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Short communication: Decreasing the dietary ratio of omega-6 to omega-3 fatty acids increases the omega-3 concentration of peripheral blood mononuclear cells in weaned Holstein heifer calves

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ABSTRACT

Utilization of nutrients to improve overall heifer health is of interest because of the importance of replacement heifers to the dairy industry. The objective of our study was to compare the effect of supplementation of dietary n-3 and n-6 fatty acids (FA) on FA concentrations in peripheral blood mononuclear cells (PBMC) of Holstein calves. Twenty-seven Holstein heifer calves (107 ± 2.6 d of age; 142.6 ± 6.5 kg of body weight) from the university research and teaching herd were randomly assigned to a common TMR supplemented with 1 of 3 treatments: Ca salts of flaxseed FA (Virtus Nutrition, Corcoran, CA) containing 35% 18:3 n-3 (N3), Ca salts of soybean FA (Virtus Nutrition) containing 50% 18:2 n-6 (N6), or a 50:50 mix of N3 and N6. Treatments were supplemented with FA at 4% of dietary dry matter and fed for 30 d. Feed intake was recorded daily, and body weight, wither height, and body condition score were measured weekly throughout the study. On d 28 heifers were vaccinated with a *Pasteurella* vaccine and the temperature response to the vaccine was recorded. Blood was collected on d 0 and 28 for PBMC isolation. After total lipid extraction and FA methyl ester preparation, FA composition of PBMC was measured. We observed no effect of treatment on body weight gain, body condition score change, or wither height change. Heifers receiving the N3 diet had a lower temperature response to *Pasteurella* challenge compared with both the mix and N6 diets. Heifers consuming the N3 diet had a greater content of total n-3 FA, α-linolenic acid, and eicosapentaenoic acid in PBMC compared with heifers fed the N6 and mix diets. Heifers receiving the N3 diet also had a lower content of total n-6 FA, linoleic acid, and arachidonic acid in PBMC than heifers fed the N6 and mix diets. In conclusion, our study determined that feeding weaned female Holstein heifers a diet high in n-3 FA increased concentrations of n-3 FA in PBMC.
Key words: fatty acid, heifer, peripheral blood mononuclear cell

Short Communication

The health and survival of replacement heifers is critical to dairy operations. Dairy managers rely on replacement heifers to increase herd size, replace cows culled, increase milk production, and enhance profitability. Consequently, it is of interest to utilize nutrients to prevent or decrease incidence of disease and improve overall health of heifers.

Supplementation of α-linolenic acid (**ALA**; 18:3 n-3 series) and linoleic acid (**LA**; 18:2 n-6 series), both essential PUFA, can influence animal performance when included in ruminant diets. Calder (2005) reported that ALA has anti-inflammatory and proresolution properties. Feeds commonly rich in n-3 fatty acids (**FA**) fed to dairy cows include flaxseed, fish meal oil, fish oil, and fresh grass and alfalfa. Conversely, feeds higher in n-6 FA include corn grain, corn and alfalfa silage, corn distillers grain, soybean meal, and whole cottonseeds (National Research Council, 2001).

Dietary n-3 FA, such as ALA, serve as precursors to docosahexaenoic acid (**DHA**; 22:6 n-3) and eicosapentaenoic acid (**EPA**; 20:5 n-3). Treatment of human leukemic promonocytes with DHA and EPA decreased cell expression of several proinflammatory cytokines, including tumor necrosis factor (**TNF**)-α and IL-1β (Chu et al., 1999). Linoleic acid has both pro- and anti-inflammatory roles (Fritsche, 2008). Arachidonic acid (**ARA**; 20:4 n-6) can be synthesized from LA and can lead to the formation of prostaglandins and thromboxanes that have proinflammatory properties (Ricciotti and FitzGerald, 2011). Alternatively, production of prostaglandin E2, a derivative of ARA, inhibits production of the proinflammatory cytokines TNF-α and IL-1 (Calder, 2006).

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Calder and Yaqoob (2007) reported that FA could affect immune function in numerous ways, including controlling gene expression and modifying lipid mediator production. To date, there are limited reports on the effect of dietary FA with respect to altering the immune response in dairy calves (Hill et al., 2011a; Karcher et al., 2014). Feeding preweaned calves diets with 2% flax oil or fish oil, both high in n-3 FA, tended to moderate the fever response after *Pasteurella* vaccine challenge and reduced blood cell expression of key proinflammatory cytokines (Karcher et al., 2014).

