Extruded linseed alone or in combination with fish oil modifies mammary gene expression profiles in lactating goats \_ Y. Faulconnier, L. Bernard, C. Boby, J. Domagalski, Y. Chilliard and C. Leroux

# Table S1 *Ingredient and chemical composition of the ingested experimental diet*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Treatment | | |  |  |
|  | CTRL1 | EL2 | ELFO3 | SEM4 | *P*-value |
| Ingredient |  |  |  |  |  |
| Natural grassland hay (kgDM/d) | 1.25 | 1.19 | 1.34 | 0.09 | 0.57 |
| Barley (kgDM/d) | 1.11a | 0.49c | 0.57b | 0.02 | 0.01 |
| Soya bean meal (kgDM/d) | 0.08a | 0.03b | 0.06a | 0.01 | 0.01 |
| Extruded Linseeds (kgDM/d) | 0.00c | 0.50a | 0.34b | 0.01 | 0.01 |
| Fish Oil ( kg/d) | 0.00b | 0.00b | 0.04a | 0.01 | 0.01 |
| Forage percentage (%) | 51 | 54 | 57 | 1.90 | 0.13 |
| Chemical composition ( g/kgDM) |  |  |  |  |  |
| Organic matter | 936a | 934ab | 932b | 1.06 | 0.08 |
| Acid Detergent Fiber | 170b | 190a | 191a | 3.85 | 0.05 |
| Neutral Detergent Fiber (NDF) | 364 | 376 | 382 | 6.01 | 0.15 |
| Crude protein | 116c | 131a | 126b | 1.34 | 0.01 |
| Starch | 251a | 169b | 163b | 7.28 | 0.01 |
| Diethyl Ether Extract | 17b | 72a | 68a | 2.22 | 0.01 |

1 CTRL: natural grassland hay control diet (*n* = 5).

2 EL: CTRL supplemented with 530 g/d of extruded linseed (*n* = 4).

3 ELFO: CTRL supplemented with 340 g/d of extruded linseed plus 39 g/d of fish oil (*n* = 5).

4 SEM for *n* = 14.

a, b c Mean values for each treatment within a row sharing a common superscript differ (*P* < 0.05)

