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Liver gene expression in periparturient dairy goats fed diets enriched with stearate or PUFA

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Background

- Different fat sources have been shown to differentially impact adipose and liver tissue in ruminants (Thering et al., 2009).
- Studies in dairy goats characterizing lipid metabolism in body tissues and the influence of different fat sources are still scant, especially in fresh dairy goats when they are in NEB.
- In a previous experiment we showed the effectiveness of hydrogenated palm oil , mainly containing C16:0 and C18:0, in enhancing expression of PPARA and selected target genes in liver of transition dairy goats in comparison with fish oil (Agazzi et al., 2010).

Aim

The goal of the present trial was to study the expression of genes involved in lipid metabolism in liver of peripartal dairy goats fed diets supplemented with saturated (calcium-stearate-ST) or unsaturated (fish oil-FO) sources of fatty acids.

Materials & Methods

- 23 second-parity twins-diagnosed alpine dairy goats were either fed stearic acid (ST, n.7) or fish oil (FO, n.8) C (n.8)
- Diet supplemented from the last week of gestation until 21 days on milk, kids were suckling during the 21 d post partum
- 30g/head/d before and 50g/head/d supplemented fatty acids after kidding
- Liver biopsies (SCD, SREBF2, SOD, CAT, ACOX1, ACAA1, PPARA) were harvested on day -7, 7 and 21 relative to kidding date, and blood samples at -7, -2, 0, +2, +7, +14, +21 d

FO = EPA 10.4%,
DHA 7.8%
ST= C16:0 26%,
C18:0 69.4%

Materials & Methods

	Experimental Diets					
	C		FO		ST	
	Pre-kidding	Post-kidding	Pre-kidding	Post-kidding	Pre-kidding	Post - kidding
Ingredient %of DM						
Alfalfa hay	**0.0**	31.2	0.0	29.8	0.0	30.7
Hay	**62.3**	15.3	59.6	14.6	61.4	15.1
Concentrate	**31.9**	46.8	30.5	44.8	31.4	46.2
Steam-flaked corn	**5.3**	6.2	5.0	5.9	5.2	6.2
Fish oil	**0.0**	0.0	4.4	4.3	0.0	0.0
Calcium Stearate	**0.0**	0.0	0.0	0.0	2.0	1.9
$CaCO_3$	**0.5**	0.5	0.5	0.5	0.0	0.0
Composition						
DM, %	88.42	89.26	88.70	89.51	88.57	89.40
CP	12.35	17.79	11.94	17.16	12.17	17.54
EE	2.93	3.22	4.92	5.16	4.55	4.79
NDF	43.90	33.70	43.76	34.00	43.27	33.22
Ash	6.35	7.17	6.53	7.31	6.04	6.85
Ca	0.77	1.09	0.82	1.13	0.89	1.20
P	0.39	0.81	0.38	0.78	0.39	0.80
NE_L (Mcal/d)	1.61	1.67	1.66	1.72	1.67	1.72

Fatty acids profile of the three experimental diets (g/100g of FAMEs)

Fatty acids	Dry off diet			Lactation diet		
	C	FO	ST	C	FO	ST
C12:0	0.17	0.14	0.15	0.06	0.07	0.08
C14:0	0.48	2.98	1.08	0.28	2.69	0.98
C16:0	18.69	17.41	21.36	16.55	16.21	20.12
C16:1	0.42	3.85	0.27	0.27	3.54	0.17
C18:0	4.92	4.36	28.46	3.40	3.49	28.36
C18:1n9	17.86	17.23	11.34	24.41	21.19	15.18
C18:2n6	37.98	24.26	24.12	46.34	30.16	28.82
C18:3n3	15.14	9.36	9.62	5.52	3.98	3.43
C20:5n3	0.07	**4.43**	0.04	0.09	**4.16**	0.05
C22:6n3	0.00	**3.29**	0.00	0.00	**3.07**	0.00

Materials & Methods

- LBW, DMI were assessed weekly
- Milk production and milk composition were assessed weekly by separating the kids from the mothers for two consecutive milkings, starting from the night before.
- Cholesterol, NEFA, BOHB content in blood were measured (ILAB 300plus, Instrumentation Laboratory, Milan; Randox, UK)
- Biopsied tissues were snap frozen in liquid nitrogen
- RNA was extracted by Qiagen miRNA kit
- Quality assessed by Bioanalyzer, Agilent, only samples with RIN>5 were used

