Modifying the Acute Phase Response of Jersey Calves by Supplementing Milk Replacer with Omega-3 Fatty Acids from Fish Oil

M. A. Ballou,*¹ G. D. Cruz,* W. Pittroff,* D. H. Keisler,† and E. J. DePeters*²

*Department of Animal Science and Nutritional Biology Graduate Group, University of California, Davis 95616

†Division of Animal Sciences, University of Missouri, Columbia 65211

ABSTRACT

Fifty-one Jersey bull calves (5 ± 1 d old) were assigned to 1 of 3 milk replacers to determine the effects of increasing doses of n-3 fatty acids from fish oil on the acute phase response after an endotoxin challenge. All calves were fed a 22.5% crude protein and 18% lipid milk replacer (Calva Products, Acampo, CA) supplemented with an additional 2% fatty acids. Treatments differed only in the supplemental lipid source and included a 3:1 mix of corn and canola oils, a 1:1 blend of fish oil (Omega Proteins, Houston, TX) and the 3:1 mix of corn and canola oils, and fish oil only. On d 23, each calf was injected subcutaneously with 4 μ g/kg of body weight of *Salmonella* Typhimurium endotoxin. Clinical, hematological, and biochemical parameters were measured at 0, 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 24, and 72 h post endotoxin challenge. Endotoxin caused a dramatic rise in respiratory rate; feeding fish oil significantly attenuated the increase. Heart rate and rectal temperature were not affected by treatment. Feeding fish oil attenuated the change in serum iron concentration over time. Endotoxin caused severe hypoglycemia, reaching a nadir at 4 h. Calves supplemented with fish oil had reduced concentrations of serum glucose for 8 to 24 h. Furthermore, calves supplemented with fish oil alone had reduced serum insulin at 12, 28, and 24 h. In contrast, endotoxin caused an acute increase in blood urea nitrogen and nonesterified fatty acids; there were significant linear effects of fish oil on both blood urea nitrogen and nonesterified fatty acids. Serum triglycerides were elevated beginning at 12 h after the endotoxin challenge and returned to baseline values within 72 h. Fish oil suppressed the rise in triglycerides during this period, and the effect was linear with increasing fish oil. Serum concentrations of leptin decreased after the endotoxin challenge; however, the treatment did

not influence the response. There was no treatment effect on serum aspartate aminotransferase or lactate dehydrogenase activity. Adding fish oil to milk replacer attenuated many aspects of the acute phase response, and the effect was linear in the range of 5 to 10% of the lipid replaced as fatty acids from fish oil. Adding fish oil might provide a better balance between a necessary versus an excessive acute phase response.

Key words: calf, fish oil, inflammation, septicemia

INTRODUCTION

Septicemia occurs when bacteria transverse the physical barriers, evade the immune system, and enter the bloodstream. Septicemia is a major cause of mortality among dairy calves; Lofstedt et al. (1999) found 31% of scouring calves to be septicemic, and survival rates were extremely low, only 12%. In agreement, Fecteau et al. (1997) reported that the incidence of septicemia in severely ill calves was between 24 and 31%. The mortality rate of the severely ill calves was much greater for calves with associated septicemia, 57.4% of blood culture-positive calves versus 15.1% of blood culture-negative calves. The high mortality associated with septicemia is attributed to an overaggressive systemic acute phase response. There are 2 stages in the pathogenesis of the acute phase response (Lanza-Jacoby et al., 2001). The first is a period of hyperinflammation, followed second by immune paralysis caused by a strong counter antiinflammatory response. The systemic release of cytokines by macrophages in the liver, spleen, and other sites causes vasodilatation and increases vascular permeability, which results in a loss of plasma volume and blood pressure that could lead to septic shock. In addition, activation of the clotting cascade during septic shock results in disseminated intravascular coagulation, which further compromises perfusion of blood to vital organs, including the heart, liver, lungs, and kidneys. Often, irreversible organ failure results, and if the calf survives the acute phase overreaction, subsequent immune paralysis severely compromises its defenses against the infecting pathogen (Lanza-Jacoby et al., 2001). Therefore, strategies

Received January 15, 2008.

Accepted May 28, 2008.

¹Current address: Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409.

²Corresponding author: ejdepeters@ucdavis.edu

that attenuate the pathogenesis of the acute phase response during septicemia hold promise for significantly reducing calf mortality.

Feeding fish oil (FO) as the sole source of lipid [15% (wt/wt) of the diet] compared with safflower oil improved survival (87 vs. 63%) at 20 h after an LPS challenge in guinea pigs and decreased the accompanying acute phase response (Mascioli et al., 1989). Furthermore, rats supplemented with FO at 1 mL/d for 14 d increased survival after cecal ligation and puncture compared with rats supplemented with corn oil at the same dose (Johnson et al., 1993). These studies attributed the observed responses mainly to supplemental FO attenuating the hyperinflammation. On the other hand, data from septicemic rats and humans provide evidence that supplemental FO reduces the immune-suppressive effect on both innate and adaptive immune responses during the immune paralysis phase of septicemia (Lanza-Jacoby et al., 2001; Mayer et al., 2003). Thus, supplemental FO may lead to a more balanced acute phase response, circumventing the hyperinflammation as well as the immune paralysis response. No data exist on dairy calves that describe the effects of supplemental FO on the acute phase response. Therefore, the objectives of this study were to determine the effects of increasing doses of n-3 fatty acids (FA) from FO on the acute phase response after an LPS challenge. Clinical and biochemical acute phase responses were determined.

