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Differences in liver functionality indexes in peripartal dairy cows fed rumen-protected methionine or choline are associated with performance, oxidative stress status, and plasma amino acid profiles

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ABSTRACT

The liver functionality index (LFI) represents an assessment of transition cow metabolic health by measuring changes in biomarkers associated with liver plasma protein synthesis (albumin), lipoprotein synthesis (cholesterol), and heme catabolism (bilirubin). The present analysis was conducted to determine the role of peripartal rumen-protected Met or choline (CHOL) supplementation on LFI groupings, and to assess relationships with performance, inflammation, oxidative stress status, and plasma AA profiles. A cohort of 40 multiparous Holstein cows that were part of a randomized complete block design with 2 × 2 factorial arrangement of Met (Smartamine M, Adisseo NA, Alpharetta, GA) and CHOL (ReaShure, Balchem Inc., New Hampton, NY) level (with or without) were used. From −21 d to calving, cows received the same close-up diet and were assigned randomly to each treatment. From calving to 30 d, cows were on the same postpartal diet and continued to receive the same treatments until 30 d. Addition of Met was adjusted daily at 0.08% dry matter of diet and CHOL was fed at 60 g/cow per day. Liver (−10, 7, 20, and 30 d) and blood (−10, 4, 8, 20, and 30 d) samples were harvested for biomarker analyses. Cows were ranked retrospectively and assigned to low (LLFI, LFI <0) and high (HLFI, LFI >0) LFI groups regardless of Met or CHOL supplementation. Compared with cows in LLFI, close-up and lactation DMI, milk yield, milk fat yield, and milk protein yield were greater in HLFI cows. As expected, cows in LLFI had lower plasma cholesterol and albumin but greater bilirubin concentrations around parturition. Plasma haptoglobin concentration was also lower in HLFI cows, but plasma paraoxonase and hepatic total and reduced hepatic glutathione concentrations were greater. Although higher concentrations of His, Met, and Trp, as well as a tendency for greater Ile, were observed in HLFI cows, overall essential AA concentrations did not differ with LFI status. In contrast, overall concentrations of nonessential AA were greater in HLFI cows due to greater circulating concentrations of Ala, Asn, Gln, Pro, and Ser. Similarly, overall concentrations of total AA and total sulfur-containing compounds were greater in cows with HLFI. Feeding Met compared with CHOL led to a tendency for more cows classified as HLFI. Overall, results support the broader application of the LFI in the management of transition cows. In that context, the fact that precalving concentrations of compounds such as reduced glutathione, total sulfur-containing compounds, Met, Tau, and homocysteine differed between HLFI and LLFI independent of Met or CHOL feeding also underscores their potential for monitoring cows that might be at a greater risk of developing health problems after calving. Further studies on the applicability of these biomarkers to monitor transition success appears warranted.

Key words: transition cow, nutrition, methyl donors

INTRODUCTION

Around parturition, the metabolic stress inflicted by negative energy and nutrient balance contributes greatly to the occurrence of most metabolic and infectious diseases of dairy cows during the first few weeks of lactation (Goff and Horst, 1997). In fact, various diseases manifested at later stages of lactation also are related to metabolic insults during the peripartal period (Bertoni et al., 2008). It is noteworthy that metabolic stress around parturition may impair production and reproduction performance of dairy cows, even in the absence of serious infections or other pathologies (Bionaz et al., 2007). Hence, assessing metabolic sta-

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tus around parturition can help diagnose nutritional management-related issues on dairy farms.

Although metabolic profiling can be a useful diagnostic tool to prevent metabolic disorders, it has not been routinely adopted owing to cost and insensitivity to nutritional inadequacies (Bertoni and Trevisi, 2013). In contrast, a composite index, such as the liver functionality index (**LFI**), using changes in plasma concentrations of several blood biomarkers (i.e., albumin, cholesterol, and bilirubin) has promise for diagnosis and correction of management and nutritional problems on dairy farms (Bertoni and Trevisi, 2013). In fact, results from previous work have demonstrated the applicability of the LFI to assess immune and inflammatory status as well as metabolic profiles in the periparturient period (Calamari et al., 2014; Zhou et al., 2016b). For instance, a low LFI (**LLFI**) is indicative of a pronounced inflammatory response and less favorable circulating AA profile, which together suggest a more difficult transition from gestation to lactation; in contrast, a high LFI (**HLFI**) is suggestive of a smooth transition (Trevisi et al., 2012).

It is noteworthy that feed additives, such as rumen-protected Met and choline (**CHOL**), and ionophores are often supplemented to transition dairy cows in an effort to improve not only EAA and gluconeogenic precursor availability, but also hepatic fatty acid metabolism. As such, these approaches result in physiologic profiles associated with a smoother transition into lactation (Zahra et al., 2006; Swyers et al., 2014; Osorio et al., 2016). Although it is possible to assess changes in metabolic and inflammatory status in response to feed additive supplementation (Batistel et al., 2016; Osorio et al., 2016; Zhou et al., 2016a,c), the cost and time needed to obtain results make it a less desirable approach for evaluating effectiveness of feed additives. In addition, due to differences in nutrition and management strategies among farms, variation in effectiveness of such feed additives are commonly observed, even among research conducted in well-controlled environments (Pinotti et al., 2003; Guretzky et al., 2006; Chen et al., 2011; Zom et al., 2011; Osorio et al., 2013; Leiva et al., 2015).

A composite index, such as the LFI, which can be determined at a lower cost without invasive procedures, can potentially help evaluate the effectiveness of feed additives within a farm-specific environment. However, it is unknown whether feed additive-induced changes in metabolic and inflammatory profiles of transition dairy cows are manifested in the LFI. Our general hypothesis was that favorable changes in response to peripartal Met and CHOL supplementation lead to higher LFI, indicating a more favorable immunometabolic status around parturition. Therefore, the objectives of the present study were to determine (1) if Met or CHOL alter the LFI response in cows, and (2) if high and low LFI cows have distinct oxidative stress status in addition to lactation performance and plasma AA profiles.

