



Effects of monensin and starch level in early lactation diets on indices of immune function in dairy cows

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ABSTRACT

The objective of this study was to evaluate the effect of dietary starch level and monensin on immune function. Prior to parturition, primiparous ($n = 21$) and multiparous ($n = 49$) Holstein cows were fed a common controlled energy close-up diet with a daily topdress of either 0 or 400 mg/d monensin. From 1 to 21 d in milk (DIM), cows were fed a high-starch (HS; 26.2% starch) or low-starch (LS; 21.5% starch) total mixed ration with a daily topdress of either 0 or 450 mg of monensin/d continuing with prepartum topdress assignment. From 22 through 63 DIM, all cows were fed HS and continued with assigned topdress treatment until 63 DIM. Endometrial cytology and whole-blood immune function were assessed at 8 DIM and on 1 d between 40 and 60 DIM. At 8 DIM, cows fed HS had an increased percentage (%) of phagocytic monocytes and tended to have a greater phagocytosis index (% of positive cells \times mean fluorescence intensity) in monocytes compared with cows fed LS. At 8 DIM, cows fed HS also tended to have a higher percentage of monocytes involved in oxidative burst and a higher monocyte oxidative burst index compared with LS cows. At 8 DIM, blood polymorphonuclear neutrophils (PMN) isolated from cows fed monensin during the periparturient period tended to have higher PMN glycogen content compared with control cows. At 40 to 60 DIM, the incidence of cytological endometritis as diagnosed by uterine cytology was not affected by dietary treatment. However, at 40 to 60 DIM, cows fed monensin had an increased percentage of *Escherichia coli*-stimulated PMN, tended to have a greater percentage of monocytes involved in oxidative burst, and tended to have an increased *E. coli*-stimulated monocyte oxidative burst index. At 40 to 60 DIM, blood PMN isolated from cows

fed HS during early lactation had higher PMN glycogen content compared with cows fed LS during early lactation. Overall, results suggest that feeding higher starch diets postpartum and peripartal supplementation with monensin may have some beneficial effects on immune function, although uterine cytology was not affected by treatment.

Key words: monensin, starch, immune function, endometritis

INTRODUCTION

During the transition period, immune function is generally decreased (Mallard et al., 1998; Waller, 2000; Rainard and Riollot, 2006), and as a result dairy cows have increased susceptibility to infection, contributing to the comparatively high incidence of disease around parturition (Waller, 2000; Ingvarsen et al., 2003; Rainard and Riollot, 2006). Both polymorphonuclear neutrophils (PMN) and the activation of monocytes are critical for the initial defense against invading microbial pathogens (Burton et al., 2005; Rinaldi et al., 2008). When PMN are activated, they are able to kill bacteria through an oxygen-independent mechanism via the production of microbicidal peptides and proteases, and through the oxygen-dependent generation of reactive oxygen species (Rinaldi et al., 2008), which are produced by oxidative burst activity (Rainard and Riollot, 2006). Functional competences of PMN, including oxidative burst activity, are known to be reduced during the periparturient period (Dettloux et al., 1995; Burvenich et al., 2003; Rainard and Riollot, 2006). Monocytes also have been shown to be more responsive to inflammatory stimulation during the transition period than during other physiological states, resulting in greater cytokine production (Sordillo et al., 1995), which may suggest an increased importance in the role of monocytes as a part of the immune response during the transition period.

Uterine defenses rely on innate immunity at the initial stages of infection (Wathes et al., 2009). Gilbert et al.