MATERIALS AND METHODS

Experimental Design, Calves, and Diets

Fifty-one Jersey bull calves (1 ± 1 d of age) were acquired from local commercial dairy herds over a period of 3 d. On arrival at the University of California at Davis research facility, calves were examined and weighed, 20 mL of blood was sampled into evacuated tubes containing either no additive or K₂EDTA, and calves were given 0.1 mL/kg of BW of prophylactic long-acting oxytetracycline (LA200, 200 mg/mL oxytetracycline s.c., Pfizer Animal Health, New York, NY). Calves were individually housed in wire pens (1.5 × 3 m) that were bedded with rice hulls.

All calves were fed a commercial milk replacer for a 4-d adaptation period. During this period, all calves were trained to drink from buckets. After the adaptation period, calves were assigned randomly to 1 of 3 treatment diets, differing only in the FA composition of the milk replacer. To ensure adequate randomization, BW and total serum protein were evaluated after the assignment of treatments. The basal diet given to all calves was a 22.5% CP and 18% lipid industry-standard

milk replacer (Calva Products, Acampo, CA). The treatments were applied by supplementing the basal diet with an additional 2% FA. Treatments included a 3:1 (wt/wt) blend of corn and canola oils (control diet), a 1:1 (wt/wt) mix of FO (Omega Proteins, Houston, TX) and the control oil (blend diet), and FO only (fish diet). The oils for each treatment were blended 3:1 (wt/wt) with silica dioxide (Rhodia Silica Systems, Lyon, France) to make the oil more powder-like. Twenty-five percent of the milk replacer was added slowly to the silica oil blend and mixed well for 2 min in a Hobart mixer (Hobart Corp., Troy, OH). An additional 25% of the milk replacer was added and mixed for another 2 min. Finally, the remaining milk replacer was added and mixed for 5 min. All milk replacer was sacked in approximately 50-lb (22.7-kg) bags, sealed, and stored at 4°C to minimize peroxidation of the FA. No additional emulsifying agents were added; preliminary studies showed that the supplemental oil emulsified well in the milk replacer. All treatments were supplemented with 150 mg of vitamin E/kg of milk replacer, which is approximately 3 times the minimum recommended dose published by the NRC (2001). The additional vitamin E was added by the manufacturer. Calves were fed according to NRC recommendations for calves fed only milk replacer (NRC, 2001). Calves were fed twice daily at 0700 and 1600 h and had free access to fresh water. Intake of milk replacer was adjusted once weekly to account for changes in BW. Predicted ADG for wk 1 and 2, 3 to 5, and 6 to 8 were 200, 400, and 600 g/d, respectively. Samples of milk replacer were collected every Monday, Wednesday, and Friday a.m. and composited by week for proximate analyses and FA determination. All animal care was approved by the Animal Care and Use Committee of the University of California at Davis.

LPS Challenge

On d 23 after the initiation of dietary treatments, each calf was injected subcutaneously with 4 µg/kg of BW of *Salmonella* Typhimurium LPS (Sigma-Aldrich Co., St. Louis, MO) at 0600 h. The quantity of LPS used in the challenge was determined from a preliminary study. The dose of LPS caused an acute change in all the variables evaluated in the current study, with limited risk of mortality. The LPS was reconstituted with nonpyrogenic PBS. Calves were not fed during the first 24 h of data collection because postprandial metabolic changes may have confounded the effects of LPS on blood metabolites and hormones (Hüsier and Blum, 2002). Furthermore, anorexia is a well-known response during an acute phase response, and restricting milk intake during the challenge is more physiologically relevant.

Observations and Sampling

General attitude, appetite, rectal temperature, and heart and respiration rates were assessed before and at 1, 2, 3, 4, 5, 6, 8, 10, 12, 15, 18, 24, and 72 h post LPS challenge. Approximately 20 mL of peripheral blood was collected at each time point into evacuated tubes containing either no additive or K₂EDTA to evaluate changes in clinical and biochemical parameters. General attitude was visually classified as 0 = alert, normal; 1 = alert, depressed; 2 = lethargic, responds slowly to stimuli; or 3 = morbid, little or no response. The suckling response of each calf, as an objective measure of appetite, was gauged based on the intensity with which the calf suckled the observer's finger. Appetite was classified as 0 = normal, strong suckle reflex; 1 = moderately anorectic, weak suckle reflex, licks finger; and 2 = completely anorectic, no suckle reflex or interest in the observer's finger. All attitude and appetite data were collected by 1 observer throughout the experiment. Heart and respiration rates were recorded with the aid of a stethoscope. Rectal temperatures were taken with thermometers that were calibrated before the challenge.

Serum Metabolite Analyses

Glucose and BUN were analyzed colorimetrically with a Technicon Auto Analyzer (Technicon Corp., Ardsley, NY). Glucose was determined by using the ferricyanide method and BUN was determined via a modified carbamido-diacetyl reaction. Triacylglycerol (TG) and NEFA were analyzed colorimetrically by using commercially available kits (Wako USA, Richmond, VA). Triacylglycerol was measured by using a micro-method described by the company, and NEFA was measured by using a micro-method described by Johnson and Peters (1993).

Plasma lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities were measured enzymatically by using commercial reagents (StanBio Labs, San Antonio, TX). All methods were adapted for use in a microplate and were standardized by using commercially available control sera (Bio-Rad, Hercules, CA). Briefly, the volumes of serum used were 2 μ L for LDH and 10 μ L for AST. The AST assay was based on the linear increase in reduced nicotinamide adenine dinucleotide production, and the LDH assay was based on the linear extinction of reduced nicotinamide adenine dinucleotide. The ratios of serum to reagent used were according to the manufacturer's instructions. All colorimetric and enzymatic assays were measured with a VersaMax microplate reader spectrophotometer (Molecular Devices, Sunnyvale, CA).

