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Circulating amino acids during the peripartal period in cows with different liver functionality index

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ABSTRACT

The liver functionality index (LFI) measures the changes of albumin, cholesterol, and bilirubin concentrations between 3 and 28 d postpartum. This composite index, based on variables with direct relevance to liver-specific plasma protein synthesis (albumin), hepatic/intestinal lipoprotein synthesis (cholesterol), and clearance of breakdown products of heme catabolism (bilirubin), provides a tool for evaluating manifestations of hepatic disease. Both energy and protein metabolism are likely to be affected by various physiological challenges in this period but have not been tested systematically. The present study was conducted to profile AA in cows with high or low LFI during the peripartal period and relate this to production outcomes. Eighteen multiparous cows were used from –21 through 28 d around parturition and divided retrospectively into the high or low LFI group. Blood samples were obtained on –21, –14, –7, 1, 3, 7, 10, 14, 17, 21 and 28 d relative to calving, and biomarkers and AA in plasma were measured. Grouping based on LFI resulted in 8 cows with high LFI (HLFI) and 10 cows with low LFI (LLFI). Although the temporal response in dry matter intake (DMI, 16.3 kg/d) and body condition score (2.56) did not differ, cows with high compared with low LFI had greater overall milk production (37.9 vs. 32.9 kg/d) although energy-corrected milk yield did not differ (42.6 vs. 38.7 kg/d). As expected, cows grouped as LLFI had lower cholesterol and albumin but greater bilirubin after calving compared with HLFI animals. Despite similar temporal responses in DMI between groups, concentrations of total AA were greater in HLFI, particularly after calving. Although concentrations of total essential AA (EAA) and branched-chain AA did not differ with LFI status, cows in HLFI had greater concentrations of Thr and Ile postpartum. Nearly all plasma AA con-

centrations followed the general trend of a nadir at 1 d after calving followed by a gradual increase to prepartal levels before 28 d. Glycine was the only AA exhibiting a gradual increase in concentration through the transition, with a maximum at 7 d postpartum followed by a gradual decrease. We detected no effect of LFI status on plasma Lys, which decreased markedly from –21 d to calving, followed by an increase to prepartal values by d 7. In contrast, concentrations of Met and His decreased markedly between –21 and 10 d and did not reach prepartal values by 28 d. The marked decrease in Gln concentration after calving regardless of LFI might compromise immune function during this period. Overall, the results indicate the existence of an association among inflammation, liver function postpartum, and AA plasma concentrations, irrespective of temporal differences in DMI. Cows with better indices of liver function produced more milk and maintained greater concentrations of total AA and some EAA such as Thr and Ile. Whether these AA played a direct role in the greater milk production remains to be determined.

Key words: transition period, nutrition, liver function, amino acid metabolism

INTRODUCTION

The periparturient or transition period, defined as 3 wk before through 3 wk after parturition, is the most challenging period of the lactation cycle (Drackley, 1999). Cows are susceptible to various health complications during this time because of marked changes in endocrine status and immunosuppression, followed by decreased feed intake (Mallard et al., 1998; Drackley, 1999). Inflammatory responses occur during the periparturient period and may impair liver function and performance of dairy cows even without serious infections or other pathologies (Bionaz et al., 2007; Bertoni et al., 2008). Therefore, multiple indices have been proposed to assess the metabolic, immune, and inflammatory status of dairy cows in the periparturient period (Bertoni and Trevisi, 2013).

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Using changes in plasma concentrations of albumin, cholesterol, and bilirubin, the liver functionality index (**LFI**) characterizes the extent of the inflammatory response and helps predict its likely consequences on health and well-being of the cow (Bertoni and Trevisi, 2013). Previous work utilizing blood and milk samples has confirmed the usefulness of this approach to assess immune and inflammatory status (Trevisi et al., 2010b). For instance, a low LFI (**LLFI**) value is indicative of a pronounced inflammatory response, suggestive of a more difficult transition from gestation to lactation, whereas a high LFI (**HLFI**) is suggestive of a smooth transition (Trevisi et al., 2012).

