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Effects of fish oil supplementation and supplementation period on adipose tissue generation sites and the gene expression of enzymes involved in metabolizing adipose tissue in Holstein bulls under various forage types

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ABSTRACT

To investigate the effects of alfalfa hay (AH) proportion, fish oil (FO) supplementation, and the length of FO supplementation on lipoprotein lipase (LPL), peroxisome proliferator activated receptorγ (PPARγ) and stearoyl-COA desaturase (SCD) gene expression in various adipose tissue depots, 36 Holstein bulls (initial body weight of 345 ± 61 kg) were randomly assigned to 6 dietary treatments in a 2 × 3 factorial arrangement, with 2 levels of AH (10 and 20% of dietary dry matter) and 3 levels of FO (0, 1.05 and 2.1% of dry matter). Samples of adipose tissue were collected at the middle and end of the period (day 90), after slaughtering. The RT-qPCR technique was used for gene expression analyses. The relative mRNA abundance of lipogenic genes was not correlated with AH proportion and FO interaction. Supplementation of FO increased LPL and SCD gene expression in visceral and subcutaneous adipose tissues regardless of AH proportion ($P < 0.05$). The gene expression of PPARγ was not affected by dietary treatments. Supplementation of FO increased the mRNA abundance of SCD1 gene more in subcutaneous than visceral adipose tissue ($P < 0.01$). Gene expression of PPARγ and SCD enzymes increased as a result of prolonged FO supplementation ($P < 0.01$), whereas LPL mRNA abundance was lower at the end of period comparing to the middle period ($P < 0.01$). The results demonstrate that FO supplementation increases the abundance of adipose tissue SCD mRNA, which in the case of enhancement in UFAs concentration as a result of higher mRNA abundance and enzyme activity, can benefit both human and animal health. Moreover, the duration of FO supplementation was found to be an important factor in regulating enzymes involved in adipose tissue metabolism.

1. Introduction

In rodents, lipogenesis occurs in both liver and adipose tissues, whereas, in pig and non-lactating ruminants, adipose tissues are the primary site for lipogenesis. There is evidence that the fatty acids in fish oil (FO) such as docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids and fatty acids in the conjugated linoleic acid (CLA) series decrease preadipocyte proliferation in cell lines and reduce adiposity in rodents and ruminants, respectively (Azain, 2004; Smith et al., 2009). Fatty acids or their metabolites, such as the prostaglandins, are ligands for peroxisome proliferator activated receptors (PPARs) (Kliewer et al., 1997; Hertzel and Bernlohr, 1998; Sessler and Ntambi, 1998). The CLA isomers are also ligands for PPAR (Ding et al., 2000; Belury et al., 2002) and have been shown to affect adipose tissue development and gene expression in vitro. In addition, dietary fat inhibits lipogenesis in ruminants (Deeth and Christie, 1979; Page et al., 1997), but it is not clear if there are differences in potency for different degree of unsaturation. It has been reported that fat supplements enriched with oleic and linoleic acids reduce de novo lipogenesis, whereas linolenic-acid-enriched fat supplements increase lipogenesis in adipose tissue of growing cattle (Shingfield et al., 2013), which is not in agreement with results obtained in rodents (Flachs et al., 2009).

The polyunsaturated fatty acids (PUFAs), especially DHA, in FO escape from rumen biohydrogenation (BH) which indicate that FO fatty acids can have their effects on tissues metabolism when they are

Abbreviations: AH, alfalfa hay; FO, fish oil; LPL, lipoprotein lipase; PPARγ, peroxisome proliferator activated receptorγ; SCD, stearoyl-COA desaturase; UFAs, unsaturated fatty acids; SFAs, saturated fatty acids; DHA, docosahexaenoic acids; EPA, eicosapentaenoic acids; CLA, conjugated linoleic acid; PUFAs, polyunsaturated fatty acids; BH, biohydrogenation; DMI, dry matter intake; UXT, ubiquitously-expressed transcript; EIF3K, eukaryotic translation initiation factor3, subunit k; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; MUFA, monounsaturated fatty acid.

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used in unprotected form. In addition, FO predominant fatty acids inhibit the last step of ruminal BH for other dietary PUFAs (AbuGhazaleh and Jenkins, 2004) and subsequently increase the flow of vaccenic acid and *cis*-9,*trans*-11 CLA (common linoleic and linolenic acid BH intermediates) to the small intestine, which can influence adipose tissue metabolism. The ability of unprotected FO in inhibiting the last step of biohydrogenation possibly is higher than protected FO due to its more interaction with rumen microflora.

In addition, alfalfa hay (AH) has been shown to form a stable rumen environment and subsequently allow more complete rumen BH of unsaturated fatty acids. Onetti et al. (2002) reported increased concentration of C18:0 and reduced *trans*-10 C18:1 and total trans fatty acids in milk fat with increased proportion of alfalfa silage relative to corn silage; hence, type of forage can be a factor affecting the fatty acid profile of dietary fat. Moreover, dietary fatty acids have been shown to have site-specific effects on gene expression of enzymes involved in adipose tissue metabolism of various species (Adams et al., 1997; Barber et al., 2000; Archibeque et al., 2005; Herdmann et al., 2010). In dairy goats, dietary supplementation of FO and sunflower oil caused a depot-specific response of lipogenic enzymes mRNA abundance (Toral et al., 2013). It has been reported that adipose tissue depots in dairy cows response differently in relation to the energy density of the diet (Ji et al., 2014a, 2014b). In addition, dietary inclusion of FO reduced dry matter intake (DMI) in several studies (Rule et al., 1994; Whitlock et al., 2002), which is not desired. Defining an optimum length of FO supplementation to obtain favourable FO-induced results would prevent further deleterious effects of reduced DMI as a consequence of extended FO supplementation.

