Effects of Heat Stress and Dietary Organic Acids and Botanicals on Hepatic One-carbon Metabolism

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Introduction

Reductions in milk protein content and yield motivated to investigate the impact of heat exposure on protein metabolism in dairy cows (Gao et al., 2017). McGuire et al., (1989) confirmed that heat stress (HS) reduces the intestine absorptive capacity of amino acids (AA), and this is probably explained by the loss in intestinal integrity (Koch et al., 2019). Very recently, our group has demonstrated that heat-stressed lactating dairy cows develop with an increased total-tract gut permeability (Fontoura et al., 2022). This condition leads to leakage of bacteria and their endotoxin (e.g., lipopolysaccharide [LPS]) into the bloodstream, which in turn triggers an immune response. This is associated with hepatic removal and utilization of AA to produce acute phase and heat-shock proteins (Rius et al., 2019). In addition, the activation of the immune system increases glucose consumption (Kvidera et al., 2017). It is well known that heat-stressed dairy cows have reduced feed intake, which partially explains the lowered production responses (Baumgard and Rhoads, 2013). Despite this hypophagia, increasing levels of circulating insulin concentrations are common in heat-stressed cows (Wheelock et al., 2010; Fontoura et al., 2022). Insulin is a hormone that inhibits lipolysis and might induce muscle protein breakdown to support gluconeogenesis. The end product of AA catabolism is urea. Robust increases in plasma levels of urea-nitrogen are a repeatedly observed response in heat-stressed dairy cows (Wheelock et al 2010; Gao et al., 2017; Fontoura et al., 2022).

Excessive circulating urea can cause toxicity, even in ruminants (Whitehair, 1989). Urea can damage cells by disrupting the osmotic balance and as a consequence require osmoprotective responses to counteract it. Research from human and rodent species tells us that under hyperosmotic conditions, liver and kidney cells accumulate methylamine osmolytes such as betaine or glycerophosphocholine (GPC; Okazaki et al., 2018). The abundance of betaine transporters increases under osmotic stress (Kempson et al., 2014). In response to changing levels of NaCl and urea, Burg and Gallazzini (2009) identified a reduction in the activity of glycerophosphocholine phosphodiesterase (GPC-PDE), the enzyme that degrades GPC to choline, and as a result they observed an intracellular accumulation of GPC. The literature reports higher accumulations of GPC rather than betaine, and presumably it is due to a lower metabolic cost. The inhibition of an enzyme doesn't require extra energy whereas betaine transporters are against gradient concentration (Burg and Peters, 1998).

GPC is synthesized from the degradation of phosphatidylcholine (PC) and broken down into choline and α-glycerophosphate. The inhibition of GPC-PDE can

reduce choline recovery and negatively affect the CDP pathway to support PC synthesis. Choline also has a one-carbon unit that is called methyl group, which can be used in the one carbon metabolism. Choline can enter the methionine cycle through the oxidation into betaine. The methionine cycle is coupled to the folate cycle to drive the synthesis of *S*-adenosyl methionine (SAM; the Universal Methyl Donor). SAM can then provide methyl groups to be used for DNA synthesis, PC synthesis via the PEMT pathway or to maintain the redox status through the transsulfuration pathway (McFadden et al., 2020).

We need to develop nutritional strategies to mitigate heat stress effects and gut-liver axis consequences. Dietary supplementation of organic acid and pure botanicals (OA/PB) has been shown to improve animal performance by enhancing gastrointestinal health in swine and poultry species (Hassan et al., 2020, Grilli et al., 2015b). Dietary OA/PB supplementation was also investigated in dairy calves experiencing moderate heat stress (Fontoura, 2022b), and it was observed that dietary OA/PB supplementation partly restored dry matter intake (DMI).

