



## **EFFECTS OF DIETARY SUPPLEMENTATION WITH DIFFERENT POLYUNSATURATED FATTY ACIDS ON EXPRESSION OF GENES RELATED TO SOMATOTROPIC AXIS FUNCTION IN THE LIVER, SELECTED BLOOD INDICATORS, MILK YIELD AND MILK FATTY ACIDS PROFILE IN DAIRY COWS**

Essa Dirandeh<sup>1,2</sup>, Armin Towhidi<sup>2\*</sup>, Zarbakht Ansari<sup>1</sup>, Saeed Zeinoaldini<sup>2</sup>, Mehdi Ganjkanlou<sup>2</sup>

<sup>1</sup>Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Sari, Iran

<sup>2</sup>Department of Animal Science, University of Tehran, Alborz, Iran

\*Corresponding author: atowhidi@ut.ac.ir

### **Abstract**

The objective of this study was to investigate whether dietary supplementation with different polyunsaturated fatty acids (PUFAs) affects expression of genes related to somatotropic axis and the plasma concentrations of insulin, glucose, non-esterified fatty acids (NEFA), beta hydroxyl butyrate acids (BHBA) and insulin-like growth factor 1 (IGF1) and milk fatty acids profile. Right after calving, Holstein cows (n=45) were randomly assigned to one of three diets supplemented with roasted whole soybean as a source of omega-6 PUFA (omega-6, n=15), linseed as a source of omega-3 PUFA (omega-3, n=15) or palm oil (control, n=15). Each cow was in the study over a period of 70 days. Blood samples were collected every two weeks from day 1 to 70 of lactation and plasma concentrations of insulin, glucose, NEFA, BHBA and IGF1 were determined. Liver samples were taken from a subset of 18 cows (6 per diet) at day 70 postpartum and hepatic mRNA level of total growth hormone-receptor 1A (GHR1A), insulin receptor (INSR), IGF1 and insulin-like growth factor binding protein (IGFBP2) was assessed. Experimental diets did not affect milk yield. Plasma glucose and insulin concentrations were greater for omega-3 treatment compared to omega-6 and control treatments. Cows fed diets enriched in omega-3 exhibited greater INSR and GHR1A mRNA expression, and a tendency for greater IGF1 mRNA expression in the liver compared to omega-6 and control cows. Plasma IGF1 concentration was significantly higher in omega-3 treatment compared with omega-6 and control treatments. Results of this study suggest that feeding omega-3 PUFAs during early postpartum couples with the somatotropic axis, leading to an increase in plasma IGF1 concentration in dairy cows.

**Key words:** dairy cows, PUFA, GHR1A, INSR, IGF1, IGFBP2 mRNA

The periparturient period in dairy cows is associated with insulin resistance, which increases the availability of glucose, amino acids and fatty acids (FA) to mammary gland (Bell and Bauman, 1997; Renaville et al., 2002). Conceptually, exaggerated insulin resistance may enhance the degree of FA mobilization from adipose tissue, and further increase plasma NEFA concentration and decrease IGF1, which is associated with poor reproductive performance (Pires et al., 2007).

Growth hormone (GH) directly acts on liver and adipose tissue (e.g. increases gluconeogenesis and decreases lipogenesis) as well as indirectly through insulin-like growth factor 1 (IGF1) and insulin-like growth factor binding proteins (IGFBP) on muscle and mammary gland (e.g. increases utilization of NEFA and increases mammary blood flow; Renaville et al., 2002). However, it has been shown that in some metabolic situations during early lactation period, elevated plasma growth hormone (GH) concentrations fail to stimulate an increase in hepatic IGF1 synthesis (Fenwick et al., 2008; Lucy, 2008). In cattle, both insulin and IGF1 interact synergistically with gonadotrophin hormones to promote follicular growth, steroidogenesis, and enhance maximum size of the pre-ovulatory follicle (Wathes et al., 2003). Perturbations in metabolic status, indicated by changes in circulating concentrations of IGF1, have been reported to be associated with reproductive efficiency (Wathes et al., 2003; Patton et al., 2007).

