

Modulation of adipose tissue gene expression and fatty acid composition by dietary fat in pigs

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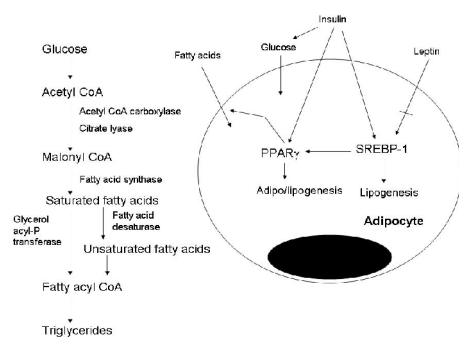
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Introduction

- Fatty acid synthesis regulated by hormones as well as dietary fat
- Fatty acid synthase (FAS) and stearoyl-coenzyme desaturase (SCD) form key enzymes determine fatty acid composition
- Desaturation forms the first regulatory step in formation of long chain of unsaturated fatty acids
- Lipogenesis-highly responsive to changes in the diet (Madsen *et al.*, 1992) .

Fatty acid synthesis in adipocyte



Objectives

- To study the effect of dietary fat on adipose tissue gene expression
- To study the effect of dietary fat on serum lipid profile
- To study the effect of dietary fat on subcutaneous adipose tissue fatty acid composition

Materials and methods

- Eighteen crossbred (Hampshire x Assam local) male grower pigs (n=6)
- Fed with isocaloric ration containing either sunflower oil or coconut oil.
- Oil contributed 10% of the total feed energy.
- The control group on standard farm ration.
- The experimental feeding continued for two months.

Materials and methods (contd.)

- **Blood was collected and body weight was measured at ten day intervals to assess the following serum parameters**
 - Glucose
 - Cholesterol
 - Total triglycerides
 - High density lipoprotein, low density lipoprotein, very low density lipoprotein

Materials and methods (contd.)

- At the beginning and end of the study the subcutaneous adipose tissue to study gene expressions following RNA isolation, reverse transcription and real time polymerase chain reaction using gene specific primers.
- Enzymes (fatty acid synthase, Stearoyl CoA desaturase)
- Transcription factors (SREBP-1c, CEBP α , PPAR γ)
- A part of adipose tissue collected at the time of slaughter analyzed for fatty acid composition using a gas chromatograph.

Materials and methods (contd.)

- Forty cycles of amplifications with the following thermal cycling conditions: initial denaturation-94°C for 6 minutes, cycling- 94°C for 40sec, 59°C for 60sec and 72°C for 40 sec and final extension for 6 minutes at 72°C except for SCD, where annealing temperature was kept at 56°C.
- The relative quantification of target genes expression were calculated using $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001).
- All gene expression values normalized to 18S and control values taken arbitrarily as 100

Materials and methods (contd.)

- Serum lipid profile, glucose analysis using standard kits
- Fatty acid composition was determined using GC using and comparing with known fatty acid methyl ester standards

Statistical analysis

- The data was analyzed using Graphpad prism software and quantification software supplied along with PCR machine. All data are presented as means $\pm$ SEM.

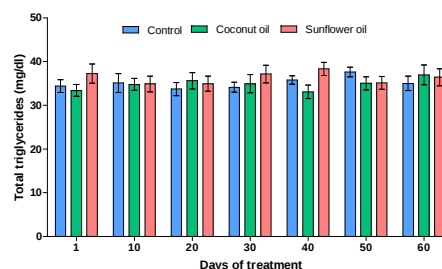
Results

Average daily gain (kg) of pigs fed with oil (Mean $\pm$ SE)

Treatment	Period of treatment (days)					
	10	20	30	40	50	60
Sunflower oil	0.28 $\pm$ 0.03	0.29 $\pm$ 0.05	0.32 $\pm$ 0.04	0.31 $\pm$ 0.02	0.34 $\pm$ 0.02	0.39 $\pm$ 0.03
Coconut oil	0.29 $\pm$ 0.03	0.31 $\pm$ 0.02	0.31 $\pm$ 0.03	0.34 $\pm$ 0.03	0.37 $\pm$ 0.03	0.40 $\pm$ 0.02
Control	0.26 $\pm$ 0.03	0.29 $\pm$ 0.02	0.29 $\pm$ 0.05	0.33 $\pm$ 0.03	0.36 $\pm$ 0.03	0.38 $\pm$ 0.02

