J. Dairy Sci. 100:1–11
https://doi.org/10.3168/jds.2016-12079

Regulatory effect of dietary intake of chromium propionate on the response of monocyte-derived macrophages from Holstein cows in mid lactation

M. Garcia,* Y. Qu,* C. M. Scholte,* D. O'Connor,† W. Rounds,† and K. M. Moyes*[1]
*Department of Animal and Avian Sciences, University of Maryland, College Park 20740
†Kemin Industries Inc., Des Moines, IA 50317

ABSTRACT

Chromium (Cr) has been reported to enhance immune function and improve insulin sensitivity and performance in beef and dairy cattle. However, its effect on bovine macrophage inflammatory and metabolic response is unknown. The objective of this study was to characterize the effect of dietary Cr on the inflammatory and metabolic response of polarized macrophages ex vivo. Twelve primiparous and 16 multiparous healthy Holstein cows in mid lactation (143 ± 37 d in milk) were enrolled in this study. Cows were fed a common total mixed ration once per day that was top-dressed with 200 g of ground corn containing 1 of 2 dietary treatments: control (CTL, no Cr supplementation) or Cr propionate (CrP, 8 mg of Cr/cow per day) for 35 d. At d 1, 17, and 35 of treatment, blood monocytes were isolated and cultured to obtain 3 monocyte-derived macrophage (MDM) phenotypes: M0 (non-polarized), M1 (pro-inflammatory; IFN-γ polarized) and M2 (anti-inflammatory; IL-4 polarized). The experiment was set in a randomized complete block design. Neither dry matter intake nor milk yield was affected by treatment. Plasma concentrations of metabolites and the metabolic and inflammatory response of MDM in spent media were not affected by treatment. Neither the whole blood cell population nor the specific proportion of leukocytes was affected by the main effect of treatment. However, we did observe a trend for fewer circulating neutrophils in cows fed CrP than in cows fed CTL for 35 d, which may be partly attributable to a greater influx of neutrophils into peripheral tissues, a reduced pro-inflammatory response during disease, or both; this warrants future study. Expression of *IGFI* was increased in MDM-M0, and expression of *CXCL11* tended to increase in MDM-M2 from cows fed CrP compared with cows fed CTL. Expression of *SLC2A3* also tended to increase in MDM-M2 from cows fed CrP compared with cows fed CTL at 17 d. Our results suggest that CrP has minimal effect on the inflammatory and metabolic response of MDM for Holstein dairy cows in mid lactation. Future studies are warranted to evaluate the differential regulation of Cr on the inflammatory and metabolic response of leukocytes from dairy cows at different stages of lactation and parity.
Key words: chromium propionate, Holstein cows, immune function, monocyte-derived macrophage

INTRODUCTION

Lactating dairy cows require high levels of nutrients to meet their needs for milk production. During early lactation, insulin resistance is one mechanism dairy cows use to repartition glucose away from skeletal muscle and adipose tissue and toward the mammary gland to synthesize milk and milk components (Bauman and Currie, 1980; Aschenbach et al., 2010). The failure of cows to meet their glucose demands for lactation may lead to impaired immune response and increased risk of disease that may affect milk production and profitability (Ingvartsen and Moyes, 2013).

Dietary supplementation with Cr appears to enhance glucose use by improving insulin sensitivity and glucose metabolism in growing heifers and periparturient cows (Hayirli et al., 2001; Sumner et al., 2007; Spears et al., 2012). Studies evaluating the effect of Cr supplementation on immune response have focused mainly on lymphocyte and neutrophil response. Chromium supplementation enhanced antibody production and mononuclear cell proliferation (Burton et al., 1993). For neutrophils, Cr supplementation did not affect phagocytic activity (Chang et al., 1996), but it did influence the expression of pro-inflammatory genes (Yuan et al., 2014). However, no studies have examined how dietary Cr supplementation alters macrophage response.

Macrophages play a critical role in the innate immune response; they are the first immune cells to encounter pathogens and can lead to an orchestrated innate-adaptive response, as well playing a role in metabolism

Received September 30, 2016.
Accepted April 2, 2017.
[1]Corresponding author: kmoyes@umd.edu

and homeostasis (Murray and Wynn, 2011; Italiani and Boraschi, 2014). The M1 phenotype is linked to induction of a pro-inflammatory state, reduction of fatty acid oxidation, and insulin resistance. The M2 phenotype is associated with an anti-inflammatory state, induction of fatty acid oxidation, and insulin sensitivity (Epelman et al., 2014). We hypothesized that dietary supplementation with Cr propionate would regulate the ability of macrophages to respond to tissue signaling by controlling the pro-inflammatory response. The objective was to characterize the effect of dietary Cr propionate on the response of polarized macrophages from Holstein cows in mid lactation.