In addition to GH, it has been suggested that insulin is an important endocrine factor regulating somatotrophic axis (Butler et al., 2003). In this regard, insulin treatment has been shown to restore liver GH receptor expression and IGF1 release in dairy cows (Butler et al., 2003). Moreover, concomitant insulin and glucose intravenous infusion compared with infusing separately with either saline or glucose caused an increase in plasma IGF1 concentrations in cows (Léonard and Block, 1997). Other studies have demonstrated that GH binding to hepatic microsomal membranes is enhanced in insulin-treated cows, and that GH binding is correlated with plasma insulin concentrations (Leonard et al., 2001).

Dietary FA profile can modulate the response to insulin in non-ruminants; particularly, omega-3 PUFA, such as C18:3n-3, C20:5n-3 and C22:6n-3, may prevent the development of insulin resistance in humans and rodents (Clarke, 2000; Delarue et al., 2004). In agreement with these findings, abomasal infusion of fish oil (4% of DMI) to beef steers increased key intermediaries of intracellular insulin signaling cascade in muscle, enhanced whole body insulin-induced disposal of amino acids and glucose, compared to the infusion of a control fat source composed of 60% cotton oil and 40% olive oil (Gingras et al., 2007). As a result, dietary PUFA supplementation in dairy cows, particularly omega-3 fatty acids, may decrease the probability of insulin resistance, and finally affect somatotrophic axis action.

The aim of this study was to determine the effect of different dietary PUFAs supplementation on mRNA expression of genes related to the somatotrophic axis function in the liver of dairy cows. In addition, plasma concentrations of insulin, glucose, NEFA, BHBA and IGF1 as well as milk yield and milk fatty acid profiles were investigated.

## Material and methods

### Cows and treatments

This experiment was performed according to the procedures laid down by the Iranian Ministry of Agriculture (experimental permission no. 858).

Table 1. Ingredient and chemical composition of diets based on the palm oil (Control, n=15), linseed as a source of omega-3 PUFA (Omega-3, n=15) or roasted whole soybean as a source of omega-6 PUFA (Omega-6, n=15)

Ingredient (% of dry matter)	Treatments		
	control	Omega-3	Omega-6
Alfalfa hay (mid bloom)	26.72	26.72	26.72
Corn silage	22.1	22.1	22.1
Dehydrated beet pulp	2.97	2.97	2.97
Ground corn grain	9.74	9.74	9.74
Rolled barley grain	9.65	9.65	9.65
Molasses	0.78	0.76	0.78
Wheat bran	0.32	0.20	0.71
Soybean meal (48% crude protein)	8.81	7.93	3.03
Roasted whole soybean	0	0	7.31
Corn gluten meal (60% crude protein)	1.83	1.83	1.83
Cottonseed meal	3.39	3.08	3.14
Whole cottonseed	1.51	1.48	1.51
Extruded linseed	0	4.06	0
Canola meal (solvent)	4.6	3.26	4.4
Wheat rolled	2.64	2.69	2.69
Palm oil	1.41	0	0
Limestone	0.45	0.45	0.45
Vitamin and mineral premix <sup>1</sup>	0.89	0.89	0.89
Dicalcium phosphate	0.54	0.54	0.54
Magnesium oxide	0.20	0.20	0.20
Salt	0.45	0.45	0.45
Sodium bicarbonate	1.00	1.00	1.00
Chemical composition			
Net energy for lactation (Mcal/kg of dry matter)	1.58	1.58	1.58
Fat (% of dry matter)	4.00	4.00	4.00
Crude protein (% of dry matter)	17.90	17.85	17.85
Neutral detergent fiber (% of dry matter)	34.40	33.90	34.10
Acid detergent fiber (% of dry matter)	20.40	20.50	20.40

<sup>1</sup>Contained (per kilogram): 16,000,000 IU of vitamin A; 3,200,000 IU of vitamin D; 48,000 IU of vitamin E; 24.0 g of Mn; 24.0 g of Zn; 24.0 g of Fe; 12.8 g of Cu; 1.44 g of I; 0.32 g of Se; and 0.32 g of Co.

