# The effect of feeding level on genes related to lipid metabolism in the mammary tissue of sheep

**E. Tsiplakou[1], E. Flemetakis[2] and G. Zervas[1]**

[1]Department of Nutritional Physiology and Feeding, [2]Department of Agricultural Biotechnology, Agricultural University of Athens, Iera Odos 75, 118 55 Athens, Greece.

- Lactation is a highly demanding process for lipid synthesis.

- The mammary epithelium cells are endowed with an enormous capacity to synthesize and secrete fatty acids (FA) with the involvement of many enzymes, encoded from the respective genes

- More specifically, the acetyl-Co A carboxylase (**ACC**) and the fatty acid synthatase (**FAS**) are involved in the metabolic pathways for the *de novo* FA synthesis in the mammary gland, whereas the lipoprotein lipase (**LPL**) is responsible for the FA uptake from the plasma.

- These FA could be desaturated by the stearoyl-Co A desaturase (**SCD**), resulting in synthesis of *cis-9* unsaturated FA

- In addition, several transcription factors, such as sterol regulatory element binding protein-1 (**SREBP-1**) and peroxisome proliferator activated receptor γ (**PPARγ)**, are also associated with the milk FA synthesis

- More specifically, the isoform c of SREBP-1 (SREBP-1c) gene is involved in triglycerides synthesis while the isoform $\gamma_2$ of PPARγ (PPAR$\gamma_2$) alter lipogenic genes networks in goat mammary epithelial cells

- Further to that, new data also confirm the involvement of hormone sensitive lipase (**HSL**) gene on lipid and energy metabolism in mammary epithelial cells

- However, despite these genes involvement on milk FA metabolism, little is known concerning the nutritional regulation of these genes in the mammary gland.

- Until now, the majority of the nutritional studies have been done in lactating cows and have been focused mainly on a few main lipogenic genes

- Taking into account that small ruminants, particularly in the Mediterranean basin, exhibit sequences of feed shortage (under-feeding) or surplus (over-feeding) due to a number of reasons such as climatic conditions, seasonality of vegetation growth etc

# The objective of this study was:

to determine the effects of long term under- and over- feeding on the expression of genes (ACC, FAS, LPL, SCD, PPAR$\gamma_2$, SREBP-1c and HSL) related to fatty acids metabolism in sheep mammary tissue (MT)

# Experimental Design

- ✓ Twenty four Friesian cross-bred dairy sheep were used for the experiment
- ✓ Three months post partum the sheep were divided into three homogenous sub-groups (n=8) balanced by body weight and milk yield
- ✓ Each group were fed the same ration, but in quantities which covered 70% (under-feeding), 100% (control) and 130% (over-feeding) of their respective energy and crude protein requirements
- ✓ The quantities of food offered to the three groups were adjusted at the 0, 12, 24, 31 and 52 experimental day according to their requirements based on their body weight and milk yield