Materials and Methods

- Primers were designed using Primer express 2.0
- Quantitative PCR using SYBR green
- Relative expression using a 6-point standard curve
- Normalization with 3 internal control genes (ICG):

  RPS9, RPS15A and UXT (Bionaz and Loor, 2007)

- Data normalized using log transformation
- Data were analyzed by a MIXED repeated model in SAS 9.2

Results

C=Control, no added fat
FO=Fish Oil
ST=Calcium Stearate

		C	FO	ST
Milk (kg/d)	7	2.85	3.52	3.36
	14	3.52	4.24	3.79
	21	3.66	4.21	3.88
Mik fat (g/100 ml)	7	4.84	4.50	4.51
	14	3.05	3.59	3.51
	21	3.40	3.51	2.93
Milk protein (g/100 ml)	7	4.17	4.19	4.36
	14	3.59	3.68	3.66
	21	3.41	3.31	3.61
Milk SCC (log)	7	5.49	5.46	5.39
	14	5.66	5.52	5.62
	21	5.74	5.62	5.48

Energy balance (Mcal/d)			
	C	FO	ST
7	0.34[a]	-0.91[b]	-1.12[b]
14	-0.2	-0.05	0.46
21	-0.16	0.89	0.82

Differently from dairy cows inclusion of fish oil does not determine a reduction of milk fat (MFD) (Agazzi et al., 2010; Chilliard et al., 2014)

A,B P<0.01
a,b P<0.05
(a),(b) P<0.1

Results

Plasma cholesterol (mg/100 ml)			
DIM	C	FO	ST
-7	53.34	54.09	63.84
-2	54.63	51.87	56.71
0	47.17	50.56	51.55
+2	49.98	51.82	55.55
+7	82.44 ^{Aa}	64.57^{b}	58.43^{B}
+14	64.94$^{(b)}$	77.43$^{(a)}$	64.77 $^{(b)}$
+21	62.04^{B}	87.20 $^{A(a)}$	71.21 $^{(b)}$

A decrease of plasma cholesterol at kidding (Agazzi et al. 2010, Bronzo et al., 2010) could be related to a low export rate from the liver or to an increased transfer into milk (Kessler et al, 2014)

C=Control, no added fat
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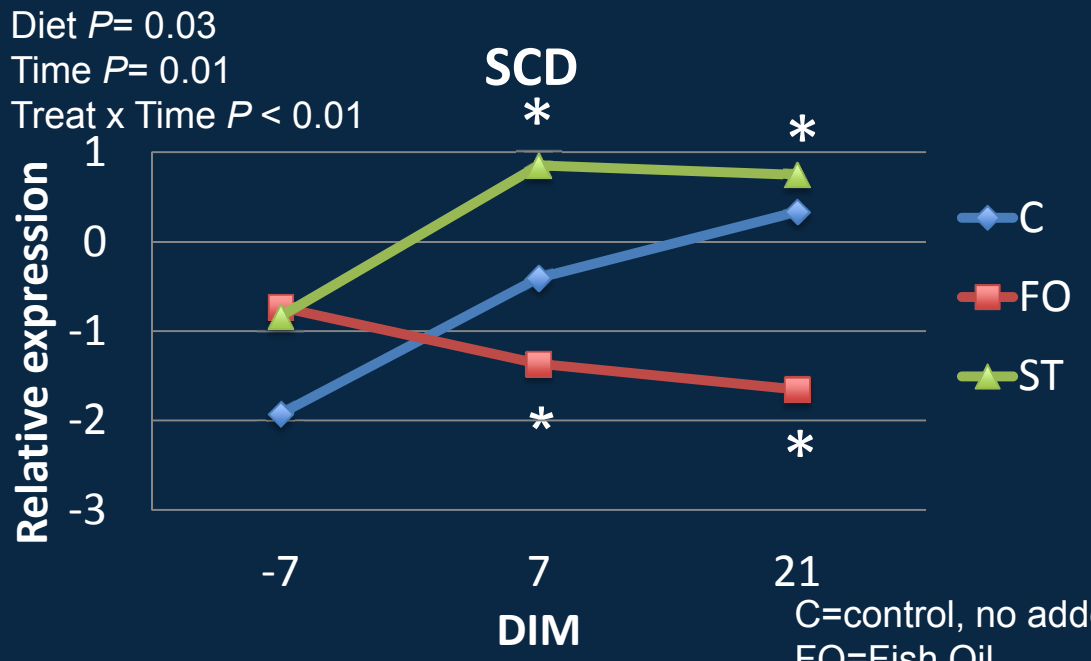
Plasma NEFA (mmol/l)			
DIM	C	FO	ST
-7	0.54 ^{a}	0.28 ^{b}	0.31 ^{ab}
-2	0.46 ^{a}	0.25 ^{b}	0.20 ^{b}
0	0.73	0.57	0.73
+2	0.38 ^{b}	0.25 ^{ab}	0.66 ^{a}
+7	0.28 ^{B}	0.50 ^{Aa}	0.36 ^{b}
+14	0.24 ^{b}	0.66 ^{a}	0.33 ^{ab}
+21	0.35	0.30	0.28