**Table S2** *Primer and probe sequences and conditions used for real-time RT-PCR*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Gene | Encoded protein | Nucleotide sequence (5’ – 3’) | | T (°C)1 | | | Source | | |
| *CD36* | Platelet glycoprotein 4 | F2: ACA GAT GTG GCT TGA GCG TG  R3: ACT GGG TCT GTG TTT TGC AGG | 58 | | Ollier *et al*., 2007 | | |
| *ACACA* | Acetyl-CoA carboxylase alpha | F: CAT GGA AAT GTA CGC GGA CC  R: GGT GGT AGA TGG GAA GGA GGA  P4: CGA GCG GAA GGA GCT GGA GAG CA | 58 | | Bernard et al., 2005 | | |
| *FASN* | Fatty acid synthase | F: ACA GCC TCT TCC TGT TTG ACG  R: CTC TGC ACG ATC AGC TCG AC  P: ATC TGG AGG CGC GTG TGG CAG CC | 60 | | Bernard *et al.*, 2005 | | |
| *LPL* | Lipoprotein lipase | F: TTC AGA GGC TAT TAC TGG AAA TCC  R: ATG TCA ATC ACA GCA TTC ATT CTA CT  P: TTC CAG TGG TGC CGG AAC ACT CCT TC | 60 | | Bonnet *et al*., 2000 | | |
| *SCD1* | Stearoyl-CoA desaturase 1 | F: TGC TGA CAA CTT ATC TGG ATG C  R: AAG GAA TCC TGC AAA CAG CTA  P: CCA GAG CCT GCA GAA GTG GCT GGT ATA A | 60 | | Bernard *et al.*, 2005 | | |
| *SCD5* | Stearoyl-CoA desaturase 1 | F: AGA AGG GGA GGA AGC TTG AC  R: GGA GGC CAG GAA GTA GGA GT | 62 | | Lengi and Corl, 2007 | | |
| *SREBF1* | Sterol regulatory element binding transcription factor 1 | F: CCA GCT GAC AGC TCC ATT GA  R: TGC GCG CCA CAA GGA | 62 | | Harvatine and Bauman 2006 | | |
| *ACSBG1* | Acyl-CoA Synthetase Bubblegum Family Member 1 | F: ATT GCC GCC CAG ATC TAT GA  R: GAA GAA GTG CTG TGT CTC CGC | 62 | | Ollier et al. 2007 | | |
| *ACSL1* | Acyl-CoA Synthetase Long-Chain Family Member | F: TGG AAA ACT CAT TTC CTG GGA  R: GCA GTA AAA GTG AAA TGC GGC | 60 | | Faulconnier et al. 2011 | | |
| *FABP3* | Fatty acid binding protein 3, muscle and heart | F: CCT CTC CTT CCA CTG ACT GC  R: TTG ACC TCA GAG CAC CCT TT | 58 | | Jurie *et al*., 2007 | | |
| *AZGP1* | Alpha-2-Glycoprotein 1, Zinc-Binding | F: CCT CTC CTT CCA CTG ACT GC  R: TTG ACC TCA GAG CAC CCT TT | 60 | | Ollier et al. 2007 | | |
| *MFG-E8* | Milk Fat Globule-EGF Factor 8 Protein1 | F: TGA GTA GGT CTG GGA TGG AC  R: GGA AGC TGC CTG TGT ACT CT | 60 | | Ollier et al. 2007 | | |
| *GPAT* | Glycerol-3-phosphate acyltransferase 1, mitochondrial | F: ACC AGC AGT TCA TCA CCT TC R: GTA CAC GGC AAC CCT CCT CT | 58 | | Ollier *et al*., 2007 | | |
| *PPIA* | Peptidyl-prolyl *cis*-*trans* isomerase A or cyclophilin A | F: GGA TTT ATG TGT CCA GGG TGG TGA  R: CAA GAT GCC AGG ACC TGT ATG  P: TCT CCC CAT AGA TGG ACT TGC CAC CAG T | 60 | | Bonnet *et al.*, 2000 | | |
| *XDH* | Xanthine Dehydrogenase | F: GCC CTG CAG AAC ATG AAT CT  R: GCA CAA ATA CTT CCT ACA CCT | 60 | | Ollier *et al*., 2009 | | |
| *UXT* | Ubiquitously expressed prefoldin like chaperone | F: TGT GGC CCT TGG ATA TGG TT  R: GGT TGT CGC TGA GCT CTG TG | | 60 | | Bonnet *et al.*, 2013 | | |
| *EIF3K* | Eukaryotic translation initiation factor 3 subunit K | F: CCA GGC CCA CCA AGA AGA A  R: TTA TAC CTT CCA GGA GGT CCA TGT | | 60 | | Bonnet *et al.*, 2013 | | |

*1T* = PCR annealing temperature (°C).

*2*F = forward primer.

*3*R = reverse primer.

*4*P = Taqman probe.

**References**

Bernard L, Leroux C, Bonnet M, Rouel J, Martin P and Chilliard Y 2005. Expression and nutritional regulation of lipogenic genes in mammary gland and adipose tissues of lactating goats. Journal of Dairy Research 72, 250-255.

Bonnet M, Leroux C, Faulconnier Y, Hocquette JF, Bocquier F, Martin P and Chilliard Y 2000. Lipoprotein lipase activity and mRNA are up-regulated by refeeding in adipose tissue and cardiac muscle of sheep. Journal of Nutrition 130, 749-756.

Harvatine KJ and Bauman DE 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. Journal of Nutrition 136, 2468–2474.

Jurie C, Cassar-Malek I, Bonnet M, Leroux C, Bauchart D, Boulesteix P, Pethick DW and Hocquette JF 2007. Adipocyte fatty acid-binding protein and mitochondrial enzyme activities in muscles as relevant indicators of marbling in cattle. Journal of Dairy Science 85, 2660–2669.

Lengi AJ and Corl BA 2007. Identification and characterization of a novel bovine stearoyl-CoA desaturase isoform with homology to human SCD5. Lipids

42, 499–508.

Ollier S, Leroux C, de la Foye A, Bernard L, Rouel J and Chilliard Y 2009. Whole intact rapeseeds or sunflower oil in high-forage or high-concentrate diets affects milk yield, milk composition, and mammary gene expression profile in goats. Journal of Dairy Science 92, 5544-5560.