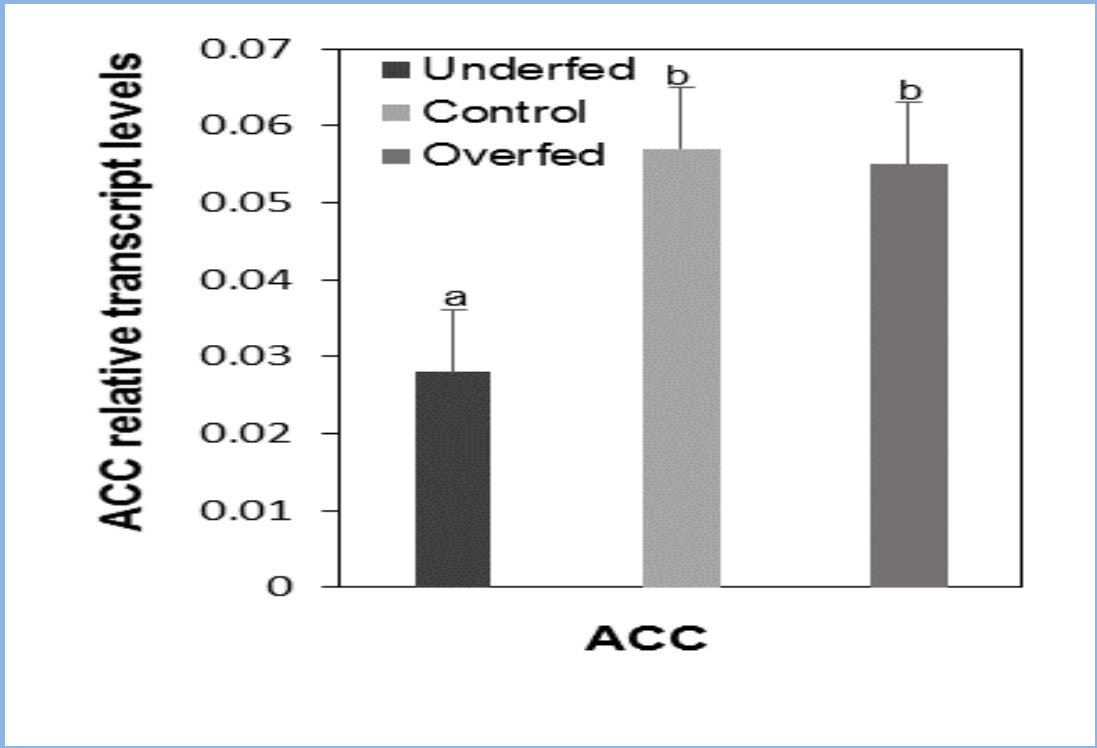
**Table 1** Average daily feed intake (kg/sheep) by sheep under the three dietary treatments throughout the experimental period

| | | ***Experimental day*** | | | | | |
|---|---|---|---|---|---|---|---|
| | ***Feedstuff*** | **0** | **12** | **24** | **31** | **39** | **52** |
| 70% | Alfalfa hay | 0.33 | 0.29 | 0.25 | 0.25 | 0.23 | 0.21 |
| | Concentrate | 0.33 | 0.29 | 0.25 | 0.25 | 0.23 | 0.21 |
| 100% | Alfalfa hay | 0.46 | 0.48 | 0.43 | 0.42 | 0.39 | 0.36 |
| | Concentrate | 0.46 | 0.48 | 0.43 | 0.42 | 0.39 | 0.36 |
| 130% | Alfalfa hay | 0.59 | 0.66 | 0.61 | 0.63 | 0.61 | 0.59 |
| | Concentrate | 0.59 | 0.66 | 0.61 | 0.63 | 0.61 | 0.59 |

- Mammary tissue samples were taken at the $30^{th}$ and $60^{th}$ day from the beginning of the experiment by biopsy after the morning milking

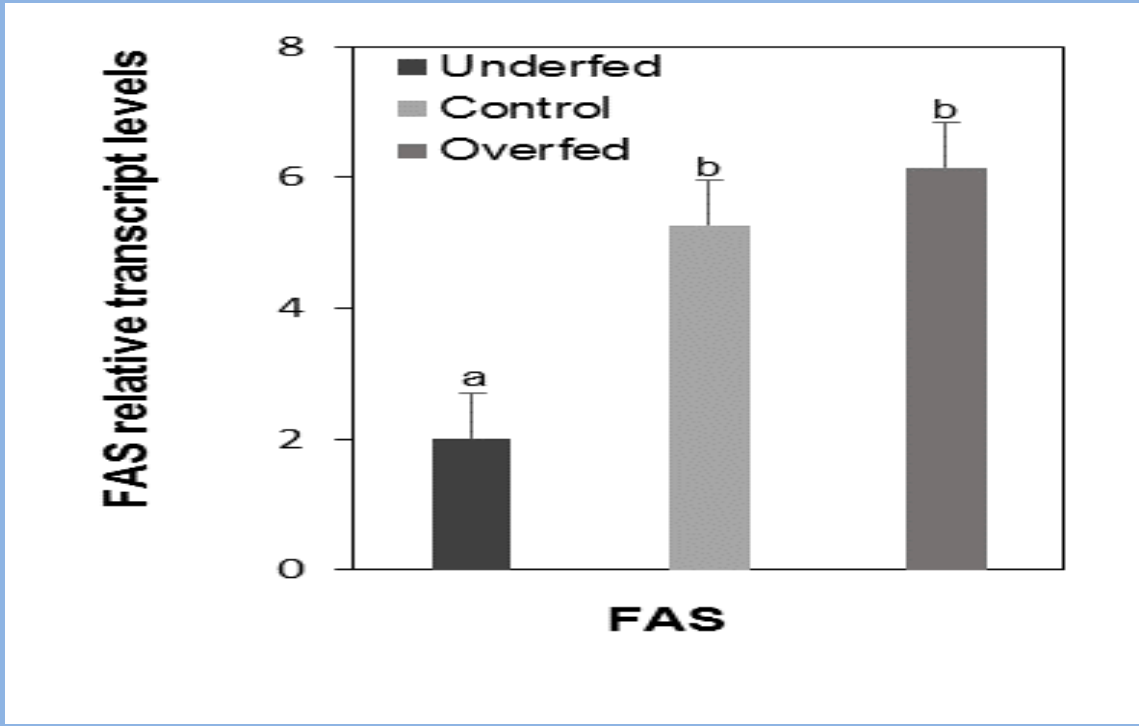
- The experimental data were analysed using the SPSS statistical package with a general linear model (GLM) for repeated measures analysis of variance with dietary treatments (T) and sampling time (S) as fixed effects according to the model: $Y_{ijk}=\mu+T_i+S_j+(TxS)_{ij}+e_{ijk}$

