ROLE OF LIPID SUPPLEMENTATION ON NEONATAL GROWTH IN PIGS

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ABSTRACT

Medium-chain fatty acids (MCFA) are used in infant formula to improve fat absorption and provide energy. We previously reported that dietary MCFA causes steatosis with concomitant changes in fatty acid metabolic enzymes in neonatal pigs. The aim of this project was to investigate whether changes fatty acid metabolic enzymes precedes steatosis in response to MCFA feeding. Pigs (*n*=18) were fed isocaloric formulas containing either MCFA or LCFA. Pigs were sacrificed at 7, 14 or 21 days. Hepatic steatosis was evident in pigs fed MCFA throughout the study compared with LCFA fed counterparts. mRNA expression of fatty acid binding protein 1 (FABP-1) was greater for MCFA fed pigs compared with those in the LCFA group. mRNA expression of carnitine palmitoyltransferase I (CPT1), long-chain acyl-CoA dehydrogenase (LCAD) and peroxisome proliferator activated receptor (PPARα) were greater in livers of pigs fed MCFA compared with those fed LCFA. The expression of ACACA and FASN were higher in MCFA group. Pigs fed MCFA had greater hepatic laurate and myristate compared to LCFA fed pigs, however palmitate levels were comparable between the two groups. These data suggest that although expression of fatty acid metabolic enzymes coincides with steatosis as early as 7 days the magnitude of these changes are greater after prolonged exposure to dietary MCFA.

INTRODUCTION

- Infant formulas are administered when breastfeeding is not possible
- Lipids are used in infant formulas as a dense energy source
- Lipids account for 45-55 % energy in those formulas
- Medium-chain fatty aids (MCFA) are frequently used due to their high digestibility and bioavailability, and improved weight gains compared with long-chain fatty acids (LCFA)
- The benefits of MCFA are attributed to their faster absorption rates compared with LCFA
- Evidence suggest that MCFA may cause hepatic steatosis, but it is unclear what physiological changes in the liver lead to MCFA accumulation

OBJECTIVES

The objectives of this study were to determine:

a. The development and progression of hepatic steatosis in neonatal pigs fed MCFA or LCFA rich formulas

b. The temporal changes in mRNA expression of genes involved in fatty acid metabolism

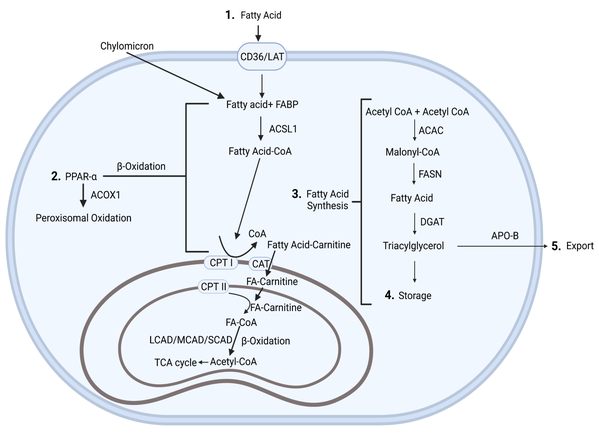
MATERIALS AND METHODS

- *Animals and sampling*
- 36 neonatal pigs (males and females), fed either a MCFA or LCFA rich formulas
- On days 7, day 14 and day 21 liver samples were collected for histology or snap frozen for molecular work
- *Analyses*
- mRNA expression of genes measured by RTqPCR and relative abundance was quantified using the $\Delta\Delta C_T$
- Fat was solvent extracted and quantified
- Fatty acid profile in the liver was determined by methylation followed by quantification on GCMS
- *Statistical analysis*
- MIXED procedure of SAS