MATERIALS AND METHODS

Experimental Design and Treatments

All procedures for this study (protocol no. 13023) were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of Illinois. Details of the experimental design and the feeding regimen (nutrient composition and evaluation) have been described previously (Zhou et al., 2016d). Briefly, the experiment was conducted as a randomized, complete, unbalanced, block design with 2 × 2 factorial arrangement of Met (Smartamine M, Adisseo NA, Alpharetta, GA) and CHOL (ReaShure, Balchem Inc., New Hampton, NY) level (with or without) using a total of 81 cows. Cows within each block were balanced for parity, previous lactation milk yield, and BCS before the close-up diet groups were assigned. Per IACUC guidelines, only half of the cows in each treatment were used for routine blood sampling. Thus, for the present study, 40 cows were used. Treatments were control (**CON**; n = 10), with no Met or CHOL supplementation; Smartamine (**SMA**; n = 10), CON plus Met at a rate of 0.08% of DM; ReaShure (**REA**; n = 10), CON + CHOL at 60 g/d; or Smartamine and ReaShure (**MIX**; n = 10), CON + Met + CHOL. Dosage of Met was based on Osorio et al. (2013), whereas CHOL was supplemented following the manufacturer's recommendations (http://balchem.com/sites/default/files/Feeding,%20Storage%20&%20Handling%20Guidelines.pdf). All cows received the same far-off diet from −50 to −22 d before expected parturition, close-up diet from −21 d to expected parturition, and lactation diet from parturition through 30 DIM. The Met and CHOL supplements were both top-dressed from −21 ± 2 to 30 DIM once daily at the a.m. feeding using approximately 50 g of ground corn as carrier for all treatments. Supplementation of SMA (0.08% DM of TMR offered) was calculated daily for each cow. Smartamine M was supplied as small beads containing a minimum of 75% DL-Met, physically protected by a pH-sensitive coating, which is considered to have a Met bioavailability of 80% (Graulet et al., 2005); therefore, per 10 g of SMA, the cows received 6 g of metabolizable Met. With Met supplementation, the amount of Met in prepartal diets increased from 1.86 to 2.37% of MP. Similarly, the amount of Met in postpartal diets also increased from 1.79 to 2.30% of the MP. The REA supplement is

reported to contain 28.8% choline chloride and is protected by microencapsulation. Although the product is considered to have CHOL bioavailability of 72% (Benoit, 2009; i.e., per 60 g of REA the cows should have received 12.4 g of metabolizable choline chloride), a recent study estimated the relative bioavailability (i.e., net absorption of choline in ReaShure from intestine) at 8% (de Veth et al., 2016). Thus, using that value to calculate the amount of choline that was bioavailable from ReaShure in our study gives 1.03 g of free choline (60 g/d ReaShure × 28.8% choline-chloride content × 74.6% choline in choline-chloride × 8% bioavailability). Clearly, these discrepancies should be taken into account when evaluating the present data in the context of choline adequacy. To our knowledge, neither SMA nor REA have specific characteristics that may affect palatability of diets.

The LFI was determined for all cows based on plasma concentration of albumin, cholesterol, and bilirubin, as described in Bertoni and Trevisi (2013), and this used to rank the cows retrospectively. The LFI calculation was carried out in 2 steps. First, we considered the concentration values (V) of the 3 parameters detected on d 3 (**V3**) and changes in concentrations between d 3 and 28 (**V28**). The albumin and cholesterol index was calculated as 0.5 × V3 + 0.5 × (V28 − V3), with albumin and cholesterol level on d 3 postpartum representing 50% and the reduction between d 3 and 28 the remaining 50% of the partial LFI index. Similarly, the bilirubin index was calculated using concentration value as 0.67V3 + 0.33 (V28 − V3), with bilirubin level on d 3 postpartum representing 67% and the reduction between d 3 and 28 the remaining 33% of the partial LFI index. In the second step, the LFI was calculated according to the formula: LFI = [(albumin index − 17.71)/1.08 + (cholesterol index − 2.57)/0.43 − (bilirubin index − 6.08)/2.17] (Zhou et al., 2016b). Cows were ranked retrospectively according to LFI and assigned to a low- (**LLFI**) and a high-LFI group (**HLFI**) regardless of Met or CHOL supplementation. Cows with a positive LFI (31 cows, mean ± SEM: 2.04 ± 0.24) were considered as the HLFI group and cows with a negative LFI (9 cows, mean ± SEM: −3.63 ± 0.76) were considered as the LLFI group.

Animal Management

Full details have been reported previously (Zhou et al., 2016d). Briefly, dry cows were housed in a ventilated enclosed barn during the dry period and fed individually once daily at 0630 h using an individual gate system (American Calan Inc., Northwood, NH). After parturition, cows were housed in a tiestall barn and were fed a common lactation diet once daily. Feed offered was adjusted daily to achieve ∼10% refusals.

Feed and Milk Sample Collection and Analyses

Full details have been reported previously (Zhou et al., 2016d). Briefly, DMI and milk yield were recorded daily. Consecutive morning, midday, and evening milk samples were taken weekly until 30 DIM. Composite milk samples were prepared in proportion to milk yield at each milking, preserved (800 Broad Spectrum Microtabs II; D & F Control Systems Inc., San Ramon, CA), and analyzed for contents of fat, protein, lactose, SNF, MUN, and SCC by mid-infrared procedures in a commercial laboratory (Dairy Lab Services, Dubuque, IA).

Blood and Liver Sample Collection and Biomarker Analyses

Full details have been reported previously (Zhou et al., 2016a). Briefly, blood was sampled for biomarker analysis from the coccygeal vein on −10 d relative to expected calving date and on 4, 8, 20, and 30 d relative to actual calving date before the morning feeding. For plasma AA and derivative analyses, blood was sampled from the coccygeal vein on −30 and −10 d relative to expected parturition date and on 4, 14 and 28 d relative to actual parturition date before the morning feeding. Samples were collected into evacuated tubes (BD Vacutainer; BD and Co., Franklin Lakes, NJ) containing either clot activator or lithium heparin for serum and plasma, respectively. Liver was sampled via puncture biopsy from cows under local anesthesia at approximately 0800 h on d −10, 7, 20, and 30 d relative

Table 1. Frequency of peripartal multiparous Holstein cows supplemented (+) or not (–) with rumen-protected Met and choline (CHOL) in low- (LLFI) or high-liver functionality index (HLFI) groups

	Met		CHOL		*P*-value		
Item	+Met	–Met	+CHOL	–CHOL	Met	CHOL	Met × CHOL
LLFI	2	7	5	4	0.06	0.71	0.80
HLFI	18	13	15	16			

Table 2. Frequency of occurrence of health problems during the peripartal period in multiparous Holstein cows retrospectively grouped into low (LLFI) or high liver functionality index (HLFI)

Variable	LLFI	HLFI	*P*-value
Cows	9	31	—
Ketosis[1]	6	0	<0.01
Displaced abomasum	1	0	0.06
Retained placenta[2]	2	2	0.17
Mastitis	0	1	0.59

[1]Defined as cows having moderate (~40 mg/dL) or large ketone concentrations (>80 mg/dL) in urine and treated by veterinarians with oral propylene glycol or intravenous dextrose.

