J. Dairy Sci. 107:1397–1412
https://doi.org/10.3168/jds.2024-23546

Observational study on the associations between milk yield, composition, and coagulation properties with blood biomarkers of health in Holstein cows

D. Giannuzzi,[1] F. Piccioli-Cappelli,[2] S. Pegolo,[1]* V. Bisutti,[1] S. Schiavon,[1] L. Gallo,[1] A. Toscano,[1] P. Ajmone Marsan,[2,3] L. Cattaneo,[2] E. Trevisi,[2] and A. Cecchinato[1]
[1]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Legnaro (PD) IT-35020, Italy
[2]Department of Animal Science, Food and Nutrition (DIANA) and the Romeo and Enrica Invernizzi Research Center for Sustainable Dairy Production (CREI), Faculty of Agricultural, Food and Environmental Sciences, Catholic University of the Sacred Heart, Piacenza IT-29122, Italy
[3]Nutrigenomics and Proteomics Research Center (PRONUTRIGEN), Catholic University of the Sacred Heart, Piacenza IT-29122, Italy

ABSTRACT

The considerable increase in the production capacity of individual cows owing to both selective breeding and innovations in the dairy sector has posed challenges to management practices in terms of maintaining the nutritional and metabolic health status of dairy cows. In this observational study, we investigated the associations between milk yield, composition, and technological traits and a set of 21 blood biomarkers related to energy metabolism, liver function or hepatic damage, oxidative stress, and inflammation or innate immunity in a population of 1,369 high-yielding Holstein-Friesian dairy cows. The milk traits investigated in this study included 4 production traits (milk yield, fat yield, protein yield, daily milk energy output), 5 traits related to milk composition (fat, protein, casein, and lactose percentages and urea), 11 milk technological traits (5 milk coagulation properties and 6 curd-firming traits). All milk traits (i.e., production, composition, and technological traits) were analyzed according to a linear mixed model that included the days in milk, the parity order, and the blood metabolites (tested one at a time) as fixed effects and the herd and date of sampling as random effects. Our findings revealed that milk yield and daily milk energy output were positively and linearly associated with total cholesterol, nonesterified fatty acids, urea, aspartate aminotransferase, γ-glutamyl transferase, total bilirubin, albumin, and ferric-reducing antioxidant power, whereas they were negatively associated with glucose, creatinine, alkaline phosphatase, total reactive oxygen metabolites, and proinflammatory proteins (ceruloplasmin, haptoglobin, and myeloperoxidase). Regarding composition traits, the protein percentage was negatively associated with nonesterified fatty acids and β-hydroxybutyrate (BHB), while the fat percentage was positively associated with BHB, and negatively associated with paraoxonase. Moreover, we found that the lactose percentage increased with increasing cholesterol and albumin and decreased with increasing ceruloplasmin, haptoglobin, and myeloperoxidase. Milk urea increased with an increase in cholesterol, blood urea, nonesterified fatty acids, and BHB, and decreased with an increase in proinflammatory proteins. Finally, no association was found between the blood metabolites and milk coagulation properties and curd-firming traits. In conclusion, this study showed that variations in blood metabolites had strong associations with milk productivity traits, the lactose percentage, and milk urea, but no relationships with technological traits of milk. Specifically, increasing levels of proinflammatory and oxidative stress metabolites, such as ceruloplasmin, haptoglobin, myeloperoxidase, and total reactive oxygen metabolites, were shown to be associated with reductions in milk yield, daily milk energy output, lactose percentage, and milk urea. These results highlight the close connection between the metabolic and innate immunity status and production performance. This connection is not limited to specific clinical diseases or to the transition phase but manifests throughout the entire lactation. These outcomes emphasize the importance of identifying cows with subacute inflammatory and oxidative stress as a means of reducing metabolic impairments and avoiding milk fluctuations.

Key words: blood metabolites, dairy cows, lactose, milk yield

INTRODUCTION

Milk is a valuable source of energy, high-quality protein, and several crucial minerals and vitamins in human nutrition (Bauman et al., 2006). To meet the growing demand for milk and dairy products in the past 3 decades, the global production of bovine milk has greatly increased (by around 60%) and the production capacity of individual cows has been enhanced

Received March 29, 2023.
Accepted July 31, 2023.
*Corresponding author: sara.pegolo@unipd.it

(Górska-Warsewicz et al., 2019). Advances in knowledge on the biology of lactation and the regulation of milk biosynthesis, in addition to a better understanding of the relationships between the nutritional and health status of cows and their performance and milk quality, have led to improved management practices and considerable increases in milk production and individual productivity (Gross and Bruckmaier, 2019).

Various blood biochemical constituents are used as indicators of the nutritional status and metabolic health of cows, and these constituents can be monitored through analysis of the blood metabolic profile (Puppel and Kuczyńska, 2016). Such analysis is generally performed to estimate the prevalence and risk levels of specific metabolic disorders in the herd (Calamari et al., 2016). In addition, the association between alterations in blood metabolites due to clinical metabolic disorders, such as ketosis or hepatic lipidosis, and the detrimental effects of these disorders on milk production and composition are well known; for example, an increase in circulating nonesterified fatty acids (**NEFA**) and BHB is associated with a reduction in milk yield (**MY**) and an increase in the milk fat-to-protein ratio (Guliński, 2021). Moreover, the association between a decrease in lactose and udder health status has been repeatedly described, with low lactose having been reported as a reliable predictor of subclinical mastitis (Bobbo et al., 2016). Notably, lactose is a water-soluble component in milk and is consequently highly sensitive to the variation of mammary homeostasis due to local inflammatory processes. A body of research has shown that high-yielding dairy cows that are apparently clinically healthy can exhibit increased levels of systemic inflammation, oxidative stress, or hepatic overload throughout lactation and up to the dry period owing to the differing abilities of individuals to maintain homeostasis and counteract the negative energy balance of the postpartum period (Omidi et al., 2017; Giannuzzi et al., 2021).

However, published studies focus on only a small number of blood metabolites that are mainly energy related, or are conducted on a small number of individuals. The effects of variations in circulating metabolites on the milk productivity, composition, and technological traits of dairy cattle without overt signs of disease have never been explored with a relatively large sample size. A common limitation of these types of study is that blood samples are seldom collected at a time close to milk sampling. Moreover, protocols and procedures for animal sampling should be consistent over batches to obtain reliable inferences regarding putative associations between milk and blood traits.

Hence, in the present observational study, we evaluated the associations between a set of 21 blood metabolites, including ones related to energy metabolism, liver function or hepatic damage, oxidative stress, and inflammation or innate immunity, and milk productivity, composition, and technological traits. This study was performed in a population of 1,369 high-yielding Holstein-Friesian dairy cows to determine whether variations in blood biomarkers of health were associated with milk productivity, composition, and technological traits in clinically healthy lactating cows.

MATERIALS AND METHODS

Field Data

This study included 1,369 Holstein-Friesian lactating cows from 5 herds located in the Veneto (3 herds, n = 346 cows) and Emilia Romagna (2 herds, n = 1,023 cows) regions in northern Italy. The sampled cows were of different parities (1–4) and stages of lactation (5–400 DIM); the colostrum was excluded. The distribution of different parities and stages of lactation was well balanced within farms. The cows were housed in sand-bedded free stalls and fed twice daily on TMR based on corn and sorghum silage supplemented with concentrates. The cows were sampled once (one herd per day) during the evening milking after medical checks, and any animals having clinical disease or receiving pharmaceutical treatment were excluded. Further details on the data, which were collected between May 2019 and September 2020, are reported in Pegolo et al. (2022) and Giannuzzi et al. (2023).

The research was approved by the Committee for the Protection and Welfare of Experimental Animals (Organismo Preposto al Benessere degli Animali) of the Catholic University of the Sacred Heart and by the Italian Ministry of Health (protocol number 510/2019-PR of July 19, 2019).

Milk Sampling and Analysis

Milk samples were collected in 21 batches (herd and date combinations): 16 in 2019 (1,027 cows) and 5 in 2020 (342 cows). Large herds were sampled on more than one day as the laboratory could only process around 65 milk samples per day (see Pegolo et al., 2021). The individual milk samples were divided into 2 aliquots and maintained at 4°C until laboratory analysis (within 24 h). Bronopol preservative was added to 1 aliquot, which was then transferred to the laboratories of the Breeders' Association of the Veneto Region (Padova, Italy), where milk composition, including protein, casein, fat, and lactose percentages and urea content (mg/100 g), was analyzed with an FT6000 Milkoscan infrared analyzer (Foss A/S, Hillerød, Denmark). The other

aliquot, without preservative, was transferred to the cheese-making laboratory of our department (Legnaro, Padova, Italy) for analysis of milk technological traits.

Analysis of Milk Coagulation Properties and Curd Firm Modeling. Milk coagulation properties (**MCP**) were measured in duplicate using 2 mechanical lactodynamographs (Formagraph; Foss Electric A/S). Analyses were carried out simultaneously by the same operator under identical experimental conditions, following the procedure described in Bisutti et al. (2022). Briefly, 200 μL of a rennet solution (Hansen Standard 215 with 80 ± 5% chymosin and 20 ± 5% pepsin; Pacovis Amrein AG, Bern, Switzerland) diluted to 1.2% (wt/vol) with distilled water was added to thawed milk samples (10 mL), which were then heated to 35°C. The following traditional single-point MCP traits were obtained directly from each lactodynamograph: (1) rennet coagulation time (**RCT**), the time (min) from rennet addition to the start of coagulation; (2) curd-firming time ($\mathbf{k_{20}}$), the time (min) to reach a curd firmness of 20 mm; and (3) curd firmness (mm) at 30, 45, and 60 min after rennet addition (**a30**, **a45**, **a60**). In addition, the extent of coagulation was recorded by the instruments every 15 s for 60 min, for a total of 240 measurements per milk sample. These measurements were then used in the equation proposed by Bittante et al. (2015) to model the following curd firming and syneresis parameters: RCT_{eq} (min), RCT estimated using the individual curd-firming equations; k_{CF} (%min), the curd-firming instant rate constant; k_{SR} (%min), the curd syneresis instant rate constant; CF_{max} (mm), the maximum curd firmness reached within 45 min; and t_{max}, the time needed to reach CF_{max}. All the analyses were done in duplicate for each animal, and duplicates were averaged prior to the statistical analysis. The number of records for MCP were slightly lower with respect to other milk traits because samples had to be from fresh milk for analysis, which limited the maximum number of samples that could be analyzed in a day.

