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Intermittently-induced endotoxaemia has no effect on post-challenge plasma metabolites, but increases body temperature and cortisol concentrations in periparturient dairy cows

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ABSTRACT

This study evaluated the responses of plasma cortisol, metabolites and body temperature to intermittently-induced endotoxaemia in periparturient cows. Sixteen Holstein cows were randomly allocated to one of the two treatment groups. Cows were infused intravenously either with saline solution (control) or with the same solution containing 3 increasing doses of lipopolysaccharide (LPS) for 3 consecutive weeks around parturition as follows: 0.01 μg LPS/kg body weight (BW) on *d* −14 and −10 prepartum, 0.05 μg LPS/kg BW on *d* −7 and −3 prepartum, and 0.1 μg LPS/kg BW on *d* 3 and 7 postpartum. Blood samples were measured shortly before and in 8 time-points after (up to 6 h) the challenges on *d* −14, −7, 3, and 7 to evaluate the post-challenge plasma profile. Results showed greater concentrations of plasma cortisol, in particular after the second and third LPS challenge. An increase in body temperature was recorded after administration of the greatest LPS dose, but this effect diminished during the very last LPS challenge. A biphasic response of glucose was observed; a linear increase up to 60 min after the second LPS challenge followed by a rapid decrease thereafter. Other plasma variables like lactate, cholesterol, non-esterified fatty acids, and beta-hydroxybutyrate were not affected by treatment. In conclusion, LPS administrations did not notably affect post-challenge metabolic responses in periparturient dairy cows but increased the level of plasma cortisol and the body temperature after the highest LPS challenge.

1. Introduction

During the periparturient period, dairy cows are immunosuppressed and are affected by various infectious diseases such as mastitis and endometritis (Mallard et al., 1998). These diseases increase the likelihood of cows to encounter various immunogenic substances such as lipopolysaccharide (LPS) early postpartum (Ametaj et al., 2012). The LPS is a cell-wall component of all Gram-negative bacteria, commonly known as endotoxin, and has highly immunogenic properties. Endotoxin also may enter into the systemic circulation from the disturbed gastrointestinal tract in response to high-grain feeding (Emmanuel et al., 2008; Plaizier et al., 2012) early postpartum.

Recent investigations have shown that the intravenous administration of single and large doses of LPS (i.e., 2.5 μg LPS/kg body weight) – simulating acute LPS insults – initiated an inflammatory response and perturbation of various metabolic pathways, related to host responses against LPS in cattle (Carroll et al., 2009). Administrations of single LPS doses modified the concentration of multiple plasma variables including non-esterified fatty acid (NEFA) and beta-hydroxy butyric acid (BHBA) in heifers (Werling et al., 1996; Steiger et al., 1999) and dairy cattle (Waldron et al., 2003). Additionally, infusion of LPS to heifers resulted in a biphasic response of plasma glucose with an initial peak and a later decline (Steiger et al., 1999). The same study also reported an increased concentration of lactate and a short-term increase in the concentration of cortisol in the plasma (Steiger et al., 1999).

Although much is known about metabolic responses to a single challenge with high doses of LPS (i.e., >1 μg LPS/kg body weight), there is a lack of information on the role of chronic experimental endotoxaemia on plasma metabolic profile in periparturient dairy cows. To our best knowledge, only one study evaluated long-term effects of intermittent endotoxaemia on metabolic health status of dairy cows (Zebeli et al., 2011). In that study, cows challenged with low but increasing doses of LPS (i.e., 0.01, 0.05 and 0.1 μg LPS/kg body weight) had a higher susceptibility to metabolic disorders postpartum, and these cows showed disturbances of plasma

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metabolites and immune variables up to 4 weeks postpartum. However, the diurnal metabolic changes relative to the LPS challenges were not evaluated in that study. Therefore, the objective of this study was to determine post-challenge responses of selected plasma metabolites, cortisol, and body temperature of periparturient dairy cows to intermittent intravenous administration of LPS.

2. Materials and methods

2.1. Animals, study design and treatments

Sixteen clinically healthy, primiparous (6 cows) and multiparous (10 cows) Holstein cows at 14 days before the expected day of parturition, were used in this study. All experimental procedures were approved by the University of Alberta Animal Care and Use Committee for Livestock, and animals were cared for in accordance with the guidelines of the Canadian Council on Animal Care (1993). Veterinary supervision was provided to the animals throughout the experiment. Cows were blocked by parity and the anticipated day of calving, and were randomly allocated to one of the two treatment groups, with eight cows each, according to a randomized block design. Details of the administration protocol of LPS, management of animals and diet ingredients were described previously (Zebeli et al., 2011). In brief, the eight cows assigned to intravenous saline (0.9% w/v NaCl) infusion constituted the control group (CTR). The other eight cows allocated to the LPS group received intravenous infusion of LPS (*Escherichia coli* 0111:B4; Sigma–Aldrich, Mississauga, Ontario, Canada). Three different increasing doses of LPS, dissolved in sterile saline solution, were administered to the cows before and after parturition. The doses and the schedule of LPS administration were as follows: (1) 0.01 μg LPS/kg body weight (BW) on *d* −14 and −10 prepartum, (2) 0.05 μg LPS/kg BW on *d* −7 and −3 prepartum and (3) 0.1 μg LPS/kg BW on *d* 3 and 7 postpartum. The mean difference between actual calving date and the expected calving date was 1.6 *d* (±3.2 *d* as standard deviation). Because the aim was to simulate a state of low-degree endotoxaemia, similar to conditions of LPS translocated by gastrointestinal tract (Plaizier et al., 2012) and not acute infection insults with Gram-negative bacteria, the LPS dose infused was chosen to be low 0.01–0.1 μg/kg BW. Animals were fed a total mixed ration (TMR) at 08:00 h.

