***Animal* Journal**

**Supplementary Material S1**

**Review: Nutrigenomics of marbling and fatty acid profile in ruminant meat**

M. M. Ladeira1§, J. P. Schoonmaker2, K. Swanson3, S. K. Ducket4, M. P. Gionbelli1, L. M. Rodrigues1, P. D. Teixeira1

**Table S1***Papers published in the last 10 years about fatty acids concentration in ruminant meat and human health*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Reference | Diets | EE1 on diet | Fatty acids | Effect |
| van Cleef et al. ([2017](#_ENREF_25)) | levels of crude glycerin: 7.5% | 2.60% | CLA2 | no effect |
|  | 15% | 2.30% | Oleic | no effect |
|  | 22.50% | 2.10% | Hypercholesterolemics | no effect |
|  | 30% | 1.80% | n-6/n-33 | no effect |
| [Carvalho et al. (2017)](#_ENREF_5) | palm oil | 104 g/kg DM | CLA | greater to linseed oil |
|  | linseed oil | 94 g/kg DM | Oleic | greater to whole soybeans |
|  | rumen protected fat | 97 g/kg DM | Hypercholesterolemics | greater to palm oil |
|  | whole soybeans | 116 g/kg DM | n-6/n-3 | reduced to palm and linseed oil |
| [Correia et al. (2016)](#_ENREF_8) | Levels of Peanut cake (biodiesel byproduct) |  | CLA | positive quadratic to 100% |
|  | 33% | 2.48% | Oleic | no affect |
|  | 66% | 3.0% | Hypercholesterolemics | decreased with 100% of peanut cake |
|  | 100% | 3.51% | n-6/n-3 | quadratic effect |
| [Ribeiro et al. (2016)](#_ENREF_22) | Corn silage | 2.64% | CLA | no effect |
|  | Sugar cane | 2.12% | Oleic | no effect |
|  | Sugar cane bagasse | 2.60% | Hypercholesterolemics | no effect |
|  |  |  | n-6/n-3 | no effect |
| [Rivaroli et al. (2016)](#_ENREF_23) | inclusion of essential oils |  | CLA | no effect |
|  | 3.5 g/animal/day | 3.12% | Oleic | no effect |
|  | 7.0 g/animal/day | 2.80% | Hypercholesterolemics | no effect |
|  |  |  | n-6/n-3 | no effect |
| [Rabelo et al. (2016)](#_ENREF_21) | 60:40 F:C4 untreated | 43.4 g/kg DM | CLA | no effect |
|  | 60:40 F:C inoculated with Lactobacillus buchneri | 42.2 g/kg DM | Oleic | no effect |
|  | 40:60 F:C untreated | 47.8 g/kg DM | Hypercholesterolemics | no effect |
|  | 40:60 F:C inoculated with Lactobacillus buchneri | 47.0 g/kg DM | n-6/n-3 | no effect |
| [Yasuda et al. (2016)](#_ENREF_27) | soybean curd residue + soy sauce cake | 5.34% | CLA | no effect |
|  |  |  | Oleic | no effect |
|  |  |  | Hypercholesterolemics | no effect |
|  |  |  | n-6/n-3 | not analyzed |
| [Rossi et al. (2016)](#_ENREF_24) | high starch with ground soybean | 5.87% | CLA | no effect |
|  | high starch without ground soybean | 2.48% | Oleic | no effect |
|  | low starch with ground soybean | 6.46% | Hypercholesterolemics | no effect |
|  | low starch without ground soybean | 3.14% | n-6/n-3 | no effect |
| [Comparin et al. (2015)](#_ENREF_7) | pasture | 22.07 g/kg DM | CLA | no effect |
|  | supplement | 38.62 g/kg DM | Oleic | no effect |
|  | protected fat | 850 g/kg DM | Hypercholesterolemics | no effect |
|  |  |  | n-6/n-3 | not analyzed |
| [da Silva Lima et al. (2015)](#_ENREF_9) | 2.5% cottonseed | 3.57% | CLA | not analyzed |
|  | 11.5% cottonseed | 5.18% | Oleic | no effect |
|  | 3.13% cottonseed + protected lipid | 5.11% | Hypercholesterolemics | no effect |
|  |  |  | n-6/n-3 | not analyzed |
| [Machado Neto et al. (2015)](#_ENREF_18) | ground soybean | 6.48% | CLA | greater to soybean |
|  | ground soybean with vitamin E | 6.48% | Oleic | greater to soybean |
|  | ground cottonseed | 6.56% | Hypercholesterolemics | no affect |
|  | ground cottonseed with vitamin E | 6.56% | n-6/n-3 | greater to cottonseed |
| [Andrade et al. (2014)](#_ENREF_2) | Without Rumen Protected Lipids | 3.20% | CLA | no effect |
|  | With Rumen Protected Lipids | 1.65% | Oleic | no effect |
|  |  |  | Hypercholesterolemics | no effect |
|  |  |  | n-6/n-3 | no effect |
| [Albertí et al. (2014)](#_ENREF_1) | linseed | 5.60% | CLA | no effect |
|  | linseed + vitamin E | 5.60% | Oleic | no effect |
|  |  |  | Hypercholesterolemics | no effect |
|  |  |  | n-6/n-3 | reduced with linseed |