[2]Defined as fetal membranes retained >24 h after calving.

to parturition (Zhou et al., 2016a). Liver was frozen immediately in liquid nitrogen and stored until analysis.

Plasma was analyzed for creatinine, bilirubin, aspartate aminotransferase, gamma-glutamyl transferase, cholesterol, paraoxonase (**PON**), albumin, ceruloplasmin, haptoglobin, myeloperoxidase, reactive oxygen metabolites, and ferric-reducing ability of plasma (**FRAP**) using kits purchased from Instrumentation Laboratory (Lexington, MA) following the procedures described previously (Jacometo et al., 2015) using the clinical auto-analyzer (ILAB 600, Instrumentation Laboratory). Bovine IL-1β (Cat. No. ESS0027; Thermo Scientific, Rockford, IL) plasma concentration was determined using commercial kits. Plasma total glutathione (**GSH**), as well as liver total and reduced GSH, were measured using a commercial kit (Cat. No. NWH-GSH01; Northwest Life Science Specialties LLC, Vancouver, WA).

Analyses of Plasma AA and Their Derivatives

Plasma was used to analyze the concentrations of free Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, Val, Asn, Asp, Ala, Glu, Gln, Gly, Pro, Ser, Tyr, Cit, carnosine, Orn, sarcosine, cystathionine, Cys, homocysteine, taurine, α-aminoadipic acid, α-aminobutyric acid, β-alanine, γ-aminobutyric acid, hydroxylysine, hydroxyproline, phosphoserine, 1-methyl histidine, and 3-methyl histidine at the University of Missouri Agriculture Experiment Station Chemical Laboratories (Columbia, MO) using HPLC (Deyl et al., 1986; Fekkes, 1996). The EAA pool included Arg, His, Ile, Leu, Lys, Met, Phe, Thr, Trp, and Val; the NEAA pool included Asn, Asp, Ala, Gln, Glu, Gly, Pro, Ser, and Tyr. Total AA (**TAA**) was the sum of EAA and NEAA; the total sulfur-containing compounds (**TSC**) included Met + Cys + cystathionine + homocysteine + taurine + GSH.

Statistical Analysis

Data were assessed for normality of distribution using the Shapiro-Wilk test. When the normality assumption was rejected, data were log-transformed before statistical analysis. Back-transformed data were reported in Tables and Figures for ease of interpretation. Data were analyzed using PROC MIXED of SAS 9.4 (SAS Institute Inc., Cary, NC). The model for data analysis contained LFI, day, and their interactions as fixed effects, and cow nested within LFI as random effect. For the analysis of all variables, parity was kept in the model as covariate when significant. For the analysis of various AA and derivatives, concentrations obtained at −30 d were maintained in the model as covariates when significant ($P < 0.05$). Amino acids and biomarkers in plasma and liver were analyzed at various time points that were not equally spaced. Therefore, the first-order antedependence covariance structure, ANTE (1), were used for repeated measures. Frequency of Met- and CHOL-supplemented cows in the HLFI or LLFI group and health data were analyzed with PROC GLIM-

Table 3. Dry matter intake and milk production in peripartal Holstein cows retrospectively grouped into low (LLFI) or high liver functionality index (HLFI)

				P-value		
Parameter (kg/d)	LLFI	HLFI	SEM[1]	LFI	Day	LFI × day
Prepartum						
DMI	11.59	14.62	0.74	<0.01	<0.01	0.68
Postpartum						
DMI	16.14	20.21	0.96	<0.01	<0.01	0.21
Milk yield	36.24	46.30	2.20	<0.01	<0.01	0.07
Fat (%)	3.90	3.56	0.20	0.15	<0.01	0.80
Protein (%)	3.12	3.29	0.11	0.18	<0.01	0.83
Milk fat yield	1.35	1.59	0.08	0.02	<0.01	0.34
Milk protein yield	1.09	1.48	0.07	<0.01	<0.01	0.06

[1]Greatest SEM.

MIX (distribution = binary and link = logit) of SAS. Least squares means separation between time points was performed using the PDIFF statement. Statistical differences were declared significant at $P \leq 0.05$ and tendencies at $P \leq 0.10$.

RESULTS

Distribution of Met- or CHOL-Supplemented Cows in LFI Groups

Distribution Met- or CHOL-supplemented cows in LLFI and HLFI groups is summarized in Table 1. Among the 20 Met-supplemented cows, only 2 cows had an LFI <0. In contrast, 7 cows without Met supplementation during the peripartal period were in the LLFI. Hence, although the majority (65%) of cows without Met supplementation ended up with a HLFI, a tendency for a greater ($P = 0.06$) number of Met-supplemented cows in the HLFI was observed. In contrast, peripartal CHOL supplementation resulted in a similar number of cows in the LLFI (4 vs. 5) and HLFI (15 vs. 16).

Animal Health and Performance

Health-related problems that occurred during the experiment and production performance are summarized in Table 2 and Table 3, respectively. As expected, cows with LLFI had greater incidence of health-related problems, as indicated by more cases of ketosis ($P < 0.01$) and a tendency for more cases of displaced abomasum (1 vs. 0; $P = 0.06$). Both prepartal ($P < 0.01$; Figure 1A) and postpartal ($P < 0.01$; Figure 1B) DMI was greater in cows with HLFI. Similarly, compared with LLFI cows, overall milk yield ($P < 0.01$; Figure 1C), milk protein yield ($P < 0.01$), and milk fat yield ($P = 0.02$) were also greater in cows with HLFI.