Serum iron was determined by atomic absorption spectrophotometry (PerkinElmer, Norwalk, CT). Briefly, 400 μ L of serum was diluted with equal volumes of 20% TCA. The mixture was heated at 90°C for 15 min and then centrifuged; 150 μ L of the supernatant and each standard were removed in duplicate and placed into 500- μ L microcentrifuge tubes. Working standards (0.5 to 5 mg/L) were prepared in 10% TCA. The 150- μ L sample and standards were discontinuously nebulized into an air-C₂H₂ flame. Real-time peak heights (mm) were recorded for each standard and sample.

Serum concentrations of leptin were determined in triplicate and were quantified by using a competitive liquid-liquid phase, double-antibody leptin RIA procedure described previously (Delavaud et al., 2000), with one modification. The primary antiserum reported by Delavaud et al. (2000) was substituted with rabbit anti-ovine leptin primary antiserum no. 7105 (D. K. Keisler, University of Missouri, Columbia). Serum concentrations of insulin were measured in triplicate and quantified by using a specific double-antibody, equilibrium RIA as described previously (Kolath et al., 2006). The intraassay coefficients of variation were 1.75 and 1.95%, whereas the interassay coefficients of variation were 4.65 and 3.82% for the insulin and leptin assays, respectively.

Statistical Analyses

The repeated ordinal variables, attitude and appetite, were analyzed by generalized estimating equations with a multinomial distribution by using the GenMod procedure of SAS (version 9.1, 2003, SAS Institute Inc., Cary, NC). Calf was the repeated unit and the independent covariance structure was used to fit the model. Orthogonal comparisons were performed to determine the linear and quadratic effects of supplemental FO.

All repeated continuous data were analyzed by restricted maximum likelihood ANOVA for a completely randomized design by using the MIXED procedure of SAS (version 9.1, 2003, SAS Institute). A linear, mixed model with the fixed effects of treatment, time, and the interaction of treatment $\times$ time was fitted. The mean model was run with all available covariance structures for the within-subject measurement. The appropriate covariance structure for unequal time spacing was chosen for each analysis based on Schwarz's Bayesian information criterion. Degrees of freedom for *F*-tests of the fixed effects were estimated by using the Kenward-Rogers approximation. Orthogonal comparisons were performed to determine the linear and quadratic effects of supplemental FO. Repeated data were tested for normality of the residuals by evaluating the Shapiro-Wilk statistic, normal probability plots of the residuals, and

Table 1. Chemical composition of treatment milk replacers¹ (least squares means)

Item	Treatment ²			SEM ³
	Control	Blend	Fish	
DM, %	95.4	95.4	95.4	0.1
Ash, % of DM	7.97	8.14	8.03	0.15
CP, % of DM	21.6	21.7	21.8	0.3
Fatty acids, ⁴ % of DM	19.4	19.6	19.7	0.2
Arachidonic acid, g/100 g of fatty acid	0.222	0.262	0.309	0.009
Eicosapentaenoic acid, g/100 g of fatty acid	0.010	0.645	1.267	0.078
Docosaheptaenoic acid, g/100 g of fatty acid	0.010	0.560	1.103	0.066
n-6, ⁵ g/100 g of fatty acid	15.6	13.7	11.4	0.1
n-3, ⁶ g/100 g of fatty acid	1.5	2.8	4.1	0.2
n-6:n-3	10.1	4.8	2.9	0.1

¹Source of milk replacer was Calva Products (Acampo, CA).

²Control = 3:1 mix of corn and canola oils; blend = 1:1 mix of fish oil and control oil; fish = fish oil only.

³SEM was determined from analysis of weekly composite samples.

⁴Total fatty acids were determined by GC.

⁵n-6 = C18:2, C20:3, C20:4, C22:4, and C22:5n-6.

⁶n-3 = C18:3, C20:5, C22:5n-3, and C22:6.

histograms of the residuals with the univariate procedure of SAS (version 9.1, 2003, SAS Institute). Data that were not normally distributed were transformed and normalized before statistical analyses. Means separation was performed at each time for significant treatment $\times$ time interactions by using a sliced-effect multiple comparison approach with a Tukey-Kramer adjustment (version 9.1, 2003, SAS Institute). As a result of baseline variability in the concentrations of various blood metabolites and hormones before the injection of LPS, subsequent analyses of these response variables after the LPS injection were expressed relative to the baseline. To address the acute effects of LPS on serum metabolites, the time interval from 0 to 6 h was analyzed for serum glucose, BUN, and NEFA. Serum TG was not elevated until 10 h after the injection of LPS, so the interval from 10 to 72 h was additionally analyzed. Finally, the complete intervals, baseline to the time when serum metabolic and biochemical variables returned to baseline concentrations, were analyzed for all variables. Least squares means ($\pm$ SEM) are reported throughout. A treatment difference of $P \leq 0.05$ was considered significant, and $0.05 < P \leq 0.10$ was considered a tendency.

RESULTS

Calves

Before the LPS challenge, 9 calves died: 2 in the control group, 4 in the blend group, and 3 in the fish group. During the first week of the study, there was a highly virulent attaching and effacing *Escherichia coli* outbreak, which accounted for the high mortality.

