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Thiamine supplementation facilitates thiamine transporter expression in the rumen epithelium and attenuates high-grain-induced inflammation in low-yielding dairy cows

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ABSTRACT

An experiment was conducted to uncover the effects of increasing dietary grain levels on expression of thiamine transporters in ruminal epithelium, and to assess the protective effects of thiamine against high-grain-induced inflammation in dairy cows. Six rumen-fistulated, lactating Holstein dairy cows (627 ± 16.9 kg of body weight, 180 ± 6 d in milk; mean ± standard deviation) were randomly assigned to a replicated 3 × 3 Latin square design trial. Three treatments were control (20% dietary starch, dry matter basis), high-grain diet (HG, 33.2% dietary starch, DM basis), and HG diet supplemented with 180 mg of thiamine/kg of dry matter intake. On d 19 and 20 of each period, milk performance was measured. On d 21, ruminal pH, endotoxic lipopolysaccharide (LPS), and thiamine contents in rumen and blood, and plasma inflammatory cytokines were detected; a rumen papillae biopsy was taken on d 21 to determine the gene and protein expression of toll-like receptor 4 (TLR4) signaling pathways. The HG diet decreased ruminal pH (5.93 vs. 6.49), increased milk yield from 17.9 to 20.2 kg/d, and lowered milk fat and protein from 4.28 to 3.83%, and from 3.38 to 3.11%, respectively. The HG feeding reduced thiamine content in rumen (2.89 vs. 8.97 μg/L) and blood (11.66 vs. 17.63 μg/L), and the relative expression value of thiamine transporter-2 (0.37-fold) and mitochondrial thiamine pyrophosphate transporter (0.33-fold) was downregulated by HG feeding. The HG-fed cows exhibited higher endotoxin LPS in rumen fluid (134,380 vs. 11,815 endotoxin units/mL), and higher plasma concentrations of lipopolysaccharide binding protein and pro-inflammatory cytokines when compared with the control group. The gene and protein expression of tumor necrosis factor α (TNFα), IL1B, and IL6 in rumen epithelium increased when cows were fed the HG diet, indicating that local inflammation occurred. The depressions in ruminal pH, milk fat, and protein of HG-fed cows were reversed by thiamine supplementation. Thiamine supplementation increased thiamine contents in rumen and blood, and also upregulated the relative expression of thiamine transporters compared with the HG group. Thiamine supplementation decreased ruminal LPS (49,361 vs. 134,380 endotoxin units/mL) and attenuated the HG-induced inflammation response as indicated by a reduction in plasma IL6, and decreasing gene and protein expression of pro-inflammatory cytokines in rumen epithelium. Western bottling analysis showed that thiamine suppressed the protein expression of TLR4 and the phosphorylation of nuclear factor kappa B (NFκB) unit p65. In conclusion, HG feeding inhibits thiamine transporter expression in ruminal epithelium. Thiamine could attenuate the epithelial inflammation during high-grain feeding, and the protective effects may be due to its ability to suppress TLR4-mediated NFκB signaling pathways.
Key words: thiamine, high-grain feeding, transporter, inflammation, nuclear factor kappa B

INTRODUCTION

Dairy cows are often fed high-grain diets to maximize energy intake and to support high milk production. However, overfeeding cows with grain-rich diets results in several disorders such as SARA and liver abscesses (Emmanuel et al., 2008). Feeding high amounts of rapidly fermenting carbohydrates decreased rumen pH, altered rumen microbial population, and increased concentrations of endotoxin (LPS) in rumen fluid (Khafipour et al., 2009; Plaizier et al., 2014). The altered ruminal LPS and pH could act synergistically to disrupt barrier function (Emmanuel et al., 2007), and once the epithelium has been breached, mucosa-associated lymphoid tissue cells respond by triggering local inflammation via LPS/toll-like receptor 4 (**TLR4**) signaling

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pathway, and the pro-inflammatory cytokines would be excessively produced (Kurashima et al., 2013; Zhang et al., 2016). Moreover, the impaired barrier function of gastrointestinal epithelium facilitates the translocation of LPS from the digestive tract into the circulation, and may trigger systemic inflammation by promoting the release of pro-inflammatory cytokines (Dong et al., 2013).