Blood Collection and Analysis. Concurrent with the milk sampling, blood was collected from the jugular vein of each animal in the morning before TMR distribution and placed in 5-mL vacuum tubes containing 150 USP units of lithium heparin (Vacumed; FL Medical, Torreglia, Padua, Italy). A small portion of the blood was used to perform the hematocrit (packed-cell volume) test using the microhematocrit method (ALC Centrifugette 4203; 15,300 × *g*, 12 min). The blood metabolic profiles of these samples were then obtained with the protocols described by Mezzetti et al. (2019). Briefly, the tubes were kept on ice until centrifugation at 3,500 × *g* for 16 min at 6°C (Hettich Universal 16R Centrifuge), which was performed within 2 h of collection, and the plasma obtained was stored at −20°C until analysis. An ILAB-650 clinical auto-analyzer (Instrumentation Laboratory, Werfen, Bedford, MA) was used to measure the concentrations of 21 blood metabolites: glucose, NEFA, BHB, urea, creatinine, aspartate aminotransferase (**AST**), γ-glutamyl transferase (**GGT**), alkaline phosphatase (**ALP**), total proteins, haptoglobin, ceruloplasmin, albumin, total bilirubin (**BILt**), total cholesterol, total reactive oxygen metabolites (**ROMt**), advanced oxidation protein products, ferric-reducing antioxidant power (**FRAP**), total thiol groups of plasma (**SHp**), paraoxonase, and myeloperoxidase. The globulin concentration was calculated as the total protein concentration minus the albumin concentration.

Statistical Analyses: Productivity Indicators

To evaluate the impact of blood metabolites on the amount of energy excreted by each individual, we computed the energy content of the milk (**NEl**, MJ/kg) according to the following equation (NASEM, 2021):

$$NEl = 0.3887 \times fat + 0.2301 \times protein + 0.1653 \times lactose,$$

where *fat*, *protein*, and *lactose* are the percentages of fat, protein, and lactose in 1 kg of milk. To obtain the output indicators other than MY (kg/d), the daily production levels of fat, protein, and NEl were computed by multiplying the daily MY by the corresponding trait derived from the milk analysis. The resulting traits were fat yield (**FY**, kg/d), protein yield (**PY**, kg/d), and daily milk energy output (**dMEO**, MJ/d).

Mixed Model Analyses

As stated in the objectives of the study, the experimental hypothesis tested was that hematochemical (**HC**) parameters are related to changes in the productivity of dairy cows without overt disease (i.e., the cows contributing to the overall productivity and milk quality of the herd). The blood metabolites were therefore all treated as explanatory variables, and milk production, productivity indicators, milk composition, and technological traits as response variables. To study this association, we adopted a conservative approach assuming no linear relationship between the response and independent variables. To better assess the pattern of different blood metabolites and to obtain balanced classes, the explanatory variables (i.e., the HC parameters) were discretized, and classes were created on the basis of the 25th, 50th, and 75th percentiles.

We assumed that the animals belonging to the extreme classes (i.e., below the 25th percentile or above the 75th percentile), were potentially at risk of developing subclinical or clinical disease.

A preliminary exploratory data analysis was performed to check the assumptions required for model fitting, hypothesis testing, and handling extreme values as needed. To better assess the relevance of the HC parameters and avoid potential multicollinearity among them, we decided to assess the associations between milk traits and HC parameters by introducing the parameters into the model one at a time.

The associations between the HC parameters and milk productivity indicators (MY, FY, PY, and dMEO), milk composition traits (fat, protein, casein, lactose, and urea), and milk technological traits (RCT, k20, a30, a45, a60, RCT_{eq}, t_{max}, CF_{max}, CF_p, k_{CF}, and k_{SR}) were investigated using the following linear mixed model in PROC MIXED (SAS Institute Inc., Cary, NC, USA):

$$y_{ijklm} = \mu + DIM_i + parity_j + HC_k + herd/date_l + e_{ijklm},$$

where y_{ijklm} is the observed phenotype (i.e., milk productivity, composition, and technological traits); μ is the overall mean; DIM_i is the fixed effect of the ith class of days in milk ($i = 11$ classes: class 1 $\leq$ 30 [n = 61]; 30 $<$ class 2 $\leq$ 60 [n = 116]; 60 $<$ class 3 $\leq$ 90 [n = 109]; 90 $<$ class 4 $\leq$ 120 [n = 129]; 120 $<$ class 5 $\leq$ 150 [n = 126]; 150 $<$ class 6 $\leq$ 180 [n = 111]; 180 $<$ class 7 $\leq$ 210 [n = 115]; 210 $<$ class 8 $\leq$ 240 [n = 120]; 240 $<$ class 9 $\leq$ 270 [n = 158]; 270 $<$ class 10 $\leq$ 300 [n = 113]; class 11 $>$ 300 [n = 211]); $parity_j$ is the fixed effect of the jth parity ($j = 4$ classes; class 1 = 1 [n = 551]; class 2 = 2 [n = 391]; class 3 = 3 [n = 228]; class 4 $>$ 3 [n = 197]); HC_k is the fixed effect of the kth class of the HC parameters discretized on the basis of the 25th, 50th, and 75th percentiles; $herd/date_l$ is the random effect of the lth herd and date ($l = 1$ to 21); and e_{ijklm} is the random residual. Herd and date and the residuals were assumed to be normally distributed with a mean of zero and variances of σ_h^2 and σ_e^2, respectively. A given effect was declared significant at $P < 0.05$. Polynomial contrasts ($P < 0.05$) were estimated to describe the pattern of variation in the milk traits using the variation in the HC variables, with the first-, second-, and third-order comparisons used to measure the linear, quadratic, and cubic relationships, respectively. Only significant results ($P < 0.05$) are reported. Interactions among the HC parameters and the effects of DIM and parity were also explored. To better interpret the pattern of the least squares means, only interactions with $P < 0.001$ are displayed in Supplemental Figure S1 (https://doi.org/10.6084/m9.figshare.23849007.v1; Giannuzzi, 2023).

RESULTS

Descriptive Statistics

The descriptive statistics for milk traits are reported in Table 1, and those for blood metabolites are reported in Table 2 together with the range of variation in each of the 4 blood metabolite classes (quartiles). In the investigated population, serum BHB levels were greater than 1 mmol/L in less than 3% of the cows, while around 1% had high blood NEFA concentrations (>0.7 mmol/L). Among the hyperketotic cows, only 0.4% (n = 6) also had high NEFA concentrations. With regard to blood protein alterations, 20% of the cows had elevated globulin concentrations (>50 g/L), while only 1% had low albumin concentrations (<30 g/L). Twenty-seven percent of the cows had increased blood haptoglobin levels (≥0.35 g/L), while 10% had high ceruloplasmin levels (>3.2 mmol/L). The urea concentration threshold of ≥6.78 mmol/L was exceeded in 29% of cows, while 5% had elevated BILt levels (>4 mmol/L).

Blood Metabolites and Milk Productivity Traits

The blood metabolic profile had strong associations with all the milk productivity indicators. The results of the ANOVA are shown in Table 3.

Energy-Related Metabolites. Increasing levels of blood glucose were associated with a linear decrease in MY (−14% going from the first to the fourth class of glucose; Figure 1a), PY (−12%), FY (−16%), and dMEO (−14%; Figure 1b; $P < 0.001$). Similarly, an increase in blood creatinine concentrations was associated with linear decreases in MY (−7%, $P < 0.001$; Figure 1a), PY (−7%, $P < 0.001$), FY (−7%, $P < 0.05$), and dMEO (−8%, $P < 0.001$; Figure 1b). In contrast, increasing concentrations of serum cholesterol and urea were associated with linear increases ($P < 0.001$) in MY (+12 and +9%, respectively; Figure 1a), PY (+10 and +9%, respectively), FY (+9%), and dMEO (+12 and +9%, respectively; Figure 1b). An increase in blood NEFA was associated with linear increases in MY (+6%; Figure 1a) and dMEO (+4%; Figure 1b; $P < 0.001$). In contrast, increasing concentrations of blood BHB was associated with a linear increase in FY (+6%, $P < 0.05$).

Liver Function and Hepatic Damage Metabolites. Increases in serum AST, GGT, and albumin were associated with linear increases in MY (+6%, +7%,

Table 1. Descriptive statistics for milk production, composition, and technological traits

Trait[1]	N	Mean	SD	P1[2]	P99[2]
Production					
Milk yield, kg/d	1,369	33.18	9.27	11.7	56.5
Protein yield, kg/d	1,368	1.12	0.28	0.43	1.80
Fat yield, kg/d	1,363	1.20	0.37	0.35	2.15
dMEO, MJ/d	1,366	99.37	26.41	37.56	160.20
Composition					
Protein, %	1,367	3.43	0.34	2.69	4.32
Casein, %	1,367	2.67	0.28	2.07	3.4
Fat, %	1,360	3.68	0.81	1.58	5.69
Lactose, %	1,361	4.86	0.23	4.09	5.30
Urea, mg/100 g	1,367	27.44	5.72	14.5	40.7
Traditional MCP					
RCT, min	973	23.06	8.09	10.0	50.72
k20 min	927	8.09	4.35	2.73	21.65
a30, mm	994	20.14	14.57	0	51.87
a45, mm	994	29.91	12.35	0	55.76
a60, mm	994	30.32	11.93	0	56.8
Curd firming					
RCT_{eq}, min	960	22.94	7.08	10.45	42.54
t_{max}, min	975	50.33	9.15	26.0	60.0
CF_{max}, min	975	34.67	10.25	3.94	57.44
CF_p, mm	975	46.46	13.74	5.27	76.97
k_{CF}, % × min^{-1}	966	8.68	3.03	4.71	18.38
k_{SR}, % × min^{-1}	899	0.75	0.41	0.04	1.85

[1]dMEO = daily milk energy output; MCP = milk coagulation properties; RCT = rennet coagulation time; k20 = curd-firming rate as the time to a curd firmness of 20 mm; a30, a45, a60 = curd firmness at 30, 45, and 60 min from rennet addition; RCT_{eq} = rennet coagulation time estimated using the equation; CF_p = asymptotic potential curd firmness; k_{CF} = curd-firming instant rate constant; k_{SR} = syneresis instant rate constant; CF_{max} = maximum curd firmness achieved within 45 min; t_{max} = time at achievement of CFmax. All traditional MCP and curd-firming related traits were obtained in duplicate. Descriptive statistics refer to the averaged data.

