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Short communication: Iodine content in bovine milk is lowly heritable and shows limited genetic variation

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ABSTRACT

Milk and dairy products are considered important sources of iodine in several countries. Despite this, there is a paucity of studies that have investigated sources of variation of milk iodine, especially on a large scale. So far, it is not clear if milk iodine content could be increased through breeding in dairy cattle. Recently, a mid-infrared spectroscopy prediction model has been developed for an indirect quantification of iodine content in cow milk, as it is a faster and less expensive method that allows the prediction at population level. The model has coefficient of determination and ratio of performance to deviation in external validation of 0.57 and 1.44, respectively, and it was used in the present study to predict the iodine content from historical milk spectral data to investigate phenotypic and genetic aspects in the Italian Holstein cattle. Based on the accuracy of the model, the prediction was interpreted as proxy for the real milk iodine concentration (IOD_P). The data set comprised 33,776 test-day records with IOD_P from 4,072 cows. Data of IOD_P were transformed through natural logarithm to achieve a normal distribution. The effect of parity, lactation stage, and month of sampling were investigated, and genetic parameters were estimated using a test-day repeatability animal model. Milk IOD_P decreased with parities and was the lowest in early lactation. Heritability of IOD_P was low (0.025) and it was positively genetically correlated with milk yield and negatively with fat content. Results suggested that it would be challenging to directly improve this trait through breeding strategies in dairy cattle, because IOD_P is mainly affected by temporary environmental factors and thus, cannot be easily improved through genetics. Although preliminary, findings of this study suggest that it would be more convenient to develop feeding and management strategies to drive milk iodine level than to put efforts and resources into breeding strategies. Further studies should validate IOD_P as an indicator trait of milk iodine content by improving reference data and estimating genetic correlation between predicted and measured values.
Key words: dairy cattle, iodine deficiency, genetic correlation, Holstein

Short Communication

Among essential nutrients in human beings, iodine is responsible for thyroid hormone biosynthesis (Fuge and Johnson, 2015). Due to specific environment and availability of minerals in the earth's crust, people from many countries suffer from iodine deficiency (Fuge and Johnson, 2015; Censi et al., 2020). Indeed, iodine-fortified salt and fortification plans are present worldwide to limit the incidence of iodine deficiency disorders (Rohner et al., 2014). One example is the European project EUthyroid of Horizon 2020, coordinated by the Faculty of Medicine of the University of Greifswald (https://cordis.europa.eu/article/id/239956-first-european-map-on-iodine-deficiency). Recently, an Italian case study has been reported by Censi et al. (2020), who found that dietary habits have changed in the last 20 yr, but iodine sufficiency is not covered.

Milk and dairy products are considered an important source of iodine in several countries (Flachowsky et al., 2014). Some studies have investigated factors affecting milk iodine content and report that feed, supplements, and udder sanitizers influence the iodine measured through inductively coupled plasma mass spectrometry (van der Reijden et al., 2018). As for other minerals, the quantification of iodine on a large scale is quite expensive (around $18/sample) and time-consuming (Niero et al., 2019); for this reason, large-scale phenotypic and genetic studies are difficult to perform and, to the best of our knowledge, are not already available. Recently, a mid-infrared spectroscopy prediction model has been developed for an indirect quantification of iodine in milk, as it is a less consuming method in terms of costs and time of analysis (De Marchi et al., 2014). Briefly,

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iodine (μg/kg) was measured with the gold standard inductively coupled plasma mass spectrometry in milk of 147 Holstein cows farmed in 4 herds, and a partial least squares procedure with backward interval was used to develop the prediction model. The coefficient of determination in external validation was 0.57, and the ratio of performance to deviation in external validation was 1.44 (Niero et al., 2020). In the present study, the iodine content was predicted from stored mid-infrared spectra of individual milks using the calibration model proposed by Niero et al. (2020) to investigate phenotypic and genetic aspects in the Italian Holstein cattle.

