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# The effect of dietary fat on fatty acid composition, gene expression and vitamin status in pre-ruminant calves

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ABSTRACT

Dietary saturated (SFA) and unsaturated fatty acids (UFA) alter fatty acid (FA) composition of various tissues, serum, and circulating immune cells. The objective of this study was to examine the effect of dietary SFA and UFA on adipose, liver, serum, polymorphonuclear (PMN) and peripheral blood mononuclear cells' (PBMC) FA profiles, gene expression of selected inflammatory mediators, and their relation with serum lipid-soluble vitamin in pre-ruminant calves. Twelve Holstein male calves were randomly assigned to one of two treatments. Starting at 3 d of age, six calves received 120 mL palm oil/day (SFA), and six calves received 80 mL flaxseed oil plus 40 mL conjugated linoleic acid/day (UFA). After 50 d, all animals were euthanized and samples were obtained. Fatty acid composition of non-esterified fatty acid (NEFA), neutral lipid (NL), and phospholipids (PL) was analyzed by gas-chromatography. High-performance liquid chromatography was used to detect α-tocopherol and retinol in liver, as well as α-tocopherol, retinol, and β-carotene in serum. In addition, liver and adipose tissue were analyzed for gene expression of interleukin (IL)-1β, IL-6, IL-8, IL-10, IL-12, interferon-γ, peroxisome proliferator-activated receptor-γ, TNF-α, retinol binding protein-4, and NF-κB. The PBMC were examined for IL-1β, IL-6, TNF-α and intercellular adhesion molecule-1; PMN cells were analyzed for caspase-1, IL-8 receptor, and L-selectin (L-SEL) expression. Data were analyzed using the Proc TTEST of SAS with significance declared at $P \leq 0.05$. Results showed that the UFA had greater α-linolenic acid compared to SFA in all three (NEFA, NL, and PL) lipid fractions of liver, adipose and serum, as well as PBMC and PMN. The greater content of α-LA in calves fed UFA resulted in greater eicosapentaenoic acid (EPA) in all three lipid fractions of serum, as well as NL and PL in adipose tissue. The UFA however, had lower γ-linolenic acid compared with SFA in all three lipid fractions of liver, as well as NL and PL in serum. Dietary UFA also increased total PUFA in all lipid fractions of serum and adipose. In addition, PBMC and PMN had greater EPA in calves fed UFA. Lipid-soluble vitamins in serum were reduced by dietary UFA. In contrast, UFA resulted in upregulation of L-SEL expression in PMN. This may indicate that UFA elevated the substrate for PUFA biosynthesis, but possibly degraded lipid-soluble vitamins to protect these FA from oxidation. Greater circulating PUFA may influence the migration of PMN from the blood to tissues, affecting overall inflammatory responses.

*Abbreviations:* AA, arachidonic acid; CLA, conjugated linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; IL, interleukin; LA, linoleic acid; NEFA, non-esterified fatty acid; NL, neutral lipid; PBMC, peripheral blood mononuclear cells; PL, phospholipid; PMN, polymorphonuclear neutrophils; PUFA, polyunsaturated fatty acid; UFA, unsaturated fat; SFA, saturated fat

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## 1. Introduction

Pre-ruminant calf diseases increase the cost of production including veterinary, mortality, reduced growth, and delay the age of first calving (Windeyer et al., 2014). According to the National Animal Health Monitoring System, digestive and respiratory problems caused 56.5% and 22.5% of the total mortality in pre-weaned heifers, respectively (USDA, 2007). These two major diseases cause an annual loss of $33.5 and $14.7 per pre-weaned calf, respectively (Kaneene and Hurd, 1990).

A pre-ruminant calf is fed whole milk or milk replacer with the high content of fat (200–230 g/kg DM; Graulet et al., 2000). Feeding fat has nutritional and economic advantages to satisfy energy requirement and replace carbohydrates with inexpensive lipid sources. In addition, dietary polyunsaturated fatty acid (PUFA) can modulate the immune system to improve growth performance and health. Research showed that milk replacer containing fish oil weakened acute phase responses and the effect was linear in response as the fish oil FA replacement increased from 5 to 10% (Ballou et al., 2008).