[2]P1, P99 = 1st and 99th percentiles, respectively.

and +11%, respectively; Figure 2a), PY (+7%, +7%, and +11%, respectively), FY (+6%, +7%, and +9%, respectively), and dMEO (+6%, +7%, and +11%, respectively; Figure 2b; $P < 0.001$; $P < 0.01$ for FY and AST, GGT). In addition, increases in blood BILt levels over 1.99 μmol/L were associated with increases in MY, FY, and dMEO ($P < 0.01$; Figures 2a and 2b). Alkaline phosphatase blood levels were associated only with MY, with milk production decreasing with ALP serum concentrations higher than 50.21 U/L ($P < 0.05$; Figure 2a). Notably, an increase in serum paraoxonase concentrations was associated with linear increases in MY (+8%, $P < 0.001$; Figure 2a), PY (+8%, $P < 0.001$), and dMEO (+6%, $P < 0.01$; Figure 2b).

Oxidative Stress Metabolites. Oxidative stress metabolites appeared to significantly affect milk productivity traits. An increase in oxidative stress, measured as ROMt serum levels, was associated with a linear decrease in MY (−17%; Figure 3a), PY (−17%), FY (−12%), and dMEO (−15%; Figure 3b; $P < 0.001$). In contrast, the increase in antioxidant capacity, measured as FRAP blood levels, was associated with a linear increase in MY (+9%, $P < 0.001$; Figure 3a), PY (+9%, $P < 0.001$), FY (+8%, $P < 0.05$), and dMEO (+9%, $P < 0.001$; Figure 3b). With regard to the other measure of antioxidant power, the associations between blood levels of SHp and the traits MY, PY, FY, and dMEO tended to follow a quadratic pattern in which increases in the milk productivity indicator values were greater going from low to medium SHp levels than from medium to high SHp levels ($P < 0.05$; dMEO: $P < 0.01$; Figures 3a and 3b).

Inflammation and Innate Immunity Metabolites. An increase in ceruloplasmin concentrations was associated with a sharp linear decrease in MY (−20%; Figure 4a), PY (−20%), FY (−14%), and dMEO (−18%; Figure 4b; $P < 0.001$). All the productivity indicators were affected by the variations in globulins, with the increases in milk productivity indicators being greater going from the low to medium globulin blood levels than from the medium to high levels ($P < 0.001$, FY: $P < 0.01$; Figures 4a and 4b). Milk yield, PY, and dMEO decreased with increasing levels of serum haptoglobin, with the decreases in the values for the milk productivity indicators being greater going from the medium to high blood levels than from the low to medium levels ($P < 0.001$, dMEO: $P < 0.01$; Figures 4a and 4b). In contrast, the increase in myeloperoxidase serum levels was associated with a linear decrease in MY (−10%, $P < 0.001$; Figure 4a), PY (−9%, $P < 0.001$), and dMEO (−7%, $P < 0.01$; Figure 4b).

Blood Metabolites and Milk Composition Traits

The associations between the blood metabolites and milk composition traits are shown in Table 4. Energy-related blood metabolites had strong associations with milk protein and casein contents, with the latter decreasing with increasing NEFA and BHB concentrations ($P < 0.001$; Figure 1c). Increasing BHB hematic levels were also associated with a linear increase in milk fat (+6%, $P < 0.05$; Figure 1d), while increasing levels of BILt were associated with decreases in milk protein ($P < 0.05$) and casein ($P < 0.01$; Figure 2c). In addition, an increase in blood PON concentrations over 93.98 U/mL was associated with a decrease in milk fat ($P < 0.01$; Figure 2d), whereas with increasing levels of blood ceruloplasmin over 2.62 μmol/L, milk fat increased ($P < 0.05$).

Blood Metabolites and Milk Lactose Percentages

The results of the ANOVA of the blood metabolites and lactose are reported in Table 4. Lactose increased linearly with increasing cholesterol concentrations (+1%, $P < 0.001$; Figure 1e) and with increasing albu-

Table 2. Descriptive statistics for the blood metabolic profile

Trait[1]	N	Mean	SD	Quartile			
				0–25th	25th–50th	50th–75th	75th–100th
Energy-related metabolites							
Glucose, mmol/L	1,369	4.18	0.47	<3.93	3.93–4.25	4.25–4.50	>4.50
Cholesterol, mmol/L	1,365	5.29	1.27	<4.43	4.43–5.32	5.32–6.14	>6.14
NEFA, mmol/L	1,365	0.13	0.16	<0.075	0.075–0.094	0.094–0.137	>0.137
BHB, mmol/L	1,369	0.55	0.21	<0.42	0.42–0.51	0.51–0.63	>0.63
Urea, mmol/L	1,369	6.04	1.21	<5.24	5.24–6.05	6.05–6.87	>8.87
Creatinine, µmol/L	1,369	84.05	7.18	<79.34	79.34–83.30	83.30–88.26	>88.26
Liver function/hepatic damage							
AST, U/L	1,369	104.88	27.26	<87.27	87–27–99.08	99.08–115.49	>115.49
GGT, U/L	1,369	29.95	13.65	<23.90	23.90–28.46	28.46–33.32	>33.32
BILt, µmol/L	1,345	2.14	1.28	<1.58	1.58–1.98	1.98–2.42	>2.42
Albumin, g/L	1,369	37.25	2.27	<36.11	36.11–37.47	37.47–38.71	>38.71
ALP, U/L	1,369	53.99	20.38	<39.01	39.01–50.21	50.21–64.26	>64.26
PON, U/mL	1,369	96.40	19.60	<82.6	82.6–93.9	93.9–108.4	>108.4
Oxidative stress metabolites							
ROMt, mg H_2O_2/100 mL	1,369	13.73	4.17	<11.16	11.16–13.27	13.27–15.63	>15.63
AOPP, µmol/L	1,365	50.38	14.61	<42.29	42.29–48.3	48.3–55.01	>55.01
FRAP, µmol/L	1,364	190.1	55.83	<159.1	159.1–185.3	185.3–213	>213
SHp, µmol/L	1,369	411.31	97.52	<357.1	357.1–387.6	387.6–436.2	>436.2
Inflammation/innate immunity							
CPl, µmol/L	1,369	2.15	0.77	<1.61	1.61–2.05	2.06–2.62	>2.62
PROTt, g/L	1,369	82.29	5.64	<78.66	78.66–81.63	81.63–85.23	>85.23
Globulins, g/L	1,369	45.04	6.35	<40.73	40.73–43.85	43.85–47.85	>47.85
Hp, g/L	1,369	0.31	0.28	<0.16	0.16–0.20	0.20–0.30	>0.30
MPO, U/L	1,365	457.89	88.95	<403.5	403.5–453.9	453.9–503.2	>503.2

[1]NEFA = nonesterified fatty acids; AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; BILt = total bilirubin; ALP = alkaline phosphatase; PON = paraoxonase; ROMt = total reactive oxygen metabolites; AOPP = advanced oxidation protein products; FRAP = ferric reducing antioxidant power; SHp = total thiol groups; CPl = ceruloplasmin; Hp = haptoglobin; PROTt = total proteins; MPO = myeloperoxidase.

min serum levels (+1%, $P < 0.01$; Figure 2e). Regarding stress oxidative blood metabolites, lactose decreased linearly with increasing ROMt concentrations (−1%, $P < 0.01$; Figure 3c), but increased linearly with increasing FRAP values (+1%, $P < 0.01$; Figure 3c). Inflammation indicators showed consistent trends with each other and exhibited various relationships with lactose. Lactose decreased linearly with increasing blood levels of total proteins (−1%, $P < 0.01$), globulins (−1%, $P < 0.001$), haptoglobin (−1%, $P < 0.001$), and ceruloplasmin (−2%, $P < 0.001$; Figure 4c).

Blood Metabolites and Milk Urea

The results of the ANOVA between the blood metabolites and milk urea are shown in Table 4. Milk urea was associated with variations in all the energy-related metabolites (Figure 1f). With increasing levels of serum glucose, it increased up to 4.25 mmol/L, then decreased ($P < 0.05$), whereas with increasing levels of blood cholesterol, it increased linearly (+3%, $P < 0.05$). Milk urea tended to increase with increasing NEFA concentrations up to 0.14 mmol/L, but tended to decrease with higher values, showing a cubic trend ($P < 0.05$). It increased linearly with increases in serum BHB, urea, and creatinine ($P < 0.01$, $P < 0.001$, and $P < 0.001$, respectively) and with increasing levels of serum albumin (+4%, $P < 0.01$), and it decreased with ALP concentrations higher than 64.26 mmol/L ($P < 0.01$, Figure 2f). Regarding associations with the oxidative stress metabolites, milk urea decreased linearly with increasing amounts of hematic ROMt (−5%; $P < 0.001$), but increased linearly with increasing levels of FRAP (+4%; $P < 0.05$). It tended to decrease linearly with serum SHp concentrations below 357.1 mmol/L ($P < 0.01$; Figure 3d), and it also decreased with increasing levels of inflammation metabolites (Figure 4d), with a linear trend in the case of ceruloplasmin (−8%, $P < 0.001$) and myeloperoxidase (−4%, $P < 0.05$), and with less marked trends in the case of haptoglobin ($P < 0.01$, from 0.21 g/L) and globulins ($P < 0.01$, from 43.85 g/L).