Thiamine plays a critical role in carbohydrate metabolism and is essential for normal cellular functions and growth. Breves et al. (1981) and Miller et al. (1986) reported that ruminants have no dietary thiamine requirements because of the ability of the rumen microbes to synthesize thiamine. However, Dabak and Gul (2004), Karapinar et al. (2010), and Pan et al. (2016) found that thiamine deficiency occurred when sheep or cattle had subacute or acute ruminal acidosis, and it is associated with the increasing thiamine degradation by thiaminase and the decreasing microbial thiamine synthesis activity under high-grain-induced SARA (Brent, 1976). Our previous study found that thiamine supplementation in high-grain diet could increase ruminal pH and decrease rumen lactate concentration (Pan et al., 2016), and thiamine regulated the structure of rumen microbial community of SARA cows by reducing the population of *Streptococcus bovis* and prompting the growth of *Megasphaera elsdenii* (Wang et al., 2015).

In our previous study (Pan et al., 2016), SARA induced by a high-grain diet decreased the blood thiamine contents, and we attributed it to the low ruminal contents and abnormal absorption from the gastrointestinal tract. It is widely accepted that thiamine absorbed in the intestine, but whether rumen wall is permeable to thiamine has remained controversial. Hoeller et al. (1977) and Garnsworthy (2013) found that rumen wall mucosa has low permeability to thiamine. However, McDowell (2012) reported that the rumen wall is just not permeable for bound thiamine or for thiamine contained in rumen microorganisms, but ruminants can absorb free thiamine from the rumen by active transport mechanism. The active absorption of thiamine is mediated by transporter-1 and transporter-2 (**THTR1** and **THTR2**), which are coded by SLC19A2 and SLC19A3, respectively (Zhao and Goldman, 2013; Zhu et al., 2015). In recent human research, Ganapathy et al. (2004) demonstrated that SLC19A2 and SLC19A3 are expressed ubiquitously, and thiamine influx into cells via THTR1 and THTR2 is enhanced by an outwardly directed H^+ gradient ($pH_{out} > pH_{in}$). Under high-grain feeding, the accumulation of acids would decrease the outwardly directed H^+ gradient and may influence the ruminal epithelium uptake of free thiamine. The first hypothesis of our study is that thiamine absorption in rumen epithelium can be blocked by high-grain feeding.

In addition to carbohydrate metabolism, thiamine holds a key position in regulation of oxidative stress, excitotoxicity, and inflammation (Hazell and Butterworth, 2009). Thiamine deficiency prompts the release of pro-inflammatory cytokines via regulation of nuclear factor kappa B (**NFκB**) signaling pathways (Jhala et al., 2014). Investigations from human and rat have indicated that thiamine administration has a positive effect on inflammation and immune response (Gonzalez-Ortiz et al., 2011; Shoeb and Ramana, 2012). Yadav et al. (2010) and Bozic et al. (2015) found that benfotiamine, a fat-soluble vitamin B_1 analog, could modulate the macrophage inflammatory response against LPS-induced inflammation by inhibiting translocation of NFκB p65 into the nucleus. However, to our knowledge, research about anti-inflammation of thiamine in dairy cows has not been conducted. The second objective of this study is to evaluate the anti-inflammatory properties of thiamine supplementation in high-grain-fed dairy cows.

MATERIALS AND METHODS

Animals and Experimental Design

Animal care and procedures were in accordance with the Chinese guidelines for animal welfare and approved by Animal Care and Use Committee of the Chinese Academy of Agricultural Sciences. Six Chinese Holstein dairy cows in second parity fitted with 10-cm ruminal cannulas (Bar Diamond, Parma, ID) were allocated to a replicated 3 × 3 Latin square design. Cows had an average milk yield of 24.4 ± 3.1 kg/d and 180 ± 6 DIM (mean ± SD), and an average BW of 627 ± 16.9 kg (mean ± SD) at the beginning of the experiment. They were paired according to initial weight, DIM, and milk production. The 3 periods consisted of a 18-d adaptation period, followed by a 3-d period used for data and sample collection. Treatments included a control diet (**CON**; 20% starch, DM basis), high-grain diet (**HG**, 33.2% starch, DM basis), and HG diet supplemented with 180 mg of thiamine/kg of DMI (**HG+T**). This dose of thiamine was selected based on our previous dose-response studies in dairy cows (Zhang et al., 2014; Wang et al., 2015). Thiamine (Thiamine hydrochloride, purity ≥99%; Wanrong Science and Technology Development Co., Ltd., Wuhan, China) was administered via the ruminal cannula twice daily after supplying diets.

Experimental Diets and Feeding

The diets were formulated according to NRC (2001) to meet or exceed the energy requirements of Holstein dairy cows yielding 20 kg of milk/d with 3.5% milk