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 TGR3: ACT GGG TCT GTG TTT TGC AGG | 58 | Ollier *et al*., 2007 |
| *ACACA* | Acetyl-CoA carboxylase alpha | F: CAT GGA AAT GTA CGC GGA CCR: GGT GGT AGA TGG GAA GGA GGAP4: CGA GCG GAA GGA GCT GGA GAG CA | 58 | Bernard et al., 2005 |
| *FASN* | Fatty acid synthase | F: ACA GCC TCT TCC TGT TTG ACGR: CTC TGC ACG ATC AGC TCG ACP: ATC TGG AGG CGC GTG TGG CAG CC | 60 | Bernard *et al.*, 2005 |
| *LPL* | Lipoprotein lipase | F: TTC AGA GGC TAT TAC TGG AAA TCCR: ATG TCA ATC ACA GCA TTC ATT CTA CTP: 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 CR: AAG GAA TCC TGC AAA CAG CTAP: 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 ACR: 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 GAR: 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 GAR: 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 GGAR: 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 GCR: 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 TGAR: CAA GAT GCC AGG ACC TGT ATGP: 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 TTR: 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 AR: 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.

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**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 |