Plasma BOHB (mmol/l)			
DIM	C	FO	ST
-7	0.34	0.26	0.33
-2	0.39	0.25	0.29
0	0.60	0.33	0.36
+2	0.60	0.48	0.67
+7	0.64	0.59	0.76
+14	0.51	0.50	0.55
+21	0.49	0.37	0.64

A,B P<0.01
a,b P<0.05
(a),(b) P<0.1

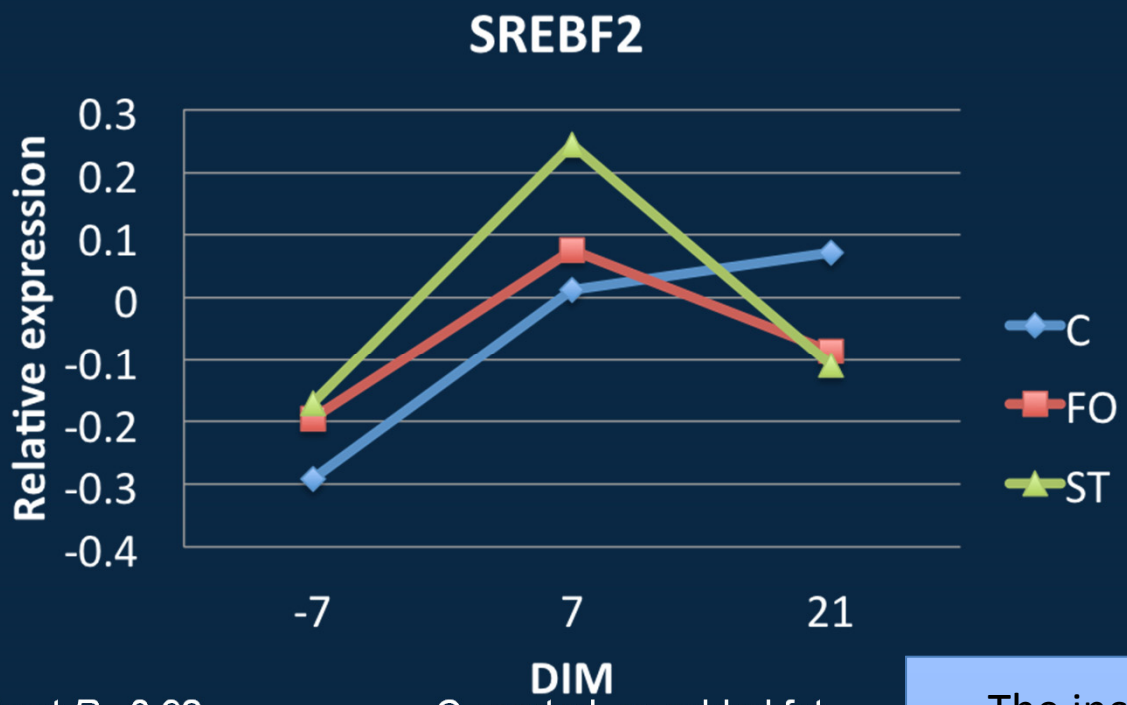


Stearoyl-CoA desaturase (SCD) is a key enzyme in FA metabolism, catalyzes a rate limiting step in the synthesis of unsaturated FA, the main product of SCD is oleic acid that is formed by desaturation of stearic acid

C=control, no added fat
FO=Fish Oil
ST=Calcium Stearate

Toral et al. (2013) in late lactating dairy goats in positive energy balance, observed that FO reduced the mRNA abundance of SCD1.