However, by the second week the outbreak was controlled. After the LPS challenge, an additional 4 calves died during the challenge, 2 each in the control and fish groups. All 4 calves had pathologies that were consistent with endotoxemia. After elimination of the dead calves, a total of 38 calves, 13 in both the control and blend groups and 12 in the fish group, were available for statistical analyses.

Diets

There were no differences in the composition of the 3 milk replacers, except for the anticipated differences in FA composition (Table 1). Supplementing milk replacer with FO increased the concentrations of arachidonic (C20:4n-6), eicosapentaenoic (C20:5n-3), and docosaheptaenoic (C22:6n-3) acids. Additionally, supplementing FO in milk replacer decreased the total n-6 FA and increased the total n-3 FA; consequently, the n-6:n-3 FA ratio was significantly decreased in the FO-supplemented milk replacers.

LPS: Clinical and Physiological

Measures of both attitude and appetite were altered after the LPS challenge. Supplementing FO attenuated a change in attitude, and the effect was linear ($P < 0.05$; data not shown). However, there were no linear ($P = 0.75$) or quadratic ($P = 0.22$) effects of supplementing FO on appetite (data not shown). Challenge with LPS caused a biphasic increase in body temperature, which peaked at approximately 5 and 12 h (Figure 1A). The individual response curves were variable, with many calves becoming hypothermic. No treatment effects

on body temperature were apparent. An initial drop in heart rate was observed during the first 2 h, which was followed by a moderate return (Figure 1B). There were no treatment effects on heart rate. Lipopolysaccharide caused an immediate increase in respiration rate (Figure 1C), which was sustained over 6 h. The intensity of the response was attenuated in calves fed FO. Lipopolysaccharide increased serum AST activity, which peaked between 10 and 12 h and had not completely returned to baseline within 72 h (Figure 1D). Within the first 4 h, LPS transiently decreased serum LDH activity, followed by a modest increase (Figure 1E). There were no treatment effects on either serum AST or LDH activity.

LPS: Biochemical

Serum iron concentrations were altered after the injection of LPS. Concentrations decreased to 72 h (Figure 2A). There was a tendency ($P = 0.06$) for an interaction between treatment and time; calves in the fish group had an attenuated response.

Lipopolysaccharide treatment caused a dramatic and sustained decrease in serum concentrations of glucose, reaching a nadir at 4 h and returning to baseline by 72 h after initiation of the challenge (Figure 2B). The decreased serum glucose was greater ($P < 0.01$) in calves in the blend and fish groups over the 1- to 72-h interval; however, there were no treatment differences during the acute interval. Sliced effects revealed that the reduced serum glucose in calves in the blend and fish groups occurred during the 8- to 24-h interval. Serum concentrations of insulin decreased after the injection with LPS and remained below baseline at 24 h (Figure 2C). The changes in insulin concentrations were influenced by treatment, as demonstrated by the interaction ($P < 0.01$) between treatment and time. Insulin concentrations of calves in the control and blend groups reached a nadir at 4 and 5 h, respectively, but those of calves in the fish group continued to decrease and reached a nadir at 8 h. Sliced effects indicated that there was a tendency for calves in the fish group to have decreased serum concentrations of insulin at 12, 18, and 24 h after the initiation of the LPS challenge.

There was an acute rise in BUN concentrations within the first 6 h after LPS injection, and an interaction of treatment $\times$ time ($P < 0.02$) was detected; calves in the blend and fish groups had attenuated responses within the first 4 h after the LPS challenge compared with those in the control group. However, at 6 h blood concentrations of BUN in calves in the blend group had increased to a greater extent (Figure 2D). There was a tendency ($P < 0.06$) for a treatment $\times$ time interaction when the 1- to 72-h interval was analyzed. The

sliced effects indicated that the treatment differences occurred within the first 8 h after the LPS challenge.

Serum concentrations of NEFA increased after the injection of LPS. There was an interaction of treatment $\times$ time ($P < 0.001$) during the 1- to 72-h interval. Sliced effects and the acute analysis revealed that the treatment differences occurred within the first 6 h after the challenge (Figure 2E). Calves in the control group demonstrated a biphasic response, peaking at 6 and 15 h, and calves in the blend and fish groups had a monophasic response that peaked between 15 and 18 h after LPS injection. Serum concentrations of TG were elevated beginning at 10 h and returned to baseline by 72 h after injection with LPS (Figure 2F). There was a linear effect ($P < 0.03$) of supplemental FO on serum TG during this period, whereby supplemental FO attenuated the increase in serum concentrations of TG.

Serum concentrations of leptin markedly decreased over 6 to 8 h after injection of LPS, which was followed by a partial recovery; however, concentrations remained below baseline at 24 h (Figure 2G). There was no treatment effect on changes in serum concentrations of leptin after the LPS challenge.

DISCUSSION

We investigated the impacts of supplementing milk replacer with FO on the acute phase response after an LPS challenge. The general demeanor of LPS-challenged calves was characterized as depressed, lethargic, and anorectic. Supplementing FO attenuated the general changes in attitude but had no effects on the measures of appetite in the current study. The etiology of what is now considered "sickness behavior," which includes depression, anorexia, fatigue, and anxiety, is not well understood. Cytokines, eicosanoids, peripheral signals, and neurotransmitters have all been identified as possible regulators of sickness behavior, and these indicators are not mutually exclusive. The fact that the n-3 FA in FO modulates the synthesis and action of cytokines as well as the synthesis of eicosanoids suggests that the pathogenesis of sickness behavior can be ameliorated by supplementing calves with FO. Furthermore, this was supported by the observation that supplementing rats with ethyl esters of eicosapentaenoic acid at either 0.2 or 1% of the diet was antiinflammatory and improved various measures of the altered behavior (Song et al., 2004). The effects of FO on appetite during endotoxemia are not well known. Data from the present study indicated that during endotoxemia in Jersey calves, appetite was not altered when replacing 5 to 10% of the lipid as FA from FO.

