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# Grain challenge affects systemic and hepatic molecular biomarkers of inflammation, stress, and metabolic responses to a greater extent in Holstein than Jersey cows

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## ABSTRACT

Long-term feeding of high-grain diets to dairy cows often results in systemic inflammation characterized by alterations in acute-phase proteins and other biomarkers, both in plasma and immune-responsive tissues like the liver. The molecular and systemic changes that characterize an acute grain feeding challenge remain unclear. The current study involved 6 Holstein and 6 Jersey cows in a replicated $2 \times 2$ Latin square. Periods (10 d) were divided into 4 stages (S): S1, d 1 to 3, served as baseline with total mixed ration (TMR) ad libitum; S2, d 4, served as restricted feeding, with cows offered 50% of the average daily intake observed in S1; S3, d 5, a grain challenge was performed, in which cows were fed a TMR ad libitum without (CON) or with an additional pellet wheat-barley (1:1; HIG) at 20% of dry matter intake top-dressed onto the TMR; S4, d 6 to 10, served as recovery during which cows were allowed ad libitum access to the TMR. Among the 28 biomarkers analyzed in blood 12 h after grain challenge on d 5, the concentrations of fatty acids and bilirubin increased in HIG Holstein but not Jersey cows. In Holsteins, feeding HIG also increased total protein and albumin while decreasing ceruloplasmin, myeloperoxidase, and alkaline phosphatase concentrations. At the molecular level, hepatic genes associated with inflammation (*IL1B*, *IL6*, *TNF*, *TLR4*, *MYD88*, and *NFKB1*) were upregulated in Holstein cows fed HIG versus CON. Despite such response, expression of the acute-phase proteins *SAA* and *HP* in Holsteins fed HIG compared with CON was markedly downregulated. In Holsteins fed HIG versus CON, the marked downregulation of *SCD*, *ELOVL6*, and *MTTP* along with upregulated *CPT1A*, *ACOX1*, and *APOA5* indicated alterations in fatty acid and lipoprotein metabolism during grain challenge. Genes related to ketogenesis (*HMGCS2* and *ACAT1*) were upregulated in Jerseys, and gluconeogenic genes (*PDK4* and *PCK1*) were upregulated in Holstein cows fed HIG, suggesting alterations in ketone body and glucose production. Expression of phosphorylated p70S6K1, RPS6, and 4EBP1 proteins, as well as total mechanistic target of rapamycin (mTOR) protein, decreased in Holsteins fed HIG, whereas phosphorylated mTOR and 4EBP1 proteins increased in Jerseys fed HIG. From a metabolic and inflammatory biomarker standpoint, data indicate that Jersey cows better tolerated the acute grain challenge. Alterations in mTOR signaling proteins in both Jerseys and Holsteins fed HIG suggest a potential role for exogenous AA in the hepatic adaptations to grain challenge. It remains to be determined if these acute responses to a grain challenge can elicit long-term liver dysfunction, which could negatively affect welfare of the cow.

**Key words:** acidosis, immune response, inflammation, mechanistic target of rapamycin

## INTRODUCTION

Feeding high-grain diets has been adopted as a strategy for meeting the increased requirements for energy of high-yielding dairy cows (Lawrence et al., 2015). As a result, the increased proportions of fermentable substrates rapidly change the ruminal environment and could induce SARA, which negatively affects the health of cows (Plaizier et al., 2008; Gao and Oba, 2014; McCann et al., 2016). Subacute ruminal acidosis is widely defined as a clinical disorder that is characterized by a ruminal pH between 5.6 and 5.8 for more than 3 h (Plaizier et al., 2008). This lower ruminal pH is directly related to the accumulation of VFA by reducing fibrolytic bacteria and increasing amylolytic bacteria (Tajima et al., 2001). As an outcome of the low pH, hyperosmolarity elicits the release of endotoxins from lysed bacteria and in turn could lead to impairment of the barrier function of ruminal epithelium (i.e., hy-

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perkeratosis) and further leading to systemic metabolic alterations (Emmanuel et al., 2007; Liu et al., 2013). Another systemic effect of acidosis is laminitis, which is caused through the release of vasoactive substances, such as histamine (Nocek, 1997).