The initial data set accounted for 72,582 milk spectra of 9,195 Italian Holstein cows. Spectra were collected in the laboratory of the South Tyrolean Dairy Association (Bolzano, Italy) between January 2011 and December 2017, during the monthly official milk recording scheme performed by the Breeders Association of Bolzano Province (Bolzano, Italy). For each milk spectra, the ID and birth date of cow, test-day milk yield (kg/d), DIM, parity, and herd were available. Content of fat, CP, casein, and lactose were assessed using MilkoScan FT6000 (Foss, Hillerød, Denmark) until March 2017 and MilkoScan FT7 (Foss) afterward. The principal component analysis on spectra was used to test if significant differences between the 2 instruments were present, and standard samples were routinely used to ensure the comparability of spectra, following manufacturers' instructions. Milk SCC (cells/μL) was determined through Fossomatic 5500 (Foss) and transformed to SCS using the formula of Ali and Shook (1980): SCS = $\log_2$(SCC/100,000) + 3.

According to the most recent literature (Wang and Bovenhuis, 2019; Benedet et al., 2020), models with fitting statistics similar to those reported for the calibration equation used to predict iodine in the current study have been reported for other traits and have been considered accurate enough for genetic purposes on a large scale. Considering the accuracy of the model proposed by Niero et al. (2020) and recent findings by Grelet et al. (In press), the prediction of iodine can be considered as a proxy of milk iodine concentration (**IOD_P**), because it allows discrimination between low and high values but cannot be used for precise quantification and quality control. The Mahalanobis distance between the data point (spectrum) and the centroid of spectra included in the calibration set was used to identify and remove spectral outliers from the data set. All the predicted values of IOD_P were $\log_e$-transformed to reach a normal distribution of the data. The transformed IOD_P values averaged 4.97 (SD = 0.67) and were within the window mean ± 3 standard deviations (**SD**, range from 3.19–6.42).

Spectra of milk test-day records outside the window 6 to 305 DIM and lactations with fewer than 5 test-day records were discarded. Cows with more than 10 lactations and with unknown parents were removed. At least 5 cows had to be sampled in each contemporary group, defined as cows that were sampled in the same herd and test-date (**HTD**). Values of milk yield (kg/d) and composition traits deviating more than 3 SD from the respective mean were treated as missing. Milk SCC was restricted to be between 1 and 10,000 cells/μL. Such restrictions led to a final data set of 33,776 test-days from 4,072 cows in 221 herds and 3,393 HTD. Cows were offspring of 733 sires.

Sources of variation of IOD_P were investigated using the HPMIXED procedure of the SAS software v. 9.4 (SAS Institute Inc., Cary, NC), according to the following linear model:

$$y_{ijklmn} = \mu + Parity_i + DIM_j + Month_k + Year_l + (Parity \times DIM)_{ij} + Herd_m + Cow_n + e_{ijklmn},$$

where y_{ijklmn} is milk IOD_P; μ is the overall intercept of the model; $Parity_i$ is the fixed effect of the ith parity of the cow (i = 1–5, with class 5 including parities 5–10); DIM_j is the fixed effect of the jth class of DIM of the cow (j = 1–30, each class being 10 d wide); $Month_k$ is the fixed effect of the kth month of sampling (k = 1–12); $Year_l$ is the fixed effect of the lth year of sampling (l = 2011–2018); $(Parity \times DIM)_{ij}$ is the fixed interaction effect between parity and DIM class; $Herd_m$ is the random effect of the mth herd $\sim N\left(0, \sigma_h^2\right)$, where σ_h^2 is the herd variance; Cow_n is the random effect of the nth cow $\sim N\left(0, \sigma_{cow}^2\right)$, where σ_{cow}^2 is the cow variance; and e_{ijklmn} is the random residual $\sim N\left(0, \sigma_e^2\right)$, where σ_e^2 is the residual variance. A multiple comparison of least squares means (**LSM**) of IOD_P for the fixed effects was performed using Bonferroni's test ($P < 0.05$).