Serum AST and LDH activities were altered by LPS, which indicated soft tissue damage. Despite increased

AST and LDH, there were no significant effects of FO. In agreement with this result, Vollmar et al. (2002) found that rats consuming diets enriched with 9% FO for 8 wk were not protected from LPS-induced

hepatic microvascular dysfunction. The dramatic decrease in serum iron is well documented in models of both acute and chronic inflammation, and appears to be due to an increased storage of iron by cells of the

Figure 1. A) Effects of supplemental fish oil on the febrile responses of neonatal calves after an LPS challenge. ● = Control group; ○ = blend group; ▼ = fish group. No effects of treatment were evident. Error bars represent $\pm$ SEM. B) Effects of supplemental fish oil on the heart rate of neonatal calves after an LPS challenge. ● = Control group; ○ = blend group; ▼ = fish group. No effects of treatment were evident. Error bars represent $\pm$ SEM. C) Effects of supplemental fish oil on the respiratory rate of neonatal calves after an LPS challenge. ● = Control group; ○ = blend group; ▼ = fish group. There was a treatment effect ($P < 0.02$). Sliced time effects reported as $*P < 0.10$ and $**P < 0.05$. Error bars represent $\pm$ SEM. D) Effects of supplemental fish oil on serum aspartate amino transferase activity of neonatal calves after an LPS challenge. ● = Control group; ○ = blend group; ▼ = fish group. No effects of treatment were evident. Error bars represent $\pm$ SEM. E) Effects of supplemental fish oil on the change over baseline in serum lactate dehydrogenase activity of neonatal calves after an LPS challenge. Baseline concentration mean was 761 U/L. ● = Control group; ○ = blend group; ▼ = fish group. No effects of treatment were evident. Error bars represent $\pm$ SEM.

Figure 2. A) Effects of supplemental fish oil on the change over baseline in serum iron concentrations of neonatal calves after an LPS challenge. Baseline concentration mean was 3.49 mg/L. ● = Control group; ○ = blend group; ▼ = fish group. A tendency ($P < 0.06$) for a treatment $\times$ time interaction was evident. Sliced time effects are reported as $*P < 0.10$ and $**P < 0.05$. Error bars represent $\pm$ SEM. B) Effects of supplemental fish oil on the change over baseline in the serum glucose concentrations of neonatal calves after an LPS challenge. Baseline concentration mean was 74.7 mg/dL. ● = Control group; ○ = blend group; ▼ = fish group. No treatment effects were evident during the acute interval from 1 to 6 h; however, there was a linear treatment effect ($P < 0.01$) during the 1- to 72-h interval. Sliced time effects are reported as $*P < 0.10$ and $**P < 0.05$. Error bars represent $\pm$ SEM. C) Effects of supplemental fish oil on the change over baseline in the serum insulin concentrations of neonatal calves after an LPS challenge. Baseline concentration mean was 3.6 ng/mL. ● = Control group; ○ = blend group; ▼ = fish group. A treatment $\times$ time interaction ($P < 0.01$) was evident during the 1- to 24-h interval. Sliced time effects are reported as $*P < 0.10$ and $**P < 0.05$. Error bars represent $\pm$ SEM. D) Effects of supplemental fish oil on the change over baseline in the BUN concentrations of neonatal calves after an LPS challenge. Baseline concentration mean was 7.44 mg/dL. ● = Control group; ○ = blend group; ▼ = fish group. A treatment $\times$ time interaction ($P < 0.02$) was evident during the acute interval from 1 to 6 h. Further, there was a tendency ($P < 0.06$) for a treatment $\times$ time effect during the 1- to 72-h interval. Sliced time effects are reported as $*P < 0.10$ and $**P < 0.05$. Error bars represent $\pm$ SEM. E) Effects of supplemental fish oil on the change over baseline in the serum NEFA concentrations of neonatal calves after an LPS challenge. Baseline concentration mean was 0.247 mEq/L. ● = control group; ○ = blend group; ▼ = fish group. A linear treatment effect ($P < 0.0001$) was evident during the acute interval from 1 to 6 h. Furthermore, during the 1- to 72-h interval, there was a treatment $\times$ time interaction ($P < 0.001$). Sliced time effects are reported as $*P < 0.10$ and $**P < 0.05$. Error bars represent $\pm$ SEM. F) Effects of supplemental fish oil on the change over baseline in the serum triacylglycerol concentrations of neonatal calves after an LPS challenge. Baseline concentration mean was 22.3 mg/dL. During the 1- to 72-h interval, no treatment effect was evident; however, the interval from 1 to 10 h revealed a linear effect ($P < 0.03$) of supplemental fish oil. ● = Control group; ○ = blend group; ▼ = fish group. Sliced time effects are reported as $*P < 0.10$ and $**P < 0.05$. Error bars represent $\pm$ SEM. G) Effects of supplemental fish oil on the change over baseline in the serum leptin concentrations of neonatal calves after an LPS challenge. Baseline concentration mean was 9.87 ng/mL. ● = Control group; ○ = blend group; ▼ = fish group. No treatment effects were evident. Sliced time effects are reported as $*P < 0.10$ and $**P < 0.05$. Error bars represent $\pm$ SEM.

reticulo-endothelial system. Kwak et al. (1995) found the transcription of ferritin to be regulated by nuclear factor- κ B. Therefore, the attenuated response in serum iron observed for calves in the fish group was possibly a consequence of reduced nuclear factor- κ B-induced ferritin synthesis.