Biomarkers of Muscle Mass, Inflammation, Liver Function, and Oxidative Stress

Effects of LFI on blood and liver biomarkers are summarized in Table 4. Although plasma BHB did not differ between LLFI and HLFI cows, a tendency ($P = 0.09$) for lower fatty acid concentrations was observed in HLFI cows. Greater ($P = 0.05$; Figure 2A) circulating 3-methyl histidine concentration was observed in LLFI cows. In agreement with 3-methyl histidine, a tendency for lower ($P = 0.06$; Figure 2B) creatinine concentration was also shown in plasma of LLFI cows. As expected, greater albumin ($P < 0.01$; Figure 2C) and cholesterol ($P < 0.01$; Figure 2D), as well as lower

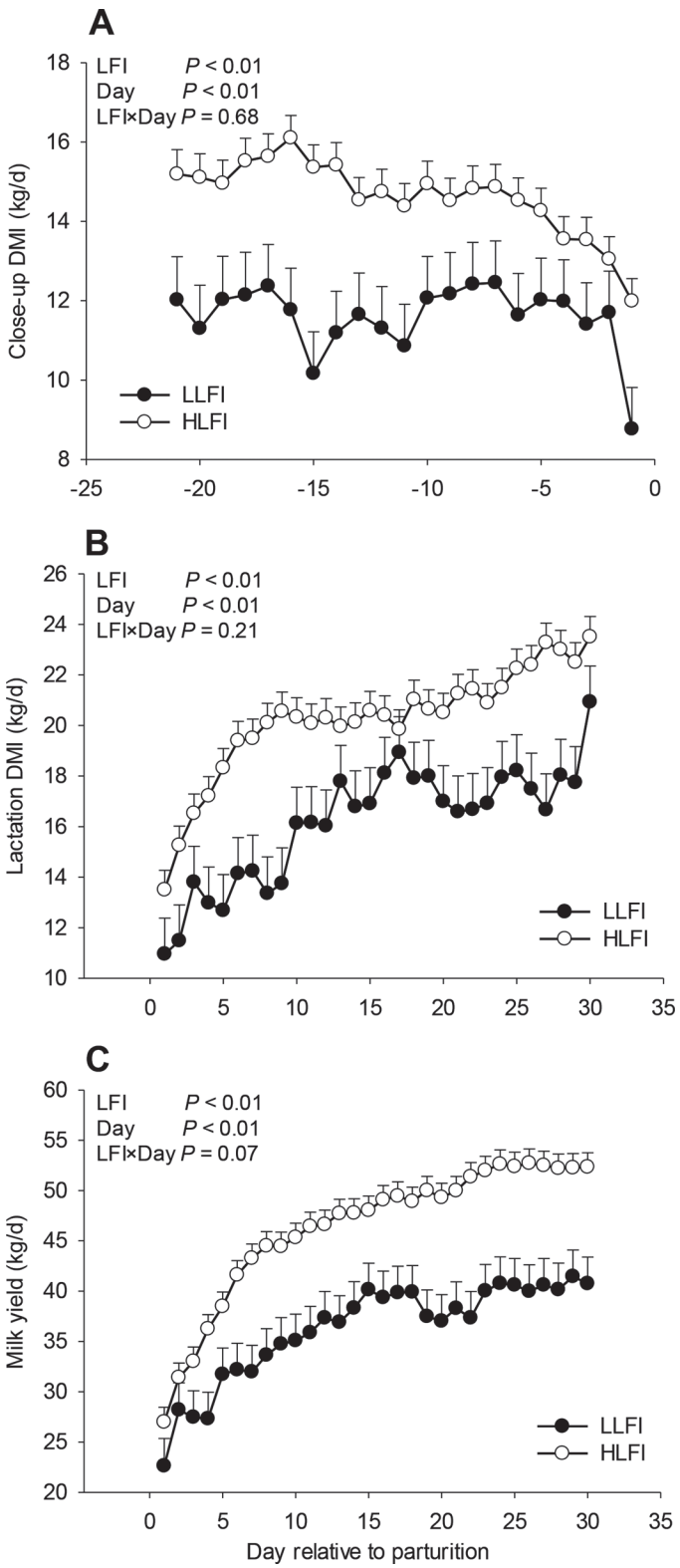

Figure 1. Dry matter intake and milk yield (means and SE) during the periparturient period in cows retrospectively grouped into high (H) and low (L) liver functionality indexes (LFI).

bilirubin ($P = 0.05$; Figure 2E), were observed in HLFI cows; in addition, overall plasma haptoglobin concentration also was lower ($P = 0.02$; Figure 2F) in HLFI cows. Although overall circulating concentrations did not differ between LLFI and HLFI cows, an LFI × day interaction ($P < 0.01$) was observed for ceruloplasmin, which increased to a greater extent in cows with LLFI on d 30 postpartum.

Among the oxidative stress biomarkers determined in the present study, HLFI cows had greater ($P < 0.01$; Figure 2G) overall circulating PON concentrations compared with LLFI cows. In agreement with PON, overall hepatic total ($P = 0.05$) and reduced ($P = 0.05$; Figure 2H) GSH levels in HLFI cows were doubled compared with cows in the LLFI group. Although overall plasma FRAP values did not differ between HLFI and LLFI cows, an LFI × day interaction ($P < 0.01$) was observed due to a greater level in LLFI cows on d 30 postpartum.

AA and Derivatives

Effects of LFI on plasma AA and derivatives are summarized in Table 5 and Table 6, respectively. Although similar ($P > 0.05$) levels of circulating EAA were observed in LLFI and HLFI cows, HLFI cows had greater circulating His ($P = 0.02$) and Trp ($P = 0.03$), as well as a tendency ($P = 0.06$) for greater plasma Ile. Similarly, plasma Met also was greater ($P = 0.01$; Figure 3D) in cows with HLFI due to more ($P = 0.06$) MET-supplemented cows in this group. Although overall plasma Arg did not differ between HLFI and LLFI cows, an LFI × day interaction ($P = 0.10$) was observed due to greater level of Arg on d 4 in HLFI cows. In contrast, an LFI × day interaction ($P = 0.02$) was observed due to a lower level of Phe on d 28 in HLFI cows.

Unlike EAA, circulating concentrations of most NEAA were either greater (Ala, Asp, Gln, Pro, and Ser; $P < 0.05$) or tended to be greater (Tyr; $P = 0.09$) in HLFI cows. In fact, despite similar circulating levels of EAA between LLFI and HLFI cows, overall circulating TAA was greater ($P = 0.01$; Figure 3A) in HLFI cows due mainly to greater ($P = 0.01$; Figure 3B) overall circulating NEAA concentrations in these cows. It is noteworthy that HLFI cows also tended to have greater ($P = 0.08$) overall percentage of Met in the total AA pool (Met as a percent of TAA). In contrast, an LFI

Table 4. Blood and liver biomarkers in peripartal Holstein cows retrospectively grouped into low or high liver functionality index (LFI)

