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The effect of grape seed and grape marc meal extract on milk performance and the expression of genes of endoplasmic reticulum stress and inflammation in the liver of dairy cows in early lactation

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ABSTRACT

During the periparturient phase, cows are typically in an inflammation-like condition, and it has been suggested that inflammation associated with the development of stress of the endoplasmic reticulum (ER) in the liver contributes to the development of fatty liver syndrome and ketosis. In the present study, we investigated the hypothesis that feeding grape seed and grape marc meal extract (GSGME) as a plant extract rich in flavonoids attenuates inflammation and ER stress in the liver of dairy cows. Two groups of cows received either a total mixed ration as a control diet or the same total mixed ration supplemented with 1% of GSGME over the period from wk 3 prepartum to wk 9 postpartum. Dry matter intake during wk 3 to 9 postpartum was not different between the 2 groups. However, the cows fed the diet supplemented with GSGME had an increased milk yield and an increased daily milk protein yield. Cows supplemented with GSGME moreover had a significantly reduced mRNA abundancy of fibroblast growth factor (*FGF*) 21, a stress hormone induced by various stress conditions, in the liver in wk 1 and 3 postpartum. In contrast, mRNA abundances of a total of 3 genes involved in inflammation and 14 genes involved in ER stress response, as well as concentrations of triacylglycerols and cholesterol, in liver samples of wk 1 and 3 postpartum did not differ between the 2 groups. Overall, this study shows that supplementation of GSGME did not influence inflammation or ER stress in the liver but increased milk yield, an effect that could be due to effects on ruminal metabolism.
Key words: dairy cow, liver, inflammation, grape seed and grape marc meal extract

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INTRODUCTION

The transition period spanning the time period between wk 3 prepartum and wk 3 postpartum represents the most critical period in the productive life of high-yielding dairy cows. During this period, the liver experiences pronounced metabolic stress due to a marked influx of NEFA, which are mobilized from adipose tissue triacylglycerol (**TAG**) stores due to a severe negative energy balance (Drackley, 1999). Approximately one-third of the whole-body NEFA flux is taken up into the liver and exceeds its oxidation capacity and, thus, promotes liver-associated diseases, such as fatty liver and ketosis (Drackley et al., 2001). Besides this metabolic stress, the liver of early lactating cows is exposed to diverse inflammatory challenges, such as microbial components, pro-inflammatory cytokines, and reactive oxygen species. These inflammatory challenges are resulting from infectious diseases, such as mastitis, endometritis, but also from subacute rumen acidosis and abomasal displacement (Plaizier et al., 2008; Vels et al., 2009; Zebeli and Metzler-Zebeli, 2012), which frequently occur during parturition, the onset of lactation, or both. As a consequence of this, transition dairy cows develop an inflammation-like condition in the liver (Bionaz et al., 2007; Vels et al., 2009), which is evident from the induction of an acute phase response. The acute phase response is characterized by the production of positive acute phase proteins, such as serum amyloid A, haptoglobin, or C-reactive protein, which compete with the production of essential liver proteins, also called negative acute phase proteins, such as albumins, enzymes, lipoproteins, transferrin, or carriers of vitamins and hormones (Carroll et al., 2009). The role of inflammation during transition to lactation for health and performance of dairy cows has been recently highlighted in a review in this journal (Bradford et al., 2015). It has been found that metabolic and inflammatory stress induces stress of the endoplasmic reticulum

(**ER**) in the liver of early lactating cows (Gessner et al., 2014). Endoplasmic reticulum stress is defined as an imbalance between the folding capacity of the ER and the protein load, with the consequence that unfolded or misfolded proteins accumulate in the ER lumen, thereby disturbing ER homeostasis (Cnop et al., 2012). It is known from studies in humans and rodents that this causes activation of an adaptive response, termed unfolded protein response (**UPR**), which aims to decrease the unfolded protein load and restore ER function (Cnop et al., 2012). The activation of the UPR, such as observed in rat models of obesity or diabetes or induced by application of chemical ER stress inducers, leads to a variety of symptoms in the liver, which are similar to those observed in periparturient dairy cows. This includes the development of fatty liver (Gentile et al., 2011; Lee et al., 2012; Pagliassotti, 2012), an enhancement of the antioxidant and cytoprotective capacity by activation of Nrf2 (Cullinan et al., 2003; Cullinan and Diehl, 2006; Gessner et al., 2013b), an induction of inflammation (Zhang and Kaufman, 2008; Rath and Haller, 2011), and an induction of FGF21 (Schaap et al., 2013). Therefore, it has been proposed that ER stress-induced UPR contributes to the pathophysiologic conditions commonly observed in the liver of periparturient cows, such as the development of fatty liver, ketosis, or inflammation (Ringseis et al., 2015).