Forty-five multiparous Holstein cows (milk =  $35 \pm 2.1$  kg/d) from a commercial dairy herd were blocked by predicted calving date and parity and randomly allocated to one of three experimental diets supplemented with: 1) palm oil (control;  $n=15$ ); 2) rolled linseed (omega-3;  $n=15$ ) or; 3) whole roasted soybean (omega-6;  $n=15$ ). The diets were formulated to meet or exceed nutrient requirements for lactating cows (NRC, 2001) and to be isonitrogenous and isoenergetic (Table 1). Fatty acid analyses were made for whole roasted soybean, linseed and palm oil before starting the study. The total mixed ration (TMR) was also sampled and analyzed for chemical composition (for details see below). Supplementation of FA was at 1.5% of dietary dry matter.

Diets were fed twice daily (08:00 and 16:00 h) for *ad libitum* intake (10% of refusals on as fed basis) from calving until 70 days postpartum (dpp). Total mixed rations were sampled every 2 wk and pooled each month. Feed samples were dried at 65°C for 24 h and then ground to pass through a 1-mm screen (Retsch SM 100, Retsch GmbH, Haan, Germany). Cows were milked three times per day at 07:00, 14:00, and 24:00 h and yields were recorded automatically (BouMatic, Madison-Wisconsin, USA).

### Body condition score

Cows were evaluated for BCS at calving, 20, 40 and 70 days post-partum (dpp). Scores were given by one experienced veterinarian based on a 1 (thin) to 5 (obese) predetermined scales using a quarter point system (Edmonson et al., 1989). Changes in BCS were obtained by subtracting BCS at 20 dpp from BCS at parturition, BCS at 40 dpp from BCS at 20 dpp, and BCS at 70 dpp from BCS at 40 dpp.

### Milk fatty acid profiles

Milk was sampled from 3 consecutive milkings on each day. Fatty acid extraction was done according to the Folch method (Folch et al., 1957) with some modifications. Briefly, chloroform/methanol (2:1, v/v) containing 0.005% butylated hydroxytoluene (as antioxidant) was added (usually 5 mL solvent added to 50–100  $\mu$ l sample) and vortexed for 1 min and then left at 4°C overnight. One mL of 0.9% NaCl was added and mixed again. The chloroform phase (e.g. lower phase) was transferred to a clean tube and care was taken not to transfer any remaining aqueous phase along with the chloroform phase. Remaining FA in original tubes was extracted twice with 2.0 mL of chloroform and these two extractions were then combined to the first one. Fatty acid methyl esters were prepared according to methods described previously (Morrison and Smith, 1964; Salem et al., 1996) and using the BF<sub>3</sub>/methanol reagent (1 mL of 14% BF<sub>3</sub>/MeOH reagent).

### Blood collection and insulin, glucose, NEFA, BHBA and IGF1 analyses

Blood samples were collected from all animals every two weeks from d 1 to 70 of dpp via venipuncture from the coccygeal vein before the morning meal. Blood was collected in vacutainers (BD, Franklin Lakes, NJ) containing EDTA and samples were centrifuged at  $1,500 \times g$ , 20 min within 2 h after collection. Plasma was harvested and stored at -20°C until further processing. Plasma concentrations of glu-

cose (Glucotrend, Roche, England), NEFAs (Cat. No. FA 115, Randox Laboratories Ltd, UK) and BHBA (Abbott Diabetes Care Ltd, Rang Road, Witney, Oxon, OX29 OYL, UK) were determined enzymatically using a spectrophotometer (Shimadzu 2100, Kyoto, Japan). Intra- and inter-assay coefficients of variation were <5%. Plasma insulin was measured using a commercially available bovine ELISA kit supplied by Mercodia AB (Mercodia, Uppsala, Sweden). Analytical range was 0.05–3.0 µg/L, with a sensitivity of 0.025 µg/L and intra-assay coefficient of variation was <4%. Concentrations of total IGF1 were measured using the DSL-10-2800 ACTIVE non-extraction IGF1 ELISA kit (Diagnostic Systems Laboratories, Webster, Texas, USA).