Blood Metabolites and Milk Technological Traits

Few associations were observed between blood metabolites and technological traits, mostly with small significance or erratic patterns (Supplemental Tables S1 and S2; https://doi.org/10.6084/m9.figshare.23849007.v1; Giannuzzi, 2023). Increased glycemia (≥3.93

Table 3. Results from linear mixed model (*F*-values and RMSE) for the productivity traits

Trait[1]	Milk yield, kg/d		Protein yield, kg/d		Fat yield, kg/d		dMEO, MJ/d	
	F	RMSE[2]	*F*	RMSE	*F*	RMSE	*F*	RMSE
Hematochemical parameters								
Energy-related metabolites								
Glucose, mmol/L	16.13***	7.15	12.04***	0.24	12.79***	0.31	15.41***	21.4
Cholesterol, mmol/L	17.45***	7.16	17.01***	0.24	7.49***	0.31	15.27***	21.45
NEFA, mmol/L	6.13***	7.25	2.44	0.24	2.08	0.31	3.66*	21.73
BHB, mmol/L	0.27	7.29	0.89	0.24	3.38*	0.31	1.03	21.80
Urea, mmol/L	9.07***	7.23	9.21***	0.24	5.91***	0.31	8.65***	21.65
Creatinine, µmol/L	5.93***	7.25	5.84***	0.24	3.62*	0.31	6.14***	21.69
Liver function/hepatic damage								
AST, U/L	6.88***	7.24	7.77***	0.24	5.04**	0.31	6.10***	21.67
GGT, U/L	6.91***	7.24	8.16***	0.24	4.80**	0.31	6.62***	21.66
BILt, µmol/L	4.47**	7.26	3.22**	0.24	1.23	0.31	2.68*	21.76
Albumin, g/L	16.16***	7.18	16.38***	0.24	7.49***	0.31	15.10***	21.48
ALP, U/L	2.89*	7.27	2.28	0.24	1.81	0.31	2.51	21.76
PON, U/mL	9.00***	7.22	9.25***	0.24	1.44	0.31	4.70**	21.71
Oxidative stress metabolites								
ROMt, mg H_2O_2/100 mL	28.05***	7.10	28.34***	0.23	9.71***	0.31	20.56***	21.37
AOPP, µmol/L	0.73	7.29	1.51	0.24	0.19	0.31	0.58	21.81
FRAP, µmol/L	8.27***	7.24	6.19***	0.24	3.60*	0.31	6.76***	21.67
SHp, µmol/L	2.90*	7.27	2.91*	0.24	3.64*	0.31	4.34**	21.72
Inflammation/innate immunity								
CPl, µmol/L	30.32***	7.08	33.78***	0.23	10.95***	0.31	24.55***	21.30
PROTt, g/L	2.27	7.28	2.17	0.24	1.00	0.31	1.59	21.79
Globulins, g/L	7.34***	7.24	7.24***	0.24	5.14**	0.31	7.98***	21.65
Hp, g/L	7.53***	7.24	9.71***	0.24	2.19	0.31	5.19**	21.71
MPO, U/L	7.53***	7.25	6.87***	0.24	1.29	0.31	3.90**	21.76

[1]All traits are included as class effect according to the 25th, 50th, and 75th percentiles. NEFA = nonesterified fatty acids; AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; BILt = total bilirubin; ALP = alkaline phosphatase; PON = paraoxonase; ROMt = total reactive oxygen metabolites; AOPP = advanced oxidation protein products; FRAP = ferric reducing antioxidant power; SHp = total thiol groups; CPl = ceruloplasmin; Hp = haptoglobin; PROTt = total proteins; MPO = myeloperoxidase.

[2]RMSE = root mean square error.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

mmol/L) was associated with an increase in RCT and RCT_{eq} ($P < 0.05$) and a decrease in a30, a45, a60, CF_p, and CF_{max} ($P < 0.05$, for a_{60} $P < 0.01$). An increase in blood urea levels was associated with an increase of a_{45} and a_{60} ($P < 0.05$). With increasing PON blood levels (>82.6 U/mL), k_{CF} linearly decreased ($P < 0.05$). Further, increased levels of haptoglobin (>0.2 g/L) were associated with increasing k_{SR} ($P < 0.05$). With increasing blood levels of advanced oxidation protein products up to 55.01 µmol/L, k_{CF} increased and t_{max} decreased ($P < 0.05$). With increased blood globulin concentration, k_{20} linearly increased, whereas CF_p and CF_{max} linearly decreased ($P < 0.05$).

Interactions Between Metabolites and Individual Sources of Variation

Significant ($P < 0.001$) interactions were detected between groups of metabolites and individual sources of variation (i.e., DIM and parity). The first interactions were between NEFA and parity for MY and dMEO, with primiparous cows in the highest class of serum NEFA concentrations having lower productivity than multiparous cows (Supplemental Figure S1a, and S1b, respectively). We also observed an interaction between ALP and DIM for MY, with the cows in the 2 lowest classes of serum ALP (up to 50.2 U/L) having greater productivity than those in the higher classes up to the peak of lactation (90–120 DIM), with the trend then reversing with advancing lactation (Supplemental Figure S1c).

DISCUSSION

In this study, we aimed to assess potential associations between the blood metabolite profile and milk productivity, composition, and technological traits in a population of 1,369 Holstein-Friesian cattle without overt signs of disease. As the descriptive statistics show, the average values for the milk production and composition traits and the blood metabolites were consistent with previous studies on clinically healthy Holstein cows with comparable yields and welfare conditions (Pegolo et al., 2021; Premi et al., 2021). The range of variation in blood metabolites was in line with the expected values in physiological conditions, and it

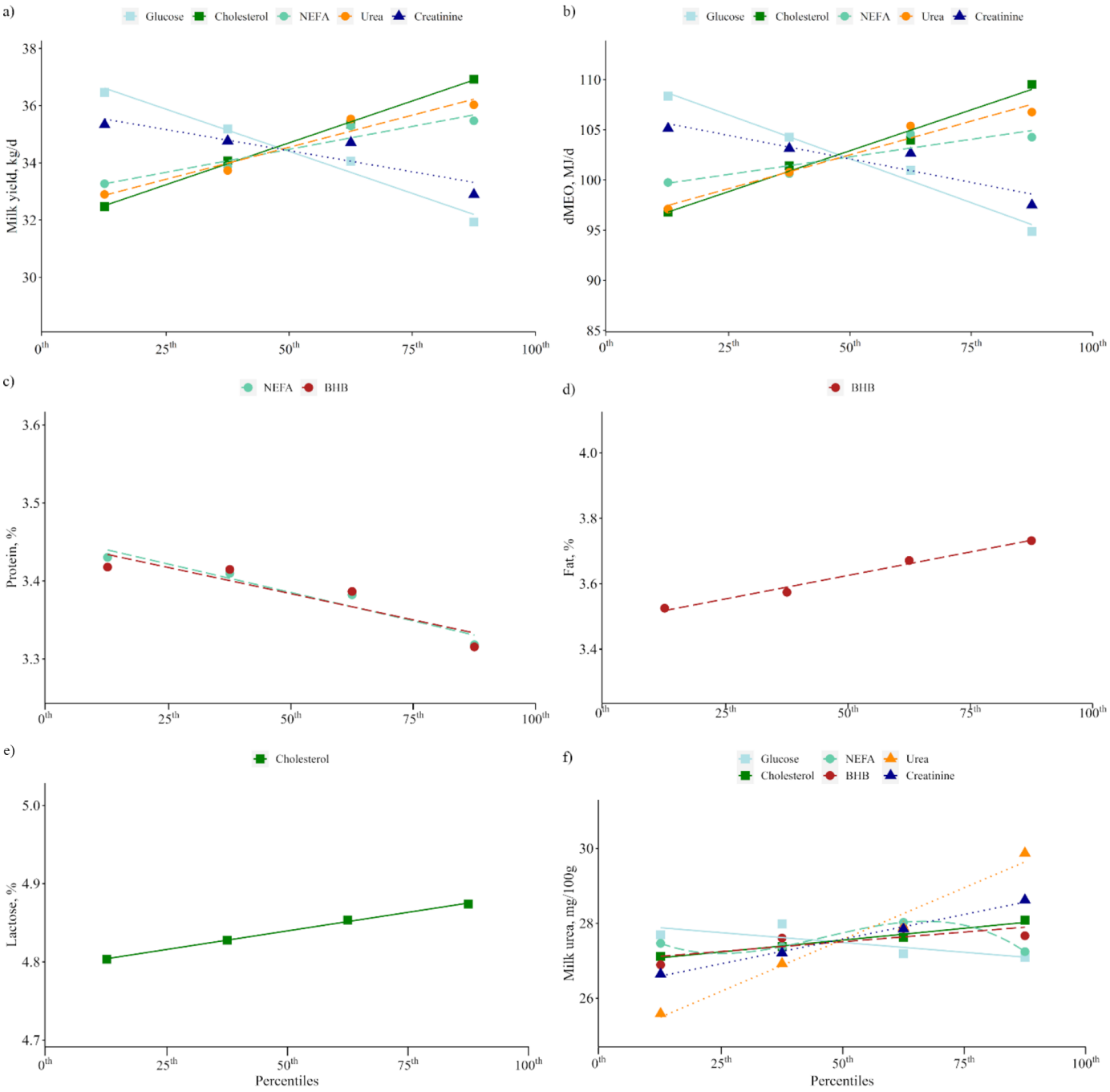

Figure 1. Least squares means (LSM) for milk yield (a), dMEO (b), protein percentage (c), fat percentage (d), lactose percentage (e), and milk urea (f) across the classes of energy-related metabolites. Symbols indicate the LSM, whereas the lines describe the linear, quadratic, or cubic pattern of variation of the dependent variable as the independent variable increases. dMEO = daily milk energy output; NEFA = non-esterified fatty acids.

was mainly attributable to a homeorhetic shift during lactation. For this reason, we chose a classification using quartiles, which placed individuals with serum metabolite values potentially indicative of subclinical disorders in the 2 extreme classes (below the 25th or above the 75th quartile, depending on the metabolite). Exceptions were haptoglobin, which showed that a proportion of the individuals in the studied population were in a low-grade, subclinical systemic inflammatory state (≥0.35 g/L; Bertoni and Trevisi, 2013; Martins et