Activation of inflammatory immune cells causes changes in nutrient partitioning and growth (Kinsbergen et al., 1994). The acute phase response requires nutrients to be supplied to tissues involved in host defense. As discussed earlier, the acute phase response causes anorexia, which decreases nutrient availability and impairs growth. However, anorexia alone could not completely explain the decreased growth or alterations in nutrient partitioning (Laurin and Klasing, 1987). There were dramatic and sustained decreases in serum concentrations of glucose. Whole-body glucose utilization increased after activation of the acute phase response, and tissues rich in macrophages, including the liver, spleen, and lungs, had the greatest and most sustained increase in glucose utilization on a percentage basis (Lang et al., 1993). However, total glucose utilization by tissues such as skin, intestines, and muscle also account for a large increase in glucose utilization during the acute phase response. Despite dramatic, acute reductions in serum concentrations of glucose (Figure 2B), feeding FO had no effect compared with feeding the control diet. The severity of LPS-induced hypoglycemia in these calves was atypically severe when compared with other animal models; therefore, any dietary effect may have been masked by the sensitivity of these calves to endotoxin-derived hypoglycemia. However, when glucose was analyzed over the entire 72-h period (Figure 2B), calves in the blend and fish groups showed a more pronounced hypoglycemia, which was particularly apparent after 6 h. The attenuated hypoglycemia in calves in the control group could be attributed to an increased gluconeogenesis mediated by increased glucocorticoids, insulin insensitivity, or both. It is unknown whether glucose utilization by immune cells is altered during the latter stages of endotoxemia and septicemia. This latter point is of significance because a decrease in the utilization of glucose by immune cells, compounded by low blood glucose, could be linked to altered competence of immune cells during septicemia. In fact, genetic differences in the decrease in serum glucose concentrations of pigs after an LPS challenge were inversely related to mortality rates (Leininger et al., 2000a).

Serum concentrations of insulin decreased after the injection of LPS (Figure 2C). In contrast, previous studies reported a rapid and transient increase in serum insulin followed by a rapid decrease to concentrations below baseline (Kenison et al., 1991; Kinsbergen et al.,

1994). Kenison et al. (1991) suggested the transient increase in serum insulin was due to the initial period of hyperglycemia; however, the hyperinsulinemia observed by Kinsbergen et al. (1994) was not associated with a transient hyperglycemia. Whether the hyperinsulinemia in previous reports was a direct reflection of changes in blood glucose concentrations or was due to an indirect, glucose-independent mechanism is unknown. The lack of a transient period of hyperglycemia and hyperinsulinemia in the present study and the fact that the changes in serum concentrations of glucose and insulin paralleled each other over the first 24 h of the LPS challenge support the hypothesis that changes in serum glucose affected serum insulin during endotoxemia, with concomitant anorexia.

It is imperative that microbial septicemia be recognized and the appropriate treatment be initiated early in the pathogenesis to increase the likelihood of calf survival. However, differentiating between septicemic and nonsepticemic scouring calves is difficult because many of the clinical signs of septicemia can be mistaken for dehydration (Fecteau et al., 1997). Accordingly, finding a fast, sensitive, and easy biological marker that positively identifies septicemic calves would be beneficial. The severity of the endotoxin-induced hypoglycemia suggested that blood glucose concentrations may be a specific marker by which to classify ill Jersey calves as septicemic versus nonsepticemic; however, more research is needed to determine the specificity and sensitivity of blood glucose as a marker of septicemic Jersey calves.

After the LPS challenge, serum concentrations of BUN were elevated (Figure 2D), which could be attributed to accelerated rates of skeletal muscle degradation, upregulated urea synthesis, or renal damage (Nielsen et al., 2005). The liver increases the uptake of amino acids from skeletal muscle to synthesize acute phase proteins. In a recent study, pectoralis cationic amino acid transporter-2 mRNA was dramatically increased at 4 h after the LPS challenge, but by 8 h activity had returned to baseline (Humphrey and Klasing, 2005). Increased expression of cationic amino acid transporter-2 was associated with the efflux of cationic amino acids attributable to catabolism; furthermore, cationic amino acid transporter-2 mRNA in rat kidney is under the regulation of nuclear factor- κ B. Adding FO to milk replacer significantly attenuated the acute rise in serum concentrations of BUN, and the effect was linear within the first 6 h. These acute effects of FO on serum concentrations of BUN could be due to the inhibitory effects on nuclear factor- κ B expression, thereby decreasing the supply of amino acids to the liver and amino-nitrogen for urea synthesis. There was an interaction of treatment $\times$ time because serum

concentrations of BUN in calves in the blend group increased to a greater extent at 6 and 8 h; however, there were no treatment differences on serum concentrations of BUN after 8. In rats given a high dose of LPS, the in vivo capacity to synthesize both urea and serum concentrations of BUN was lower at 24 h than in rats given a moderate dose of LPS. This reflected functional liver failure in rats given the high dose (Nielsen et al., 2005). The reason no treatment differences were evident during the later period in the present study could be related to the moderate dose of LPS administered, which did not cause functional liver failure. Further, proteolysis of skeletal muscle was probably a primary source of carbons for gluconeogenesis during this period, because calves were fasted and glycogen stores were likely depleted.