	LFI			*P*-value		
Parameter	Low	High	SEM[1]	LFI	Day	LFI × day
Energy metabolism						
Glucose (mmol/L)	3.70	3.76	0.10	0.62	<0.01	0.01
Fatty acids (mmol/L)	0.55	0.46	0.05	0.09	<0.01	0.74
BHB (mmol/L)	0.80	0.76	0.07	0.55	<0.01	0.11
Muscle body mass						
Creatinine (μmol/L)	86.35	91.91	2.42	0.06	<0.01	0.28
3-Methyl histidine[2]	7.23	6.25	0.45	0.05	<0.01	0.71
Liver function markers[3]						
Bilirubin (μmol/L)	3.83	2.51	0.71	0.05	<0.01	0.61
GOT (U/L)	97.78	98.91	4.57	0.83	<0.01	0.36
Cholesterol (mmol/L)	2.60	3.35	0.14	<0.01	<0.01	<0.01
GGT (U/L)	25.07	22.72	2.34	0.36	<0.01	0.93
Inflammation and acute phase proteins						
Albumin (g/L)	34.36	36.46	0.48	<0.01	0.64	<0.01
Ceruloplasmin (μmol/L)	2.71	2.67	0.15	0.84	<0.01	<0.01
Haptoglobin (g/L)	0.37	0.25	0.05	0.02	<0.01	0.17
IL-1β (pg/mL)	5.49	4.67	1.08	0.48	<0.01	0.17
Oxidative stress						
Ferric-reducing ability of plasma (μmol/L)	131.81	132.59	4.28	0.87	0.08	0.05
Myeloperoxidase (U/L)	441.57	396.56	28.99	0.16	0.21	0.60
Paraoxonase (U/mL)	71.49	90.35	4.32	<0.01	<0.01	<0.01
Reactive oxygen metabolites (mg of H_2O_2/100 mL)	13.65	12.97	0.67	0.37	<0.01	0.19
Liver total glutathione (μmol/g of protein)	12.26	25.11	4.27	0.05	<0.01	0.97
Liver reduced glutathione (μmol/g of protein)	11.75	24.35	4.21	0.05	<0.01	0.98

[1]Greatest SEM.

[2]Log_2 back-transformed data reported.

[3]GOT = aspartate aminotransferase; GGT = gamma-glutamyl transferase.

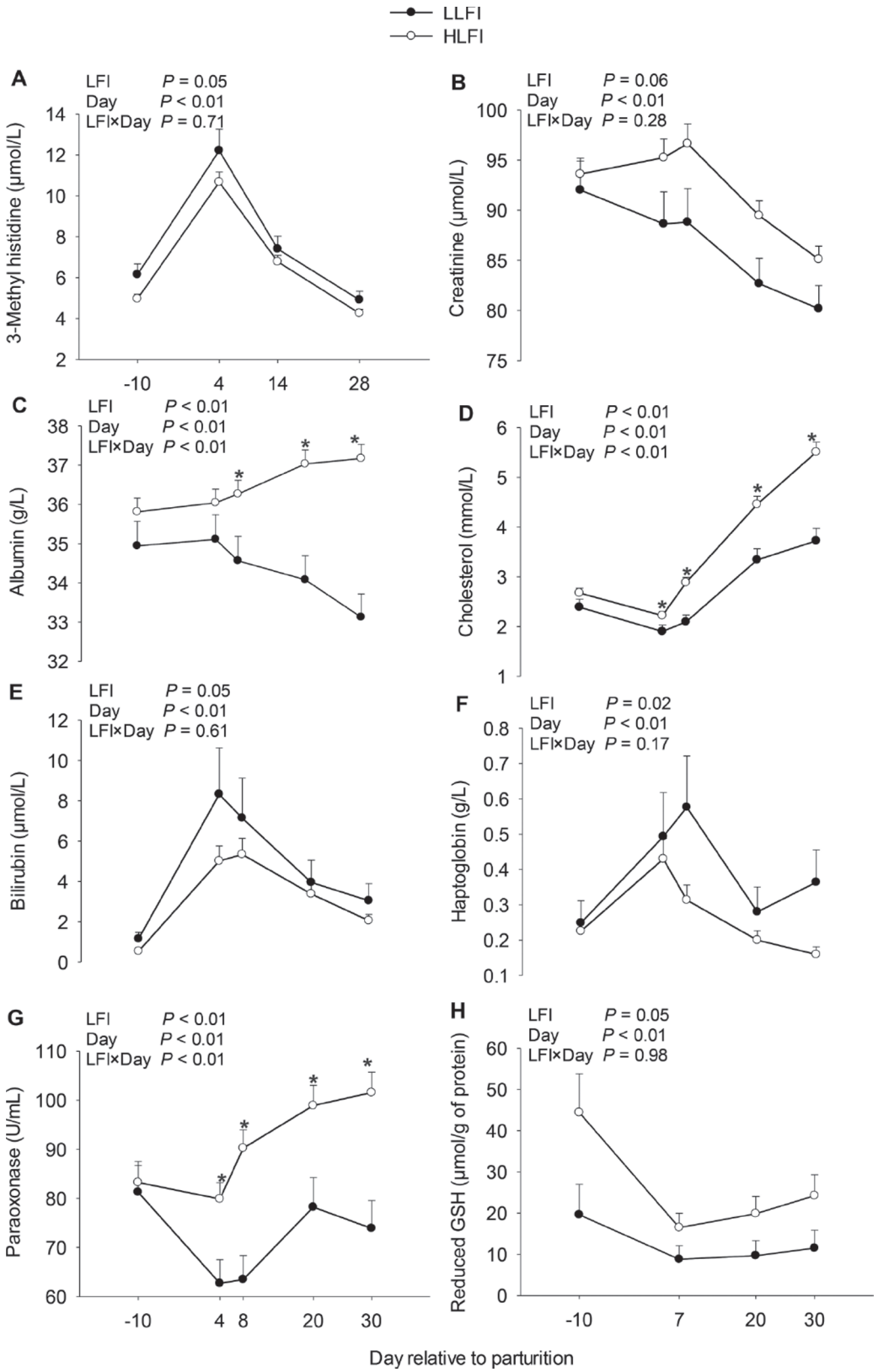

Figure 2. Plasma concentrations (LSM and SE) of 3-methyl histidine, creatinine, albumin, cholesterol, bilirubin, haptoglobin, paraoxonase, and hepatic-reduced glutathione (GSH) concentration during the periparturient period in cows retrospectively grouped into high (H) and low (L) liver functionality indexes (LFI). *$P < 0.05$ (LFI × day).

× day interaction was observed due to a lower ($P = 0.03$) percentage of Lys in the total AA pool (Lys as a percent of TAA) on d 4 in HLFI cows.

