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Effects of whole flaxseed, raw soybeans, and calcium salts of fatty acids on measures of cellular immune function of transition dairy cows

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ABSTRACT

The objective of the current study was to evaluate the effects of supplemental n-3 and n-6 fatty acid (FA) sources on cellular immune function of transition dairy cows. Animals were randomly assigned to receive 1 of 4 diets: control (n = 11); whole flaxseed (n-3 FA source; n = 11), 60 and 80 g/kg of whole flaxseed [diet dry matter (DM) basis] during pre- and postpartum, respectively; whole raw soybeans (n-6 FA source; n = 10), 120 and 160 g/kg of whole raw soybeans (diet DM basis) during pre- and postpartum, respectively; and calcium salts of unsaturated FA (Megalac-E, n-6 FA source; n = 10), 24 and 32 g/kg of calcium salts of unsaturated FA (diet DM basis) during pre- and postpartum, respectively. Supplemental FA did not alter DM intake and milk yield but increased energy balance during the postpartum period. Diets containing n-3 and n-6 FA sources increased phagocytosis capacity of leukocytes and monocytes and phagocytosis activity of monocytes. Furthermore, n-3 FA source increased phagocytic capacity of leukocytes and neutrophils and increased phagocytic activity in monocytes and neutrophils when compared with n-6 FA sources. Supplemental FA effects on adaptive immune system included increased percentage of T-helper cells, T-cytotoxic cells, cells that expressed IL-2 receptors, and CD62 adhesion molecules. The results of this study suggest that unsaturated FA can modulate innate and adaptive cellular immunity and trigger a proinflammatory response. The n-3 FA seems to have a greater effect on phagocytic capacity and activity of leukocytes when compared with n-6 FA.
Key words: adaptive immunity, innate immunity, phagocytosis, unsaturated fatty acid

INTRODUCTION

The immune system is composed of innate and adaptive components, in addition to the anatomical and physiological barriers. The components of innate immunity are white blood cells (leukocytes), such as macrophages, neutrophils, and natural killer cells. Innate immunity is the first line of defense against microbial infection (Aderem, 2003). On the other hand, the adaptive immune system is composed of T and B lymphocytes, dependent on a clonal system for activation and proliferation, and may take 4 to 7 d to take effect (Turvey and Broide, 2010).

Fatty acids (**FA**) can modify the immune response by several mechanisms, which include inhibition of arachidonic acid metabolism, induction of antiinflammatory mediators, modification of intracellular lipids, and activation of nuclear receptors (Yaqoob, 2004; Calder, 2006). Studies in cultured cells, animal models, and human subjects have shown that both the amount and type of FA influence the immune response (Kelley and Rudolph, 2000). For example, Silvestre et al. (2011) observed that feeding calcium salts of safflower oil (rich in n-6 FA) instead of palm oil (rich in saturated FA) during the early postpartum period resulted in greater phagocytic and oxidative burst activity in neutrophils.

The transition from late gestation to early lactation is the period of greatest risk for metabolic and infectious diseases and even death in dairy cows (Grummer et al., 2004). Many types of evidence exist that the immune system is not functioning at an optimal level during the transition period, including decreased mitogen-induced proliferation of lymphocytes (Kimura et al., 2002), decreased antibody response (Mallard et al., 1997), decreased chemotaxis and adhesion molecule expression by neutrophils (Weber et al., 2001), and decreased capacity of neutrophils to kill pathogens (Hammon et al., 2006). One strategy to improve the metabolic status of transition cows is supplementing FA, which can

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increase dietary energy density (Grummer et al., 2004) and modulate immune cell function and inflammatory response (Greco et al., 2015). However, published scientific studies are relatively lacking on the effects of specific FA on cellular immune function in transition cows.

The current study was designed to evaluate the effects of diets containing oilseeds or calcium salts of FA rich in n-3 FA (flaxseed) or n-6 FA (whole raw soybeans and calcium salts of unsaturated FA) on phagocytic capacity and activity and leukocyte profile of dairy cows during the transition period and early lactation. Our hypothesis was that supplemental fat would modulate the immune system in dairy cows during the transition period and the response would depend on the FA source.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

This study was approved by the Bioethics Committee of the School of Veterinary Medicine and Animal Sciences, University of Sao Paulo, in accordance with the ethical principles of animal experimentation. The experiment was conducted in the Dairy Cattle Research Laboratory, Pirassununga, Brazil.

