Preliminary data mining of downregulated genes in lactating ewes showing trans-10 cis-12 CLA- or fish oil-induced milk fat depression

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Take home message
Comparison of genes downregulated in lactating ewes showing CLA- or fish oil-induced milk fat depression highlights the involvement of several genes related to mammary lipogenesis.

Introduction
Milk fat depression (MFD) is a phenotype shown in dairy sheep supplemented with both marine lipids (fish oil, FO) and trans-10 cis-12 CLA (an antilipogenic conjugated linoleic acid isomer). Nutrigenomic research on MFD has been mainly focused on the evaluation of candidate genes associated with lipid metabolism. However, the application of high-throughput RNA sequencing approaches to elucidate the functional regulation of complex phenotypes has proven to be very useful as this methodology evaluates all the transcripts present in the studied tissue (Suárez-Vega et al., 2017). This study focuses on common downregulated genes when the mammary gland transcriptome of sheep suffering from fish oil- or CLA-induced MFD is compared to that from control animals.

Materials & methods
We used 14 lactating Assaf ewes fed a total mixed ration without supplementation (n=4; Control animals without MFD), supplemented with 2.4% FO (n=4; FO-MFD) or supplemented with 1% of rumen-protected trans-10 cis-12 CLA (n=6; CLA-MFD). Compared to the Control, decreases in milk fat concentration averaged 36% in FO-MFD and 30% in CLA-MFD. The protocol for milk sampling and total RNA extraction was previously described by Suárez-Vega et al. (2015). Independent RNA-Seq bioinformatics workflows were followed to obtain the differentially expressed genes between FO-MFD and Control, and between CLA-MFD and Control. Samples were aligned to the Oar_v.3.1 ovine reference genome using the Oar_v.3.1_r88 annotation with STAR v.2.4.0 (Dobin et al., 2013). The quantification step was performed with RSEM v.1.3.0 (Li and Dewey, 2011), and DESeq2 (Love et al., 2014) was used for differential expression analyses. Genes with a p-adjusted < 0.05 were considered as differentially expressed (DEG). In order to elucidate key genes controlling the MFD phenotype, we looked for common downregulated DEGs identified in the two comparisons: FO-MFD vs. Control, and CLA-MFD vs. Control.

Results & discussion
A total of 237 differentially expressed genes (80 of them downregulated) were found between FO-MFD and Control, whereas 1,528 DEGs (271 of them downregulated) were detected between CLA-MFD and Control. The transcriptomes of the MFD ewes fed diets containing either FO or trans-10 cis-12 CLA had only 20 downregulated genes in common. After the functional enrichment analysis, all DEGs were clustered in fifteen GO terms. The highest enriched terms were “acyl-CoA metabolic process” (FDR=8.40E-03) and “acid-thiol ligase activity” (FDR=2.79E-05), in the GO-Biological Process and GO-Molecular Function categories, respectively. Among the common downregulated genes, we found genes associated with the activation of acetoacetate to acetoacetyl-CoA (AACS), activation with CoA (ACSS2, ACSS3), de novo synthesis (ACACA), and desaturation (FADS2) of fatty acids, which suggests that the reduction of milk fat content was mediated by their downregulation. Some genes related to cholesterol synthesis (MVD, LSS) were also downregulated.

Conclusion
Fish oil- and trans-10 cis-12 CLA-induced MFD in lactating sheep is underpinning by the downregulation of genes mainly related with mammary lipogenesis.

Acknowledgements
This work was supported by the Spanish Ministry of Economy and Competitiveness (MINECO; AGL2014-54587-R and AGL2015-66035-R). P.G. Toral and B. Gutiérrez-Gil benefit from Ramón y Cajal research contracts. Co-funding by the European Regional Development Fund is also acknowledged.

References
Li B and Dewey CN 2011. BMC Bioinformatics 12, 323.
Proceedings
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2018

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ISSN 2040-4700
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Editors
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**Preliminary data mining of downregulated genes in lactating ewes showing trans-10 cis-12 CLA- or fish oil-induced milk fat depression**

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**Take home message** - Comparison of milk somatic cell transcriptome in lactating ewes showing CLA- or fish oil-induced milk fat depression highlights the downregulation of several genes related to mammary lipogenesis.

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**INTRODUCTION**

Milk fat depression (MFD) is a phenotype shown in dairy sheep supplemented with both marine lipids (fish oil, FO) and trans-10 cis-12 CLA (an anti-lipogenic conjugated linoleic acid isomer). Nutrigenomic research on MFD has been mainly focused on the evaluation of candidate genes associated with lipid metabolism. However, the application of high-throughput RNA sequencing approaches to elucidate the functional regulation of complex phenotypes has proven to be very useful as this methodology evaluates all the transcripts present in the studied tissue.

This study compares the mammary gland transcriptome of sheep suffering MFD (induced by either fish oil or CLA) to that of control animals, and focuses on genes downregulated in both MFD conditions.

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**MATERIAL AND METHODS**

Experimental animals
14 lactating Assaf ewes
38 days on experimental diets

Experimental treatments (forage:concentrate ratio 40:60)
- Control (n=4)
- FO-MFD (n=4) supplemented with 2.4% of fish oil (FO)
- CLA-MFD (n=6) supplemented with 1% of rumen-protected CLA (rich in trans-10 cis-12 CLA)

Sampling and RNA extraction (from milk somatic cells)

RNA sequencing and bioinformatic workflow
- Illumina Hi-Seq 2000 sequencer
  - Minimum depth of 30 million reads
  - Stranded paired-end reads of 75 bp
- 1. Alignment and quantification
  - Oar_v.3.1 ovine reference genome (Oar_v.3.1_r88)
  - STAR v.2.4.0
  - RSEM v.1.3.0
- 2. Differential expression analysis
  - DESeq2
- 3. Functional annotation
  - WebGestaltR: Gene Ontology (GO)

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**RESULTS**

Comparison of transcriptome profiles

- High level of dispersion for CLA-MFD

Differentially expressed genes (DEG)

- Control vs. FO-MFD
  - 237 DEGs (80 downregulated)

- Control vs. CLA-MFD
  - 1,528 DEGs (271 downregulated)

  - 20 downregulated in common

**Genes related with lipid metabolism**
- activation of acetoacetate to acetoacetyl-CoA (AACS)
- fatty acid activation (ACSS2, ACSS3)
- de novo synthesis (ACACA)
- desaturation of fatty acids (FADS2)

**Genes related with cholesterol synthesis**
(MVD, LSS)

**Gene functional classification**

All DEGs were clustered in 15 GO terms

- Highest enriched terms:
  - acyl-CoA metabolic process
    (FDR=8.40E-03)
  - acid-thiol ligase activity
    (FDR=2.79E-05)

**CONCLUSION**

Fish oil- and trans-10 cis-12 CLA-induced MFD in lactating sheep is underpinning by the downregulation of genes mainly related with mammary lipogenesis.