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Immune response of cows fed polyunsaturated fatty acids under high ambient temperatures

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ABSTRACT

The aim of the experiment was to determine the effects of 2 different fat supplementations on immune functions of dairy cows under high ambient temperatures. The experiment involved 24 Italian Friesian cows, divided into 3 groups of 8 animals, that were subjected to fat supplementations based on whole flaxseed (FS) or microencapsulated fish oil (FO). At d 0, 45, and 90 of the experiment, lymphocyte response to phytohemagglutinin (PHA) was determined in vivo on each animal by measurement of skin-fold thickness at the site of PHA injection. A humoral response to chicken egg albumin (OVA) was established following a subcutaneous injection with OVA. To assess cows' immune responses, plasma was prepared from experimental blood samples taken at d 0, 15, 30, 45, 60, 75, and 90 of the experiment. Plasma samples were measured for the presence of anti-OVA IgG, IL-1β, IL-6, and IL-10. Results revealed greater skin-fold thickness in cows fed FS compared with the FO and the control groups, corresponding to higher mean lymphocyte proliferation following in vivo PHA injection. Cows fed FS displayed higher titers of anti-OVA IgG than the control and FO-fed cows. No effects of the diet on IL-1β or IL-6 were found, whereas IL-10 secretion was lower in FS-fed cows than in control cows. The present study demonstrates that feed supplementation of n-3 polyunsaturated fatty acids can enhance immune responses of dairy cows exposed to high ambient temperatures.

Key words: immune function, dairy cow, polyunsaturated fatty acid, high ambient temperature

INTRODUCTION

Environmental and nutritional factors such as ambient temperature and diet have been implicated in alterations of immune function. Peripheral blood mononuclear cells (**PBMC**) collected from dairy cattle experiencing temperature-humidity index (**THI**) values >72 exhibit reduced proliferation in vitro in response to mitogenic stimulation compared with PBMC from cattle experiencing THI values <72 (Lacetera et al., 2005). Furthermore, incubation of cattle PBMC at high temperature (42°C) reduces proliferation to mitogens compared with incubation at 38.5°C, an observation that is not simply due to reduced cell viability (Elvinger et al., 1991). The precise mechanisms underlying reduced cellular immune function in cattle under higher temperatures remain undefined, particularly with regard to cytokine profiles that would be indicative of a regulatory, antiinflammatory phenotype.

A balanced and adequate diet positively influences the development, maintenance, and function of the immune system. Several studies in humans have reported that increased intake of n-3 polyunsaturated fatty acids (**PUFA**) in plant and fish oils results in decreased proliferation of PBMC in response to mitogenic stimulation and decreased production of proinflammatory cytokines such as IL-1β, IL-6, and tumor necrosis factor (**TNF**)-α (Kelley, 2001). Such immunomodulatory effects have led to an interest in the therapeutic use of dietary PUFA to treat inflammatory immune disorders in humans (Calder, 2002). Consistent with the studies in humans, PUFA have also been reported to exert immunomodulatory effects in livestock. Dairy cows fed flaxseed rich in n-3 PUFA exhibit a transient reduction in mitogen-driven PBMC and a reduction in blood concentration of prostaglandin E_2 after calving (Lessard et al., 2003). The immunomodulatory effects of n-3 PUFA in cattle appear to be directed more toward T-cell and monocyte/macrophage function because there are no significant differences in antigen-specific antibody responses in cattle fed flaxseed compared with cattle fed a diet enriched with saturated fatty acids or soybeans (n-6 PUFA; Lessard et al., 2003, 2004). The infusion of fish oil or linseed to PBMC isolated from fasted dairy cows contributed to reduce the alteration of lymphocytes to mitogens (Lacetera et al., 2007).

The aim of the present study was to evaluate the effects of dietary fatty acid enrichment (flaxseed and fish

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Table 1. Ingredient and chemical composition of the experimental diets (DM basis)

Item	Diet[1]		
	Control	FO	FS
Ingredient			
Concentrate	61.4	60.7	54.8
Corn	5.7	5.7	5.7
Oat hay	32.9	32.5	33
Whole flaxseed[2]	0	0	6.5
Fish oil[3]	0	1.1	0
Chemical composition			
DM, %	92.04	92.00	92.26
Ether extract, % of DM	3.23	3.46	5.31
CP, % of DM	15.43	15.36	15.21
ADF, % of DM	22.72	22.49	22.75
NDF, % of DM	40.99	41.45	40.84
ADL,[4] % of DM	3.98	3.95	4.09
NE_L, Mcal/kg	1.68	1.69	1.65

[1]Control group had no fat supplementation; FO = group with fat supplementation based on fish oil; FS = group with fat supplementation based on flaxseed.