Ollier S, Robert-Granie C, Bernard L, Chilliard Y and Leroux C 2007. Mammary transcriptome analysis of food-deprived lactating goats highlights genes involved in milk secretion and programmed cell death. Journal of Nutrition 137, 560-567.

**Table S3** *Effects of dietary supplements of extruded linseeds alone or in combination with fish oil on milk yield and composition in lactating goats*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | CTRL1 | EL2 | ELFO3 | SEM4 | *P*-value |
| Milk yield (kg/d) | 2.87 | 2.27 | 2.35 | 0.24 | 0.21 |
| Protein |  |  |  |  |  |
| Yield (g/d) | 86 | 73 | 71 | 7.50 | 0.32 |
| Content (g/kg) | 29.9 | 32.5 | 30.1 | 1.31 | 0.39 |
| Fat |  |  |  |  |  |
| Yield (g/d) | 77 | 80 | 66 | 9.62 | 0.59 |
| Content (g/kg) | 27.0a | 36.0 b | 26.9 a | 2.52 | 0.06 |
| Lactose |  |  |  |  |  |
| Yield (g/d) | 130 | 114 | 111 | 12.61 | 0.52 |
| Content (g/kg) | 45.2 | 50.3 | 47.2 | 1.42 | 0.10 |

1 CTRL: natural grassland hay control diet (*n* = 5).

2 EL: CTRL supplemented with 530 g/d of extruded linseed (*n* = 4).

3 ELFO: CTRL supplemented with 340 g/d of extruded linseed plus 39 g/d of fish oil (*n* = 5).

4 SEM for *n* = 14.

a, b Mean values for each treatment within a row sharing a common superscript differ (*P* < 0.05).

**Table S4** *Effect of diets on milk fatty acid composition in lactating goats*

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | | Diets |  | |  | |  | |
| Fatty Acid (g/100g fatty acids) | CTRL1 | EL2 | | | ELFO3 | | *SEM4* | | *P-value* | |
| 4:0 | 1.79 | 1.77 | | | 1.85 | | 0.13 | | 0.89 | |
| 6:0 | 2.02 | 2.14 | | | 2.09 | | 0.14 | | 0.83 | |
| 8:0 | 2.30 | 2.50 | | | 2.36 | | 0.22 | | 0.84 | |
| 10:0 | 9.39 | 8.31 | | | 8.32 | | 0.92 | | 0.66 | |
| 12:0 | 5.66a | 3.71b | | | 4.78ab | | 0.51 | | 0.08 | |
| 14:0 | 12.4a | 7.55b | | | 10.16ab | | 0.86 | | 0.01 | |
| cis9-14:1 | 0.29 a | 0.10b | | | 0.17b | | 0.02 | | 0.01 | |
| 16:0 | 29.98a | 16.36b | | | 22.99ab | | 2.46 | | 0.01 | |
| cis9-16:1 | 0.85a | 0.36b | | | 0.81a | | 0.10 | | 0.02 | |
| 18:0 | 5.44b | 13.58a | | | 3.27b | | 1.21 | | 0.01 | |
| cis9-18:1+ cis10-18:1+trans-14+trans-15 | 15.19b | 21.91a | | | 9.65c | | 1.65 | | 0.01 | |
| 20:0 | 0.13 | 0.13 | | | 0.15 | | 0.03 | | 0.84 | |
| cis9,trans11-CLA | 0.603b | 0.981a | | | 2.967a | | 0.70 | | 0.08 | |
| cis11-22:1 | 0.00 b | 0.000 1b | | | 0.193a | | 0.03 | | 0.01 | |
| 22:6n3 | 0.056a | 0.032b | | | 0.103a | | 0.02 | | 0.04 | |
| ***Sums and ratios*** |  |  | | |  | |  | |  | |
| ∑SFA5 | 73.49a | 59.05b | | | 60.69b | | 2.56 | | 0.01 | |
| ∑MUFA6 | 19.98b | 31.64a | | | 27.39a | | 1.96 | | 0.01 | |
| ∑PUFA7 | 4.8 b | 8.13a | | | 9.54a | | 1.16 | | 0.04 | |
| ∑ CLA | 0.67 b | 1.181a | | | 3.202a | | 0.70 | | 0.06 | |
| ∑cis-18:1 | 15.9 b | 24.21a | | | 11.02b | | 1.76 | | 0.01 | |
| ∑trans-18:1 | 1.60b | 5.76b | | | 13.02a | | 2.02 | | 0.01 | |
| 18:3n-3/18:2n-6 | 0.29c | 1.17a | | | 0.88b | | 0.09 | | <.01 | |
| cis9-14:1/14:0+cis9-14:1 | 0.023a | 0.013b | | | 0.016b | | 0.01 | | 0.01 | |