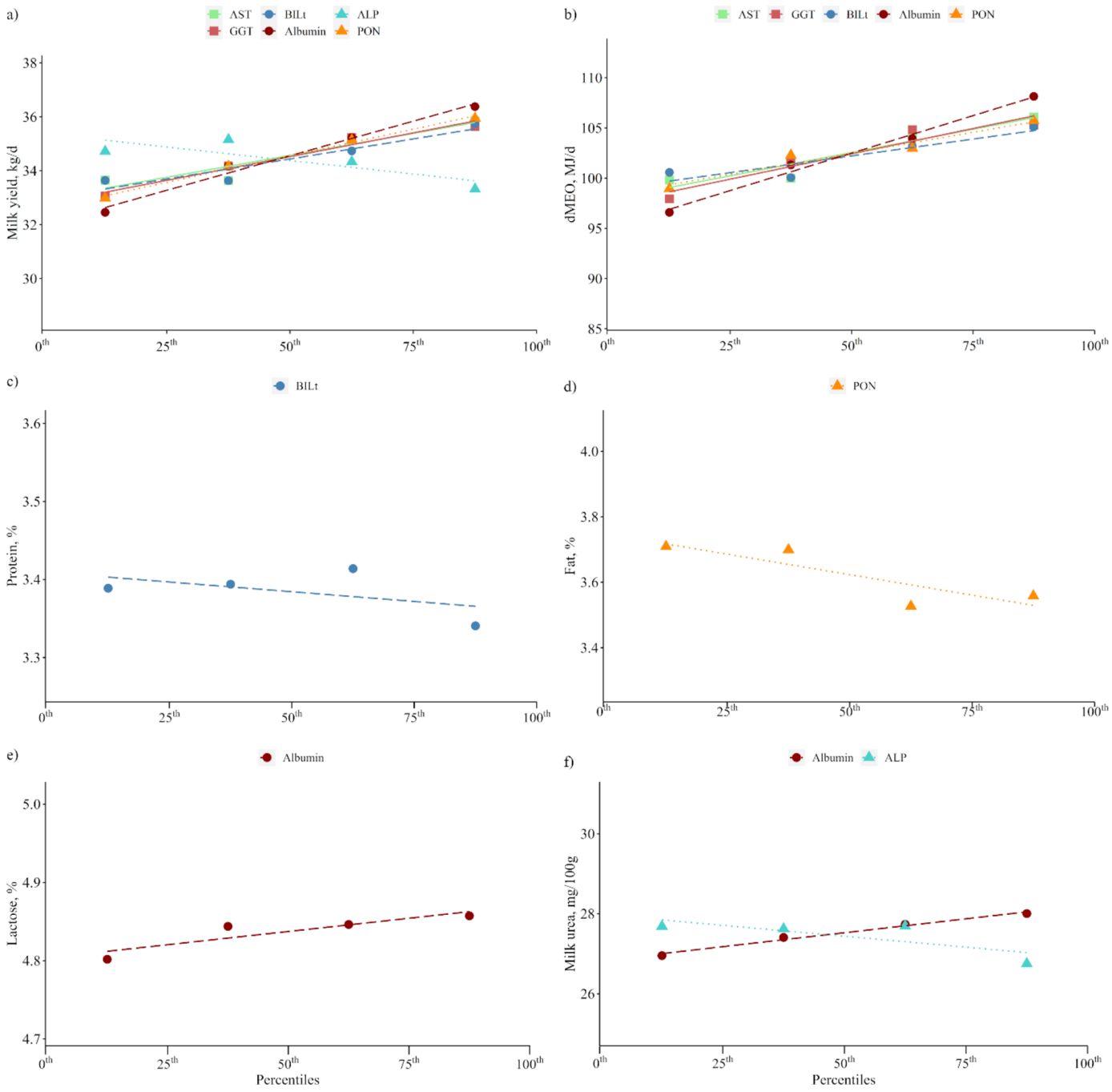

Figure 2. Least squares means (LSM) for milk yield (a), dMEO (b), protein percentage (c), fat percentage (d), lactose percentage (e), and milk urea (f) across the classes of liver function/hepatic damage metabolites. Symbols indicate the LSM, whereas the lines describe the linear, quadratic, or cubic pattern of variation of the dependent variable as the independent variable increases. dMEO = daily milk energy output; AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; BILt = total bilirubin; ALP = alkaline phosphatase; PON = paraoxonase.

al., 2021), and urea (6.78 mmol/L; Butler et al., 1996), which reflected excess dietary intake of CP (Schiavon et al., 2016). As reported previously (Colmenero and Broderick, 2006), the diets of high-producing dairy cows commonly contain CP at levels exceeding 16% to ensure maximum milk output, as was the case with the herds investigated here (Giannuzzi et al., 2022). The significance of high concentrations of blood urea in dairy cows is controversial, with various studies reporting no detrimental effects on milk production (Godden

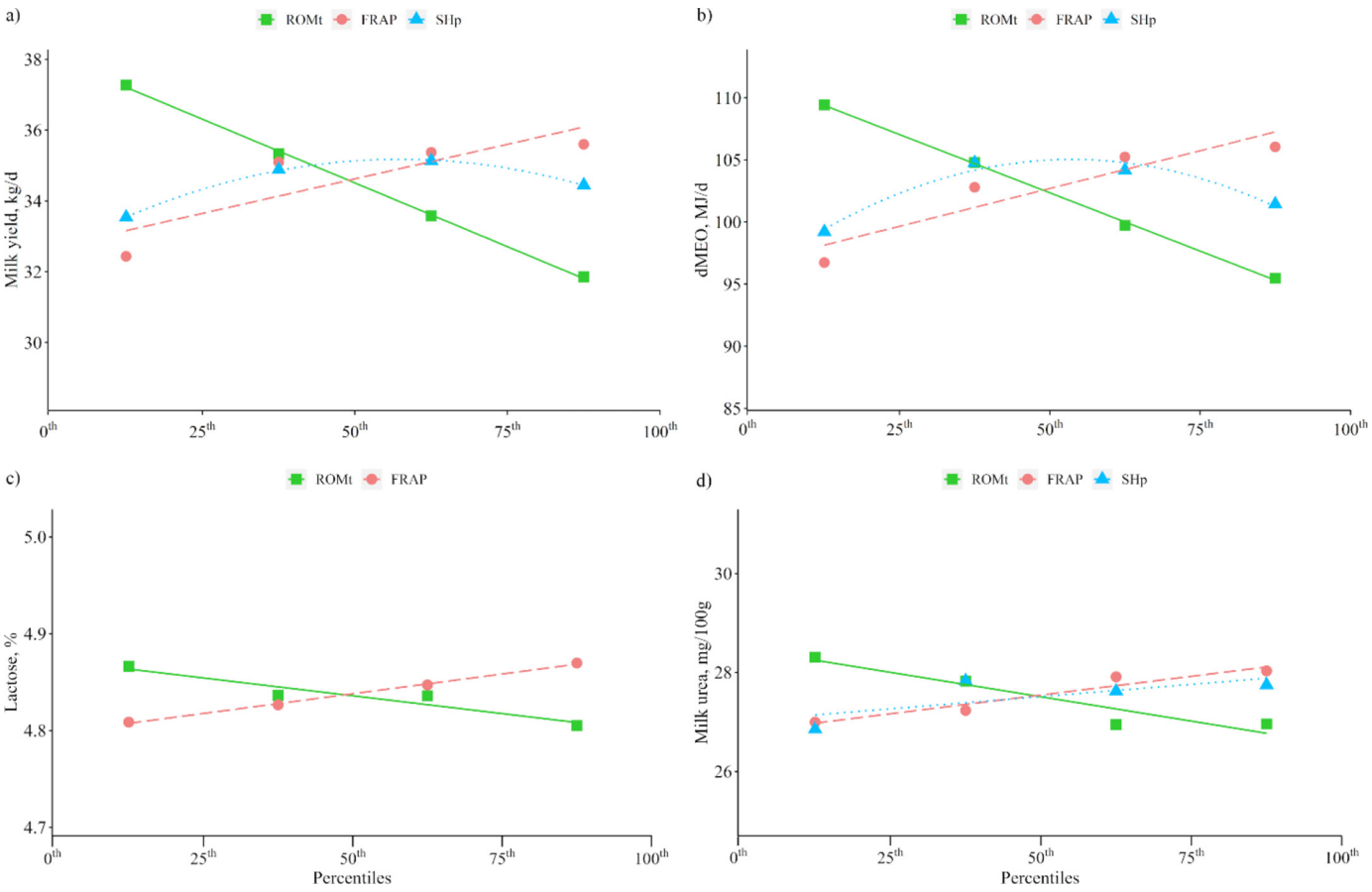

Figure 3. Least squares means (LSM) for milk yield (a), dMEO (b), lactose percentage (c), and milk urea (d) across the classes of oxidative stress metabolites. Symbols indicate the LSM, whereas the lines describe the linear, quadratic, or cubic pattern of variation of the dependent variable as the independent variable increases. dMEO = daily milk energy output; ROMt = total reactive oxygen metabolites; FRAP = ferric reducing antioxidant power; SHp = total thiol groups.

et al., 2001). Within the investigated population, cows seemed to tolerate high levels of feed protein and consequently circulating urea, thus increasing productivity.

Blood Metabolic Profile and Milk Productivity Traits

By switching the paradigm in which variations in blood metabolites are associated with the occurrence of specific diseases, we observed how in physiological conditions blood metabolites are significantly associated with the productivity of cows and consequently to their resilience and adaptability to metabolic changes. An overall picture of these associations and their magnitudes is reported in Figure 5a. Notably, when blood glucose levels rose to 3.5 mmol/L, MY and dMEO dropped, showing that when production levels are high, blood glucose availability is low. This finding suggests that high rates of utilization are needed to fulfill the requirements of the mammary gland for higher milk synthesis (Omari et al., 2020).

All the productivity traits decreased with increasing blood creatinine concentrations. Creatinine is physiologically rather constant compared with blood urea, which is more prone to fluctuations related to diet and metabolic challenge. High creatinine could be related to a decline in renal efficiency with reduced glomerular filtration capability (Prahl et al., 2022), a condition that is detrimental to high milk production. Conversely, increasing cholesterol and NEFA concentrations within physiological ranges were associated with increases in MY, PY, FY, and dMEO. In fact, increasing levels of circulating NEFA and cholesterol—and consequently lipoproteins—are associated with homeorhetic maintenance of energy metabolism (Gross et al., 2021), leading to higher production performances. The interaction between hematic NEFA levels and parity for both MY and dMEO traits suggests that multiparous cows support their higher productivity levels with high levels of lipid mobilization, whereas primiparous cows are less able to cope with high levels of circulating NEFA, prob-