SCD activity is required for efficient cholesterol esterification, as MUFA are essential for lipid esterification. Loss of SCD activity leads to an increase of free cholesterol (Paton and Ntambi, 2010)



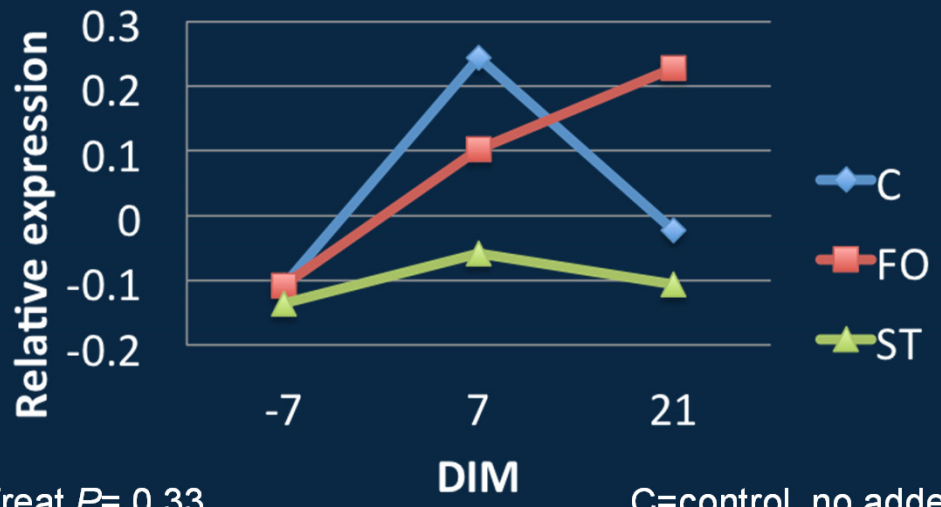
Sterol regulatory element – binding factor 2 (SREBF2) regulates the transcription of sterol-regulated genes, is required for cholesterol biosynthesis and for uptake and FA biosynthesis

Treat *P*= 0.92
Time *P*= 0.06
Treat x Time *P*= 0.73

C=control, no added fat
FO=Fish Oil
ST=Calcium Stearate

The increase in SREBF2 mRNA from pregnancy to early post partum is consistent with results of Kessler et al. (2014) in dairy cows, and can be linked to the need of increasing cholesterol availability to export liver TG, even if cholesterol plasma level is decreased from late lactation to kidding

SOD



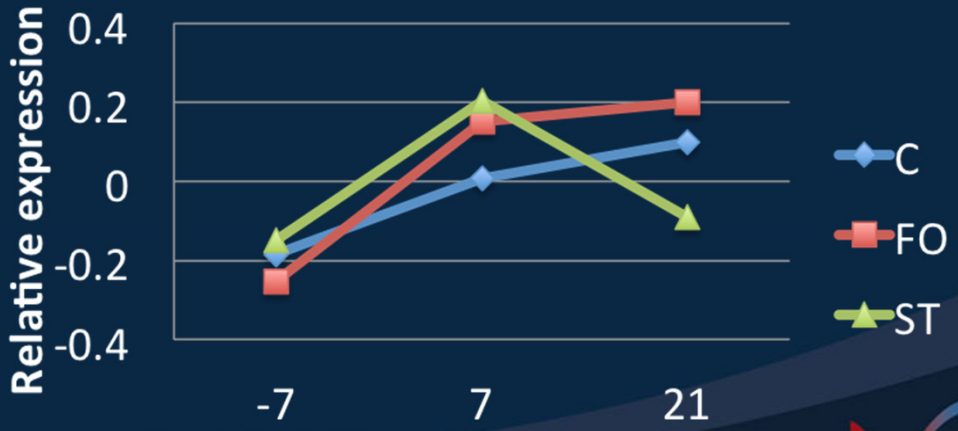
Treat *P*= 0.33
Time *P*= 0.12
Treat x Time *P*= 0.5

C=control, no added fat
FO=Fish Oil
ST=Calcium Stearate

Different fat sources do not affect the oxidative status, at least as measured as mRNA expression of super oxide dismutase (SOD), that catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide and catalase (CAT), that catalyzes the decomposition of hydrogen peroxide to water and oxygen