Serum concentrations of NEFA were dramatically elevated and sustained after the administration of LPS (Figure 2E). It is interesting to note that the biphasic response was observed only in the control group. It was likely that the initial rise in serum concentrations of NEFA was under the regulation of proinflammatory mediators, such as the stimulatory effect of tumor necrosis factor- α on hormone-sensitive lipase (Coppack, 2001). The late phase was likely predominantly a result of the autonomic nervous system because of the short-term fast (Kinsbergen et al., 1994). Therefore, the monophasic response of serum concentrations of NEFA in both the blend and fish groups might be the result of an attenuated acute release of NEFA attributable to proinflammatory mediators and a sustained capacity of adipose tissue to respond to the autonomic nervous system.

Studies on the ability of septicemic animals to use lipids as an energy source did not yield definitive conclusions. Hypertriglyceridemia, hypoketonemia, and decreased lipoprotein lipase and carnitine palmitoyltransferase activities were observed previously after the administration of LPS (Takeyama et al., 1990). These results implicate decreased whole-body utilization of lipids during septicemia. In contrast, in a study evaluating very low density lipoprotein kinetics in septicemic dogs, Wolfe et al. (1985) reported that 17% of the total production of CO₂ could be accounted for by very low density lipoprotein FA oxidation, suggesting that FA provided a substantial energy source during septicemia. The elevated serum concentrations of TG observed in the present study could be due to an increased output of very low density lipoprotein by the liver, decreased clearance of lipoproteins by the liver and peripheral tissues, or both (Takeyama et al., 1990).

Leptin limits feed intake and increases energy expenditure, resulting in a loss of body energy. In rodents, se-

rum concentrations of leptin increased after LPS challenges, which suggested that leptin might be partially responsible for the anorexia and tissue wasting (Sarraf et al., 1997). In contrast, studies with human subjects provided inconsistent responses for leptin (Koc et al., 2003; Landman et al., 2003). Likewise, studies with pigs and sheep did not demonstrate a positive relationship between endotoxemia and serum concentrations of leptin (Leininger et al., 2000b; Soliman et al., 2001). It is not known why the leptin response to LPS varies between species; however, Leininger et al. (2000b) suggested that during endotoxemia, serum concentrations of leptin reflect a balance between 2 opposing forces, the stimulatory effects of tumor necrosis factor- α and cortisol on leptin synthesis and secretion, and the inhibitory effects of energy balance. In the present study, serum leptin declined after the LPS challenge, following a pattern similar to serum concentrations of glucose and insulin. The lack of a treatment effect may be related to the fact that the net effect between the 2 opposing forces was cancelled out in each treatment.

CONCLUSIONS

Supplementing milk replacer with n-3 FA from FO attenuates many clinical and biochemical aspects of the acute phase response. A more appropriate acute phase response may protect calves from the immediately dangerous pathophysiological hyperinflammatory response as well as the subsequent immune paralysis.

ACKNOWLEDGMENTS

The authors thank S. O. Juchem and S. J. Taylor for their technical assistance. We appreciate the constructive comments of J. E. Santos and K. C. Klasing. We acknowledge the contributions of the American Jersey Cattle Association (Reynoldsburg, OH), Omega Proteins Inc. (Houston, TX), Calva Products (Acampo, CA), Western Milling (Goshen, CA), Mendes Calf Ranch (Tipton, CA), Hilmar Cheese Company (Hilmar, CA), Tech Mix (Stewart, MN), and Pfizer Animal Health (St. Louis, MO). Special thanks to the dairy producers who donated the Jersey calves.

REFERENCES

- Coppack, S. W. 2001. Pro-inflammatory cytokines and adipose tissue. *Proc. Nutr. Soc.* 60:349–356.
- Delavaud, C., F. Bocquier, Y. Chilliard, D. H. Keisler, A. Gertler, and G. Kann. 2000. Plasma leptin determination in ruminants: Effect of nutrition status and body fatness on plasma leptin concentration assessed by a specific RIA in sheep. *J. Endocrinol.* 165:519–526.
- Fecteau, G., J. Paré, D. C. Van Metre, B. P. Smith, C. A. Holmberg, W. Guterbock, and S. Jang. 1997. Use of a clinical sepsis score for