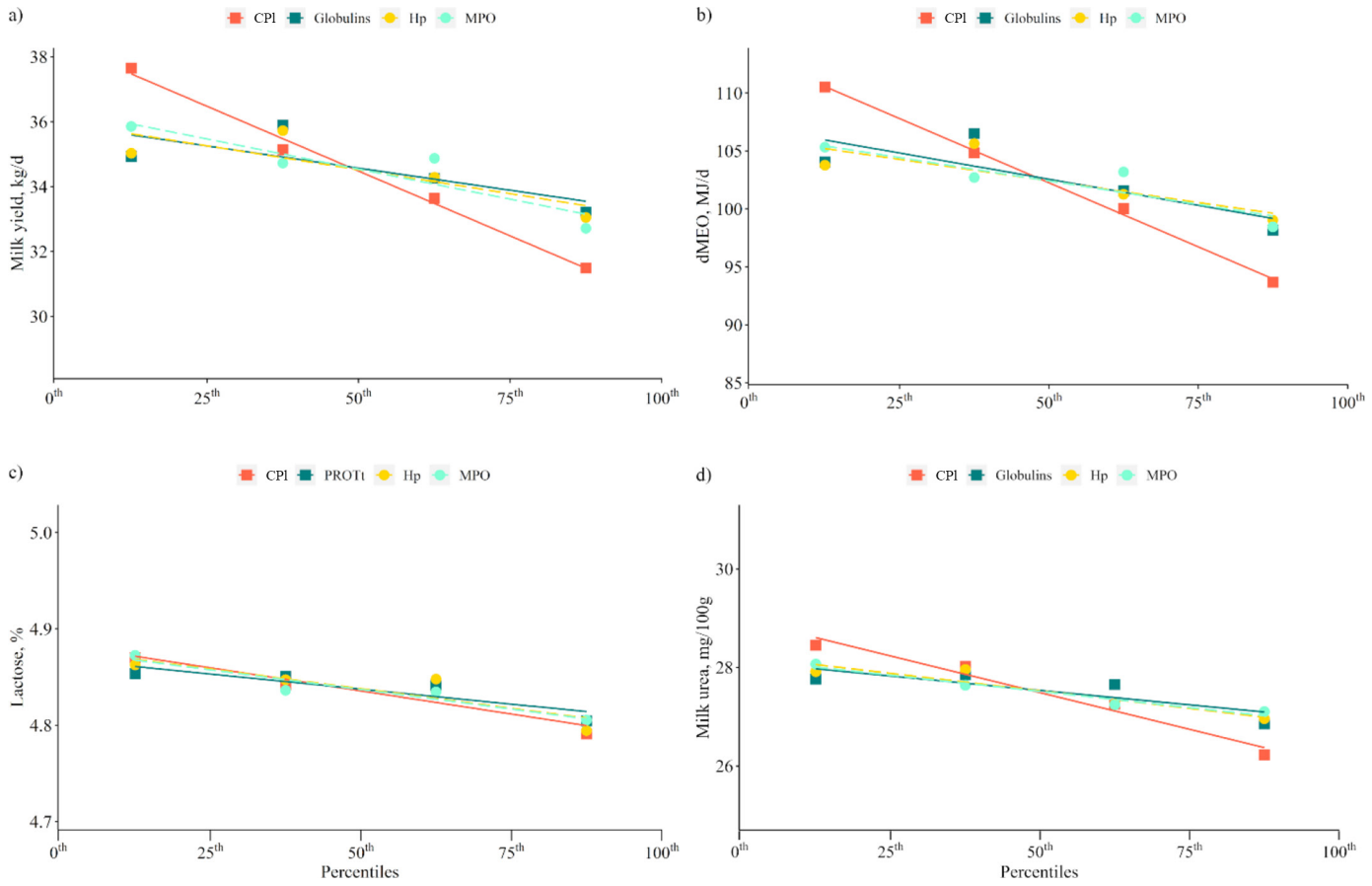

Figure 4. Least squares means (LSM) for milk yield (a), dMEO (b), lactose percentage (c), and milk urea (d) across the classes of inflammation/innate immunity metabolites. Symbols indicate the LSM, whereas the lines describe the linear, quadratic, or cubic pattern of variation of the dependent variable as the independent variable increases. CPl = ceruloplasmin; Hp = haptoglobin; PROTt = total proteins; MPO = myeloperoxidase.

ably revealing a lower energy balance (Drackley et al., 2003).

Regarding hepatic function metabolites, productivity traits increased with rising levels of GGT, AST, and BILt, revealing an increase in hepatic metabolic activity related to the lactation period (Giannuzzi et al., 2021). In addition, and consistent with previous findings (Bionaz et al., 2007), higher albumin and paraoxonase concentrations were associated with higher MY, PY, and dMEO. The 2 negative acute phase proteins (**APP**) indicated reduced serum concentration in the presence of an inflammatory or oxidative status. This finding underlines the importance of maintaining hepatic homeostasis, not only during the transition period but also throughout the entire lactation to maintain high production performance. An exception was serum ALP, which was associated with a linear decrease in productivity traits. In previous reports, an inverse relationship was observed between milk ALP and MY (Haab and Smith, 1956). Blood and milk ALP concentrations are positively correlated, with serum circulating ALP consisting of diverse isoenzymes originating from liver, bone, gut, and mammary gland (Sato et al., 2005). Specifically, mammary gland ALP has been shown to influence and contribute to an increase in the level of serum ALP activity (Sato et al., 2005), which supports our finding of an inverse association between blood ALP levels and MY. Moreover, we found a significant interaction between ALP and DIM, with lower serum ALP concentrations (up to 50.2 U/L) associated with higher MY up to the peak of lactation, and then a reversal of the tendency as lactation advanced, suggesting greater tissue destruction (and ALP release) with reduced cellular efficiency toward the end of lactation (Haab and Smith, 1956).

The determination of oxidative metabolism has become increasingly important as a complementary tool in the evaluation of metabolic status, as it is one of the factors contributing to greater susceptibility to diseases. Under physiological conditions, the animal

Table 4. Results from linear mixed model (F-values and RMSE) for the composition traits

	Protein, %		Casein, %		Fat, %		Lactose, %		Milk urea, mg/100 g	
Trait[1]	F	RMSE[2]	F	RMSE	F	RMSE	F	RMSE	F	RMSE
Hematochemical parameters										
Energy-related metabolites										
Glucose, mmol/L	2.32	0.27	1.49	0.23	0.18	0.70	1.27	0.19	3.55*	3.73
Cholesterol, mmol/L	0.78	0.27	0.80	0.23	1.33	0.70	6.35***	0.19	3.06*	3.73
NEFA, mmol/L	8.32***	0.27	7.79***	0.23	0.57	0.70	0.50	0.19	2.72*	3.73
BHB, mmol/L	9.10***	0.27	7.22***	0.23	5.03**	0.70	0.41	0.19	3.85**	3.73
Urea, mmol/L	0.13	0.27	0.004	0.23	0.60	0.70	1.79	0.19	51.93***	3.55
Creatinine, µmol/L	1.10	0.27	0.78	0.23	0.83	0.70	0.75	0.19	14.18***	3.69
Liver function/hepatic damage										
AST, U/L	1.08	0.27	1.36	0.23	2.01	0.70	0.64	0.19	1.94	3.74
GGT, U/L	0.83	0.27	0.89	0.23	0.55	0.70	0.82	0.19	1.92	3.74
BILt, µmol/L	3.56*	0.27	3.84**	0.23	2.52	0.70	0.40	0.19	0.47	3.74
Albumin, g/L	0.59	0.27	1.03	0.23	0.54	0.70	4.88**	0.19	4.36**	3.73
ALP, U/L	1.69	0.27	1.84	0.23	0.90	0.70	0.61	0.19	3.94**	3.73
PON, U/mL	0.56	0.27	0.99	0.23	5.40**	0.70	1.61	0.19	0.88	3.74
Oxidative stress metabolites										
ROMt, mg H_2O_2/100 mL	0.10	0.27	0.21	0.23	1.15	0.70	4.46**	0.19	8.69***	3.71
AOPP, µmol/L	0.67	0.27	0.49	0.23	0.09	0.70	0.23	0.19	0.54	3.74
FRAP, µmol/L	1.05	0.27	1.15	0.23	1.08	0.70	3.64*	0.19	3.24*	3.73
SHp, µmol/L	0.50	0.27	0.63	0.23	0.74	0.70	1.08	0.19	4.22**	3.73
Inflammation/innate immunity										
CPl, µmol/L	0.42	0.27	0.44	0.23	3.40*	0.70	6.43***	0.19	14.16***	3.69
PROTt, g/L	0.88	0.27	1.11	0.23	1.51	0.70	3.86**	0.19	1.80	3.74
Globulins, g/L	1.13	0.27	1.31	0.23	0.64	0.70	7.06***	0.19	4.43**	3.73
Hp, g/L	2.03	0.27	2.41	0.23	1.43	0.70	6.26***	0.19	5.19**	3.72
MPO, U/L	0.06	0.27	0.15	0.23	1.78	0.70	1.35	0.19	2.98*	3.73

[1]All traits are included as class effect according to the 25th, 50th, and 75th percentiles. NEFA = nonesterified fatty acids; AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; BILt = total bilirubin; ALP = alkaline phosphatase; PON = paraoxonase; ROMt = total reactive oxygen metabolites; AOPP = advanced oxidation protein products; FRAP = ferric reducing antioxidant power; SHp = total thiol groups; CPl = ceruloplasmin; Hp = haptoglobin; PROTt = total proteins; MPO = myeloperoxidase.

[2]RMSE = root mean square error.

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

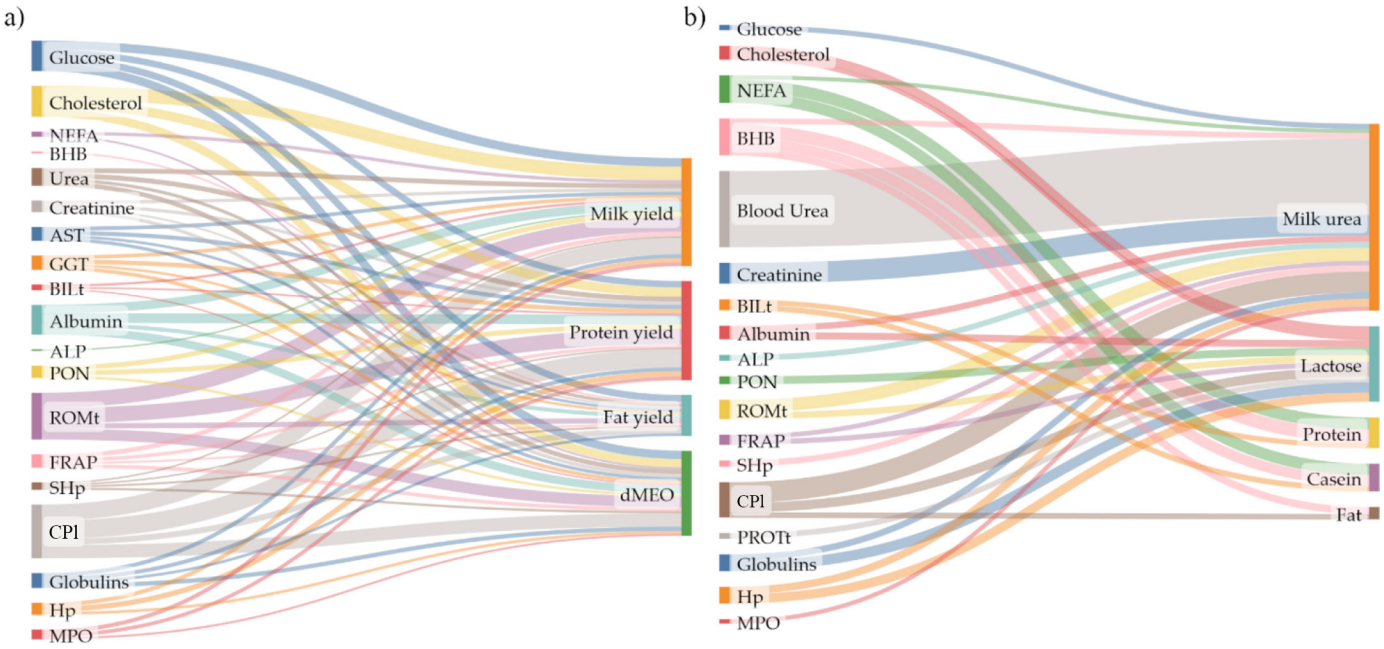

Figure 5. Sankey diagrams depicting the relationship between blood metabolites (a and b, left) and milk productivity traits (a; right) and composition traits (b; right) in Holstein cattle. The magnitude of associations for each metabolite has been depicted using the *F*-value from the linear mixed model analysis reported in Tables 3 and 4. dMEO = daily milk energy output; NEFA = nonesterified fatty acids; AST = aspartate aminotransferase; GGT = γ-glutamyl transferase; BILt = total bilirubin; ALP = alkaline phosphatase; PON = paraoxonase; ROMt = total reactive oxygen metabolites; FRAP = ferric reducing antioxidant power; SHp = total thiol groups; CPl = ceruloplasmin; Hp = haptoglobin; PROTt = total proteins; MPO = myeloperoxidase.

has sufficient antioxidant reserve to cope with the production of free radicals (Castillo et al., 2003), but individuals differ dramatically in their adaptive success, which depends on their ability to maintain or re-establish a homeostatic state while simultaneously producing high MY (Sundrum, 2015). We found that lower levels of ROMt and higher levels of antioxidant markers (i.e., FRAP and SHp) were associated with higher productivity, showing that cows that are more able to counteract oxidative conditions are more likely to have better production performances.

