Livestock Science 203 (2017) 120–123

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Gastrointestinal endotoxin and metabolic responses in cows fed and recovered from two different grain-rich challenges☆

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ARTICLE INFO

Keywords:
Endotoxin
Acute phase proteins
High-grain diet
Blood metabolites

ABSTRACT

Effects of two grain-rich feeding challenges and their subsequent recovery on gastrointestinal endotoxin concentrations, acute phase response, and blood metabolites were determined in eight rumen-cannulated, non-lactating Holstein cows. A 2 × 2 cross-over design was used. At the start of the experiment, cows were fed forage-only diet (baseline, d 0) and after a 6-d gradual adaptation, challenged with a 60% grain diet for 4 wk (d 7 – 35), either continuously (CONT) or interruptedly with a 1-wk break in the second wk (INT). Subsequently, cows were fed the forage-only diet for 9 wk to evaluate the recovery of the grain challenges. Feeding challenges markedly increased ruminal and fecal endotoxin concentrations, which returned to the baseline levels during the recovery phase. Ruminal endotoxin concentration tended to be higher in CONT than in INT cows on d 14 and 25. The concentration of acute phase proteins serum amyloid A and haptoglobin were not affected by the feeding challenges. The level of the liver enzyme aspartate aminotransferase was transiently increased on d 14 and 25 only in the CONT cows. The concentration of glutamate dehydrogenase peaked on d 14 of both challenge models and lowered thereafter. Blood glucose and urea concentrations rose due to the challenges with glucose tending to be higher in INT than in CONT cows. Both feeding challenges lowered blood cholesterol, non-esterified fatty acids, and creatinine concentrations. In conclusion, the feeding challenges promoted endotoxin release in the rumen and hindgut but did not trigger an acute phase response. All altered variables returned to baseline values within 2 wk after the both interruptedly and continuously high-grain feeding.

1. Introduction

The feeding of high amounts of grain improves milk production and sustains cost efficiency, but may keep cows at a high risk of developing subacute ruminal acidosis (SARA; Plaizier et al., 2008). Increased dietary starch contents and rumen pH drops are associated with increased concentration of cell-free lipopolysaccharides, or commonly known as endotoxins (EN), in the rumen (Plaizier et al., 2008) and the hindgut (Metzler-Zebeli et al., 2013) due to increased shedding of EN by Gram-negative bacteria (GNB). Short-term SARA conditions have been related to an activation of systemic acute phase response (APR) and the presence of EN in peripheral blood plasma (Khafipour et al., 2009), presumably due to translocation of gut-origin EN into the bloodstream (Steele et al., 2011). Notably, effects of long-term SARA and the influence of persistence of grain feeding on activation of APR have been underexplored. At the rumen level, we could demonstrate an increased severity of SARA in cows experiencing an interrupted high-grain challenge (Pourazad et al., 2016) because this feeding challenge stalled the acid-absorptive capacity of the rumen (Qumar et al., 2016) in comparison to continuous feeding of the same diet. Following these companion studies, in the present study, we hypothesized that a repeated high-grain feeding challenge increases the EN loads in the gastrointestinal tract at a greater extent than the long-term continuous model. This may increase chances for EN translocation into the blood which can be reflected by an activation of APR and perturbed metabolic response. Furthermore, the time required for a full recovery from systemic inflammation in response to different nutritional challenges in cattle is largely unknown. Therefore, we also focused on the duration of recovery from perturbations of the rumen environment and APR in cattle, which was expected to be longer than a recovery of ruminal pH after two different high-grain challenges. Our hypotheses followed the findings that although pH instantly changes with diet change, a longer duration seems to be required for structural and functional adaptation of the ruminal epithelium (Qumar et al., 2016), a stable rumen bacterial

☆ Grain-rich feeding, gut endotoxin and metabolic responses in cattle.

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http://dx.doi.org/10.1016/j.livsci.2017.07.015
Received 19 March 2017; Received in revised form 27 July 2017; Accepted 31 July 2017

community (Hook et al., 2011) and for curbing down systemic inflammation (Steele et al., 2011). To test these hypotheses we compared the effects of two different grain-rich feeding challenges and their subsequent recovery on gastrointestinal EN concentrations, activation of APR, and blood metabolites in non-lactating Holstein cows.

2. Materials and methods

2.1. Animals, experimental design, and feeding

The experiment was conducted as part of a larger research at the research farm Kremesberg of Vetmeduni, Vienna, Austria. Animal handling and treatment procedures were approved by the institutional ethics committee of the University of Veterinary Medicine (Vetmeduni) Vienna and the national authority according to §26ff of the Law for Animal Experiments, Tierversuchsgesetz 2012- TVG (GZ 68.205/0093-II/3b/2013). Non-lactating cows were chosen in order to avoid the disturbing influences related to noticeable variations in production and metabolic responses among lactating cows as well as in relation to lactation time (Humer et al., 2016). Animals, experimental design, and feeding plans are addressed in greater detail in our companion publications (Pourazad et al., 2016; Qumar et al., 2016).