Variance and covariance components were estimated in ASReml v4.1 (Gilmour et al., 2015) through univariate and bivariate repeatability animal models, respectively. For these analyses, the HTD was used as contemporary group. The general form of the linear model was as follows:

$$y_{ijklm} = \mu + Parity_i + DIM_j + HTD_k + Cow_l + Animal_m + e_{ijklm},$$

where y_{ijklm} is the investigated trait (or traits in bivariate analyses); μ is the overall intercept of the model; $Parity_i$ and DIM_j are defined as in previous phenotypic

Table 1. Descriptive statistics of the proxy for milk iodine content (IOD_P), milk yield, and quality traits

Trait	n	Mean	CV, %	Minimum	Maximum
IOD_P	33,776	5.20	11.53	3.19	6.42
Milk yield, kg/d	33,747	30.05	23.34	8.40	51.80
Milk composition, %					
Fat	33,715	3.87	15.01	2.16	5.74
CP	33,766	3.31	9.62	2.33	4.26
Lactose	33,720	4.79	3.25	4.32	5.26
SCS, units	33,776	2.74	65.54	−3.64	9.62

model; HTD_k is the fixed effect of the kth contemporary group; Cow_l is the random permanent environmental effect of the lth cow $\sim N\left(0, \mathbf{I}\sigma_w^2\right)$, where $\mathbf{I}$ is an identity matrix of appropriate order and σ_w^2 is the permanent environmental variance; $Animal_m$ is the random additive genetic effect of the mth animal $\sim N\left(0, \mathbf{A}\sigma_a^2\right)$, where $\mathbf{A}$ is the additive genetic relationship matrix of 16,236 individuals and σ_a^2 is the additive genetic variance; and e_{ijklm} is the random residual effect $\sim N\left(0, \mathbf{I}\sigma_e^2\right)$, where $\mathbf{I}$ is an identity matrix of appropriate order. To compute $\mathbf{A}$, 6 generations of ancestors were traced back starting from the cows with phenotypic information.

Phenotypic variance $\left(\sigma_p^2\right)$, heritability (h^2), repeatability (t), phenotypic correlation (r_p), and genetic correlations (r_a) were computed as:

$$\sigma_p^2 = \sigma_w^2 + \sigma_a^2 + \sigma_e^2,$$

$$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_w^2 + \sigma_e^2},$$

$$t = \frac{\sigma_a^2 + \sigma_w^2}{\sigma_a^2 + \sigma_w^2 + \sigma_e^2},$$

$$r_p = \frac{\sigma_{p12}}{\sqrt{\sigma_{p1}^2 \times \sigma_{p2}^2}}, \text{ and}$$

$$r_a = \frac{\sigma_{a12}}{\sqrt{\sigma_{a1}^2 \times \sigma_{a2}^2}},$$

where σ_{p12} and σ_{a12} are the phenotypic and the additive genetic covariances between trait 1 and trait 2, respectively; σ_{p1}^2 and σ_{p2}^2 are the phenotypic variances of traits 1 and 2; and σ_{a1}^2 and σ_{a2}^2 are the additive genetic variances of traits 1 and 2. Finally, Pearson correlations between EBV were calculated only for those animals with accuracy of EBV for IOD_P content ≥0.50 (1,690 individuals) through PROC CORR of SAS software v. 9.4 (SAS Institute Inc.).

Descriptive statistics of the final dataset are presented in Table 1. After transformation, IOD_P was characterized by a normal distribution and the coefficient of variation (**CV**, 11.53%) was intermediate between CV of fat and CP. Average milk yield and quality traits mirrored the official national statistics of Italian Holstein cattle (ANAFIJ, 2019; Costa et al., 2019a).