Along the same lines, inflammation has been linked in varying degrees to an increased risk of disease, and reduced milk production over the entire lactation (Bradford and Swartz, 2020). However, some degree of systemic inflammation helps the organism adapt to and overcome adverse stimuli to restore homeostasis. Moreover, immune cells are directly involved in metabolic physiology and exert various balancing functions (Bradford and Swartz, 2020). Indeed, we observed that animals with overall high levels of inflammation, represented in our profile by globulins, positive APP (i.e., haptoglobin and ceruloplasmin), and myeloperoxidase, exhibited lower productivity, showing that a potential impairment or excess inflammation can lead to unbalanced homeostasis and lower productivity levels (Trevisi and Minuti, 2018). Specifically, increasing levels of ceruloplasmin were associated with a reduction in productivity, which was around 6 kg/d of milk and 17 MJ/d of dMEO for the class of individuals with average ceruloplasmin levels of 3.21 μmol/L, consistent with a certain degree of alteration throughout the entire lactation (Premi et al., 2021).

Blood Metabolic Profile and Milk Composition and Technological Traits

The associations between the blood metabolic profile and milk composition traits are summarized in Figure 5b. Increasing BHB and NEFA blood levels within physiological ranges were negatively associated with the milk protein and casein percentages, as a consequence of low energy availability for rumen fermentation and thus low microbial protein synthesis (Gross and Bruckmaier, 2019). An increase in blood BHB is also associated with an increase in the milk fat percentage, which can be attributed to increased availability of BHB and fatty acids for milk fat synthesis (Duffield et al., 1998). Notably, an increase in paraoxonase of over 94 U/mL was associated with a decrease in the milk fat percentage, which is consistent with the negative relationship between paraoxonase and lipid metabolism

due to paraoxonase esterolytic activity (Kulka et al., 2016).

Several associations were detected between blood traits and the lactose percentage. With a rising inflammatory state (i.e., higher levels of ceruloplasmin, myeloperoxidase, and haptoglobin) and increasing oxidative stress parameters (i.e., higher ROMt), the lactose percentage decreased; however, it rose with increasing hematic concentrations of cholesterol, antioxidant indicator (i.e., FRAP), and negative APP (i.e., albumin). Lactose is the major compound regulating milk osmolarity and is therefore expected to be rather constant (Bobbo et al., 2016), so even minor variations reveal a degree of imbalance. Several studies have highlighted the role of lactose as an indicator of mammary gland disturbance. These studies describe how the action of bacteria and immune-response agents damages the alveolar epithelium, which increases its permeability and leads to the lactose in the alveoli being lost into the bloodstream and cleared in the urine, thus reducing the milk lactose percentage (Bobbo et al., 2016). Based on the reported risk threshold for the blood metabolites investigated in clinically healthy, high-yielding dairy cows (Premi et al., 2021), the sampled population comprised a set of individuals with a certain degree of low-grade, subclinical systemic inflammation, as evidenced by higher blood levels of positive APP and oxidation products, or lower negative APP and antioxidant compounds. Repeated systemic inflammation, even when mild, can disrupt metabolic homeostasis and trigger metabolic disorders (Bradford et al., 2015). We can therefore hypothesize that the lactose percentage is sensitive to subacute inflammatory and oxidative alterations, even when these changes are not directly related to local udder damage but are instead systemic. Milk urea exhibited various positive associations with energy-related blood metabolites, with the exception of glucose, with which it was negatively associated. Milk and blood urea levels were highly correlated, reflecting the N nutritional status of the animals (Butler et al., 1996). In addition, milk urea has been found to have positive associations with MY (Hojman et al., 2004). In high-yielding cows, when extensive milk secretion is present, homeorhetic maintenance of the physiological state requires intensive energy and protein metabolism activity, which results in higher levels of circulating lipoproteins (i.e., cholesterol), ketone substrates (i.e., BHB), and N end products (i.e., creatinine and urea), and consequently higher urea excretion in the milk. If this hypercatabolic response is controlled, homeostasis and production levels are maintained (Piazza et al., 2022). However, when the blood glucose concentrations are higher, milk urea decreases, as observed for MY, showing that high production levels go hand in hand with low blood glucose availability. We observed that milk urea decreased with increasing oxidative and inflammatory blood parameters, such as ROMt, globulins, haptoglobin, ceruloplasmin, and myeloperoxidase, and it exhibited positive relationships with increasing albumin and antioxidant compounds (i.e., SHp and FRAP), mimicking the trends of productivity traits. On the one hand, milk urea, being a measure of protein catabolism in the organism, was confirmed as a potential indicator of production levels. On the other hand, this finding underscores that even a low-grade inflammatory or oxidative imbalance can impair homeostasis, not only reducing production levels but also modulating the milk composition.

Blood metabolites showed negligible associations with traditional MCP and curd-firming traits. Specifically, none of them had an influence on the RCT trait. Our findings suggested that, in physiological conditions, milk technological traits are scarcely influenced by variations in metabolic profile.

CONCLUSIONS

This study has shown that variations in blood metabolites have strong associations with milk productivity traits, the lactose percentage, and milk urea, whereas negligible associations were observed with milk technological traits. Specifically, increasing levels of proinflammatory and oxidative stress metabolites, such as ceruloplasmin, haptoglobin, myeloperoxidase, and ROMt, as well as the reduction of some negative acute phase proteins (e.g., albumin and paraoxonase), were shown to be associated with reductions in MY, dMEO, the lactose percentage, and milk urea. These findings confirm the close connection between individual metabolic behavior and production performance. This connection is not limited to specific clinical diseases or to the transition phase, but extends to the entire lactation, showing how crucial it is to identify individuals with a low-grade subacute inflammatory and oxidative state to allow reducing metabolic impairments while preserving production performance.

ACKNOWLEDGMENTS

This research was part of the BENELAT project, funded by the Lombardy Region (Italy) under the Rural Development Program 2014–2020, and the LATSAN project, funded by the Ministry of Agricultural, Food and Forestry Policies (Rome, Italy). D. G. benefited from a research grant funded by the University of Padova BIRD202724/20 “Next generation omic tools to enhance the sustainability of dairy chain.” The authors have not stated any conflicts of interest.

REFERENCES

Bauman, D. E., I. H. Mather, R. J. Wall, and A. L. Lock. 2006. Major advances associated with the biosynthesis of milk. J. Dairy Sci. 89:1235–1243. https://doi.org/10.3168/jds.S0022-0302(06)72192-0.

Bertoni, G., and E. Trevisi. 2013. Use of the liver activity index and other metabolic variables in the assessment of metabolic health in dairy herds. Vet. Clin. North Am. Food Anim. Pract. 29:413–431. https://doi.org/10.1016/j.cvfa.2013.04.004.

Bionaz, M., E. Trevisi, L. Calamari, F. Librandi, A. Ferrari, and G. Bertoni. 2007. Plasma paraoxonase, health, inflammatory conditions, and liver function in transition dairy cows. J. Dairy Sci. 90:1740–1750. https://doi.org/10.3168/jds.2006-445.

Bisutti, V., S. Pegolo, D. Giannuzzi, L. F. M. Mota, A. Vanzin, A. Toscano, E. Trevisi, P. Ajmone Marsan, M. Brasca, and A. Cecchinato. 2022. The β-casein (CSN2) A2 allelic variant alters milk protein profile and slightly worsens coagulation properties in Holstein cows. J. Dairy Sci. 105:3794–3809. https://doi.org/10.3168/jds.2021-21537.

Bittante, G., C. Cipolat-Gotet, F. Malchiodi, E. Sturaro, F. Tagliapietra, S. Schiavon, and A. Cecchinato. 2015. Effect of dairy farming system, herd, season, parity, and days in milk on modeling of the coagulation, curd firming, and syneresis of bovine milk. J. Dairy Sci. 98:2759–2774. https://doi.org/10.3168/jds.2014-8909.

Bobbo, T., C. Cipolat-Gotet, G. Bittante, and A. Cecchinato. 2016. The nonlinear effect of somatic cell count on milk composition, coagulation properties, curd firmness modeling, cheese yield, and curd nutrient recovery. J. Dairy Sci. 99:5104–5119. https://doi.org/10.3168/jds.2015-10512.

Bradford, B. J., and T. H. Swartz. 2020. Review: Following the smoke signals: Inflammatory signaling in metabolic homeostasis and homeorhesis in dairy cattle. Animal 14(S1):s144–s154. https://doi.org/10.1017/S1751731119003203.

Bradford, B. J., K. Yuan, J. K. Farney, L. K. Mamedova, and A. J. Carpenter. 2015. Invited review: Inflammation during the transition to lactation: New adventures with an old flame. J. Dairy Sci. 98:6631–6650. https://doi.org/10.3168/jds.2015-9683.

Butler, W. R., J. J. Calaman, and S. W. Beam. 1996. Plasma and milk urea nitrogen in relation to pregnancy rate in lactating dairy cattle. J. Anim. Sci. 74:858–865. https://doi.org/10.2527/1996.744858x.