| [Carvalho et al. (2014)](#_ENREF_6) | levels of crude Glycerin (6%, 12%, and 18%) | 3.30% | CLA | no affect |
|  |  |  | Oleic | increased with 12 and 18% Glycerin |
|  |  |  | Hypercholesterolemics | no affect |
|  |  |  | n-6/n-3 | no affect |
| [Ladeira et al. (2014)](#_ENREF_17) | ground soybean | 6.50% | CLA | increased for soybean |
|  | Ground soybean + monensin | 6.50% | Oleic | greater to rumen protected fat |
|  | rumen protected fat | 6.70% | Hypercholesterolemics | no affect |
|  | rumen protected fat + monensin | 6.70% | n-6/n-3 | greater to soybean |
| [He et al. (2014)](#_ENREF_13) | DDGS5 | 3.80% | CLA | no affect |
|  | flaxseed | 7.10% | Oleic | decreased for flaxseed |
|  | DDGS +flaxseed | 7.60% | Hypercholesterolemics | not analyzed |
|  | oleate sunflower seeds | 8% | n-6/n-3 | not analyzed |
|  | oleate sunflower seeds + DDGS | 8.30% |  |  |
| [Karami et al. (2013)](#_ENREF_15) | palm oil | 4.26% | CLA | not analyzed |
|  | canola oil | 4.27% | Oleic | no affect |
|  |  |  | Hypercholesterolemics | no affect |
|  |  |  | n-6/n-3 | grater to canola |
| [Mapiye et al. (2013)](#_ENREF_19) | grass hay + flaxseed | 6.40% | CLA | greater to sunflower |
|  | grass hay + sunflower-seed | 6.60% | Oleic | greater to flaxseed |
|  | red clover silage + flaxseed | 8.20% | Hypercholesterolemics | no affect |
|  | red clover silage + sunflower-seed | 8.40% | n-6/n-3 | not analyzed |
| [Angulo et al. (2012)](#_ENREF_3) | saturated fat | 62 g/kg DM | CLA | reduced with saturated fat |
|  | linseed oil/DHA-rich algae | 62 g/kg DM | Oleic | reduced with sunflower oil/DHA-rich algae |
|  | sunflower oil/DHA-rich algae | 63 g/kg DM | Hypercholesterolemics | no affect |
|  |  |  | n-6/n-3 | reduced with linseed oil/DHA-rich algae |
| [He et al. (2012)](#_ENREF_12) | levels of WDDGS6 |  | CLA | no affect |
|  | 25% | 26 g/kg DM | Oleic | no affect |
|  | 30% | 27 g/kg DM | Hypercholesterolemics | no affect |
|  | 35% | 28 g/kg DM | n-6/n-3 | increased with WDDGS |
| [Fugita et al. (2012)](#_ENREF_11) | corn silage with low cutting and without inoculants | 2.76% | CLA | no affect |
|  | low cutting with inoculants | 2.81% | Oleic | no affect |
|  | high cutting without inoculants | 2.84% | Hypercholesterolemics | no affect |
|  | high cutting with inoculants | 2.80% | n-6/n-3 | no affect |
| [Oliveira et al. (2011)](#_ENREF_20) | soybeans | 6.10% | CLA | no affect |
|  | cottonseed | 6.70% | Oleic | no affect |
|  | linseed | 5.80% | Hypercholesterolemics | no affect |
|  |  |  | n-6/n-3 | no affect |
| [Herdmann et al. (2010)](#_ENREF_14) | grass silage with linseed oil and rapeseed cake | 4.0% | CLA | no affect |
|  |  |  | Oleic | no affect |
|  |  |  | Hypercholesterolemics | no affect |
|  |  |  | n-6/n-3 | Reduced |
| [Koger et al. (2010)](#_ENREF_16) | 20% DDGS | 6.0% | CLA | no affect |
|  | 20% WDGS | 5.0% | Oleic | no affect |
|  | 40% DDGS | 7.20% | Hypercholesterolemics | no affect |
|  | 40% WDGS | 5.20% | n-6/n-3 | not analyzed |
| [Bartoň et al. (2010)](#_ENREF_4) | maize silage | 28 g/kg DM | CLA | no affect |
|  | legume-cereal mixture silage and lucerne silage | 19 g/kg DM | Oleic | greater to maize silage |
|  |  |  | Hypercholesterolemics | no affect |
|  |  |  | n-6/n-3 | greater to maize silage |
| [Fernandes et al. (2009)](#_ENREF_10) | 40:60 F:C | 3.50% | CLA | no affect |
|  | 60:40 F:C | 4.60% | Oleic | no affect |
|  |  |  | Hypercholesterolemics | no affect |
|  |  |  | n-6/n-3 | not analyzed |
| [Warren et al. (2008)](#_ENREF_26) | Concentrate | 42.4 g/kg DM | CLA | greater to concentrate |
|  | Grass silage | 40.7 g/kg DM | Oleic | no affect |
|  |  |  | Hypercholesterolemics | no affect |
|  |  |  | n-6/n-3 | not analyzed |
|  | only pasture | 30 g/kg DM | CLA | increased with pasture + sunflower oil |
|  | pasture supplemented with sunflower oil | 142 g/kg DM | Oleic | No affect |
|  | pasture supplemented with linseed oil | 135 g/kg DM | Hypercholesterolemics | reduced with pasture + sunflower oil |
|   |  |  | n-6/n-3 | increased with pasture + sunflower oil |