Two hours before the LPS administration, cows were restrained in separate pens adjacent to each other, and their infusion area was washed with water and swabbed with 70% ethyl alcohol. To facilitate intravenous administration and blood sampling, an indwelling catheter was introduced into the jugular vein before the experiment started, as described previously (Zebeli et al., 2012). The amount of LPS was dissolved in 100 mL of sterile saline solution and was infused into the jugular vein via the catheter. The cows of the CTR group were administered the same amount of sterile saline solution according to the aforementioned schedule of the LPS administration.

2.2. Blood sampling

Starting at approximately 07:00 h each time, blood was consecutively withdrawn from the catheters at – 15, 15, 30, 60, 120, 180, 240, 300 and 360 min relative to the initiation of LPS challenge. Measurements of blood variables were conducted in multiple samples collected on days 14 and 7 prepartum as well as on days 3 and 7 postpartum, so that there were 4 sampling days in total to determine post-exposure diurnal metabolic responses. Blood samples, collected in tubes containing Na-EDTA (Preanalytical Systems Beliver Industrial Estate, Plymouth, UK), were immediately placed on ice, centrifuged at 3000*g* and 4 °C for 20 min (Rotanta 460R, Hettrich Zentrifugen, Tuttlingen, Germany), and plasma was separated and stored at −20 °C until analysis. Immediately after blood collection indwelling catheters were filled with 2–3 mL of 0.85% sterile saline containing 50 IU of heparin (Sigma–Aldrich Canada Ltd., Oakville, ON, Canada) to prevent clot formation.

2.3. Body temperature

Body temperature was measured 15 min before the LPS challenge and at 30 min and every hour thereafter, up to 6 h after

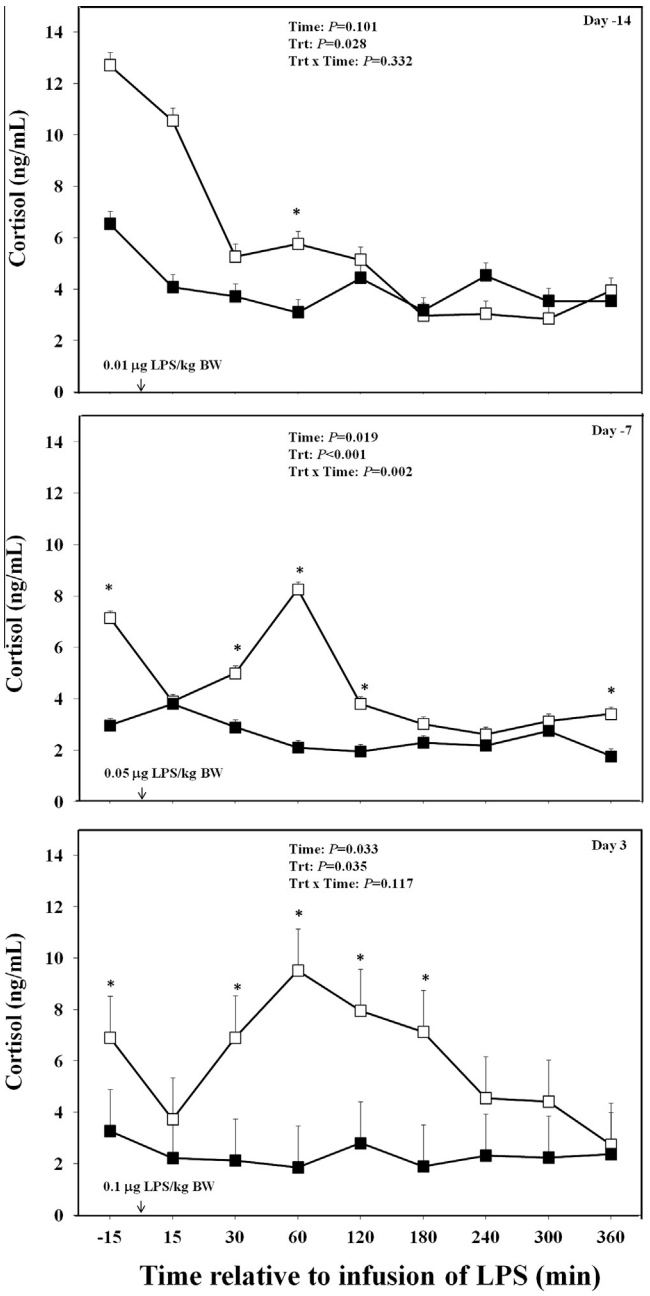

Fig. 1. The profile of cortisol in the plasma of periparturient dairy cows after repeated intravenous administration of 100 mL sterile saline alone (■) or containing lipopolysaccharide (□; LPS) at 0.01 μg/kg body weight (BW) on *d* −14 and −10, (2) 0.05 μg/kg BW on *d* −7 and −3, and 0.1 μg LPS/kg BW on *d* 3 and 7 postpartum. Cortisol was measured on days −14, −7 and 3 relative to parturition. Arrows indicate LPS administration with doses (data are shown as least-squares means and respective standard errors; $n = 8$; * indicates differences between treatments at different sampling times ($P < 0.05$)).