### **Liver biopsy**

Liver tissues (approximately 100 mg) were taken from a subset of 16 cows (six per treatment) on 70 dpp by percutaneous punch biopsy as described by Rhoads et al. (2008). Briefly, a biopsy site between the 11th and 12th ribs on the right side was shaved, and disinfected with an iodine scrub solution and 70% ethanol. Local anaesthesia (5 mL of 2% lidocaine hydrochloride solution) was administered to desensitize the incision site. An incision of approximately 1 cm was made through the skin and the biopsy instrument (Western Alloys and Metals, Vancouver, B.C) was used to pierce the intercostal muscles and peritoneum. Approximately 100 mg of liver tissue was collected, rinsed with sterile PBS, placed in a screw-cap microcentrifuge tube, snap-frozen in liquid nitrogen, and stored at –80°C until RNA extraction.

### **RNA extraction and cDNA synthesis**

Total RNA was extracted from liver samples using the RNXTM (-Plus) solution (RN7713C, Qiagen Inc., Germany) according to the manufacturer's instructions. Integrity of RNA was verified by agarose gel (1%) electrophoresis. The RNA concentration and assessment of purity (e.g., protein contamination) were determined using UV absorbance (260–280 nm). Total RNA (1 µg) was first treated with 1 U DNase (Invitrogen) to remove any contaminating DNA. The RNA was reverse transcribed in the presence of 1 mM oligo(dT) primer and 4 U Omniscript RTase (Qiagen) according to the manufacturer's instructions.

### **Measurement of mRNA expression using Real-time PCR**

Quantitative real-time PCR (qRT-PCR) analyses were used for measurement of relative mRNA abundance. PCR amplification, detection and data analyses were performed with a real-time PCR System (Rotor gene 3000, Corbett, Australia). PCR amplifications included an initial step of 10 min at 95°C, followed by 40 cycles of a two-phase PCR (denaturation at 95°C for 20 s; annealing and extension at 60°C for 30 s). Reaction mixtures (15 µL) included 1.5 µL cDNA as template, 7.5 µL SYBRGreen PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA), 1 µL of forward, 1 µL of reverse primers and 4 µL of double distilled water. The standard curves were composed of serial dilutions of cDNA. Samples were run in duplicate, and were expressed relative to 18sRNA as housekeeping gene, which was stable under the culture conditions used. Oligonucleotide primer sequences and GenBank Accession numbers for all gene transcripts are listed in Table 2.

Table 2. Oligonucleotide primer sequences for all gene transcripts

Gene	Primer	Sequence (5'to 3')	Gene accession number or reference
GHR1A	F	CCAGCCTCTGTTTCAGGAGTGT	Rhoads et al., 2008
	R	TGCCACTGCCAAGGTCAAC	
INSR	F	TCC TCA AGG AGC TGG AGG AGT	Pfafflet al., 2002
	R	GCT GCT GTC ACA TTC CCC A	
IGF1	F	TTGGTGGATGCTCTCCAGTTC	X15726
	R	GCACTCATCCACGATTCCTGT	
IGFBP2	F	GCATGGCCTGTACAACCTCAA	AF074854
	R	TCCCGGTGTTAGGGTTCACA	
18 S rRNA	F	GTAACCCGTTGAACCCCAT	DQ222453
	R	CCATCCAATCGGTAGTAGCG	

The relative mRNA abundance of each gene was calculated using the  $2^{-\Delta\Delta Ct}$  method as described in the User Bulletin #2 (Applied Biosystems 1997; ABI PRISM 7700 Sequence Detection System). The  $\Delta Ct$  value was calculated by subtracting the average (from triplicate) Ct value of the 18sRNA transcript from the average Ct value of selected genes. Then, the  $\Delta\Delta Ct$  was calculated by subtracting the  $\Delta Ct$  value of the control group from the  $\Delta Ct$  value of each treatment (average  $\Delta Ct$  value of all animals within a treatment). Then, the  $2^{-\Delta\Delta Ct}$  was calculated.