1Ether extract

2Conjugated linoleic acid: c9,t11-C18:2

3Ratio of omega-6 and omega-3

4Forage:Concentrate

5Dried distillers grains with solubles

6Wet distillers grain with solubles

**Supplementary Material S1 - Material and Methods: Duckett et al. (unpublished results)**

 Southdown ewe lambs (n = 18; 42 +5.6 kg BW) were used to assess the effects of palmitoleic acid (POA; Provinal® Purified n-7, 56% C16:1; Tersus Pharmaceuticals) or α-linolenic acid (FLAX; cold-pressed and unrefined flax seed oil, 56% C18:3, General Nutrition Company, Pittsburg, PA) supplementation on gene expression in longissimus muscle. Lambs were blocked by weight and randomly assigned to one of three treatments: 1) control (**CON**), no oil supplement, 2) flaxseed oil (**FLAX**; 56% C18:3 n-3) supplementation at 0.1% of BW, or 3) Provinal® Purified n-7 oil (**PO**; 56% C16:1 n-7) supplementation at 0.1% of BW. Provinal® Purified n-7 oil was graciously provided for this experiment by Tersus Pharmaceuticals (Bonita Springs, FL). All lambs were fed *ad libitum* the same basal diet consisting of 75% soybean hull pellets and 25% alfalfa pellets at a level to meet NRC requirements. Oil supplements were maintained at 4°C in the laboratory. Daily oil amounts were weighed out daily and hand-mixed into the basal diet just prior to feeding each lamb. Lambs were individually fed the treatment diets for 60 days.

*Gene expression*. At slaughter, longissimus muscle tissue was collected at 30 min. postmortem and immediately frozen in liquid nitrogen for storage at -80°C for subsequent gene expression analyses. Total RNA was isolated from the muscle samples using the TriZol procedure (Invitrogen; Thermo-Fisher, Waltham, MA) and were further purified using the Pure Link columns (Invitrogen). RNA was quantified using a NanoDrop spectrophotometer (Invitrogen) and quality assessed using Agilent Bioanalyzer (Agilent, Santa Clara, CA). RNA integrity number (RIN) for all samples were 7 or greater. RNA (1 ug) was reverse transcribed in duplicate using Quanta Bio qScript (Thermo Fisher) and used for qPCR according to Duckett et al. (2014). Several housekeeping genes (glyceraldehyde 3-phosphate dehydrogenase [*GAPDH*], β-actin [*ACTB*], cyclophilin [*CYC*], ribosomal subunit 9 [*RSP9*], tubulin [*TUB*], and *Thy1*) were evaluated for use to normalize the data using RefFinder, which utilizes four different computational programs (geNorm, Normfinder, BestKeeper, and comparative CT method; http://leonxie.esy.es/RefFinder/; Xie et al., 2012). The most stable housekeeping gene for both tissue types was *GAPDH*, which was used for data normalization. Normalized CT values (ΔCT= CT,gene − CT, *GAPDH*) were calculated for each sample. All qPCR results were normalized to *GAPDH* and presented as fold-change from CON according to Livick and Schmidttgen (2001). For gene expression, normalized CT values were subjected to analysis using the relative expression software tool (REST) for statistical analysis of relative gene expression by oil treatment.

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