredients found in the present study, canola, soybean and beet pulp represent the main examples. Plant varieties and technological processing (e.g., extrusion) can reduce their goitrogenic action (Niero et al., 2023) creating a huge variability within the same ingredient. In contrast, given the wide presence of commercial feedstuffs (employed in 88% of the farm diets) containing canola meal, soybean products, beet pulp, linseeds, and other goitrogens in their formula, an accurate evaluation of the amount of goitrogenic compounds in the considered diets was not feasible. The mean IOD_R content was capable to satisfy the nutritional requirements of lactating dairy cows which is ~0.42 mg/kg of DM (NASEM, 2021) and exceeded the precautionary additional levels (+100 μg of I/kg DM) reported by Borucki Castro et al. (2011) to compensate for the possible effects of goitrogens (Table 2). However, recently NASEM (2021) guidelines suggested to increase the supply up to 1.02 mg/kg DM if goitrogenic compounds. Some of the diets included in the present study showed greater values until a maximum value of 2.78 mg/kg of DM, which is below the upper tolerable limit for I (5 mg/kg DM; European Union, 2005).

Results obtained for milk composition and SCS were overall in line with Benedet et al. (2018), who evaluated bulk milk quality across Italy. Milk quality traits average and variability also agreed with previous studies using individual milk records in multibreed and single-breed herds including Holstein-Friesian in northern Italy (Gottardo et al., 2017; Visentin et al., 2018; Manuelian et al., 2019). However, those studies reported a lower SCS average with a greater variability compared with the present one. Therefore, milk samples were representative for the Italian market.

Correlations

The linear dose-response relationship between IOD_M and IOD_R content coupled with the GLM results suggested that IOD_M mainly depends on the animal's feed as reported by previous studies (Flachowsky et al., 2014; Niero et al., 2023). Flachowsky et al. (2014) performed a meta-analysis including 7 studies from 2009 to 2012 and reported a correlation coefficient of 0.71, which is similar to the one observed in the present study, while the meta-analysis by Niero et al. (2023), which included 8 studies from 2010 to 2021, reported a weaker correlation ($r = 0.56$). Rezaei Ahvanooei et al. (2021) demonstrated that I in milk, urine, and serum increased when the cows' diet was supplemented with I. The significant correlation observed between IOD_M and lactose, as well as the lack of correlation between IOD_M and other milk composition traits, pH, and SCS was previously reported by Niero et al. (2020) in Holstein-Friesian milk samples. Additionally, Denholm et al. (2022) did not find any significant correlation between IOD_M and fat and protein yields (kg).

The correlations observed between protein and casein, and between fat and SCS agreed with Visentin et al. (2018) results in individual milk samples. The lactose correlation observed with fat and SCS is in line with Costa et al. (2019), who evaluated Holstein-Friesian herds in northern Italy. Costa et al. (2019) argued that the negative correlation between lactose and SCS makes lactose an informative trait for mastitis diagnosis. The decrease in lactose content during mastitis is due to lactose acting as a substrate for the pathogens, as well as to the compromised secretory cells which have been damaged by the inflammation and infection and the disruption of the tight junctions and basal membrane permeability (Costa et al., 2019).

Fixed Effects Affecting Milk I Content

The observed variability of IOD_R and IOD_M suggests that the content of I can widely differ across farms, opening the discussion on the possibility to produce milk naturally rich in I. The broadness of IOD_R used in dairy cattle feeding was previously highlighted by Coneyworth et al. (2020) when sampling 98 herds across 6 regions in the UK. Moreover, Coneyworth et al. (2020) reported a similar IOD_M range, and Borucki Castro et al. (2012) obtained similar values during the pre-experimental and experimental period where an I sanitizer was applied. Therefore, Borucki Castro et al. (2012) results suggested that feed was a major contributor to IOD_M. In addition, the amount of IOD_M obtained in the present study agreed with those obtained applying an incomplete cleaning (IOD_M, 252 μg/kg) or using a 1% I solution (IOD_M, 218 μg/kg) in premilking (Borucki Castro et al., 2012). In contrast, Denholm et al. (2022) reported a greater IOD_M average (1,448 μg/L) and a lower variability (CV, 42%) when sampling 479 Holstein-Friesian cows representative of the UK farming systems. Niero et al. (2020) and Schöne et al. (2017) also observed a lower IOD_M content (156 μg/kg and 105 μg/kg, respectively), despite IOD_R being similar in Schöne et al. (2017). Although an earlier study observed a breed effect on IOD_M, researchers have not been able to confirm this finding, and contradictory results on the effect of stage of lactation on IOD_M have been reported (Flachowsky et al., 2014). In the present study, IOD_M