As expected, greater ($P < 0.01$; Figure 3F) homocysteine and a tendency ($P = 0.07$; Figure 3E) for greater cystathionine were observed in HLFI cows, likely due to more Met-supplemented cows in this group. Although overall taurine concentration was similar between HLFI and LLFI cows, an LFI × day interaction was observed due to a greater ($P = 0.03$) concentration of taurine on d −10 in HLFI cows (Figure 4). In addition, the proportion of Met in the TSC also was greater ($P = 0.05$; Figure 3C) in the HLFI group due to greater circulating Met in these cows. Consequently, overall circulating level of TSC also was greater in HFLI compared with LLFI cows.

Besides taurine, concentration of carnosine, another antioxidant, also was greater ($P = 0.01$) in cows with HLFI. Although overall plasma α-aminobutyric acid and γ-aminobutyric acid concentrations were similar between LLFI and HLFI cows, an LFI × day interaction was observed due to higher concentrations of α-aminobutyric acid ($P = 0.01$) and γ-aminobutyric acid ($P = 0.03$) on d 14 in HLFI cows. Although a main effect of LFI for Orn was not observed, greater Cit ($P < 0.01$) and an interaction ($P = 0.10$) for greater circulating urea on 28 d were observed in HLFI cows in addition to greater circulating concentration of Arg on 4 d in HFLI cows.

DISCUSSION

Health and Performance in Cows with HLFI and LLFI

Compared with LLFI (LFI <0), cows with HLFI (LFI >0) are considered to be at a lesser risk of health problems during the peripartal period (Bertoni and Trevisi, 2013; Zhou et al., 2016b). The fact that none of the 31 cows in the HLFI group were ketotic or experienced displaced abomasum confirmed the usefulness,

Table 5. Plasma proteinogenic AA concentrations in peripartal Holstein cows retrospectively grouped into low or high liver functionality index (LFI)

	LFI			*P*-value		
AA (μ*M*, unless noted)	Low	High	SEM[1]	LFI	Day	LFI × day
EAA						
Arginine[2]	54.95	58.29	2.47	0.26	<0.01	0.10
Histidine	50.93	55.14	1.58	0.02	0.01	0.28
Isoleucine[2]	89.39	102.66	5.56	0.06	0.30	0.24
Leucine[2]	147.88	160.40	9.20	0.26	0.15	0.52
Lysine[2]	62.74	65.34	3.57	0.53	0.03	0.14
Methionine	19.28	25.11	1.95	0.01	0.13	0.27
Phenylalanine[2]	48.98	46.26	1.63	0.14	<0.01	0.02
Threonine	70.68	77.04	3.73	0.14	0.09	0.88
Tryptophan	21.49	24.27	1.05	0.03	<0.01	0.48
Valine[2]	219.67	240.28	12.90	0.19	0.18	0.42
BCAA[2,3]	458.86	504.08	27.44	0.18	0.13	0.40
EAA[2]	789.06	858.75	36.42	0.12	0.04	0.31
Met, % EAA	2.42	2.77	0.18	0.11	0.01	0.54
Lys, % EAA	7.71	8.07	0.28	0.26	0.23	0.11
NEAA						
Alanine	177.09	202.63	7.74	0.01	<0.01	0.62
Asparagine	34.28	40.70	1.78	<0.01	<0.01	0.59
Aspartate[2]	4.41	4.52	0.30	0.75	<0.01	0.30
Glutamate[2]*	36.94	37.17	1.36	0.88	<0.01	0.11
Glutamine	235.01	258.06	9.06	0.03	<0.01	0.76
Glycine[2]	369.88	393.41	19.26	0.30	<0.01	0.72
Proline	69.25	77.02	2.96	0.03	<0.01	0.99
Serine	81.69	89.54	3.24	0.04	<0.01	0.45
Tyrosine	38.47	43.16	2.34	0.09	<0.01	0.10
NEAA	1,076.99	1,175.86	31.12	0.01	<0.01	0.70
TAA[4]	1,884.92	2,054.00	54.12	0.01	<0.01	0.86
Met,[2] % TAA	1.01	1.18	0.08	0.08	<0.01	0.54
Lys, % TAA	3.45	3.31	0.16	0.45	<0.01	0.03

[1]Greatest SEM.

[2]Log_2 back-transformed data reported.

[3]BCAA = branched-chain AA.

[4]TAA = total AA.

*$P < 0.05$.

in the present study, of LFI in identifying cows at risk, even when metabolically important feed additives such as Met or CHOL are supplemented around parturition. Considering Met supplementation led to a lower incidence of ketosis (Zhou et al., 2016d), and that more Met-supplemented cows were in the HLFI group, the overall lower incidence of ketosis observed in HLFI cows was likely due to Met supplementation.

In agreement with the better lactation performance benefits observed in HLFI cows, recent results from our group have demonstrated greater pre- and postpartum DMI, milk fat yield, and milk protein yield in response to peripartal Met, but not CHOL, supplementation (Zhou et al., 2016d). Considering only a tendency for greater milk yield was detected in a previous LFI study that did not involve Met or CHOL supplementation (Zhou et al., 2016b), the differences observed between LLFI and HLFI cows were due in large part to peripartal Met supplementation.

Inflammation and Oxidative Stress in Cows with HLFI and LLFI

Acute phase proteins of liver origin serve as useful biomarkers to evaluate chronic inflammation mainly due to the decrease of circulating concentrations of negative acute phase proteins (e.g., albumin) and the contemporary increase of positive acute phase proteins (e.g., haptoglobin; Bertoni et al., 2008, Ceciliani et al., 2012). Therefore, the substantially greater plasma concentration of albumin (2.1 g/L) and lower haptoglobin (0.12 g/L) in HLFI cows was suggestive of a less pronounced inflammatory status around parturition.

Inflammatory biomarkers in plasma are increased in the circulation of ketotic cows around parturition (Abuajamieh et al., 2016). Hence, the fact that none of the cows in the HLFI group were ketotic may have greatly contributed to the differences in inflammation status observed between the 2 cohorts of cows. In addition, rodent research revealed that limiting Met and CHOL in ketogenic diets increased expression of fatty acid oxidation and inflammatory genes, supporting a potential role of Met and CHOL supplementation on ketosis-related inflammation (Pissios et al., 2013). In agreement with this, Met-supplemented cows experienced fewer cases of ketosis and had greater plasma concentrations of albumin, cholesterol (index of lipoproteins), and lower haptoglobin (Zhou et al., 2016a,c), suggesting Met supplementation had direct bearing on the lower inflammatory status in HLFI cows. In con-

Table 6. Plasma AA derivative concentrations in peripartal Holstein cows retrospectively grouped into low or high liver functionality index (LFI)