Other than the synthesis of acute-phase proteins (**APP**) such as haptoglobin and ceruloplasmin, both the needs of the fetus prepartum and the needs of the mammary gland postpartum greatly increase the demand for AA. It is known, however, that supplies of MP and EAA are limiting around calving (Bell et al., 2000). Therefore, cows that adapt more successfully to the onset of lactation may have a better AA plasma profile. Only a few studies have attempted to characterize the AA profile during the periparturient period (Verbeke et al., 1972; Meijer et al., 1995; Doepel et al., 2002; Maeda et al., 2012; Hailemariam et al., 2014) and none in the context of LFI. Therefore, the hypothesis examined in the current study was that AA profiles would differ between cows with HLFI and LLFI.

MATERIALS AND METHODS

This study complied with Italian laws on animal experimentation and ethics.

Animal Management and Experimental Design

The experiment was carried out in the tiestall barn of the Istituto di Zootecnica (Piacenza, Italy) using 18 multiparous Holstein Friesian cows (average lactation number of 3.22 ± 1.11). During the dry period, all cows received the same diet prepartum: 6 to 8 kg of corn silage, 2 kg of dehydrated alfalfa, 9 to 10 kg of dehydrated grass, and 1 kg of concentrate. From 7 d before calving to calving, cows received an additional 1 kg of concentrate. From calving through 30 DIM, cows received a diet containing corn silage, increased at a rate of 2 kg every 4 d up to a maximum of 20 to 22 kg, 3 kg of dehydrated alfalfa, 2 kg of dehydrated grass, and a concentrate mix increased at a rate of 0.5 kg daily up to a maximum of 12 to 14 kg.

Feed intake of individual cows was measured daily. Gross analysis and nutritive value of feeds were evaluated in representative samples collected twice a week for forages, and every 2 wk for corn silage and the

concentrate. Chemical composition of the feeds was determined using standard procedures (AOAC International, 2000). Body weight was measured every 2 wk throughout the study and BCS was estimated. Milk yield at each milking was weighed and recorded from calving to 30 DIM. Based on milk sample analysis, the ECM (at 3.5% fat) was calculated as follows: $ECM = [12.82 \times \text{fat yield (kg)}] + [7.13 \times \text{protein yield (kg)}] + [0.323 \times \text{milk yield (kg)}]$ (Hutjens, 2010).

The LFI was determined for all cows based on plasma concentrations of albumin, cholesterol, and bilirubin, as described in Bertoni and Trevisi (2013), and used to rank the cows retrospectively. The LFI calculation is carried out in 2 steps; the first considers 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 indices were calculated as $0.5V3 + 0.5(V28 - V3)$. The bilirubin index was calculated 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, these partial indices were standardized according to average values observed in "healthy" cows within the same herd, and LFI was calculated according to the following formula: $LFI = [(\text{albumin index} - 17.71)/1.08 + (\text{cholesterol index} - 2.57)/0.43 - (\text{bilirubin index} - 6.08)/2.17]$. Cows with a positive LFI (mean: 1.65 ± 0.11) were considered as the HLFI group and cows with negative LFI (mean: -3.69 ± 0.64) were considered as the LLFI group.

Blood Sampling and Analysis

Blood samples were collected before concentrate and forages were offered at 0700 h from the jugular vein into heparinized vacuum tubes on -21, -14, -7, 1, 3, 7, 10, 14, 17, 21, and 28 d relative to calving. After collection, tubes were placed on ice and plasma was obtained by centrifugation at $1,900 \times g$ for 15 min at 4°C. Aliquots of plasma was frozen (-20°C) until further analysis. Metabolic biomarkers [haptoglobin, ceruloplasmin, Zn, Ca, globulin, albumin, cholesterol, bilirubin, γ -glutamyl transpeptidase (**GGT**), lactate dehydrogenase, γ -glutamyl transpeptidase (**GOT**), glucose, fatty acids, BHB, triglycerides, urea, creatinine, Mg, Na, Cl, K, and P] were analyzed by methods described in Piccioli-Cappelli et al. (2014).

Concentrations of AA (Ala, Gly, Val, Leu, Ile, Pro, Met, Ser, Thr, Phe, Asp, Cys, Glu, Lys, His, Gln, Tyr, Trp) were determined in plasma samples at the Rowett Institute for Nutrition and Health (Aberdeen, UK) by isotope dilution coupled with GC-MS (Calder et al., 1999). The area under the curve (**AUC**) approach

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