Polyphenols are members of a large family of plant-derived compounds classified as flavonoids and nonflavonoids. Polyphenols of the flavonoid class have been shown to exert several beneficial effects in humans and animals. Besides their antioxidative potential, flavonoids have strong antiinflammatory properties. Studies with various models of inflammation such as obese rats, rats fed a high-fat diet or rats challenged with endotoxins, dietary supplementation with various types of flavonoids reduced the level of inflammation in the liver and attenuated the development of hepatic steatosis, which is a typical feature in these animal models (Terra et al., 2009; Bharrhan et al., 2012; Heber et al., 2014). Recently, it has been found that flavonoids are moreover able to attenuate ER stress in liver and muscle cells (Giordano et al., 2014; Rodriguez et al., 2015).

Whereas a great number of studies dealing with antiinflammatory properties of flavonoids has been performed in humans and rodents, less information is available so far about potential beneficial effects of flavonoids in farm animals. In the present study, we investigated the hypothesis that supplementation of flavonoids could reduce inflammation and ER stress in the liver of dairy cows during early lactation. Cows were fed a diet supplemented with grape seed and grape marc meal extract (**GSGME**), an inexpensive byproduct of wine and grape juice processing, rich in flavonoids. The most abundant flavonoids in grape seeds (**GS**) are gallic acid, catechin, epigallocatechin-3-gallate, epigallocatechin, epicatechin-3-gallate, epicatechin, and proanthocyanidins (Auger et al., 2004). Grape marc (**GM**) contains fewer procyanidins (with the exception of the procyanidin dimer B1) but contains significant amounts of anthocyanins, such as cyanidine 3-glucoside, malividin 3-glucoside, cyanidin, and peonidin, which are absent in GS (Auger et al., 2004). Due to their antioxidant and free radical scavenging activities, GS meal and GM meal are used as feed additives for oxidative stabilization of feedstuff (Bonilla et al., 1999). In recent studies, grape products have been considered as supplements for ruminants, and it has been found that these products are able to reduce methane production, probably due to their high concentrations of tannins, which have inhibitory effects on methanogenic bacteria (Moate et al., 2014; Ishida et al., 2015; Khiaosa-Ard et al., 2015). In contrast, the potential effects of grape products on inflammation and ER stress in the liver of dairy cows have not yet been investigated so far.

MATERIALS AND METHODS

The study was conducted at the Educational and Research Centre for Animal Husbandry Hofgut Neumuehle in Rhineland-Palatinate (Münchweiler an der Alsenz, Germany) and were approved by the local department for animal welfare affairs (23 177–07/G 12–20–074).

Animals

Twenty-eight primiparous and multiparous Holstein cows with an average parity number of 2.8 were used as experimental animals for this study. The cows were allocated into 2 experimental groups, either a control group or a group supplemented with GSGME (GSGME group). Multiparous cows were allocated to the groups according to parity number and previous milk performance; heifers were allocated randomly. Each group was composed of 4 primiparous and 10 multiparous cows with an average parity number of 2.8 for the control group and 2.9 for the GSGME group. Body weights of the cows did not differ before parturition (wk 8 prepartum: control group, 692 ± 21 kg; treatment group, 664 ± 21 kg; mean ± SE) or after parturition (wk 2 postpartum: control group, 657 ± 28 kg; GSGME group, 632 ± 22 kg; mean ± SE) between the 2 groups. The cows were housed in a freestall barn with boxes bedded with short cut straw. Five days before the expected calving date until 5 d after calving, the cows were kept separately from the herd in calving pens bedded with short cut straw. During this time, feed intake of the individual cows could not be recorded. After cows were