1 CTRL: natural grassland hay control diet (*n* = 5)*.*

2 EL: CTRL supplemented with 530 g/d of extruded linseed (*n* = 4).

3 ELFO: CTRL supplemented with 340 g/d of extruded linseed plus 39 g/d of fish oil (*n* = 5).

4 SEM for *n* = 14.

5 SFA: Saturated Fatty Acids.

6 MUFA: MonoUnsaturated Fatty Acids

7 PUFA: PolyUnsaturated Fatty Acids.

a, b, c means within a row with superscripts differ *(P* < 0.05).

# Table S7 *Informative parts of the Ingenuity Pathway Analysis presenting the Top 5 enriched pathways*

*Table S7A**Molecular and cellular functions*

|  |  |  |
| --- | --- | --- |
| Comparison | Name | *P*-value |
| Extruded linseed **(EL)** compared to Control diets **(CTRL)** diets | Cellular growth and proliferation | 8.95 10-8 - 1.43 10-02 |
| Cellular Movement | 2.85 10-6 - 1.44 10-02 |
| Cell cycle | 2.89 10-6 - 1.51 10-02 |
| Drug metabolism | 3.43 10-6 - 1.36 10-02 |
| Molecular transport | 3.43 10-6 - 1.45 10-02 |
| Extruded linseed with fish oil **(ELFO)** compared to  CTRL diets | Cellular growth and proliferation | 8.20 10-7 - 1.40 10-02 |
| Drug metabolism | 2.73 10-6 - 1.40 10-02 |
| Molecular transport | 2.73 10-6 - 1.40 10-02 |
| RNA trafficking | 4.23 10-5 - 5.20 10-03 |
| Cellular Development | 5.30 10-5 - 1.40 10-02 |

*Table S7B**Canonical pathways*

|  |  |  |
| --- | --- | --- |
| Comparison | Name | *P*-value |
| EL compared to CTRL diets | RhoGDI Signaling | 3.13 10-4 |
| PI3K/AKT Signaling | 5.72 10-4 |
| Acute Myeloid Leukemia Signaling | 1.10 10-3 |
| Endometrial Cancer Signaling | 1.11 10-3 |
| Reelin Signaling in Neurons | 1.26 10-3 |
| ELFO compared to CTRL diets | PI3K/AKT Signaling | 1.77 10-3 |
| Tetrapyrrole Biosynthesis II | 1.91 10-3 |
| Heme Biosynthesis II | 6.62 10-3 |
| FXR/RXR Activation | 9.10 10-3 |
| Death Receptor Signaling | 5.58 10-3 |

*Table S7C**Networks*

|  |  |  |  |
| --- | --- | --- | --- |
| Comparison | Associated Network Functions | Score | |
| EL compared to CTRL diets | Protein Synthesis, Gene expression, Amino Acid Metabolism | | 59 |
| Drug Metabolism, Molecular Transport, Small Molecule Biochemistry | | 36 |
| Cell Cycle, Cellular Assembly and Organization, Cellular Growth, Proliferation | | 30 |
| Lipid Metabolism, Molecular Transport, Small Molecule Biochemistry | | 26 |
| Infectious Disease, Inflammatory Disease, Inflammatory Response | | 26 |
| ELFO compared to CTRL diets | Developmental Disorder, Hereditary Disorder, Metabolic Disease | | 71 |
| Amino Acid Metabolism, Protein Synthesis, Small Molecule Biochemistry | | 45 |
| Cell Morpho, Endocrine Syst Developt and Function, Endocrine Syst Disorders | | 35 |
| Post-Translational Modification, Hematological Disease, Hereditary Disorder | | 33 |
| Cell death and survival, Cellular Compromise, Cardiovascular Disease | | 33 |