### Statistical analyses

Plasma concentrations of IGF1, insulin and blood metabolites (i.e., BHBA, NEFA and glucose) were analyzed using repeated measures responses of the mixed model procedure of SAS (SAS Institute, 2000), with the following model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where:

$\mu$  is the population mean,

$\alpha_i$  is a population parameter corresponding to treatment (diet)  $i$ ,

$\beta_j$  is the fixed effect of sampling day or time  $j$ ,

$(\alpha\beta)_{ij}$  is interaction effects of treatment and sampling day or time,

$e_{ijk}$  is the residual error.

Data were tested for normal distribution of the residuals by the PROC UNIVARIATE procedure of SAS.

Gene expression data, calculated using  $2^{-\Delta\Delta Ct}$  method, were expressed as fold change over the control group, which was given a value of 1 (Dirandeh et al., 2015). Gene expression data was checked for adherence to a normal distribution (PROC UNIVARIATE, SAS v 9.1, 2000) and were analyzed using the SAS (version 9.1, SAS Institute, Cary, NC, USA). Differences between means were tested using Tukey test and were considered significant at  $P < 0.05$  whereas  $P < 0.10$  was interpreted as a tendency.

## Results

### Dry matter intake, milk production and body condition score

There was no difference between groups (mean  $\pm$  S.E.M.) in parity ( $3.0 \pm 1.90$ ) or body condition score (BCS) at calving ( $3.2 \pm 0.07$ ). The omega-6 to omega-3 FA ratio was 4.2, 3.2, and 1.2 for omega-6, control, and omega-3 treatments, respectively (Table 1). The greater omega-6: omega-3 ratio of the roasted whole soybean diet (omega-6) was due to the greater quantity of linoleic acid (C18:2, 58.7%), whereas the low omega-6:omega-3 ratio in the linseed diet (omega-3) was because of the greater amount of linolenic acid in linseed (C18:3, 29.70%). The FA composition of the TMR was characterized by greater proportions of linoleic acid and  $\alpha$ -linolenic acid in diets supplemented with roasted whole soybean and linseed, respectively.

Table 3. Fatty acid (FA) profile (g/100 g of FA) of milk samples, milk production, DMI and BCS in the Holstein dairy cows fed diets supplemented with palm oil (Control, n=15), linseed as a source of omega-3 PUFA (Omega-3, n=15) or roasted whole soybean as a source of omega-6 PUFA (Omega-6, n=15)

Fatty acid	Treatments		
	control	Omega-3	Omega-6
C<12	0.38	0.36	0.37
C14:0	0.47	3.03	0.50
C16:0	33.21 a	12.36 b	14.31 b
C16:1	1.01	0.93	1.05
C18:0	16.08 a	2.75 b	3.02 b
C18:1	12.18	18.47	20.58
C18:2	26.78 a	30.93 a	47.42 b
C18:3	8.33 a	29.73 b	11.25 a
C20:0/1	1.52	1.45	1.50
SCFA <sup>1</sup>	0.382	0.365	0.377
MCFA <sup>2</sup>	34.68 a	16.27 b	15.85 b
LCFA <sup>3</sup>	64.89 a	83.33 b	83.77 b
Omega-3	8.33 a	29.73 b	11.25 a
Omega-6	26.78 a	30.93 a	47.42 b
Omega-6 to omega-3 FA ratio	3.20 a	1.00 b	4.20 c
Milk yield (kg d <sup>-1</sup> )	45.57	46.05	45.50
DMI (kg d <sup>-1</sup> )	23.3	24.1	23.6
Milk fat (%)	4.00	3.65	3.85
BCS	2.86	2.93	2.75

a, b – means within a row with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>SCFA, Short-chain fatty acids: C4:0 to C12:0.

<sup>2</sup>MCFA, Medium-chain fatty acids: C12:0 to C17:0.

<sup>3</sup>LCFA, Long-chain fatty acids: >C18:0.

There was no effect of treatment on dry matter intake and body condition score ( $P>0.05$ , Table 3). The DMI increased with time but no treatment  $\times$  day interaction was detected ( $P=0.98$ ). Similarly, milk yield increased over time ( $P<0.001$ ) but was not affected by diet ( $P=0.45$ ). Milk composition was similar among diets, except milk fat percentage and yield of milk fat, which were less in the omega-3 treatment than in the other two treatments ( $P=0.01$ , Table 3).