In brief, we used 8 ruminally cannulated (100 mm i.d.; Bar Diamond, Parma, ID) non-lactating Holstein cows (initial body weight (BW)): 710 ± 118 kg, mean ± SD). The experiment was a 2 × 2 crossover design (n = 8 per treatment), testing two feeding challenge models (interrupted (INT) and continuous (CONT) model) in two sequential runs. Cows were blocked by BW and randomly allocated to both models. Before the start of the first run, all cows received a forage-only diet consisting of 50% grass silage and 50% second-cut meadow hay (dry matter (DM) basis) for 3 wk, considered as baseline period. Afterward, all cows were gradually adapted over 6 d (+10% grain-mix daily) to a diet containing 60% grain-mix and 40% forage-mix (grass silage and hay at 1:1) on DM basis. The grain-mix of the challenge diet was composed of (% of grain-mix DM) barley grain (33%), wheat (30%), maize (15%), rapeseed meal (17%), dried beet pulp (3.2%), calcium carbonate (0.5%), sodium chloride (0.3%), and mineral-vitamin premix (1%). The challenge diet (% of diet DM) contained 74.5% DM, 15.4% crude protein (CP), 31.8% neutral detergent fiber (NDF), 1.71% ether extract, 5.86% ash and 45.2% non-fiber carbohydrates. The forage-only diet consisted of 54.4% DM, 12.8% CP, 1.5% ether extracts, and 51.7% NDF (DM basis).

After the gradual adaptation, cows in the CONT model were fed the grain challenge diet continuously for 4 wk. After 1 wk of the challenge cows in the INT model were off grain for 1 wk and fed the forage-only diet as in the baseline. Thereafter, the INT cows were re-challenged for another 2 wk with 2 d of a stepwise increment of grain intake. In each run, after 4 wk of grain challenge, all cows were switched to the forage-only diet as in the baseline for the following 9 wk (termed as recovery).

During baseline and until d 4 of adaptation of each run, diet was offered at 1.5% of BW, whereas from d 5 of adaptation cows DM allowance was increased to 2% of BW, in all cases fully meeting the voluntary feed intake of the cows. All cows had access to the same fresh forage starting from 08:00 h and then to grains starting from 10:00 h. The separate feeding was essential in order to regulate a constant ratio of forage to grain ingested and to avoid interferences of concentrate sorting. Cows had full access to 7 bins containing the same feeds. During the SARA challenge, 2 bins were used for concentrate feed and 5 bins for forage. Fresh clean water and a salt licking stone were always available to animals. Individual feed intake was monitored continuously and recorded using electronic weighing scales and computer-regulated access gates (RIC system, Insentec B.V., Marknesse, The Netherlands).

2.2. Sample collection and measurements

All samples including ruminal fluid, feces, and blood samples were collected before the morning feeding at baseline (d 0), during 4 weeks of the grain challenge on d 14, 25, and 35, and at recovery on d 49 and 98 of each experimental run. Ruminal fluid samples were collected from the ventral sac of the cows, strained through four layers of medical gauze and then 2 ml of the strained sample was transferred into a pyrogen-free tube and stored at −20 °C until analysis. Fecal samples were collected through the rectum and 15 g of fecal sample was directly transferred to a pyrogen-free tube and immediately stored at −20 °C for further analysis of the fecal EN content. The concentration of cell-free EN in the free ruminal fluid and fecal samples was determined by the chromogenic kinetic Limulus amebocyte lysate (LAL) assay according to the manufacturer protocol as described by Metzler-Zebeli et al. (2013). The intra-assay coefficient of variation was < 10%.

Blood samples were collected from the jugular vein into vacutainer tubes for collecting serum (clot activator vacutainer tube) and plasma samples (lithium heparin vacutainer tube; Greiner Bio-One GmbH Kremsmünster, Austria). About 20 min after collection, the plasma was obtained by centrifuging at 3000 × *g* for 15 min and the supernatant was transferred to 2-ml aliquots. For serum collection, the blood sample was allowed to clot at room temperature for 45–60 min and then centrifuged at 3000 × *g* for 15 min. The supernatant was then transferred to 2-ml aliquots. All plasma and serum sample tubes were stored at −20 °C for further analysis

The concentration of serum amyloid A (SAA) in the serum was determined by a commercially available bovine ELISA kit (TP-802, Tridelta Diagnostics Inc., Morris Plains, NJ, USA) as described previously (Klevenhusen et al., 2013). The concentration of serum haptoglobin (Hp) was determined by a commercially available bovine ELISA kit (Genway Biotech Inc., San Diego, CA, USA). The samples were diluted 1:50 and assayed according to the manufacturer's protocol. Only for a small number a greater dilution (1:100 or 1:250) was necessary. The intra-assay variation for the acute phase protein assays was controlled by the coefficient of variations ≤ 10%.

The concentrations of non-esterified fatty acid (NEFA), beta-hydroxybutyrate (BHBA), glucose, cholesterol, urea, creatinine, aspartate aminotransferase (AST), glutamate dehydrogenase (GLDH) and gamma-glutamyl transferase (GGT) in serum and lactate in plasma were measured by a conventional large-scale analyzer for clinical chemistry at the laboratory of the Central Clinical Pathology unit, University of Veterinary Medicine, Vienna. Standard enzymatic colorimetric assays for all blood metabolites were performed on a fully automated auto-analyzer for clinical chemistry (Cobas 6000/c501; Roche Diagnostics GmbH, Vienna, Austria). The intra-assay variation for all blood chemistry assays was controlled by the coefficient of variations ≤ 5%.

2.3. Statistical analyses

Data of blood and EN parameters were analyzed as a cross-over design to test fixed effects of the sequence of the challenge model applied to the cows, experimental run, challenge model (INT and CONT), experimental day (d 0, d 14, d 25, d 49, and d 98), and challenge model × day interaction. Effects of cows were considered random. Normal distribution was evaluated with PROC UNIVARIATE of SAS (version 9.4, SAS Institute Inc., Cary, NC, USA), and because data of some variables were not normally distributed, we analyzed all data using PROC GLIMMIX of SAS. Results are reported as least square means. Significant level was set at $P \leq 0.05$ and a trend was considered when $0.05 < P \leq 0.10$.

3. Results and discussion

Results for all variables tested in the present study are listed in Table 1. On average of both challenge models, the ruminal and fecal EN