	LFI			*P*-value		
Parameter (μM)	Low	High	SEM[1]	LFI	Day	LFI × day
AA and derivatives						
1-Methyl histidine	15.79	17.10	0.96	0.24	<0.01	0.86
α-Aminoadipic acid[2]	7.09	7.95	0.48	0.14	<0.01	0.03
α-Aminobutyric acid[2]	16.06	18.06	1.20	0.18	<0.01	0.01
β-Alanine[2]	8.79	8.71	0.47	0.87	0.94	0.60
γ-Aminobutyric acid	2.33	3.13	0.49	0.16	<0.01	0.03
Carnosine[2]	14.36	18.93	1.29	0.01	<0.01	0.05
Citrulline[2]	67.12	80.24	3.43	<0.01	<0.01	0.88
Hydroxylysine[2]	0.35	0.31	0.04	0.35	0.76	0.75
Hydroxyproline	15.87	15.10	0.70	0.34	<0.01	0.01
Ornithine[2]	29.45	30.86	1.87	0.52	<0.01	0.15
Phosphoserine[2]	5.93	6.28	0.27	0.28	0.02	0.80
Sarcosine[2]	9.31	9.92	0.74	0.49	<0.01	0.60
Urea	4,246.54	4,412.14	176.69	0.41	0.01	0.10
Sulfur-containing compounds						
Cystathionine	1.64	1.93	0.13	0.07	<0.01	0.11
Cysteine	8.18	8.68	0.63	0.49	<0.01	0.71
Glutathione	4.25	4.11	0.38	0.76	0.03	0.56
Homocysteine	3.77	5.30	0.43	<0.01	0.02	0.64
Taurine	37.07	41.02	2.74	0.21	<0.01	0.04
TSC[3]*	75.22	86.57	4.92	0.05	0.06	0.29
Met (% TSC)	26.04	28.83	1.21	0.05	0.01	0.01

[1]Greatest SEM.

[2]Log_2 back-transformed data reported.

[3]Total sulfur-containing compounds (TSC) included Met + Cys + cystathionine + homocysteine + taurine + glutathione.

*$P < 0.05$.

trast, CHOL supplementation was not associated with changes in the incidence of ketosis or plasma albumin, cholesterol, and haptoglobin concentrations.

Apart from disease-induced inflammation around parturition, the lack of proper antioxidant defenses renders oxidative stress as another significant contributing factor to systemic inflammation (Sordillo et al., 2009). The fact that a hepatic intracellular antioxidant (GSH), a circulating antioxidant enzyme (PON), the FRAP-to-reactive oxygen metabolite ratio, and a circulating dipeptide (carnosine) with antioxidant properties were all greater in HLFI cows indicated an overall less pronounced state of oxidative stress (Bionaz et al., 2007; Boldyrev et al., 2013). From a mechanistic standpoint, the fact that in vivo synthesis of GSH ultimately requires sulfur from Met supports the view that the greater hepatic GSH and reduced GSH concentrations in HLFI cows were a result of the greater number of Met-supplemented cows in this group. In agreement with this, as a nonsulfur-containing compound, peri-

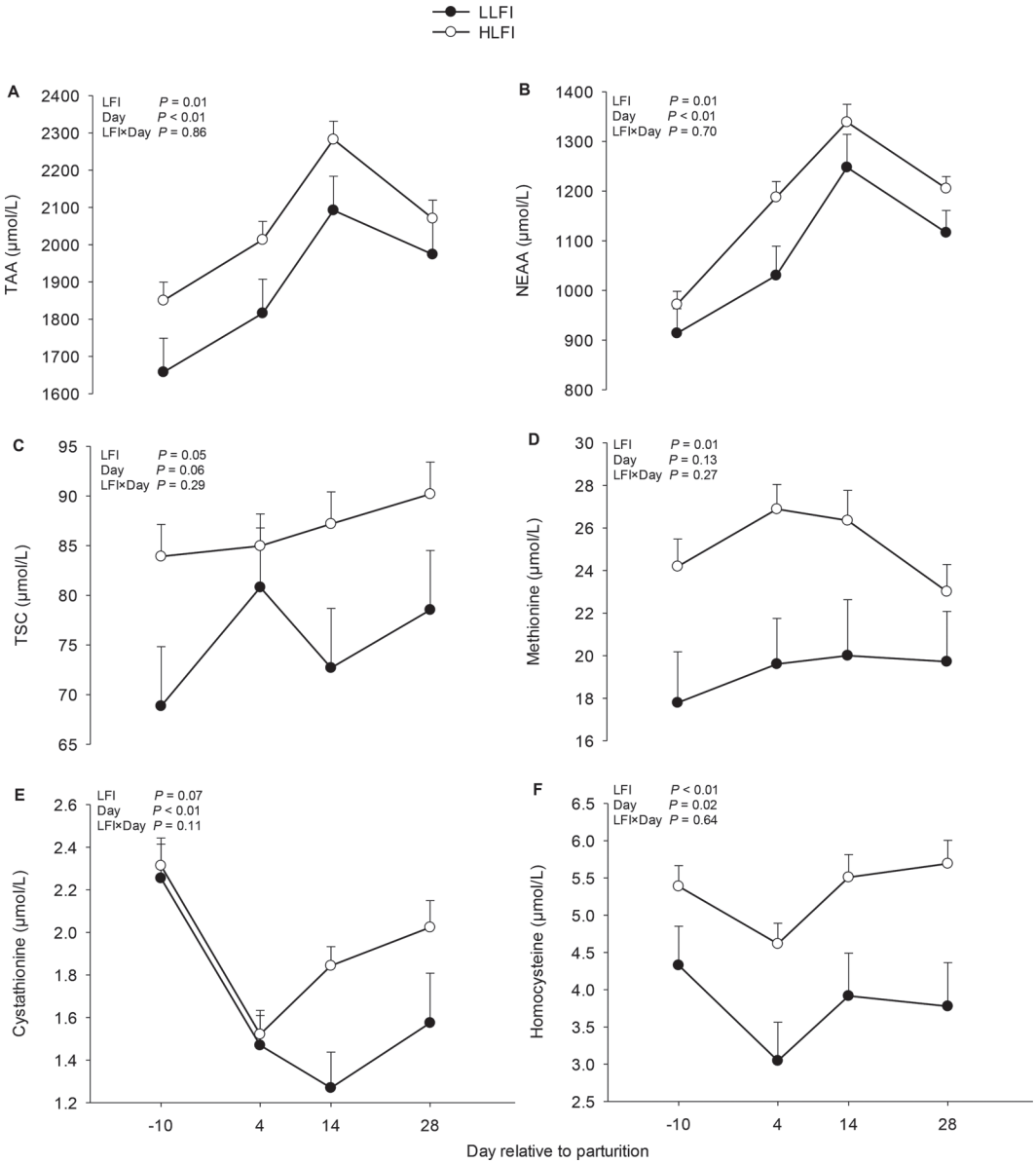

Figure 3. Plasma concentrations (means and SE) of total AA (TAA), NEAA, total sulfur-containing compounds (TSC), methionine, cystathionine, and homocysteine during the periparturient period in cows retrospectively grouped into high (H) and low (L) liver functionality indexes (LFI).