A total of 42 multiparous Holstein cows were randomly assigned to receive 1 of 4 diets during the pre- and postpartum periods: (1) control (**CON**; n = 11); (2) flaxseed (**WF**, n-3 FA source; n = 11), cows fed 60 g/kg (prepartum) and 80 g/kg (postpartum) of WF (diet DM basis); (3) whole raw soybeans (**WS**, n-6 FA source; n = 10), cows fed 120 g/kg (prepartum) and 160 g/kg (postpartum) of WS (diet DM basis); and (4) calcium salts of unsaturated FA (**CSFA**; Megalac-E, Elanco-Eli Lilly and Company, Indianapolis, IN, n-6 rumen-protected FA source; n = 10), cows fed 24 g/kg (prepartum) and 32 g/kg (postpartum) of CSFA (diet DM basis). The experimental diets were supplied from 35 d before the expected calving until 84 DIM and were formulated according to NRC (2001; Table 1).

At the start of the experiment, the CON group was composed of cows with 3.5 ± 0.5 parturitions, 762 ± 14.1 kg of BW, and 3.1 ± 0.18 BCS; the WF group was composed of cows with 4.0 ± 0.5 parturitions, 782 ± 27.1 kg of BW, and 3.25 ± 0.20 BCS; the WS group was composed of cows with 4.0 ± 1.5 parturitions, 712 ± 22.5 kg of BW, and 3.05 ± 0.15 BCS; and the CSFA group was composed of cows with 4.0 ± 1.0 parturitions, 734 ± 21.7 kg of BW, and 3.1 ± 0.15 BCS (mean ± SD).

Throughout the experiment, cows were housed in individual pens (17.5 m^2) containing forced ventilation, sand beds, and individual feeding troughs. Diets were fed daily at 0800 and 1300 h as a TMR. Amounts of feed offered and orts for each cow were weighted daily and restricted to 5 to 10% of intake on an as-fed basis. Cows were mechanically milked daily at 0630 and 1530 h, and milk yield measurements were made with an automatic milk meter that sent information to herd management software (Alpro, DeLaval, Tumba, Sweden).

Analysis of FA

The corn silage and Tifton hay were sampled weekly (17 samples), and ingredients of grain mixture (WF, WS, CSFA, ground corn, and soybean meal) were collected during the preparation of concentrate (4 samples) for chemical analysis. To analyze the FA in ingredients, lipids extraction and methylation were performed as described by Sukhija and Palmquist (1988) with minor modifications as described below. Extraction was done with 6 mL of chloroform, instead of 2 mL, and methylations were done with methanolic-HCl but using a concentration of 6.5%, instead of 10%, and a volume of 9 mL, rather than 3 mL. Thus, the ratio of extracting solvent to methylation reagent was the same as in Sukhija and Palmquist (1988). Incubation time was increased from 2 to 2.5 h, and temperature was reduced from 80 to 65°C. Tubes were continuously checked for leaks during incubation and were repeated if substantial leaks occurred.

Fatty acids were quantified by gas chromatography (GC Shimadzu 2010 with automatic injection, Shimadzu Corporation, Kyoto, Japan) equipped with a SP-2560 capillary column (100 m × 0.25 mm i.d. with 0.02-μm film thickness; Supelco, Bellefonte, PA). Oven temperature was 70°C for 4 min, increased by 13°C/min until 175°C, and then held at this temperature for 27 min. Finally, temperature was increased by 4°C/min until it reached 215°C and then was kept at this temperature for 31 min. Hydrogen (H_2) was used as the carrier gas with a flow rate of 40 cm^3/s. Four standards were used for FA identification: standard C4-C24 (TM 37, Supelco Sigma-Aldrich Group), C18:1 *trans*-11 (V038-1G, Supelco Sigma-Aldrich Group), C18:2 *trans*-10, *cis*-12 (UC-61M 100 mg, Nu-Chek Prep Inc., Elysian, MN), and C18:2 *cis*-9, *trans*-11 (UC-60M 100 mg, Nu-Chek Prep Inc.).

DMI and Energy Balance

Orts samples (12.5% of total daily orts) were collected daily from each cow and were combined into a single sample for posterior chemical analysis. Samples were stored at −20°C. Dry matter (method 950.15), ether extract (method 920.39), and CP (N × 6.25;