**Figure 1.** Relative transcript accumulation of Acetyl-Co A Carboxylase (ACC) in the mammary tissue of sheep under the three dietary treatments. Bars show mean ±SEM of both experimental days 30 and 60.



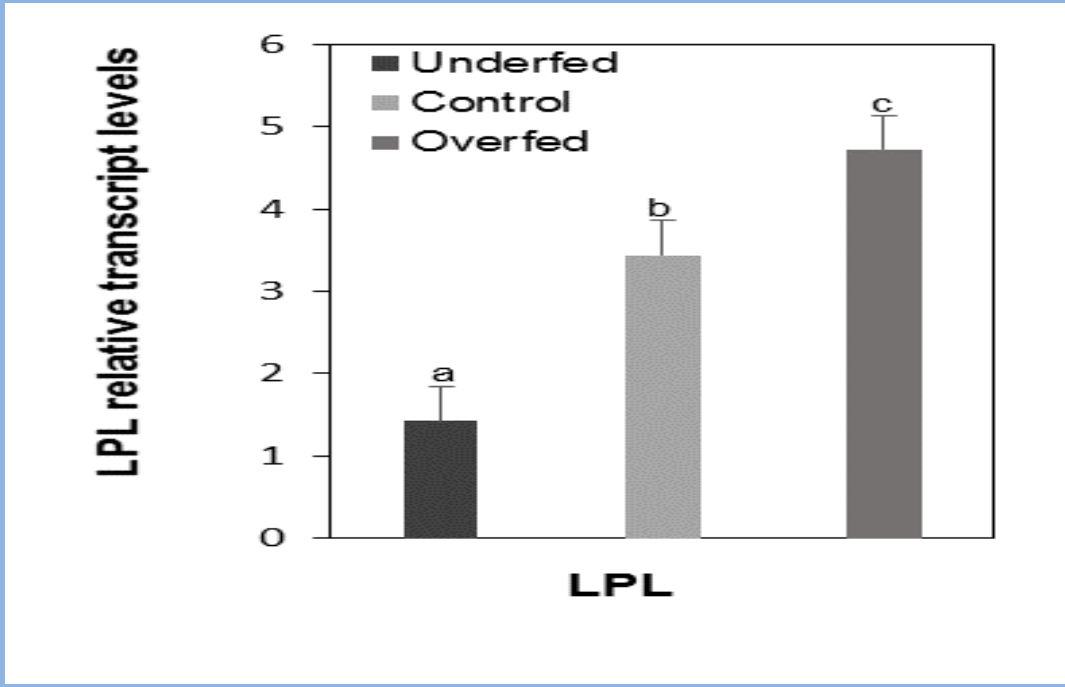

*Superscripts with out a common letter (a, b) between the three dietary treatments differ significantly (P≤0.05).*

**Figure 2.** Relative transcript accumulation of Fatty Acid Synthase (FAS) in the mammary tissue of sheep under the three dietary treatments. Bars show mean ±SEM of both experimental days 30 and 60.