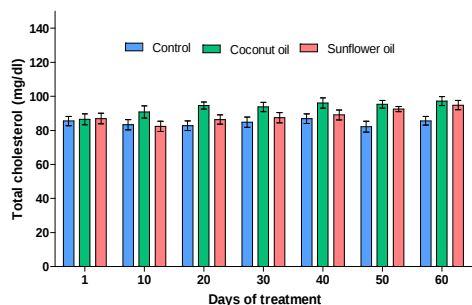
All values within same column does not differ significantly ($p < 0.05$)

Change in serum total triglycerides following oil feeding



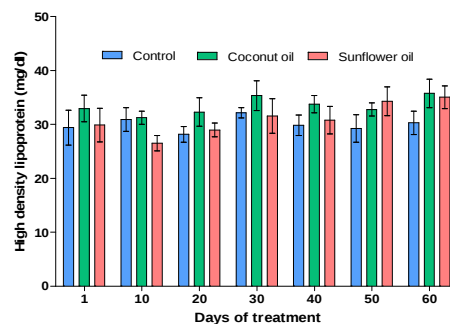
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Change in serum total cholesterol following oil feeding



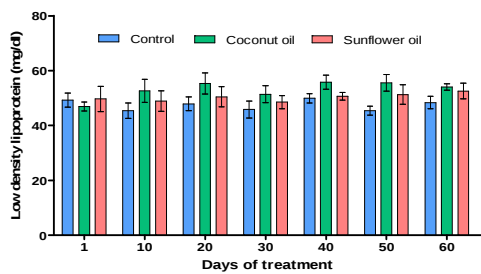
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Change in serum total HDL following oil feeding



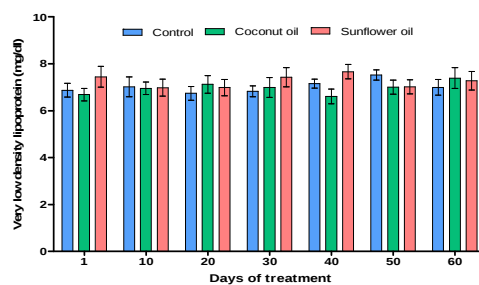
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Change in serum LDL following oil feeding



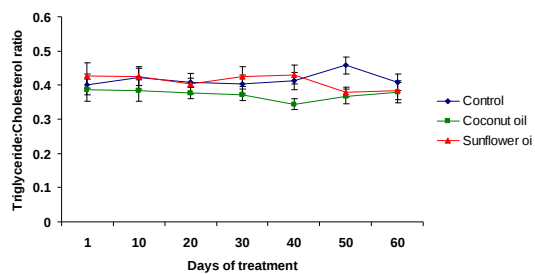
The values between groups did not differ significantly ($P>0.05$)

Change in serum VLDL



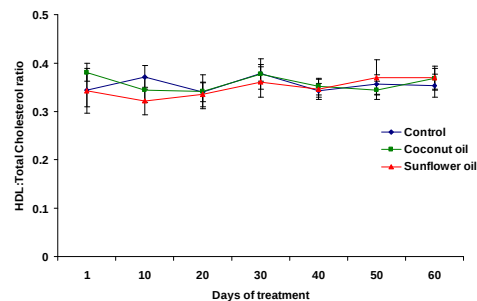
The values between groups did not differ significantly ($P>0.05$)

Serum TG: total cholesterol ratio



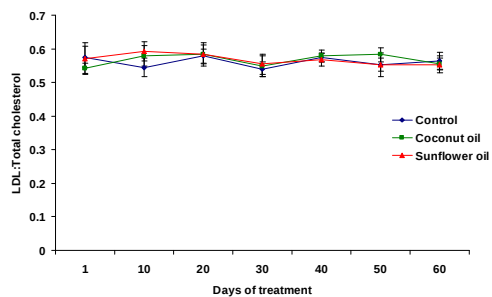
The values between groups did not differ significantly ($P>0.05$)