#### **Effects of dietary treatments on milk fatty acid profile**

The FA composition of milk was affected by diets (Table 3). There was no difference in short chain fatty acid concentrations in the milk among the dietary treatments. Lower concentrations of medium chain fatty acid were observed in cows fed with the omega-3 or omega-6 FA supplements compared with the control cows (Table 3). The proportion of C16:0 and C18:0 was lower in the milk from cows fed with omega-3 and omega-6 FA supplements compared with cows fed control treatment. Linoleic acid (C18:2) content in milk was higher for cows fed omega-6 fatty acids compared to those fed omega-3. There was no difference between treatments for saturated fatty acids (SFA) content in milk but PUFA content was higher in cows fed omega-3 or omega-6 fatty acids compared to that in control cows. The concentration of long chain fatty acids in milk was higher in cows fed omega-3 or omega-6 FA supplements than in cows fed control. The concentration of C18:3 FA was the highest in cows fed a diet supplemented with omega-3 FA than in those fed either a diet supplemented with omega-6 or control.

#### **Effects of dietary treatments on plasma insulin, glucose, NEFA, BHBA and IGF1**

Plasma glucose concentration increased with time ( $P<0.001$ , Figure 1) and was greater in the omega-3 group, compared with control and omega-6 group (Table 4). Plasma insulin increased with days in milk (DIM,  $P<0.001$ , Figure 1) and was significantly increased in cows fed the omega-3 diet compared with that in cows in the other dietary groups (Table 4). Plasma IGF1 concentration was higher for omega-6 cows compared to omega-3 or control cows ( $P=0.03$ ).

Mean plasma concentrations of NEFA and BHBA are shown in Table 4. Concentration of NEFA was affected ( $P=0.01$ ) by diets and decreased ( $P<0.001$ ) with increasing DMI (Figure 1). There was no difference among diets for BHBA concentrations.

#### **Relative mRNA abundance of studied genes**

The relative mRNA expression of GHR1A, INSR, IGF1 and IGFBP2 mRNA in liver tissue is illustrated in Figure 2. Dietary supplementation of linseed (omega-3) for 2 months increased the relative mRNA abundance of INSR and GHR1A compared with the control and omega-6 treatments ( $P<0.05$ ). The mRNA abundance of INSR and total GHR1A were not different between the control and omega-6 groups ( $P=0.202$ ). There were no differences in IGFBP2 mRNA levels among dietary treatments.