Therefore, the aim of this study was to investigate the effect of FO supplementation under different AH proportions and supplementation period on the relative gene expression of enzymes involved in the adipose tissue metabolism in subcutaneous and visceral adipose tissues.

2. Material and methods

2.1. Ethics statement

All experiments with animals were performed according to the recommendations in the Guide for the Care and Use of Laboratory Animals of the Research Station of Department of Animal Science, University of Tehran, Iran. The protocols were approved by the Animal Care and Use Committee of the University of Tehran Institutional Animal Care and Use Committee and included in a research project.

2.2. Animal, housing and diets

This experiment was conducted at the Natural Resources & Agricultural Research Farm of Tehran University, Karaj, Iran. Thirty-six Holstein bulls (initial body weight of 345 ± 61 kg and ages of 11 to 13 months) were grouped by weight and randomly assigned to 6 treatments in a 2 × 3 factorial arrangement. Bulls were housed in individual pens. The experiment began after two weeks of adaptation to experimental diets. Dietary treatments were two levels of chopped AH (10 and 20% of dry matter) combined with 3 dosages of FO (0, 1.05 and 2.1% of dietary dry matter). All diets were balanced to meet the nutrient requirements of Council N.R. (1996) and were isocaloric and isonitrogenous. The diets consisted of 30% forage and 70% concentrate mix (dry matter basis). Fish oil was added to concentrate and was prepared every 10 days to prevent fatty acid oxidation. The ingredients and chemical composition of the diets are shown in Table 1. On day 45 of the experiment, a surgical operation was used to harvest subcutaneous adipose tissue samples from the right side of the body between tuber coxae and tuber ischiadicum (Leiber et al., 2011). At the end of the period after slaughtering, samples of adipose tissue were obtained from subcutaneous (right side of the body between tuber coxae and tuber ischiadicum) and visceral (mesenteric) adipose tissues. All samples were immediately frozen in liquid nitrogen (−196 °C), transferred to the laboratory, and kept at −80 °C until analyses. Fatty acids profile of FO is presented in Table 2.

Table 1
Ingredients and chemical composition of dietary treatments.

	10%AH			20%AH		
%FO	0	1.05	2.1	0	1.05	2.1
Ingredients						
Alfalfa hay	10	10	10	20	20	20
Corn silage	20	20	20	10	10	10
Barley grain	41	41	41	41	41	41
Wheat grain	2	1	0.5	5	3	0.5
Soybean meal	2	2	2	2	2	2
Canola meal	12	13	14	10	10	11
Beet pulp	5	5	5	5	5	5
Wheat bran	5	4	2	4	5	5
Zeolite	1	1	1	1	1	1
Calcium carbonate	0.5	0.55	0.68	0.4	0.5	0.62
Sodium bicarbonate	0.7	0.7	0.7	0.7	0.7	0.7
Vitamin and mineral premix[a]	0.7	0.7	0.7	0.7	0.7	0.7
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Fish oil	0	1.05	2.1	0	1.05	2.1
Chemical composition						
DM (%)	56	56	56	56	56	56
ME[b] (MJ/kg)	10.92	10.92	10.92	10.92	10.92	10.92
EE (%)	3	4	5.1	3	4	5.1
CP (%)	15.5	15.5	15.5	15.5	15.5	15.5
NDF (%)	37	37	37	35	35	35

FO = fish oil; AH = alfalfa hay; DM = dry matter; ME = metabolizable energy; EE = ether extract, CP = crude protein.
[a] Contained per kg of diet: Ca 195 g; P 80 g; Mg 21 g; Na 50 g; Fe 3 g; Cu 0.3 g; Zn 0.3 g; Mn 22 g; I0.12 g; Co 0.1 g; Se 0.02 g; vitamin A 600,000 IU; vitamin D 200,000 IU; vitamin E 200 IU.
[b] Based on calculated value.

2.3. Total RNA extraction, clean-up, and cDNA synthesis

Total RNA was extracted according to the method of Chomczynski and Sacchi (2006) using Trizol Reagent (Invitrogen Co., Carlsbad, CA, USA). The extracted RNA was then treated with RNase-free DNase I (at 37 °C for 30 min) in order to remove the remnant genomic DNA from the samples (TaKaRa, Shuzo, Kyoto, Japan). The RNA abundance was estimated by nanodrop spectrophotometry at 260 nm, and the purities were checked by determining the absorption ratios at 260/280 nm. The $OD_{260/280}$ ratio was measured in the range of 1.72–2.13; a ratio higher than 1.8 was assumed suitable for transcriptomics analysis (Manchester, 1996). Indeed, high resolution 4% agarose gel indicated a 28S/18S ratio of 2 as classical RNA quality control. The efficiency and linearity of our RT-qPCR reactions were evaluated using 10-fold serial dilutions. The relationship between threshold cycle and the log copy numbers of cDNA for all studied genes was linear. Moreover, the PCR efficiency of our amplifications was confirmed near the theoretical optimum level of 2 (Wilkening and Bader, 2004), where the efficiency values ranged from 1.79 to 2.09. In order to test the quality of extracted

Table 2
Fatty acid profile of fish oil (%).

Fatty acids	Percentage
C14:0	6.2
C16:0	22.5
C16:1	7.9
C18:0	4.8
C18:1	25.8
C18:2	3.6
C18:3	1.3
C20:4 n−6	0.7
C20:5 n−3	8.3
C22:6 n−3	17.6