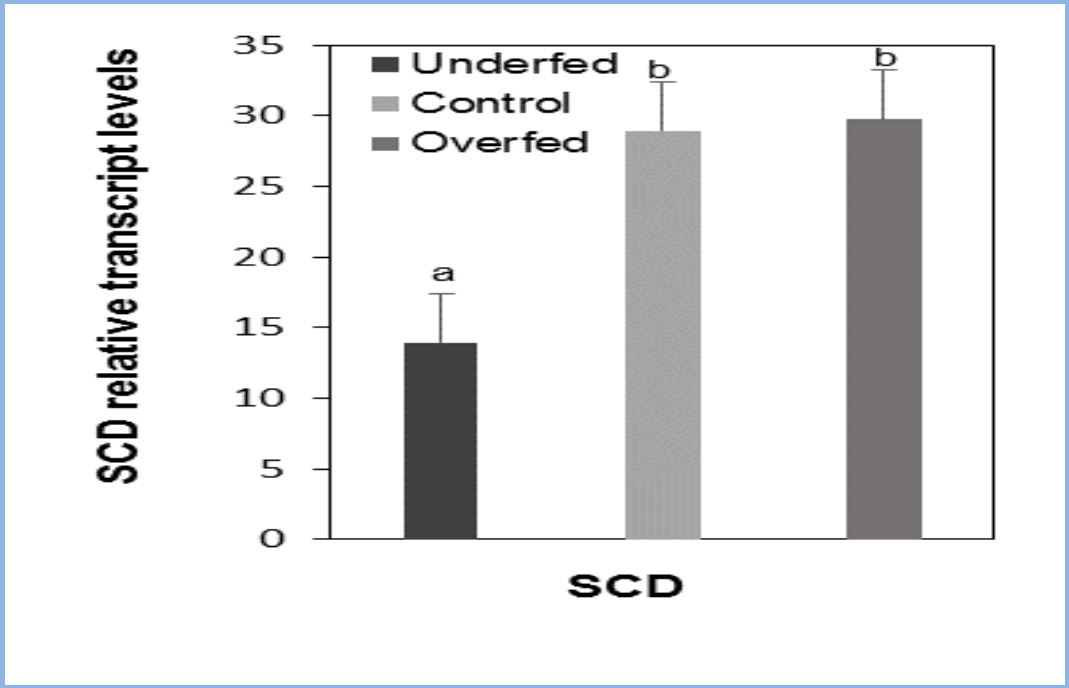

*Superscripts with out a common letter (a, b, c) between the three dietary treatments differ significantly (P≤0.05).*

**Figure 3.** Relative transcript accumulation of Lipoprotein Lipase (LPL) in the mammary tissue of sheep under the three dietary treatments. Bars show mean ±SEM of both experimental days 30 and 60.