Phospholipids are a primary component of cellular membranes, and the FA composition of the diet can influence the FA profile of the phospholipids in membranes. For example, intake of a diet high in n-6 FA can change the FA composition of the phospholipids in cell membranes such that more LA and ARA are present. This can alter gene expression toward a proinflammatory state (Calder, 2012). Conversely, consuming a diet high in n-3 FA leads to higher levels of ALA or other n-3 FA in membrane phospholipids (Calder, 2012). In this case, gene expression would favor an anti-inflammatory state (Calder, 2012).

Therefore, the objective of this study was to determine the effects of supplemental dietary n-3 and n-6 FA on the FA concentration of peripheral blood mononuclear cells (**PBMC**) of weaned Holstein heifer calves. We hypothesized that supplementing varying levels of n-3 and n-6 FA to heifers fed a TMR would alter the FA concentration of PBMC.

The Michigan State University Institutional Animal Care and Use Committee approved all animal procedures before the start of the experiment. Twenty-seven Holstein heifer calves (107 ± 2.6 d of age; 142.6 ± 6.5 kg of BW) were selected from the Michigan State University Dairy Teaching and Research Center and were housed in individual stalls (1.94 m deep, 1.13 m wide). Heifers were randomly assigned to a common TMR, fed once daily in the morning, and supplemented with 1 of 3 treatments (9 heifers/diet): Ca salts of flaxseed FA (Virtus Nutrition, Corcoran, CA) containing 35% 18:3 n-3 (**N3**), Ca salts of soybean FA (Virtus Nutrition) containing 50% 18:2 n-6 (**N6**), and a 50:50 mix of N3 and N6. Heifers were fed the common TMR for a 14-d adaptation period before the start of supplemental dietary fat inclusion and collection of data. The FA treatment was supplemented at 4% of dietary DM for 30 d and topdressed each morning at the same time the TMR was fed. Water was provided ad libitum, and calves were fed to achieve an ADG of 0.9 kg/d. The trial consisted of 3 blocks, each with a 14-d adaptation period and 30-d treatment period, with 9 heifers/block (3 heifers/treatment per block). The study was conducted from January 2012 to May 2012.

The ingredient and nutrient composition of the diets fed as a TMR and fat supplement analysis are described in Table 1. The DM concentration was determined weekly for forages, and diet was adjusted when necessary. Diet ingredients were sampled from each block and dried at 55°C in a forced-air oven for 72 h for DM determination. Dried samples were ground with a Wiley mill (1-mm screen; Arthur H. Thomas, Philadelphia, PA) and analyzed as reported by Boerman et al. (2017). The FA concentration of the fat supplements was determined as described by Lock et al. (2013).

Intake of feed from each animal was measured daily (Table 2). Feed provided was adjusted weekly to target a gain of 0.9 kg/d. Heifers were weighed at the beginning of the treatment period and weekly at d 7, 14, 21, and 28. Wither height and BCS were also measured weekly throughout the experiment. The 1-to-5 system was used for BCS (0.25-unit increments; Wildman et al., 1982), with 1 being emaciated and 5 being obese. Scores were based on changes around the vertical and transverse processes of the spine as palpated by 2 trained technicians. On d 28, heifers were vaccinated with a *Pasteurella* vaccine (OneShot; Zoetis, Madison, NJ). Rectal temperature was measured at 0, 3, 6, 9, 12, 24, 36, and 48 h relative to vaccine administration.

Blood was collected from each heifer via jugular venipuncture on d 0 and d 28 before vaccination. At each sampling, 200 mL of blood was collected in blood collection tubes containing EDTA as the anticoagulant. Samples were then placed on ice and processed within 2 h of collection.

After PBMC isolation and purification (Contreras et al., 2010), total lipids were extracted using chloroform:methanol:water (2:2:1, by vol) according to Bligh and Dyer (1959). Fatty acid methyl esters were prepared using a combination of base-catalyzed transesterification (0.5 *N* KOH in methanol) followed by an acid-catalyzed methylation (BF_3 in methanol, 10% wt/wt) and dissolved in n-hexanes (Christie, 1993). Fatty acid methyl esters were further purified by solid phase extraction silica cartridges (Supelco, Bellefonte, PA) using n-hexanes:chloroform (1:1). The FA composition analysis and GC conditions were as described by Lock et al. (2013).

Growth data were analyzed using the MIXED procedure in SAS (version 8; SAS Institute Inc., Cary, NC) as a completely randomized design. The statistical model used was $Y_{ijk} = \mu + T_i + w_j + Tw_{ij} + C_k(T_i) + \varepsilon_{ijk}$, where Y_{ijk} is the observed measurement, μ represents the overall population mean, T_i is the fixed effect of treatment i, w_j is the random effect of week j, Tw_{ij} is the interaction of treatment i and week j, $C_k(T_i)$ is the random effect of calf k nested within treatment i that was used to test the effect of treatment, and ε_{ijk} is

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