Serum HDL: total cholesterol ratio



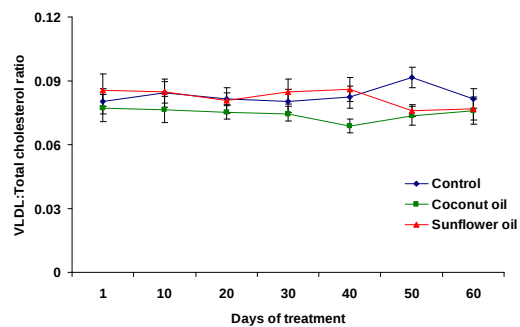
The values between groups did not differ significantly ($P>0.05$)

Serum LDL: total cholesterol ratio



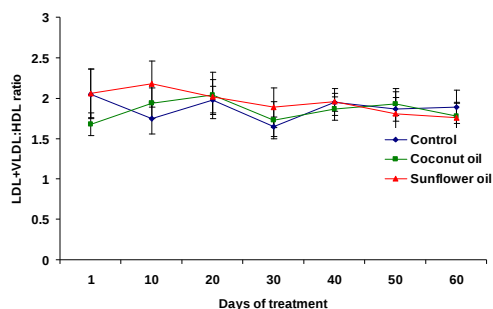
The values between groups did not differ significantly ($P>0.05$)

Serum VLDL: total cholesterol ratio



The values between groups did not differ significantly ($P>0.05$)

Serum VLDL+LDL: HDL ratio



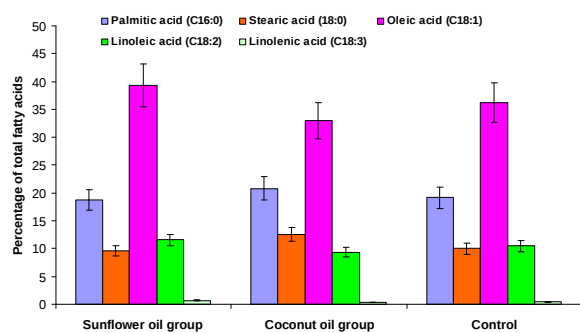
The values between groups did not differ significantly ($P>0.05$)

Subcutaneous adipose tissue gene expression

	FAS	SCD	SREBP-1c	PPAR γ	CEBP β
Coconut oil	0.84 $\pm 0.08^a$	1.08 $\pm 0.09^a$	0.99 $\pm 0.06^a$	1.02 $\pm 0.06^a$	0.95 $\pm 0.06^a$
Sunflower oil	0.39 $\pm 0.06^b$	0.51 $\pm 0.04^b$	0.47 $\pm 0.03^b$	1.18 $\pm 0.09^b$	0.96 $\pm 0.09^a$
Control	1.03 $\pm 0.14^a$	1.02 $\pm 0.05^a$	1.05 $\pm 0.08^a$	1.01 $\pm 0.08^a$	1.09 $\pm 0.08^a$

*The values with same superscript within a column did not differ significantly ($P<0.01$)

Change in fatty acid composition



Change in FA profile following oil feeding (%)

Fatty acid	Sunflower oil group	Coconut oil group
C16:0	-1.88	8.52
C18:0	-4.86	24.48
C18:1	8.52	-9.07
C18:2	10.622	-10.62
C18:3	54.7619	-19.05

Conclusions

- Replacement of 10% feed energy by oil had no significant effect on body weight gain, serum glucose, cholesterol, triglycerides and cholesterol fractions, HDL, LDL and VLDL
- Feeding of coconut oil and sunflower oil caused a shift in subcutaneous adipose tissue fatty acid composition towards saturation and unsaturation respectively
- The feeding of sunflower oil decreased FAS, SCD, SREBP-1c and CEBP β gene expression but increased PPAR γ which suggests that sunflower/PUFA tend to decrease lipogenesis as well as adipogenesis.
- Feeding of coconut oil, does not change expression of lipogenic genes but decreased CEBP β expression, suggesting that saturated fat may decrease adipogenesis

Future suggestions

- Approaches for understanding regulation of genes by nutrients-nutrigenomics/functional genomics
- Identification of pathways of gene regulation by nutrients
- Molecular regulation of FAS and SCD has implication on manipulation of carcass composition of animals
- Understanding mechanisms in obesity, diabetes, insulin resistance, atherosclerosis etc.
- Role in cellular signaling