The LSM of IOD_P for the fixed effect of parity, lactation stage, and month of sampling are depicted in Figure 1. Milk IOD_P tended to decrease with parity, and it increased with DIM being maximum in mid-late lactation. The increase of IOD_P in early lactation stages was somehow expected, and might be due to the different amount of mineral supplement distributed to dry and lactating cows. Indeed, feed supplementation of iodine is lower for dry cows and higher for lactating cows (Weiss et al., 2015). Milk IOD_P was generally greater in winter, and the lowest LSM were estimated in May and June. Similar results were reported in retail milk samples by Stevenson et al. (2018), who hypothesized that the effect of season is related to the lower amount of iodine supplementation in the summer period; the month of sampling had the greatest F-value among all fixed effects and the amount of variance due to herd random effect (12%) was double compared with cow random effect (6%). These findings indicated that climatic conditions, composition of both pasture and feed, and management were likely the most relevant factors affecting the variability of IOD_P. This is in accordance with the nature of the trait; in fact, iodine is an essential mineral absorbed in the gastrointestinal tract of ruminants.

Genetic parameters of IOD_P are reported in Table 2. Overall, IOD_P was the less heritable ($h^2 = 0.025 \pm 0.005$) and repeatable ($t = 0.207 \pm 0.011$) trait, and it exhibited low additive genetic CV (1.72%). In fact, h^2 of other traits ranged from 0.077 ± 0.016 (milk yield) to 0.364 ± 0.029 (CP content), and the t was always