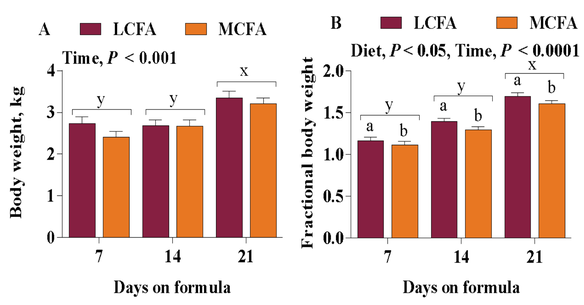
FATTY ACID PROFILE OF FED FORMULAS

Fatty Acids	LCFA		MCFA	
	mg/g	As % of Total	mg/g	As % of Total
C8:0	-	-	2.7	1.2
C10:0	0.47	0.37	9.1	4.2
C12:0	1.2	0.91	109	50
C13:0	-	-	0.08	0.04
C14:0	3.3	2.6	49	23
C16:0	30	24	21	10
C16:1	1.6	1.3		
C17:0	0.36	0.28		
C17:1	0.99	0.78		
C18:0	23	18	4.0	1.9
C18:1, n-9	55	43	17	8.1
C18:2, n-6 cis	10	8.2	3.2	1.5
C18:3, n3	0.87	0.68	0.36	0.16
C20:0	0.01	0.01	-	-
C20:1	0.46	0.36	-	-
C20:4, n6	-	-	-	-
Total Saturated	58	46	193	89
MUFA	58	45	17	8
PUFA	11	9	4	2
n-6/n-3	12	12	9	9