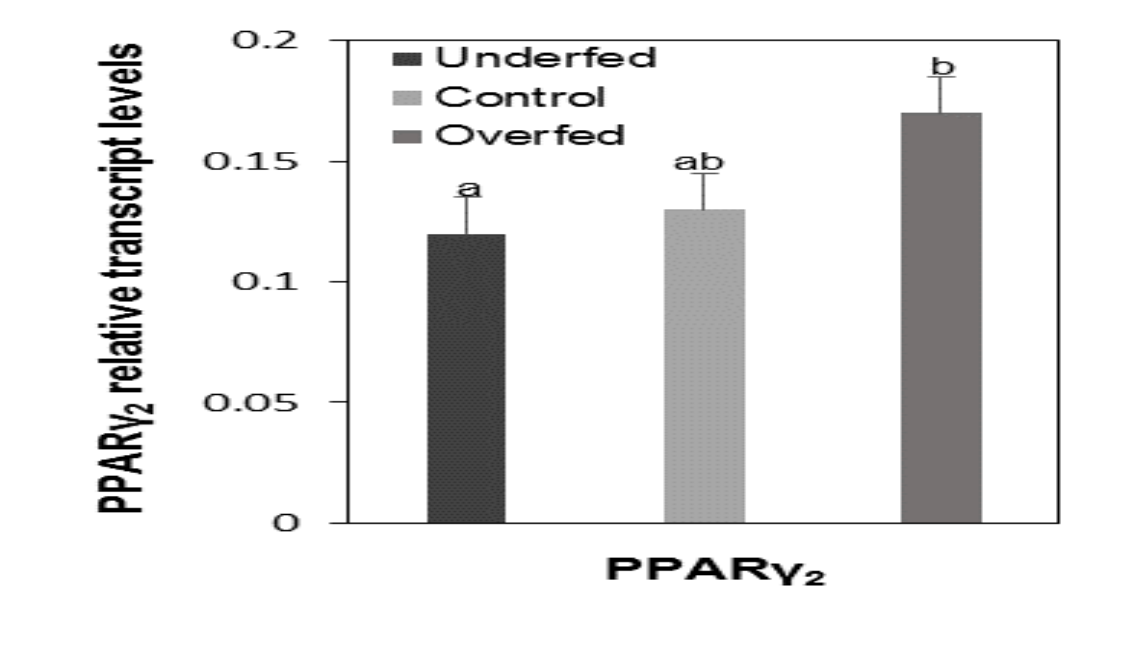

*Superscripts with out a common letter (a, b, c) between the three dietary treatments differ significantly (P≤0.05).*

**Figure 4.** Relative transcript accumulation of Steroyl-CoA Desaturase (SCD) in the mammary tissue of sheep under the three dietary treatments. Bars show mean ±SEM of both experimental days 30 and 60.



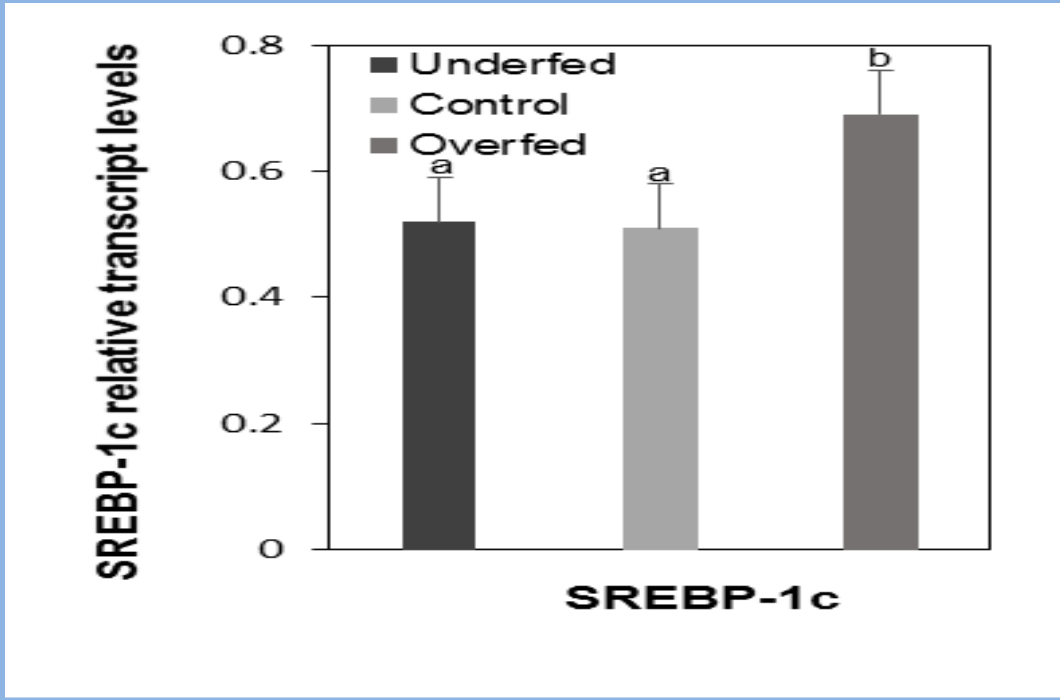

*Superscripts with out a common letter (a, b) between the three dietary treatments differ significantly (P≤0.05).*

**Figure 5.** Relative transcript accumulation of Peroxisome Proliferator Activated Receptor $\gamma_2$ (PPAR $\gamma_2$) in the mammary tissue of sheep under the three dietary treatments. Bars show mean ±SEM of both experimental days 30 and 60.