Fatty acid (FA) supplies have been demonstrated to alter immune cells function because FA alter the composition of the cells' membrane phospholipids, which relates to the membrane lipid fluidity, signal transcription factors, and bioactive synthesis of lipid mediators (Calder, 2008, 2012, 2013). Conjugated linolenic acid (CLA) is a group of derivatives from linoleic acid (LA; C18:2 *c*9, *c*12) with two conjugated double bonds. *Cis*9, *trans*11 CLA and *t*10, *c*12 CLA isomers are the most abundant isomers in natural and synthetic blends (Piras, 2014). In the mammary gland, CLA can reduce LA and arachidonic acid (AA) up to 1% and 50%, respectively; AA is the precursor of prostaglandins and leukotrienes, which are metabolized by cyclooxygenase and lipoxygenase (Banni et al., 1999). Dietary CLA can limit AA biosynthesis, and thereby limit the production of AA derivatives that can affect inflammatory transcription factors and receptors (Stachowska et al., 2007). Flaxseed oil contains high α-linolenic acid (α-LA), which is an n3 essential FA (Petit and Côrtes, 2010). Alpha-linolenic acid, similar to other PUFA, assists with lipid transport, lipoprotein enzyme activation, and contributes to cell membranes structure (McDonald et al., 2002). It is also a precursor of eicosapentaenoic acid (EPA; C20:5 n3) and docosahexaenoic acid (DHA; C22:6 n3) via desaturase and elongase processing that can alter immune functions.

Therefore, our aims were to examine the effect of dietary saturated fat (SFA) and unsaturated fat (UFA) on 1) FA profile in adipose, liver, serum, polymorphonuclear neutrophils (PMN), and peripheral blood mononuclear cells (PBMC), 2) gene expression of selected inflammatory markers in adipose, liver, PMN and PBMC, and 3) vitamin content in liver and serum of pre-ruminant calves.

## 2. Methods and materials

### 2.1. Animals, treatments and experimental design

All animal procedures were approved by the University of Idaho Animal Care and Use Committee (# 2013-77). Twelve 3-d old Holstein male calves were assigned to one of two treatments, either saturated or unsaturated fat sources in a completely randomized design. Dietary fats were started on day 3 of calves' age, and the initial body weight (BW) was 43 ± 1.2 kg. All calves were raised in individual hutches (1.47 × 0.84 m) for 50 days. Six calves were fed 4 L/day of whole milk containing 120 mL Palm Fruit Oil-Organic (Jedwards International, Braintree, MA) and labeled the SFA. The UFA was fed 4 L/day of whole milk containing 80 mL Flax Seed Oil – Virgin (α-linolenic acid C18:3n-3, ω-3; Jedwards International, Braintree, MA) plus 40 mL Lutalin® (CLA) oil (> 56% of CLA containing isomers of *cis*-9, *trans*-11 and *trans*-10, *cis*-12; BASF, New Jersey, USA). Purina® Calf Starter 18 (Purina, Missouri, USA) was fed at 0.9 kg/day from d 25 to d 50. The calves were fed twice a day at 06:00 h and 18:00 h. After the calves received the dietary treatment for 50 days, all animals were euthanized, and blood, liver and adipose tissues were obtained. The average BW at this time was 66.7 ± 0.9 kg.

### 2.2. Sample collection

Blood, liver, and perirenal adipose tissues were collected immediately after the calves were euthanized. Blood samples were collected into 50 mL conical polypropylene centrifuge tubes with 200 μL of 50 mg/mL heparin (Sigma Aldrich, St. Louis, MO). Peripheral blood mononucleocytes cells and PMN were isolated from 50 mL of blood by gradient centrifuging using Histopaque 1077 and 1119 (Sigma Aldrich, St. Louis, MO). The buffy coat was collected and washed with Hank's Balanced Salt Solution (HBSS) then and the isolated PBMC and PMN were re-suspended in HBSS, separated into two 50 mL conical polypropylene centrifuge tubes, and stored at −80 °C for RNA extraction and FA analysis, respectively. Additionally, after centrifuging blood samples at 800 × *g* for 10 min at 4 °C, serum was collected and stored at −80 °C. The liver and perirenal adipose tissue samples were collected into 2 mL micro tubes, immediately frozen in liquid nitrogen, and stored at −80 °C until RNA extraction.

### 2.3. Lipid analysis

Lipids in serum, PBMC, PMN, liver, and adipose tissues were extracted by chloroform:methanol (2:1) as previously described (Clark et al., 1982). Adipose, liver, and serum lipids were fractionated by Sep-Pak aminopropyl cartridges (Waters, Milford, MA) as previously described (Watts et al., 2013; Scholte et al., 2014). The fractionation columns were conditioned with hexane, the neutral lipid (NL) was eluted by chloroform:propanol (2:1), non esterifed FA (NEFA) eluted by 2% acetic acid in ether, and phospholipids (PL) eluted by methanol. The adipose, liver, and serum lipid fractions, and total lipids in PBMC and PMN were processed with sodium methoxide for methylation (Christie, 1982). Lipids were dissolved in 2 mL sodium methoxide, and kept in a water bath at 80 °C for 10 min with 5% methanolic hydrochloric acid. Methylated lipid samples were analyzed by Agilent 7890A gas-chromatography