We did not observe any effect of diet (FO and ST) for serum levels of MDA and 8-oxodGuo (Bellagamba et al., 2012)

CAT



Treat *P*= 0.92
Time *P*= 0.08
Treat x Time *P*= 0.74

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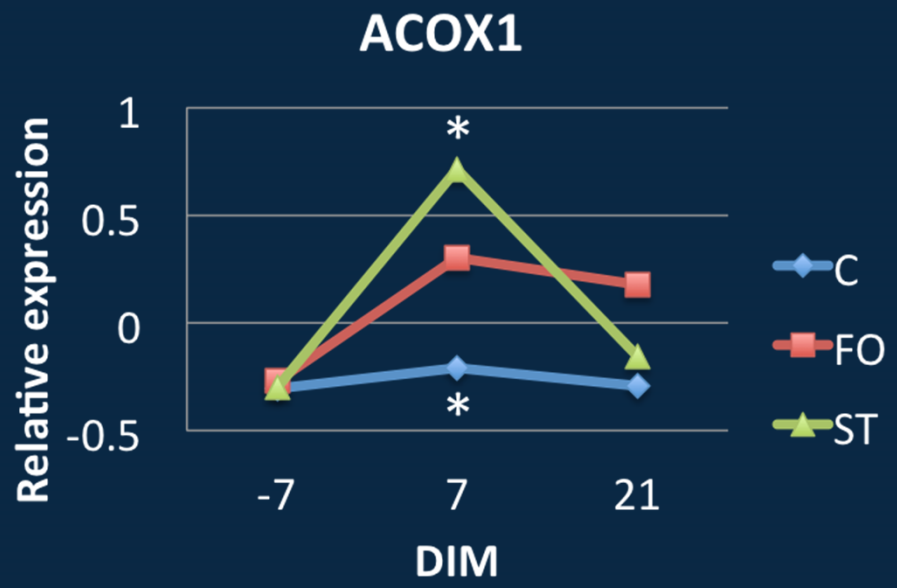
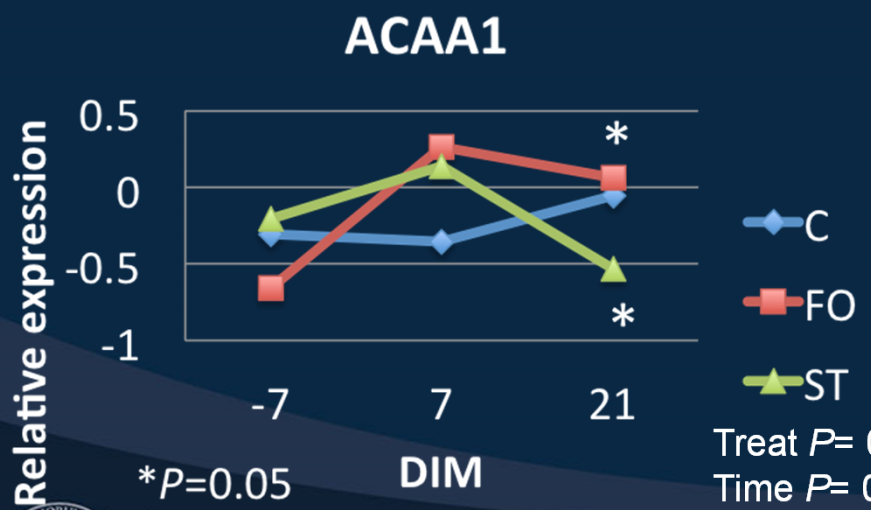