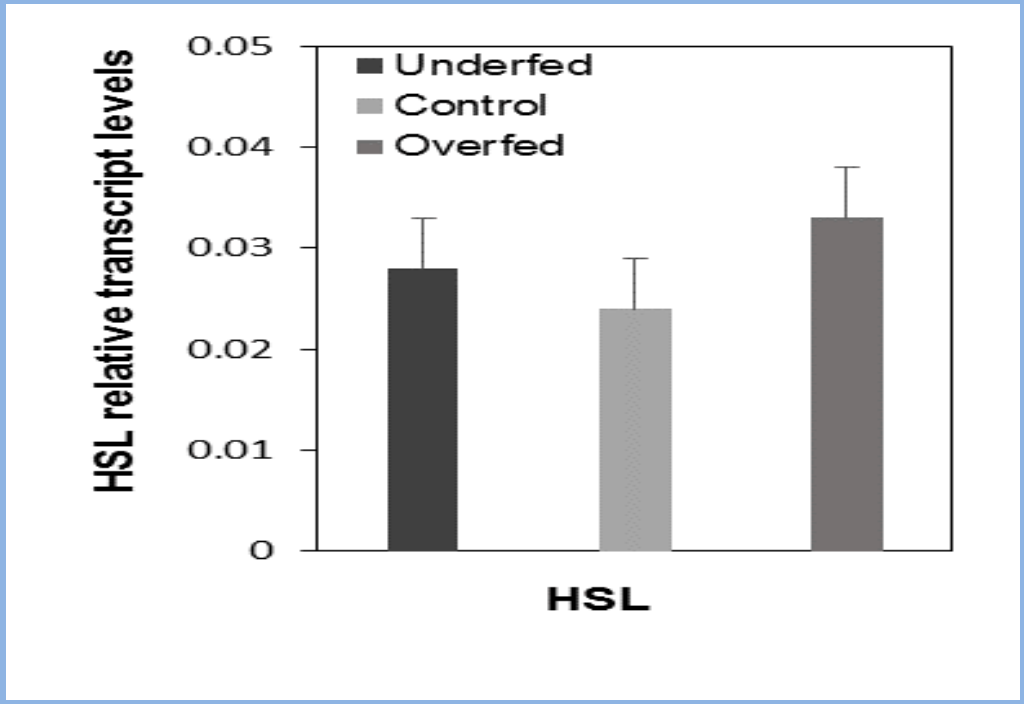

*Superscripts with out a common letter (a, b) between the three dietary treatments differ significantly (P≤0.05).*

**Figure 6** Relative transcript accumulation of Sterol Regulatory Binding Protein 1c (SREBP-1c) in the mammary tissue of sheep under the three dietary treatments. Bars show mean ±SEM of both experimental days 30 and 60.




*Superscripts with out a common letter (a, b, c) between the three dietary treatments differ significantly (P≤0.05).*

**Figure 7.** Relative transcript accumulation of Hormone Sensitive Lipase (HSL) in the mammary tissue of sheep under the three dietary treatments. Bars show mean ±SEM of both experimental days 30 and 60.




*Superscripts with out a common letter (a, b, c) between the three dietary treatments differ significantly (P≤0.05).*

**Table 1.** The mean relative transcript accumulation of genes in sheep mammary gland at the two sampling times (30th and 60th experimental day), the main effects (treatment-sampling time) and their interaction.

| | Sampling Time (S) | | | Effects | | |
|---|---|---|---|---|---|---|
| Genes | 30th | 60th | SEM | Treatment (T) | Time (S) | TxS |
| **ACC** | 0.054[a] | 0.039[b] | 0.006 | ** | * | NS |
| **FAS** | 5.67[a] | 3.28[b] | 0.628 | *** | ** | NS |
| **LPL** | 3.87[a] | 2.52[b] | 0.383 | *** | ** | NS |
| **SCD** | 28.98[a] | 19.44[b] | 2.373 | *** | *** | * |
| **$PPAR_{\gamma 2}$** | 0.15[a] | 0.13[b] | 0.009 | * | * | NS |
| **SREBP-1c** | 0.65[a] | 0.50[b] | 0.052 | * | * | * |
| **HSL** | 0.03 | 0.03 | 0.01 | NS | NS | NS |

**P<0.05, **P<0.01, ***P<0.001*

*Means with different superscript (a,b) in each row ( between sampling time) for each gene differ significantly (P≤0.05)*

# Conclusions

- The underfeeding in comparison with the overfeeding causes a significant reduction in the mRNA levels of ACC, FAS, LPL, SCD, PPAR$\gamma_2$ and SREBP-1c in sheep MT which indicates that the decrease in nutrients availability may lead to lower rate of lipid synthesis.