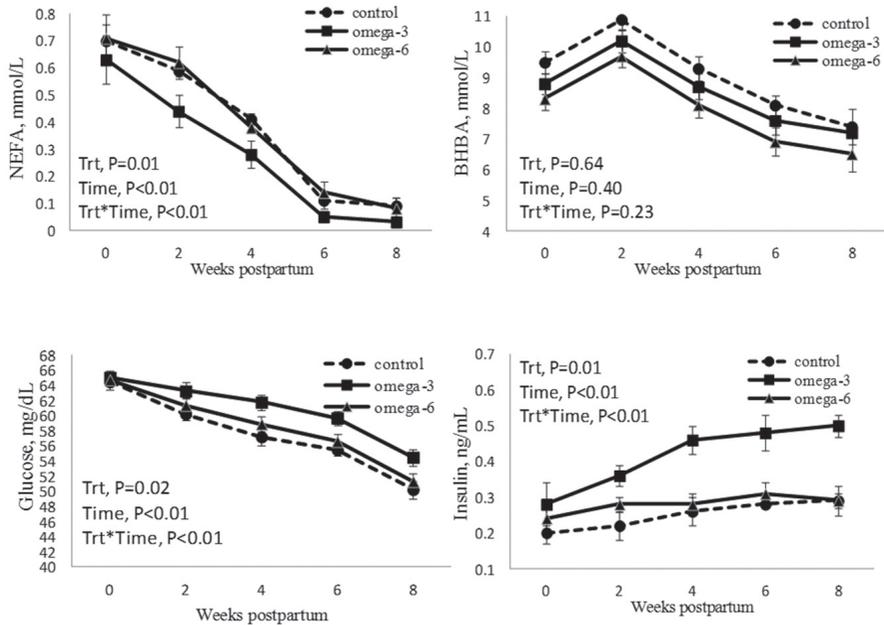
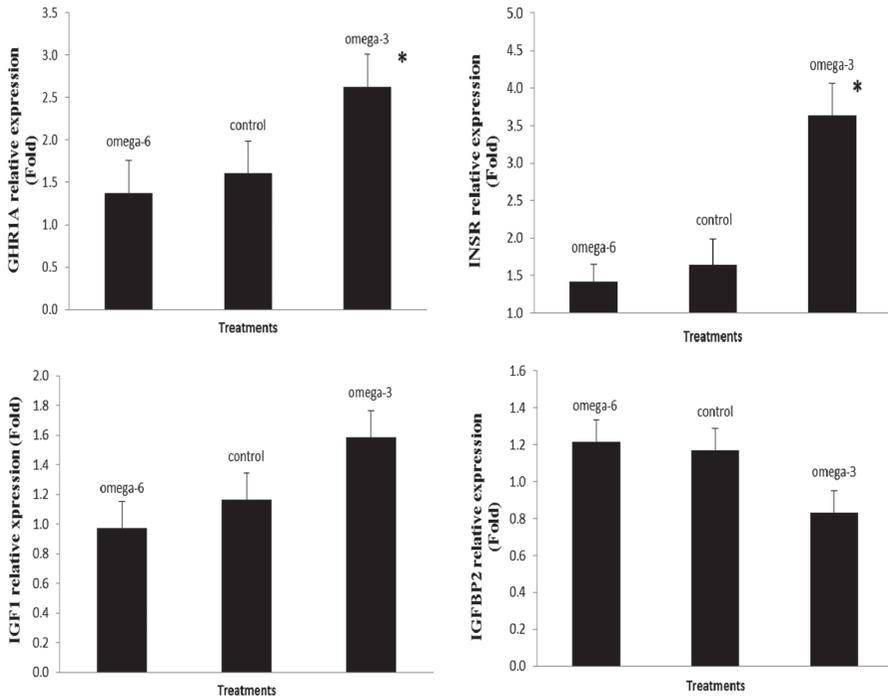


Figure 1. Mean (± SEM) plasma NEFA, BHBA, glucose and insulin concentrations in Holstein cows fed diets supplemented with palm oil (Control, n=15), linseed as a source of omega-3 PUFA (Omega-3, n=15) or roasted whole soybean as a source of omega-6 PUFA (Omega-6, n=15). Plasma samples were collected at 14 d interval from week 0 (calving) to 8 weeks postpartum

Table 4. Concentrations of plasma glucose, insulin, NEFA and BHBA in Holstein cows fed diets supplemented with palm oil (Control, n=15), linseed as a source of omega-3 PUFA (Omega-3, n=15) or roasted whole soybean as a source of omega-6 PUFA (Omega-6, n=15)

Measurement	Treatments			S.E.M	P
	control	Omega-3	Omega-6		
IGF-I (ng/ml)	100.2 a	120.1 b	108.7 a	1.3	0.03
Glucose (mg/dL)	57.53 b	60.87 a	58.59 b	0.56	0.02
Insulin (ng/ml)	0.25 a	0.42 b	0.28 a	0.01	0.01
NEFA (nmol/mL)	389 a	291 b	325 b	0.9	0.02
BHBA (mg/dL)	9.4	8.5	7.9	0.5	0.64

a, b – means within a row with different superscripts differ (P<0.05).



Significances are indicated in relation to the control group.

\* =  $P < 0.05$ .