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(2007) reported that the proportion of PMN sampled from endometrial cytology at calving was correlated negatively to the extent of bacterial infection at calving and also the proportion of PMN at 49 DIM. Cytological endometritis (**CE**) is defined by the proportion of PMN in endometrial cytology samples in the absence of clinical endometritis (Sheldon et al., 2006) and can result in decreased reproductive performance (Gilbert et al., 2005; Galvão et al., 2009; Cheong et al., 2011). The diagnosis of CE has been used as a functional-farm-relevant outcome of impaired immune function. Excessive negative energy balance (**EB**) and mobilization of body reserves during early lactation has been shown to negatively affect the postpartum interval to first ovulation and decrease conception rate (Butler, 2003), and elevated early lactation concentrations of BHB and nonesterified fatty acids (**NEFA**) have been negatively associated with subsequent reproductive function (Leroy et al., 2008; Friggens et al., 2010). Evidence suggests that incidence of CE is also associated with negative EB (Galvão et al., 2010; Cheong et al., 2011; Yasui et al., 2014b). Because the extent of negative EB has also been negatively correlated with both PMN function (Hammon et al., 2006) and PMN energy status expressed as intracellular glycogen content (Galvão et al., 2010), improving the energy status of the cow during the immediate postpartum period may have positive effects on the incidence of CE and reproductive performance via improvements in innate immunity.

A recent study observed that propionate induces degranulation of antimicrobial peptides and proteolytic enzymes in PMN in dairy cows (Carretta et al., 2013). As most of the steps in the oxygen-independent PMN bacterial killing mechanism depend on this degranulation, these data may suggest that increased ruminal propionate production could create favorable conditions for PMN function. It has been observed that cows fed more propiogenic diets during the postpartum period have increased DMI in very early lactation (Andersen et al., 2003; Rabelo et al., 2003; McCarthy et al., 2015a). Therefore, it is of interest to determine if feeding more propiogenic diets (e.g., high in fermentable starch, monensin supplementation) immediately postpartum could improve postpartum immune status by decreasing the severity of early lactation negative EB.

Monensin has been shown to decrease periparturient negative EB-associated health disorders and improve energy metabolism (Duffield et al., 2008a,b; McCarthy et al., 2015a,b). The decreased incidence of retained placenta (Duffield et al., 2002) and mastitis (Duffield et al., 2008b; Duffield, 2010) in cows fed monensin suggests that it may improve postpartum immune

status. We hypothesized that increasing starch content during the immediate postpartum period and feeding monensin throughout the periparturient period would enhance immune function through the improvement of postpartum energy metabolism.

MATERIALS AND METHODS

Experimental Animals, Treatments, and Procedures

All animal procedures were approved by the Cornell University Institutional Animal Care and Use Committee and the experiment was conducted from March to October 2012. The experimental design, treatments, and diet parameters are described more completely in a previous publication (McCarthy et al., 2015a). Briefly, the study was a completely randomized design with randomization restricted to balance for expected calving date of primiparous and multiparous cows and previous lactation 305-d mature-equivalent milk production for multiparous cows. A 2×2 factorial arrangement of postpartum treatments was used, with early lactation period feeding strategy [high starch (**HS**; 26.2% starch) vs. low starch (**LS**; 21.5% starch) diet during the first 21 DIM] and postpartum monensin level [0 mg of monensin/d (**Con**) or 450 mg of monensin/d (**Mon**); monensin; Elanco Animal Health, Greenfield, IN] as the variables of interest. In addition, cows that received Mon during the postpartum period were fed Mon (400 mg/d) initiated on 1 d between d 21 to 28 before expected parturition (average treatment of 25 d; minimum of 14 d on treatment before actual parturition was required for inclusion in the data set). The final data set included 70 cows (primiparous $n = 21$, multiparous $n = 49$). Lactating cows were dried off at least 45 d (average 53 d dry period length) before expected parturition and moved to the experimental tiestall barn 28 d before expected parturition where they began consuming the experimental close-up dry cow diet.

Diet ingredients are presented in Table 1, and nutrient composition is presented in Table 2. Procedures and methods for feed sampling and analysis are detailed in McCarthy et al. (2015a). The HS and LS experimental diets were fed from parturition until 21 DIM, after which all cows were fed HS until the end of the study at d 63 of lactation. The Mon topdress was targeted to provide 400 mg/d prepartum and 450 mg/d postpartum, whereas the actual consumptions of monensin were 392 mg/d prepartum and 438 mg/d postpartum. The analyzed topdress contained 461 g/t of Mon and were fed as a daily topdress at rates of 0.85 kg/d prepartum and 0.95 kg/d postpartum. Cows continued to receive assigned topdress treatments through 63 DIM.

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