Calamari, L., A. Ferrari, A. Minuti, and E. Trevisi. 2016. Assessment of the main plasma parameters included in a metabolic profile of dairy cow based on Fourier transform mid-infrared spectroscopy: Preliminary results. BMC Vet. Res. 12:4. https://doi.org/10.1186/s12917-015-0621-4.

Castillo, C., J. Hernández, M. López-Alonso, M. Miranda, and J. Luís. 2003. Values of plasma lipid hydroperoxides and total antioxidant status in healthy dairy cows: Preliminary observations. Arch. Tierzucht 46:227–233. https://doi.org/10.5194/aab-46-227-2003.

Colmenero, J. J., and G. A. Broderick. 2006. Effect of dietary crude protein concentration on milk production and nitrogen utilization in lactating dairy cows. J. Dairy Sci. 89:1704–1712. https://doi.org/10.3168/jds.S0022-0302(06)72238-X.

Drackley, J. K., T. M. Cicela, and D. W. LaCount. 2003. Responses of primiparous and multiparous Holstein cows to additional energy from fat or concentrate during summer. J. Dairy Sci. 86:1306–1314. https://doi.org/10.3168/jds.S0022-0302(03)73714-X.

Duffield, T. F., D. Sandals, K. E. Leslie, K. Lissemore, B. W. McBride, J. H. Lumsden, P. Dick, and R. Bagg. 1998. Efficacy of monensin for the prevention of subclinical ketosis in lactating dairy cows. J. Dairy Sci. 81:2866–2873. https://doi.org/10.3168/jds.S0022-0302(98)75846-1.

Giannuzzi, D. 2023. Supplementary_information_JDS_2023-23546. figshare. Journal contribution. https://doi.org/10.6084/m9.figshare.23849007.v1.

Giannuzzi, D., L. F. M. Mota, S. Pegolo, F. Tagliapietra, S. Schiavon, L. Gallo, P. A. Marsan, E. Trevisi, and A. Cecchinato. 2023. Prediction of detailed blood metabolic profile using milk infrared spectra and machine learning methods in dairy cattle. J. Dairy Sci. 106:3321–3344. https://doi.org/10.3168/jds.2022-22454.

Giannuzzi, D., R. Tessari, S. Pegolo, E. Fiore, M. Gianesella, E. Trevisi, P. Ajmone Marsan, M. Premi, F. Piccioli-Cappelli, F. Tagliapietra, L. Gallo, S. Schiavon, G. Bittante, and A. Cecchinato. 2021. Associations between ultrasound measurements and hematochemical parameters for the assessment of liver metabolic status in Holstein–Friesian cows. Sci. Rep. 11:16314. https://doi.org/10.1038/s41598-021-95538-x.

Godden, S. M., D. F. Kelton, K. D. Lissemore, J. S. Walton, K. E. Leslie, and J. H. Lumsden. 2001. Milk urea testing as a tool to monitor reproductive performance in Ontario dairy herds. J. Dairy Sci. 84:1397–1406. https://doi.org/10.3168/jds.S0022-0302(01)70171-3.

Górska-Warsewicz, H., K. Rejman, W. Laskowski, and M. Czeczotko. 2019. Milk and dairy products and their nutritional contribution to the average Polish diet. Nutrients 11:1771. https://doi.org/10.3390/nu11081771.

Gross, J. J., and R. M. Bruckmaier. 2019. Review: Metabolic challenges in lactating dairy cows and their assessment via established and novel indicators in milk. Animal 13(S1):s75–s81. https://doi.org/10.1017/S175173111800349X.

Gross, J. J., A. C. Schwinn, E. Müller, A. Münger, F. Dohme-Meier, and R. M. Bruckmaier. 2021. Plasma cholesterol levels and short-term adaptations of metabolism and milk production during feed restriction in early lactating dairy cows on pasture. J. Anim. Physiol. Anim. Nutr. (Berl.) 105:1024–1033. https://doi.org/10.1111/jpn.13531.

Guliński, P. 2021. Ketone bodies—Causes and effects of their increased presence in cows' body fluids: A review. Vet. World 14:1492–1503. https://doi.org/10.14202/vetworld.2021.1492-1503.

Haab, W., and L. M. Smith. 1956. Variations in alkaline phosphatase activity of milk. J. Dairy Sci. 39:1644–1650. https://doi.org/10.3168/jds.S0022-0302(56)94905-0.

Hojman, D., O. Kroll, G. Adin, M. Gips, B. Hanochi, and E. Ezra. 2004. Relationships between milk urea and production, nutrition, and fertility traits in Israeli dairy herds. J. Dairy Sci. 87:1001–1011. https://doi.org/10.3168/jds.S0022-0302(04)73245-2.

Kulka, M., J. Kołodziejska-Lesisz, and W. Kluciński. 2016. Serum paraoxonase 1 (PON1) activity and lipid metabolism parameters changes in different production cycle periods of Holstein-Friesian, Polish Red and Norwegian breeds. Pol. J. Vet. Sci. 19:165–173. https://doi.org/10.1515/pjvs-2016-0021.

Martins, L. F., P. R. Menta, L. Fernandes, V. S. Machado, and R. C. Neves. 2021. Prolonged, low-grade inflammation in the first week of lactation: Associations with mineral, protein, and energy balance markers, and milk yield, in a clinically healthy Jersey cow cohort. J. Dairy Sci. 104:6113–6123. https://doi.org/10.3168/jds.2020-19538.

Mezzetti, M., A. Minuti, F. Piccioli-Cappelli, M. Amadori, M. Bionaz, and E. Trevisi. 2019. The role of altered immune function during the dry period in promoting the development of subclinical ketosis in early lactation. J. Dairy Sci. 102:9241–9258. https://doi.org/10.3168/jds.2019-16497.

National Academies of Sciences, Engineering, and Medicine (NASEM). 2021. Nutrient Requirements of Dairy Cattle. 8th ed. The National Academies Press, Washington, DC.

Omari, M., A. Lange, J. Plöntzke, and S. Röblitz. 2020. Model-based exploration of the impact of glucose metabolism on the estrous cycle dynamics in dairy cows. Biol. Direct 15:2. https://doi.org/10.1186/s13062-019-0256-7.

Omidi, A., M. Fathi, and M. Parker. 2017. Alterations of antioxidant status markers in dairy cows during lactation and in the dry period. J. Dairy Res. 84:49–53. https://doi.org/10.1017/S0022029916000753.

Pegolo, S., L. Mota, V. Bisutti, M. Martinez-Castillero, D. Giannuzzi, L. Gallo, S. Schiavon, F. Tagliapietra, A. Revello Chion, E. Trevisi, R. Negrini, P. Ajmone Marsan, and A. Cecchinato. 2021. Genetic parameters of differential somatic cell count, milk composition, and cheese-making traits measured and predicted using spectral data in Holstein cows. J. Dairy Sci. 104:10934–10949. https://doi.org/10.3168/jds.2021-20395.

Pegolo, S., R. Tessari, V. Bisutti, A. Vanzin, D. Giannuzzi, M. Gianesella, A. Lisuzzo, E. Fiore, A. Barberio, E. Schiavon, E. Trevisi, F.

Piccioli Cappelli, L. Gallo, P. Ruegg, R. Negrini, and A. Cecchinato. 2022. Quarter-level analyses of the associations among subclinical intramammary infection and milk quality, udder health, and cheesemaking traits in Holstein cows. J. Dairy Sci. 105:3490–3507. https://doi.org/10.3168/jds.2021-21267.

Piazza, M., D. Giannuzzi, R. Tessari, E. Fiore, M. Gianesella, S. Pegolo, S. Schiavon, E. Trevisi, F. Piccioli Cappelli, A. Cecchinato, and L. Gallo. 2022. Associations between ultrasound hepatic measurements, body measures and milk production traits in Holstein cows. J. Dairy Sci. 105:7111–7124. https://doi.org/10.3168/jds.2021-21582.

Prahl, M. C., C. B. M. Müller, D. Albrecht, F. Koch, K. Wimmers, and B. Kuhla. 2022. Hepatic urea, creatinine and uric acid metabolism in dairy cows with divergent milk urea concentrations. Sci. Rep. 12:17593. https://doi.org/10.1038/s41598-022-22536-y.

Premi, M., M. Mezzetti, G. Ferronato, M. Barbato, F. Piccioli Cappelli, A. Minuti, and E. Trevisi. 2021. Changes of plasma analytes reflecting metabolic adaptation to the different stages of the lactation cycle in healthy multiparous Holstein dairy cows raised in high-welfare conditions. Animals (Basel) 11:1714. https://doi.org/10.3390/ani11061714.

Puppel, K., and B. Kuczyńska. 2016. Metabolic profiles of cow's blood; a review. J. Sci. Food Agric. 96:4321–4328. https://doi.org/10.1002/jsfa.7779.

Sato, J., M. Kanata, J. Yasuda, R. Sato, K. Okada, Y. Seimiya, and Y. Naito. 2005. Changes of serum alkaline phosphatase activity in dry and lactational cows. J. Vet. Med. Sci. 67:813–815. https://doi.org/10.1292/jvms.67.813.

Schiavon, S., G. Cesaro, A. Cecchinato, C. Cipolat-Gotet, F. Tagliapietra, and G. Bittante. 2016. The influence of dietary nitrogen reduction and conjugated linoleic acid supply to dairy cows on fatty acids in milk and their transfer to ripened cheese. J. Dairy Sci. 99:8759–8778. https://doi.org/10.3168/jds.2016-11371.

Sundrum, A. 2015. Metabolic disorders in the transition period indicate that the dairy cows' ability to adapt is overstressed. Animals (Basel) 5:978–1020. https://doi.org/10.3390/ani5040395.

Trevisi, E., and A. Minuti. 2018. Assessment of the innate immune response in the periparturient cow. Res. Vet. Sci. 116:47–54. https://doi.org/10.1016/j.rvsc.2017.12.001.

ORCIDS

D. Giannuzzi https://orcid.org/0000-0003-2975-0385
F. Piccioli-Cappelli https://orcid.org/0000-0003-1277-7821
S. Pegolo https://orcid.org/0000-0001-6390-9826
V. Bisutti https://orcid.org/0000-0001-5681-4001
S. Schiavon https://orcid.org/0000-0002-5539-8947
L. Gallo https://orcid.org/0000-0002-8908-5105
A. Toscano https://orcid.org/0000-0003-3190-2608
P. Ajmone Marsan https://orcid.org/0000-0003-3165-4579
L. Cattaneo https://orcid.org/0000-0001-6027-7536
E. Trevisi https://orcid.org/0000-0003-1644-1911
A. Cecchinato https://orcid.org/0000-0003-3518-720X