- The expression of the genes with well defined role in mammary lipid metabolism, except that of HSL, followed the lactation curve pattern which proves its significant role in maintenance of milk.

- SCD was the most abundant transcript in sheep MT which proves also its pivotal role in milk fat synthesis.

- Finally, there is a positive relationship between the mRNA levels of ACC and FAS in MT of sheep and the short– and medium chain fatty acids of their milk.

Thank you
for
your attention

**Table 2.** Primers used for real-time RT-qPCR.

| Gene | Acc. No. | Forward primer | Reverse primer |
|---|---|---|---|
| [1]*ACC* | NM_001009256 | 5′-CCGAACTGCGACTCGTTAAAT-3′ | 5′-CGGAGAGTGAGCATCACTGACT-3′ |
| [2]*FAS* | AF479289 | 5′-AAGAGAAGCTGCAGGCCAGTGT-3′ | 5′-CCAATTTCCAGGAATCGACCAT-3′ |
| [3]*LPL* | NM_001009394 | 5′-TACCCTAACGGAGGCACTTTCC-3′ | 5′-TGCAATCACACGGAGAGCTTC-3′ |
| [4]*SCD* | AJ001048 | 5′-TTCTCTTTCTCCTCATTGCCCC-3′ | 5′-TCGGCTTTGGAAGCTGGAA-3′ |
| [5]*PPAR*$\gamma_2$ | NM_001100921 | 5′-GGTTGACACAGAGATGCCGTT-3′ | 5′-TAGAAAGGTCCACGGAGCTGA-3′ |
| [6]*SREBP-1c* | XM_004013336 | 5′-CGCAAAGCCATCGACTACATC-3′ | 5′-TGAGCTTCTGGTTGCTGTGCT -3′ |
| [7]*HSL* | NM_001128154 | 5′- CAAGAGCCTGAAGCTGCATGAC -3′ | 5′-AGCTCTGGCGTGTCTGTTGTGT-3′ |
| [8]*RPS*9 | XM_004015433 | 5’-TTCGAAGGTAATGCCCTGTTG-3’ | 5’-TTCATCTTGCCCTCGTCCA-3’ |
| [9]*UXT* | XM_004022128 | 5’-TCATTGAGCGACTCCAGGAAG -3’ | 5’- CAGCCCAAATCCACTTGCAT-3’ |

*[1]ACC= acetyl-CoA carboxylase*, *[2]FAS= fatty acid synthase*, *[3]LPL= lipoprotein lipase [4]SCD= stearoyl-CoA desaturase* ,*[5]PPAR*$\gamma_2$*= peroxisome proliferator activated receptor* $\gamma_2$, *[6]SREBF-1c= sterol regulatory element binding protein-1c* ,*[7]HSL= hormone sensitive lipase*, *[8]RPS9=ribosomal proteinS9*, *[9]UXT=ubiquitously expressed transcript*

**Table 3.** The concentrations OF FA groups of sheep milk at the three dietary treatments and the two sampling times

| | Dietary treatments (T) | | | | Sampling time (S) | | | Effects | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Under-feeding (70%) | Control (100%) | Over-feeding (130%) | SEM | 39 (134)* | 60 (155) | SEM | T | S | TxS |
| SCFA | 14.58[a] | 18.50[b] | 20.98[c] | 0.972 | 18.58 | 17.45 | 0.794 | 0.000 | 0.162 | 0.642 |
| MCFA | 15.11[a] | 19.78[b] | 18.86[b] | 0.776 | 18.22 | 17.61 | 0.634 | 0.000 | 0.341 | 0.242 |
| LCFA | 42.71[a] | 40.10[a] | 36.71[b] | 1.332 | 40.47 | 39.20 | 1.088 | 0.000 | 0.251 | 0.307 |
| PUFA | 4.64[ab] | 4.09[a] | 5.39[b] | 0.368 | 4.50 | 4.92 | 0.301 | 0.003 | 0.107 | 0.776 |
| MUFA | 22.96[a] | 17.53[b] | 18.08[b] | 0.991 | 18.23[a] | 20.82[b] | 0.810 | 0.000 | 0.003 | 0.980 |