Treat *P*= 0.11
Time *P*< 0.01
Treat x Time *P*= 0.03

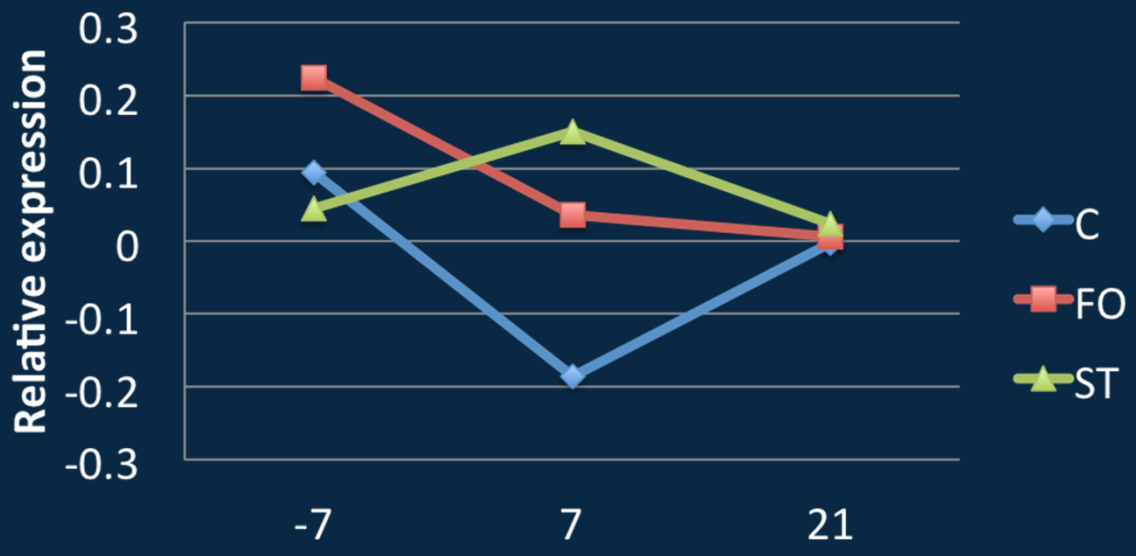
C=control, no added fat
FO=Fish Oil
ST=Calcium Stearate

Genes that encode for enzymes operative in the beta oxidation system of peroxisomes – acyl-coenzyme A oxidase 1 (ACOX1) and acetyl coenzyme A acyltransferase1 (ACAA1) followed a similar pattern at days -7 and + 7. At day 21 FO had higher expression values of ACAA1 compared to ST



*P=0.05

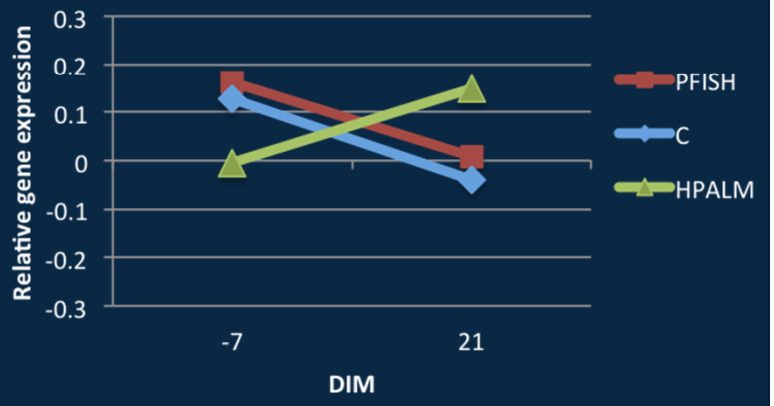
Treat *P*= 0.85
Time *P*= 0.06
Treat x Time *P*= 0.02



Different fat sources did not affect the expression of PPARA, in accordance with Toral et al, (2013)

Treat *P*= 0.68
Time *P*= 0.63
Treat x Time *P*= 0.77

C=control, no added fat
FO=Fish Oil
ST=Calcium Stearate



In a previous experiment we observed the same pattern for PPARA expression when early lactation goats were fed fish oil and an up-regulation when fed hydrogenated palm oil (Agazzi et al., 2010)

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Conclusions

- Fish oil fed to dairy goats did not decrease milk fat content

LIVER

- Feeding high saturated (ST) or unsaturated (FO) fat to dairy goats did not affect mRNA associated genes with regulation of lipogenesis (PPARA, SREBF2)
- Genes associated with FA oxidation (ACOX1, ACAA1) had an expression pattern supporting different lipomobilization
- Feeding FO and ST did not affect mRNA expression of genes involved in oxidation (SOD and CAT), moreover, in another experiment, we did not observe an effect of FO and ST on serum levels of MDA and 8-oxodGuo
- The n-3 omega FA of FO decreased the mRNA expression of SCD, suggesting that liver could be a site of delta 9 desaturation in goats (Vhamani et al., 2014, Toral et al.2013)