of multibreed farms was double compared with single-breed farms, which were mainly Holstein-Friesian. As already hypotheses by van der Reijden et al. (2018), our finding could be justify by a dilution effect which occurs in high-producing dairy cows. Moreover, Costa et al. (2021) confirmed that IOD_M was mainly affected by cows' extrinsic factors.

Fixed Effects Affecting Milk Quality

The evaluated milking systems barely affected milk quality traits, in agreement with previous studies conducted in northern Italy on individual Holstein-Friesian cow milk samples where only a significantly lower pH was observed (De Marchi et al., 2017). Moreover, Tse et al. (2018) indicated that the majority of producers did not find changes in milk fat and protein content once transitioned to AMS, independently on how long they have implemented the AMS. In contrast, Toušová et al. (2014) reported an increase in milk fat, protein content, and MUN in Czech Fleckvieh cows with the AMS and higher SCC in the milking parlor. In agreement with our results, Toušová et al. (2014) did not find differences in lactose content between AMS and milking parlor. Moreover, Innocente and Biasutti (2013) did not find differences between AMS and milking parlor in fat, protein, and lactose content in milk intended to produce Montasio cheese.

The present study identified some samples with a SCC above the limit established by the European Union (European Union, 2004) when using AMS or the milking parlor. However, only one bulk milk sample per farm was evaluated in the present study and not the geometrical average over a 3-mo period as specified by the regulation (European Union, 2004). Nevertheless, these results are in line with several studies that reported a temporary increase after the introduction of the AMS in milk SCC (Hogenboom et al., 2019), which is an indicator of udder inflammation and has been related to a worsening of milk quality. Another explanation could be that an adequate premilking teat hygiene is not always achieved with the AMS due to a lack of visual control of the milker which is the one responsible to set and adjust the cleaning procedure (Hogenboom et al., 2019). This last hypothesis could also support the observed effect of I premilking disinfectant on IOD_M.

Although no effect of herd composition on milk parameters other than IOD_M was observed in the current study, studies in northern Italy have shown differences between Holstein-Friesian and other breeds such as Brown Swiss, Alpine Gray, Simmental, and Jersey in multibreed (Manuelian et al., 2018) and single-breed (Visentin et al., 2018) herds. The lack of differences in the present study could be explained by the prevalent presence of Holstein-Friesian (88% of single-breed herds; 66% of multibreed herds). Nowadays, the TMR feeding strategy that provides dairy cows balanced nutrients over time has been implemented in most farms to optimize milk productivity and reduce single feeds animals' selection.

The results suggest that the type of teat disinfectant applied affects IOD_M as observed in previous studies (Miller and Lansing, 1991; Van Der Reijden et al., 2018; Niero et al., 2023). However, the results related to pre- and postdipping effects on IOD_M of the present study disagreed with Rezaei Ahvanooei et al. (2021) who reported a greater I in milk, urine, and serum when applying postmilking compared with premilking or no teat dipping at all. Flachowsky et al. (2014) argued that while the skin of the teat is cleaned before milking, the mammary gland is not routinely washed leaving more time for I to be absorbed. Thus, these authors indicated that the effect of I teat dipping on IOD_M is more likely due to the absorption that occurs after its application rather than a direct contamination of milk from teat skin's surface (Flachowsky et al., 2014). However, direct contamination of milk from teat skin's surface due to an incomplete cleaning could not be excluded in the current study as postdipping was not significantly affecting IOD_M.

CONCLUSIONS

The present study demonstrated that presence of AMS did not impair milk composition, including I concentration and SCS, compared with milking parlor. The results also confirm that I feeding level is the main factor affecting the concentration of this mineral in milk. In addition, farms applying I-based teat sanitizers seems to affect milk I content if used for the predipping, however further studies are needed to confirm this result. Overall, our findings suggest that the level of this mineral in cow milk can be naturally improved in dairy farms by mean of dedicated ration formulation.

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