Figure 2. Relative expression levels for INSR, GHR1A, IGF-I and IGFBP2 mRNA on day 70 pp in Holstein cows fed diets supplemented with omega-3, omega-6 or saturated fatty acid (SFA; Control)

## Discussion

The major finding of this study was that omega-3-fed cows had or tended to have higher expression of IGF1 and total GHR1A mRNAs in the liver than cows in the other two dietary treatments. In addition, cows fed a diet high in omega-3 had higher hepatic levels of INSR mRNA expression compared to control and roasted whole soybean (omega-6) fed cows. The plasma concentrations of IGF1 and insulin were also greater in cows fed omega-3 diets compared to those in the other two dietary groups.

Dietary treatments decreased NEFA concentrations that were consistent with previous studies (Badieli et al., 2014; Jahani et al., 2015). Blood concentration of NEFA is an index for body fat mobilization (Roberts et al., 1981) that is related to the energy balance of dairy cows. Bertics et al. (1992) reported that DM intake is inversely associated to concentrations of NEFA and BHBA in plasma and liver.

Dietary fatty acid profile can modulate the whole-body response to insulin. Specifically, long-chain omega-3 PUFA (derived from fish oil) may prevent development of insulin resistance in humans and rodents (Delarue et al., 2004). In agreement with these findings, abomasal infusion of fish oil enhanced insulin-induced utilization of amino acid and glucose in the bovine, and increased key intermediaries in the intracellular insulin signaling cascade in muscle (Gingras et al., 2007). Glucose-stimulated insulin secretion can be affected directly by fatty acids. For example, palm oil can induce insulin secretion (insulinogenic) compared with unsaturated fatty acids (Dobbins et al., 2002). Both *in vivo* and *in vitro* studies demonstrated these effects; for instance feeding rats with different fat sources and by perfuming rat pancreas with distinct FA, respectively (Jucker et al., 1999).

An interesting finding, in the present study, was the upregulation of the mRNA abundance of GHR1A in the liver of cows fed diets supplemented with omega-3, which is thought to be a key change during the disrupting of the somatotrophic axis in early lactation (McCarthy et al., 2009). As a consequence of coupling somatotrophic axis, we also detected increased plasma IGF1 concentration of cows fed omega-3 FA, which was consistent with results from Gross et al. (2011). These changes reflect the expected differences of hepatic IGF1 synthesis (Butler et al., 2003). In cows, down-regulation of total GHR1A gene expression after parturition causes them to become refractory to GH at this time (Butler et al., 2003). In addition, GH attenuates the lipogenic response to insulin and simultaneously amplifies the lipolytic response to  $\beta$ -adrenergic signals (Bauman and Vernon, 1993). The loss of the GHR1A is responsible for the decreased IGF1 in postpartum cows (Radcliff et al., 2003). Therefore, greater total GHR1A would lead to more IGF1 synthesis and secretion (Radcliff et al., 2004; Rhoads et al., 2004, 2007). Total GHR1A mRNA expression was significantly increased in cows fed a diet supplemented with omega-3, which resulted in greater plasma IGF1 concentrations in these cows compared to those in the control and omega-6 dietary treatments. The decrease in medium-chain fatty acids and increase in long chain fatty acids concentrations of milk in cows fed omega-3 and omega-6 fatty acids were in general agreement with several previous studies in which cows were fed diets enriched with different PUFAs (Petit et al., 2004; Dirandeh et al., 2013 a, b). In bovine mammary tissue, acetyl CoA carboxylase regulates medium- and short-chain FA synthesis (Wright et al., 2006; Bionaz and Looor, 2008), suggesting that insulin may stimulate short-chain FA synthesis in this study.

Results from the present study showed that cows fed diets supplemented with omega-3 had the lowest plasma NEFA concentrations. The major effects of insulin on adipose tissue are to reduce lipolysis and to increase lipogenesis. Accordingly, if adipose tissue is resistant to insulin, the net effect will be mobilization of body reserves and increase in plasma NEFA concentrations. This is a normal response that occurs in early lactation in high producing dairy cattle. However, it is beneficial to avoid extreme loss of body condition score in early lactation.

In conclusion, supplementation of linseed altered hepatic expression of genes related to somatotrophic axis function and concentrations of insulin, glucose and IGF1 in blood. Therefore, feeding diets supplemented with omega-3 FA during early

postpartum couples with the somatotrophic axis to increase plasma IGF1 concentration.

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