partal CHOL supplementation did not seem to increase hepatic sulfur-containing antioxidant synthesis through the transsulfuration pathway (Zhou et al., 2017). In fact, an increase in plasma cysteine in response to peripartal CHOL supplementation was likely achieved at the expense of Met (Zhou et al., 2016c). As such, we speculate that peripartal Met, but not CHOL, supplementation led to less pronounced oxidative stress status in HLFI cows by enhancing sulfur-containing antioxidant synthesis through the transsulfuration pathway.

It is noteworthy that, although differences in plasma bilirubin and cholesterol concentrations indicate a less-than-optimal liver function in LLFI cows, plasma aspartate aminotransferase and gamma-glutamyl transferase concentrations were similar between LLFI and HLFI cows and within the range considered normal for healthy cows (Bertoni et al., 2008), indicative of the absence of liver damage. Similarly, although LLFI cows had lower hepatic GSH and plasma PON, suggesting a less-than-optimal oxidative stress status, the fact that concentrations of FRAP, myeloperoxidase, and reactive oxygen metabolites in LLFI cows were similar to HLFI cows indicates that they were not suffering from severe systemic oxidative stress. Thus, although biomarker analysis in the present study indicated that LLFI cows were in a more challenging physiologic state, they were still in better overall health than those reported in previous research design to test the validity of the LFI index (Bionaz et al., 2007; Bertoni et al., 2008).

AA Profile in Cows with HLFI and LLFI

Around parturition the decrease in DMI coupled with the increase in AA requirements to sustain fetal growth and lactation lead to greater tissue protein mobilization, evidenced in part by the highest plasma 3-methyl histidine at 4 d regardless of LFI. However, the lower degree of tissue catabolism, indicated by lower 3-methyl histidine, along with the greater muscle mass, suggested by greater circulating creatinine, also revealed a lesser degree of tissue mobilization around parturition in HLFI cows. Whether such response was due to a more desirable circulating AA profile or an endocrine response induced by differences in AA is unknown. We speculate that the greater concentrations of various proteinogenic circulating EAA (His, Ile, Met, and Trp), NEAA (Ala, Asn, Gln, Pro, Ser, and Tyr), and nonproteinogenic AA (Cit) in HLFI cows not only reflect the greater pre- and postpartum DMI, but also less reliance on skeletal muscle to provide substrates for milk protein synthesis and gluconeogenesis. It remains to be determined at a more mechanistic levels what adaptations might have been induced in the skeletal muscle when Met was supplemented. In addition, the fact that concentration of circulating AA was lower in LLFI cows supports data demonstrating greater consumption of AA by the liver of steers (Burciaga-Robles, 2009) and sheep (Hoskin et al., 2016) during inflammatory events. In fact, in steers challenged with *Mannheima haemolytica* the net uptake of total AA by the liver after the challenge was estimated at ~380 g of AA (Burciaga-Robles, 2009), which at a 0.67 efficiency would be equivalent to the true protein in 8 kg of milk.

It is noteworthy that the proportion of Met in TAA (Met as a percent of TAA) was greater in HLFI cows. Concomitantly, the overall greater cystathionine and homocysteine in addition to Met were largely responsible for the greater circulating total sulfur-containing compounds. Considering that an increase in sulfur-containing compounds in a previous LFI study without Met supplementation was not observed (Zhou et al., 2016b), and that more cows with Met supplementation ended up in the HLFI group, the increase in circulating sulfur-containing compounds were likely the result of MET supplementation. Although CHOL could potentially achieve similar outcomes by promoting in vivo synthesis of Met, recent data from our group have demonstrated that in vivo CHOL supplementation did not seem to promote MET synthesis in liver, a response that could have been due to a limited level of sulfur-containing substrate around parturition (Zhou et al., 2017).

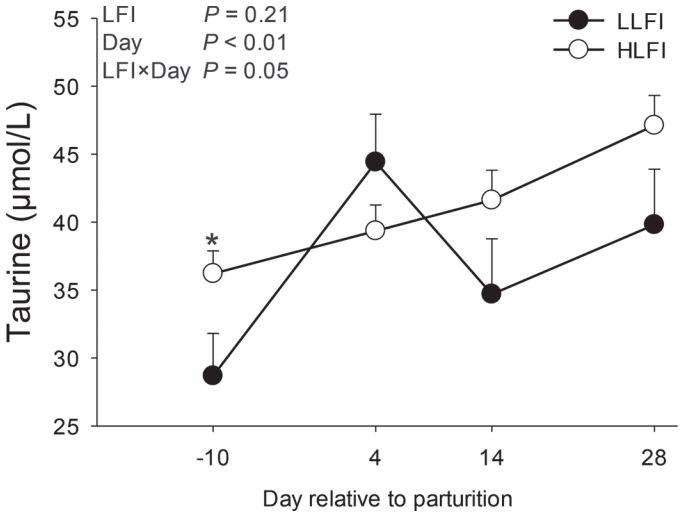

Figure 4. Plasma concentrations (means and SE) of taurine during the periparturient period in cows retrospectively grouped into high (H) and low (L) liver functionality indexes (LFI). *$P < 0.05$ (LFI × day).

CONCLUSIONS

Overall, the lower incidence of health complications, better lactation performance, and more favorable biomarker and plasma AA profiles in HLFI cows indicate a better immunometabolic status during the peripartal period. As more Met-supplemented cows were in the HLFI group, the differences observed between the 2 cohorts were due in large part to peripartal Met supplementation. Although CHOL supplementation is expected to achieve similar benefits as Met in terms of immunometabolic status, the fact that CHOL supplementation failed to improve LFI, at least in the present study, did not support this notion. However, if, as reported recently, the CHOL bioavailability from ReaShure meant that only a small fraction of the targeted dose was available to tissues, it is still possible that greater CHOL levels are able to affect LFI in a positive fashion. The LFI was able to differentiate effectiveness between Met and CHOL feeding, thus supporting its broader application in the management of transition cows. Further studies on the applicability of these biomarkers to monitor transition success appear warranted.

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