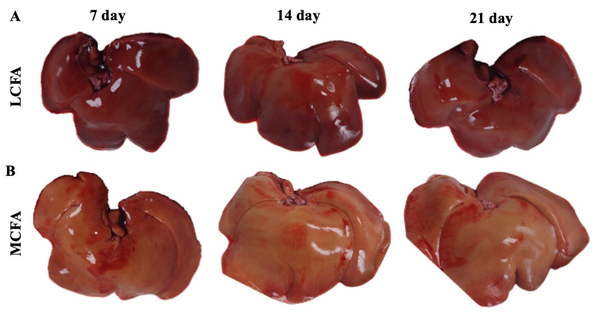
FATTY ACID METABOLISM



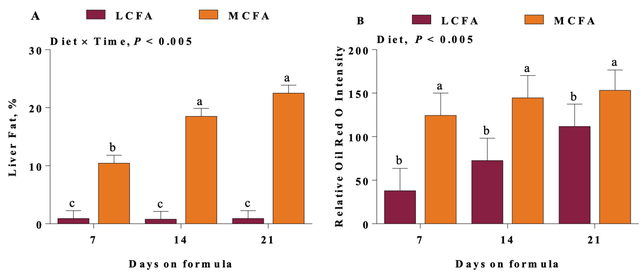
GROWTH



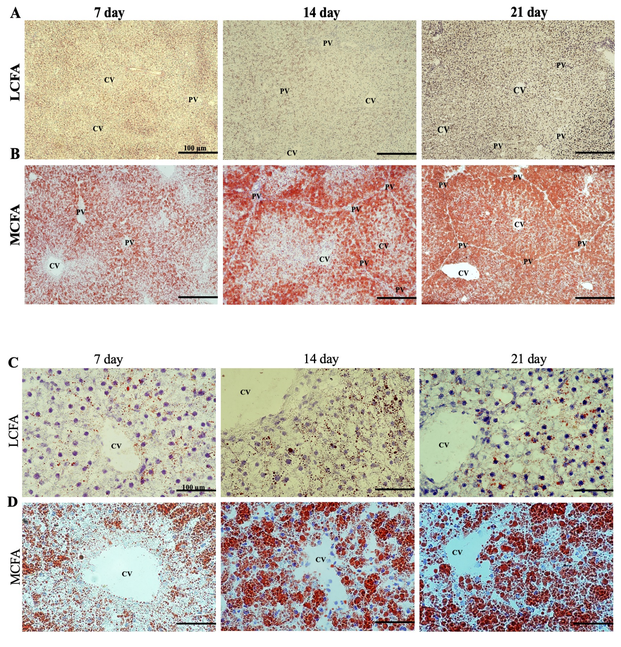
LIVER IMAGES ON 7, 14, AND 21 DAYS



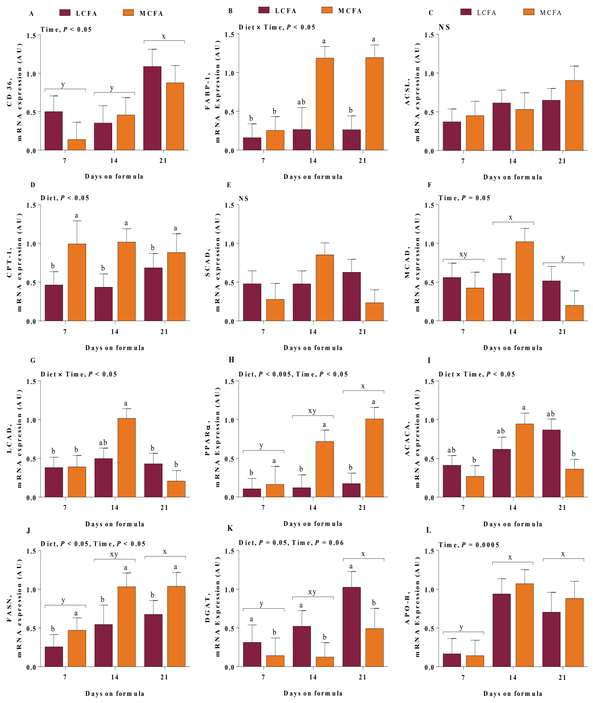
CRUDE FAT



OIL RED-O-STAINED LIVER SECTION



GENE EXPRESSION



HEPATIC FATTY ACID PROFILE

Fatty acids	LCFA			MCFA				*P - value*		
	7 days	14 days	21 days	7 days	14 days	21 days	SEM	Diet	Time	Diet x Time
C8:0	0.19		0.19	0.24	0.24	0.15	0.048	0.9	0.4	0.4
C10:0	0.60	0.57	0.65	0.80	0.85	0.57	0.165	0.4	0.9	0.6
C12:0	9.1	5.5	5.0	20	24	16	3.75	< 0.001	0.5	0.5
C14:0	13	7.3	7.9	24	30	26	4.84	< 0.001	0.9	0.5
C14:1n-5	0.20	0.33	0.24	0.24	0.23	0.28	0.047	0.9	0.4	0.3
C15:0	0.34	1.0	0.55	0.04	0.13	0.07	0.187	< 0.01	0.1	0.3
C16:0	23	23	21	23	22	27	1.12	0.2	0.4	< 0.01
C16:1n-7	1.2	0.64	0.65	1.4	1.8	2.0	0.330	< 0.01	0.9	0.2
C17:0	0.50	0.88	0.35	0.04	0.10	0.07	0.187	< 0.01	0.3	0.4
C18:0	14	15	17	8.7	5.9	8.1	2.32	< 0.001	0.6	0.6
C18:1n-9	14	18	15	11	9.0	12	1.40	< 0.0001	0.8	0.1
C18:2n-6	8	10	10	3.8	1.9	2.5	1.77	< 0.0001	1.0	0.5
C18:3n-6	5.1	5.2	6.8	2.5	1.3	1.7	1.16	< 0.001	0.7	0.6
C18:3n-3	0.24	0.57	0.60	0.11	0.08	0.05	0.152	< 0.01	0.6	0.4
C20:1n-11	0.20	0.32	0.54	0.18	0.17	0.17	0.088	< 0.05	0.2	0.2
C20:2n-6	0.80	1.03	0.95	0.12	0.10	0.05	0.098	<.0001	0.6	0.5
C 20:3n-6	0.17	0.05	0.03	0.03	0.06	0.01	0.031	0.1	0.1	0.1
C 20:3n-3	6.8	8.0	8.6	2.8	1.3	2.0	1.70	< 0.001	0.9	0.7
C 20:4n-6	2.9	2.9	4.4	1.2	0.48	0.90	0.807	< 0.01	0.5	0.6
C22:0	0.25	0.77	0.10	0.09	0.05	0.03	0.101	< 0.01	< 0.01	< 0.01
C22:6n-3	0.18	0.40	0.39	0.16	0.09	0.03	0.093	< 0.01	0.7	0.2

CONCLUSIONS

- Fractional body weight of pigs fed MCFA was less than those fed the LCFA formula
- Steatosis occurred even after 7 days of feeding in pigs fed the MCFA formula compared with their LCFA counterparts
- Although we expected to detect whether changes in fatty acid metabolic enzymes precede steatosis, differential mRNA expression of fatty acid metabolic enzymes was evident at 7 days and coincided with steatosis
- Steatosis was exacerbated following longer exposure to MCFA rich formula