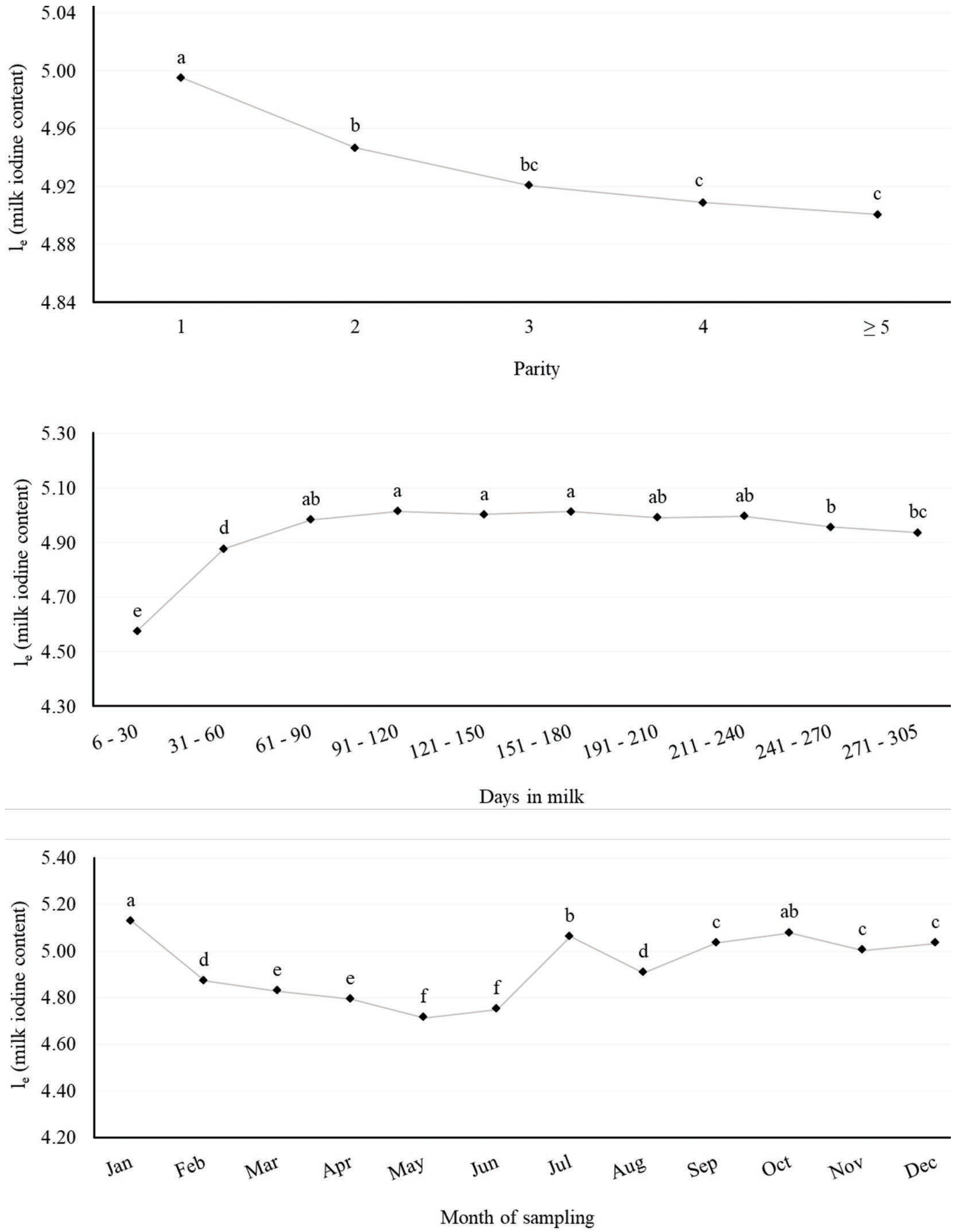

Figure 1. Least squares means of the mid-infrared predicted milk iodine content (natural logarithm, l_e) for the fixed effects of parity (SE < 0.02), lactation stage (SE < 0.02), and month of sampling (SE < 0.02). Means with different letters (a–f) within each effect are significantly different ($P < 0.05$).

greater than 0.50. The low h^2 of IOD_P confirmed the importance of environmental factors rather than genetics for this mineral. The estimate was similar to h^2 of health traits (e.g., mastitis and ketosis) in dairy cattle for which farm management, feeding, and farming conditions are the most relevant sources of variation (Egger-Danner et al., 2015; Costa et al., 2019b). Phenotypically, IOD_P was negatively correlated with fat content, whereas r_p with other traits were very weak and not different from zero. The negative association with fat content was confirmed also genetically (Table 2). The r_a between IOD_P and milk yield was positive

Table 2. Genetic parameters of the proxy for milk iodine content (IOD_P) and its associations with yield and quality traits

Item	Estimate	SE
Additive genetic variance	0.008	0.002
Residual variance	0.258	0.002
Heritability	0.025	0.005
Repeatability	0.207	0.011
Phenotypic correlations		
Milk yield, kg/d	0.012	0.015
Fat content, %	−0.240	0.010
CP content, %	−0.022	0.011
Lactose content, %	0.010	0.010
SCS, units	0.004	0.011
Genetic correlations		
Milk yield, kg/d	0.379	0.140
Fat content, %	−0.405	0.096
CP content, %	−0.169	0.108
Lactose content, %	0.140	0.111
SCS, units	−0.057	0.153
Correlations[1] between EBV for IOD_P and EBV for		
Milk yield, kg/d	0.225	
Fat content, %	−0.269	
CP content, %	−0.075	
Lactose content, %	0.066	
SCS, units	0.161	

[1]Pearson correlations ($P < 0.01$) calculated using IOD_P EBV (1,690 individuals) with accuracy ≥0.50.

and moderate (0.379 ± 0.140). Relationships between EBV confirmed results of genetic and phenotypic correlations and highlighted an association only between IOD_P and milk yield and between IOD_P and fat content (Table 2).

In conclusion, this preliminary study demonstrated that milk IOD_P decreased with parity and was the lowest in early lactation; moreover, findings confirmed that IOD_P is mainly affected by temporary environmental factors rather than by the genetic merit of the cow. Considering r_a and EBV, IOD_P was positively associated with milk yield and negatively associated with fat content. However, the heritability of IOD_P was close to zero and thus, it would be hard to directly improve this trait through breeding. As a consequence, acting on feeding and management strategies would likely be a more effective approach. According to Grelet et al. (in press), in this study, the IOD_P was considered as a proxy for the real measured milk iodine content. The role of IOD_P as indicator should be validated in future studies. In this perspective, international collaborations and specific projects would be beneficial, allowing further phenotyping. Reference data variability and quantity should improve calibration equation and prediction accuracy. With increased availability of reference data, direct estimations of genetic correlation between measured and predicted values will become possible, allowing to check if the proxy genetically represents the trait of interest.

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