[2]Lin Tech (Tecnozoo srl, Torreselle di Piombino Dese, Italy).

[3]Orovital Cod (Ascor Chimici srl, Capocolle di Bertinoro, Italy).

[4]ADL = acid detergent lignin.

oil) on the immune function of dairy cows experiencing high ambient temperatures.

MATERIALS AND METHODS

Animals and Monitoring of Environmental Conditions

The experimental site was a commercial farm located approximately 20 km northeast of Foggia, Apulia, southern Italy (latitude 41°27′6″ and longitude 15°33′5″). Air temperature and relative humidity were monitored daily using TIG2-TH thermo-hygrographs (LSI, Settala Premenugo, Milan, Italy). Data from thermo-hygrographs in conjunction with Kelly and Bond's (1971) formula were used to calculate the THI. The 12-wk trial was performed from June to September 2006, with 24 Italian Friesian cows divided into 3 groups of 8 cattle. The cows were blocked based on age, BW, DIM (100.11 ± 3.79), parity (2.46 ± 0.26), milk yield (24.37 ± 0.36 kg/d), and milk fat (3.77 ± 0.13), and protein (3.03 ± 0.08) content.

Experimental Diets and Chromatographic Analysis of Feed

All animals were individually fed with the same diet based on corn silage, oat hay, and concentrate, plus different fat supplementations based on whole flaxseed (**FS**; Lin Tech, Tecnozoo srl, Torreselle di Piombino Dese, Italy) or microencapsulated fish oil (**FO**; Orovital Cod, Ascor Chimici srl, Capocolle di Bertinoro, Italy) (Table 1). Cows were fed twice daily and feed consumption was recorded daily. Cows in all groups completely consumed the daily ration given. The DMI was 16.57 kg/d for control cows, 16.75 kg/d for FO cows, and 16.61 kg/d for FS cows. Net energy for lactation was calculated using NRC (2001). Water was available ad libitum. A sample from each experimental diet was taken weekly, frozen, and mixed for chemical analyses. The chemical composition of diets was determined by standard procedures (AOAC, 1990; Table1). Fatty acid analysis of supplementations was carried out according to Sukhija and Palmquist (1988). Fatty acid methyl esters (**FAME**) were analyzed on an Agilent 6890N gas chromatograph (Agilent, Santa Clara, CA). Separation of the FAME was performed using a DB 23 fused-silica capillary column [60 m × 0.25 mm (i.d.) with 0.25μm film thickness]. Operating conditions were helium flow rate of 1.2 mL/min; flame-ionization detector at 250°C; a split-splitless injector at 240°C; and an injection volume of 1 μL with a split ratio 1:50. The initial column temperature was set at 60°C, increased to 180°C at 25°C/min, and finally increased to 230°C at 6°C/min and held for 15 min. Retention time and area of each peak were computed using the 6890N NETWORK GC system software (Agilent). Individual FAME peaks were identified by comparing their retention times with those of defined standards (FAME mix 37 components; Matreya, Sigma-Aldrich, Milan, Italy). Results were expressed as percentage of total fatty acids analyzed (Table2).

Evaluation of the Cell-Mediated Immune Response

At d 0, 45, and 90, lymphocyte proliferation was determined in vivo in each cow by the measurement of changes in skin-fold thickness in response to intradermal injection with 1 mg/mL of phytohemagglutinin (**PHA**; Sigma) dissolved in 1 mL of sterile saline solution. At each sampling time, the injection was administered into the center of a 2-cm-diameter circle marked on shaved skin on the upper side of each shoulder. The determination of lymphocyte proliferation, measured as skin-fold

Table 2. Fatty acid composition (% of total fatty acids) of fat supplementations

Fatty acids, % of total of fatty acids	Flaxseed	Fish oil
C14:0	0.06	5.91
C16:0	5.53	17.1
C16:1	0.08	7.26
C18:0	3.56	4.29
C18:1n-9 *cis*	16.29	17.46
C18:2n-6 *cis*-9,*cis*-12	16.75	6.4
C18:3n-3	53.21	1.17
C20:5n-3 (docosahexaenoic acid)	0.01	6.15
C22:6n-3 (eicosapentaenoic acid)	0.01	6.72