A recent study conducted at Cornell University investigated the effects of heat stress conditions and dietary OA/PB supplementation in lactating Holstein dairy cows (Fontoura et al., 2022). In this study, OA/PB supplementation tended to elevate DMI and restore milk yield and energy-corrected milk. OA/PB was able to have a higher protein yield and lower milk and plasma urea, showing that it was able to improve N incorporation in the milk. OA/PB also showed a modest but real improvement in total-tract gut permeability and an improved intestinal health supported by a reduced concentration of plasma LPS-binding protein, compared to their HS control counterparts. We hypothesized that HS will develop with accumulation of glycerophosphocholine (GPC) in the liver and that dietary OA/PB will prevent it. Our objective was to evaluate the effects of HS and dietary OA/PB supplementation on liver one-carbon and phospholipid metabolism.

Materials and Methods

Liver samples from the trial Fontoura et al., (2022) were used for these analyses. Briefly, forty-six Holstein cows (208 ± 4.65 d in milk [mean ± SD], 3.0 ± 0.42 lactations, 122 ± 4.92 d pregnant) were enrolled in a study with a completely randomized design. Following a 7 d acclimation in thermoneutrality (temperature-humidity index [THI] 68), cows were assigned to 1 of 4 groups: thermoneutral conditions (TN-Con, n = 12), HS conditions (HS-Con, n = 12; diurnal THI 74 to 82), TN conditions pair-fed to match HS-Con (TN-PF, n = 12), or HS fed OA/PB (HS-OAPB, n = 10; 75 mg/kg of body weight; 25% citric acid, 16.7% sorbic acid, 1.7% thymol, 1.0% vanillin, and 55.6% triglyceride; Aviplus® R; Vetagro S.p.A) for 14 d. Cows were milked twice daily and fed a corn-silage based total mixed ration top-dressed without (triglyceride only) or with OA/PB. Liver biopsies were sampled at d 6 of acclimation (baseline) and d 13 of environmental conditioning and analyzed by liquid-chromatography mass-spectrometry (LC-MS; Division of Nutritional Sciences; Cornell University). Data were analyzed using a general linear mixed model including fixed effects of treatment and block, the random effect of

cow, and lactation, days in milk and baseline values included as covariates. Planned contrasts included HS-Con vs. TN-Con, HS-Con vs. TN-PF, and HS-Con vs. HS-OAPB. Main effects were declared significant at $P \leq 0.05$ and trending towards significance at $0.05 < P \leq 0.15$.

Results

Hepatic choline concentrations were reduced in HS-Con compared to TN-Con ($P = 0.02$) and TN-PF ($P = 0.05$). No changes were observed in hepatic phosphocholine or lysophosphatydilcholine concentrations, but HS-Con increased PC compared to TN-PF ($P < 0.01$). In agreement with our hypothesis, HS-Con accumulated greater amounts of GPC compared to thermoneutrality ($P < 0.01$) and OAPB feeding was able to significantly prevent this accumulation ($P = 0.02$). Similar results were obtained for the GPC:choline ratio (negatively correlated to the activity of the GPC-PDE), where HS-Con had greater values compared to thermoneutrality ($P < 0.01$) and HS-OAPB tended to lower the ratio ($P < 0.14$). We did not see changes in methionine or dimethylglycine but instead, betaine was increased in TN-PF group compared to HS-Con ($P < 0.01$). SAM tended to decrease in HS-Con compared to TN-Con ($P < 0.10$), which could be a consequence of the lower choline concentration. Although no differences were detected in *S*-adenosyl homocysteine (SAH), HS-Con had a lower ratio SAM:SAH compared to TN-Con ($P = 0.05$) and HS-OAPB was able to restore it ($P = 0.06$). This ratio is the marker that indicates the remethylation capacity of the liver.

Conclusion

We conclude that heat stress develops with methyl donor deficiency in parallel with an impaired N metabolism and that supplementation of OA/PB improves the remethylation capacity in the liver. On-going transcriptomic analyses will provide a better understanding of the hepatic metabolism of dairy cows exposed to heat stress.

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