- predicting bacteremia in neonatal dairy calves on a calf rearing farm. *Can. Vet. J.* 38:101–104.
- Humphrey, B. D., and K. C. Klasing. 2005. The acute phase response alters cationic amino acid transporter expression in growing chickens (*Gallus gallus domesticus*). *Comp. Biochem. Physiol.* 142:485–494.
- Hüsler, B. R., and J. W. Blum. 2002. Metabolic and endocrine changes in response to endotoxin administration with or without oral arginine supplementation. *J. Dairy Sci.* 85:1927–1935.
- Johnson, J. A. III, J. A. Griswold, and F. F. Muakkassa. 1993. Essential fatty acids influence survival in sepsis. *J. Trauma* 35:128–131.
- Johnson, M. M., and J. P. Peters. 1993. Technical note: An improved method to quantify nonesterified fatty acids in bovine plasma. *J. Anim. Sci.* 71:753–756.
- Kenison, D. C., T. H. Elsasser, and R. Fayer. 1991. Tumor necrosis factor as a potential mediator of acute metabolic and hormonal responses to endotoxemia in calves. *Am. J. Vet. Res.* 52:1320–1326.
- Kinsbergen, M., R. M. Bruckmaier, and J. W. Blum. 1994. Metabolic, endocrine, and haematological responses to intravenous *E. coli* endotoxin administration in 1-week-old calves. *J. Vet. Med.* 41:530–547.
- Koc, E., G. Ustundag, D. Aliefendioglu, E. Ergenekon, A. Bideci, and Y. Atalay. 2003. Serum leptin levels and their relationship to tumor necrosis factor- α and interleukin-6 in neonatal sepsis. *J. Pediatr. Endocrinol. Metab.* 16:1283–1287.
- Kolath, W. H., M. S. Kerley, J. W. Golden, and D. H. Keisler. 2006. The relationship between mitochondrial function and residual feed intake in Angus steers. *J. Anim. Sci.* 84:861–865.
- Kwak, E. L., D. A. Larochelle, C. Beaumont, S. V. Torti, and F. M. Torti. 1995. Role for NF- κ B in the regulation of ferritin H by tumor necrosis factor- α . *J. Biol. Chem.* 270:15285–15293.
- Landman, R. E., J. J. Puder, E. Xiao, P. U. Freda, M. Ferin, and S. L. Wardlaw. 2003. Endotoxin stimulates leptin in the human and nonhuman primate. *J. Clin. Endocrinol. Metab.* 88:1285–1291.
- Lang, C. H., Z. Spolarics, A. Ottlakan, and J. J. Spitzer. 1993. Effect of high-dose endotoxin on glucose production and utilization. *Metabolism* 10:1351–1358.
- Lanza-Jacoby, S., J. T. Flynn, and S. Miller. 2001. Parenteral supplementation with a fish-oil emulsion prolongs survival and improves rat lymphocyte function during sepsis. *Nutrition* 17:112–116.
- Laurin, D. E., and K. C. Klasing. 1987. Effects of repetitive immunogen injections and fasting versus feeding on iron, zinc, and copper metabolism in chicks. *Biol. Trace Elem. Res.* 14:153–165.
- Leininger, M. T., C. P. Portocarrero, C. A. Bidwell, M. E. Spurlock, and K. L. Houseknecht. 2000b. Leptin expression is reduced with acute endotoxemia in the pig: Correlation with glucose, insulin, and insulin-like growth factor-1 (IGF-1). *J. Interferon Cytokine Res.* 20:99–106.
- Leininger, M. T., C. P. Portocarrero, A. P. Schinckel, M. E. Spurlock, C. A. Bidwell, J. N. Nielsen, and K. L. Houseknecht. 2000a. Physiological response to acute endotoxemia in swine: Effect of genotype on energy metabolites and leptin. *Domest. Anim. Endocrinol.* 18:71–82.
- Lofstedt, J., I. R. Dohoo, and G. Duizer. 1999. Model to predict septicemia in diarrheic calves. *J. Vet. Intern. Med.* 2:81–88.
- Mascioli, E. A., Y. Iwasa, S. Trimbo, L. Leader, B. R. Bistrian, and G. L. Blackburn. 1989. Endotoxin challenge after menhaden oil diet: Effects on survival of guinea pigs. *Am. J. Clin. Nutr.* 49:277–282.
- Mayer, K., C. Fegbeutel, K. Hattar, U. Sibelius, H. J. Krämer, K. U. Heuer, B. Temmesfeld-Wollbrück, S. Gokorsch, F. Grimminger, and W. Seeger. 2003. ω -3 vs. ω -6 lipid emulsion exert differential influence on neutrophils in septic shock patients: Impact on plasma fatty acids and lipid mediator generation. *Intensive Care Med.* 29:1472–1481.
- NRC. 2001. Nutrient Requirements for Dairy Cattle. 7th rev. ed. Natl. Acad. Sci., Washington, DC.
- Nielsen, S. S., T. Grøft, N. Tygstrup, and H. Vilstrup. 2005. Effect of lipopolysaccharide on in vivo and genetic regulation of rat urea synthesis. *Liver Int.* 25:177–183.
- Sarraf, P., R. C. Frederich, E. M. Turner, G. Ma, N. T. Jaskowiak, D. J. Rivet, J. S. Flier, B. B. Lowell, D. L. Fraker, and H. R. Alexander. 1997. Multiple cytokines and acute inflammation raise mouse leptin levels: Potential role in inflammatory anorexia. *J. Exp. Med.* 185:171–175.
- Soliman, M., S. Abdelhady, I. Fattouh, K. Ishioka, H. Kitamura, K. Kimura, and M. Saito. 2001. No alteration in serum leptin levels during acute endotoxemia in sheep. *J. Vet. Med. Sci.* 63:1143–1145.
- Song, C., B. E. Leonard, and D. Horrobin. 2004. Dietary ethyl-eicosapentaenoic acid, but not soybean oil reverses central interleukin-1-induced changes in behavior, corticosterone and immune response in rats. *Stress* 7:43–54.
- Takeyama, N., Y. Itoh, Y. Kitazawa, and T. Tanaka. 1990. Altered hepatic mitochondrial fatty acid oxidation and ketogenesis in endotoxic rats. *Am. Physiol. Soc.* 259:E498–E505.
- Vollmar, B., C. Bauer, and M. D. Menger. 2002. n-3 polyunsaturated fatty acid-enriched diet does not protect from liver injury but attenuates mortality rate in a rat model of systemic endotoxemia. *Crit. Care Med.* 30:1091–1098.
- Wolfe, R. R., J. H. F. Shaw, and M. J. Durkot. 1985. Effect of sepsis on VLDL kinetics: Responses in basal state and during glucose infusion. *Am. Physiol. Soc.* 248:E732–E740.