Well-documented consequences of SARA include decreased DMI, reduced diet digestibility, depressed milk yield, reduced milk fat synthesis, gastrointestinal tract damage, and liver abscesses (Krause and Oetzel, 2006; Zebeli and Metzler-Zebeli, 2012; Kmicikewycz and Heinrichs, 2014). Although strategies to mitigate SARA and its detrimental effects have been developed, in commercial settings a considerable amount of cows still experience subclinical cases of SARA (Gao and Oba, 2015). Grain-induced SARA has been associated with an activation of the immune response (Plaizier et al., 2008) and consequently the physiologic changes that can be assessed through plasma and molecular biomarker analyses. Whether grain challenge elicits systemic responses in Jersey as in Holstein cows is still unclear.

Elevations in the concentration of the acute-phase proteins (**APP**) and other immune biomarkers in ruminants afflicted by SARA have been reported (Rodriguez-Lecompte et al., 2014; Zhang et al., 2016b). Previous studies by some of the authors have revealed a drastic change in inflammatory status in liver and mammary gland due to SARA (Chang et al., 2015; Tao et al., 2015; Xu et al., 2015). For instance, digestive LPS challenge increases oxidative stress and alters lipid metabolism in the liver, as well altering casein synthesis (Abaker et al., 2017; Zhang et al., 2016a).

In terms of the occurrence of inflammation and consequent decrease in milk yield, the available data provide valuable information regarding the etiopathology of SARA. Despite this, the effect of acute grain challenge may differ from what has been observed in longer-term studies. In particular, information is limited regarding effects of SARA induced by grain challenge on systemic and molecular biomarkers of liver function and regulation of protein synthesis via the mechanistic target of rapamycin (**mTOR**) pathway. The focus on the mTOR pathway in the liver is of importance because of its role in the regulation of protein synthesis (Laplante and Sabatini, 2013; Li et al., 2016), and the fact that inflammation is well known to shift the utilization of AA for the synthesis of positive APP. Therefore, the aim of this study was to use liver tissue from cows in the control group or cows receiving a grain challenge of 3.2 kg of wheat-barley (1:1) in the study of Luan et al. (2016) to perform analyses of systemic and molecular biomarkers of metabolism, inflammation, and oxidative stress responses.

## MATERIALS AND METHODS

### Experimental Design and Sampling

The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. The experimental design and the feeding management of cows were previously described in detail (Luan et al., 2016). Briefly, cows for the present study were 6 Holstein (2.67 parity; BW 717 ± 30 kg and 258 ± 16 DIM) and 6 Jersey (2.50 parity; 471 ± 30 and 190 ± 86 kg) fed a basal diet or the basal diet plus the highest level of grain in the study of Luan et al. (2016). Periods (10 d) were divided into 4 stages (**S**): S1, d 1 to 3, served as the baseline with TMR ad libitum; S2, d 4, served as restricted feeding, with cows offered 50% of the average daily intake observed in S1; S3, d 5, a grain challenge was performed, in which cows were fed a TMR ad libitum without (control) or with an additional 20% pellet wheat-barley (1:1; **HIG**) top-dressed onto the TMR; S4, d 6 to 10, served as recovery during which cows were allowed ad libitum access to the TMR.

### Blood Samples and Biomarker Analyses

Blood samples were collected from the coccygeal vein at 12 h on d 5 of grain challenge using evacuated tubes (BD Vacutainer, Becton Dickinson and Company, Franklin Lakes, NJ) containing either clot activator or lithium heparin for harvesting plasma. After blood collection, tubes were placed on ice and kept at 4°C until centrifugation (~30 min). Plasma was obtained by centrifugation at 1,900 × *g* for 15 min at 4°C. Aliquots of plasma were frozen (−80°C) until further analysis.

Samples were used for measuring concentrations of albumin (catalog no. 0018250040), cholesterol (catalog no. 0018250540), bilirubin (catalog no. 0018254640), glucose (catalog no. 0018250840), urea (catalog no. 0018255440), creatinine (catalog no. 0018255540), gamma-glutamyl transferase (catalog no. 0018257640), and glutamic oxaloacetic transaminase (catalog no. 0018257540) using the IL Test purchased from Instrumentation Laboratory Spa (Werfen Co., Milan, Italy) in the ILAB 600 clinical auto-analyzer (Instrumentation Laboratory, Lexington, MA). Haptoglobin was analyzed using previously described methods (Skinner et al., 1991), whereas ceruloplasmin was assessed following the method described in Batistel et al. (2016). The concentrations of paraoxonase, alkaline phosphatase, myeloperoxidase, retinol, tocopherol, fatty acids, BHB, and β-carotene were determined as described previously (Bionaz et al., 2007; Trevisi et al., 2013). Concentra-