MATERIALS AND METHODS

Cow Management and Dietary Treatments

The present experiment was approved in accordance with the regulations and guidelines set forth by the University of Maryland Animal Care and Use Committee.

Twelve primiparous and 16 multiparous (≥2 lactations) Holstein cows in mid lactation (143 ± 37 DIM) were enrolled in this study. Cows had an average milk yield of 37.8 ± 6.0 kg and an average BW of 636 ± 76 kg. All cows were free from clinical signs of disease, with an average composite milk SCC ≤100,000 cells/mL before enrollment. Cows were randomly assigned within parity to receive 1 of 2 dietary treatments: chromium propionate (**CrP**) or control (**CTL**; no CrP). Cows were individually fed a common lactation TMR once per day (~0730 h) in tie stalls fitted with mattresses that were bedded with wood shavings. Cows had continuous access to water and were milked twice per day at 0630 and 1600 h.

Feed offered was adjusted daily to achieve 5 to 10% orts. Orts were collected and weighed daily. The TMR was formulated to meet NRC (2001) requirements for lactating dairy cows (Table 1). During the first week of enrollment (i.e., the adjustment period), cows were fed the common lactation TMR, top-dressed with 200 g of ground corn. After the adjustment period, cows were fed the same TMR top-dressed with 200 g of ground corn for 35 d, with CrP (8 mg of Cr/cow per day) or without CrP (CTL). The duration of feeding was similar to those of other studies that investigated Cr supplementation in cows in early lactation (Vargas-Rodriguez et al., 2014; Yuan et al., 2014). Chromium was provided in the form of 20 g/d of KemTRACE chromium propionate 0.04% (Kemin Industries Inc., Des Moines, IA). Forages were collected once a week and analyzed for DM to adjust the as-fed TMR to maintain the formulated DM ratio of forage to concentrate. Samples of TMR were collected once per week and composited in 2 periods for nutritional analysis at a commercial laboratory (Cumberland Valley Analytical Services, Hagerstown, MD). The laboratory performed analysis of N, ADF, and minerals as described by AOAC International (2000); NDF as described by Van Soest et al. (1991); and ether extract as described by AOAC International (2006).

Table 1. Ingredients and chemical composition of basal diet fed to Holstein cows in mid lactation

Item	Value
Ingredient, % of DM	
Corn silage	37.79
Alfalfa hay	10.44
Sorghum sudangrass haylage	10.02
Ground corn	17.64
Soybean meal, 48%	12.76
Soy-plus[1]	1.51
Wheat middlings	1.34
Corn gluten meal, 60%	0.32
Megalac[2]	0.49
Premix[3]	7.70
Nutrient composition	
NE_L, Mcal/kg	1.60
CP, % of DM	15.27
NDF, % of DM	34.53
ADF, % of DM	22.43
Ether extract, % of DM	2.60
Ca, % of DM	0.88
P, % of DM	0.46
DCAD, mEq/kg of DM	282

[1]West Central (Ralston, IA).

[2]Church and Dwight Co. Inc. (Princeton, NJ).

[3]Contained (DM basis) 0.46% limestone, 0.17% Biophos (IMC-Agrico, Bannockburn, IL), 0.04% magnesium sulfate, 0.06% magnesium oxide, 0.25% sodium bicarbonate, 0.06% dynamite (Mosaic Co., Plymouth, MN), 0.25% salt-white, 0.12% Yeast XP (Diamond V Inc., Cedar Rapids, IA), 0.01% TM-433 (Southern States Cooperative Inc., Richmond, VA), 0.01% 4-plex (Southern States Cooperative Inc.), 0.01% ADE mix (5,454,545 IU/kg of vitamin A, 1,818,182 IU/kg of vitamin D, 9,091 IU/kg of vitamin E), 0.01% vitamin E (56,818 IU/kg), 0.03% selenium (0.06% selenium), 0.10% Omnigen-AF (Prince Agri Products Inc., Quincy IL), 0.03% Rumensin (Elanco, Greenfield, IN).

At d 0, 17, and 35 of feeding, jugular blood (~200 mL) was collected from each cow. Blood was drained via sterile IV catheters (Neogen Co., Lansing, MI) and collected into 15 mL sterile tubes containing 10% acid-citrate dextrose (**ACD**; Sigma-Aldrich Co., St. Louis, MO). Tubes were gently inverted ~15 times and placed on ice. Blood samples were processed within 2 h after collection. In addition, jugular blood (~10 mL) was collected with K_2EDTA (Thermo Fisher Scientific Inc., Waltham, MA) for whole blood cell composition using a ProCyte Dx hematology analyzer (Idexx Laboratories Inc., Westbrook, ME). Tubes containing K_2EDTA were centrifuged at 2,000 × *g* for 15 min at 4°C, and plasma was frozen at −20°C until further analysis.