A low-fat liquid diet increases protein accretion and alters cellular signaling for protein synthesis in 10-day-old pigs

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A low-fat liquid diet increases protein accretion and alters cellular signaling for protein synthesis in 10-day-old pigs[1]

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ABSTRACT: Previous research showed that neonatal pigs respond to decreases in energy density of liquid diets with increased feed intake, resulting in similar performance to pigs fed a more energy-dense diet. The objective of this experiment was to determine whether a high- (25%, HF) or low-fat (2%, LF) liquid diet would affect nutrient accretion rate and select proteins involved in energy homeostasis and protein synthesis in early weaned pigs. Ninety-six pigs, with an initial BW of 3,637 ± 85 g, were weaned from the sow at 10 d of age and utilized in a randomized complete block design. Pigs were blocked by BW and then assigned to pens (8 pigs/pen). Diets were formulated to provide a constant AA:ME ratio and were fed for 10 d. Pigs were killed at 10, 15, and 20 d of age, at which time blood and LM were collected, and carcasses were prepared for body composition analysis. Blood was analyzed for plasma urea nitrogen (PUN) and NEFA. Longissimus dorsi was analyzed via western immunoblot for mammalian target of rapamycin (mTOR) and adenosine 5′ monophosphate-activated protein kinase (AMPK) phosphorylation. Pigs gained 347 ± 11 g/d, which resulted in an ending BW of 6,858 ± 135 g, regardless of dietary treatment ($P > 0.49$). Pigs fed the LF diet consumed 25% more milk than pigs fed the HF diet (2,853 ± 86 vs. 2,269 ± 79 g dry feed·pen^{-1}·d^{-1}; $P < 0.01$), which resulted in similar calculated ME intakes between dietary treatments (9.9 ± 0.2 vs. 10.5 ± 0.2 Mcal·pen^{-1}·d^{-1}; $P > 0.5$). Feed conversion (G:F) was 24% greater in HF-fed compared with LF-fed pigs ($P < 0.01$). Circulating NEFA (40 ± 14 vs. 138 ± 21 μEq/L; $P < 0.01$) and PUN (3.0 ± 0.6 vs. 17.7 ± 0.8 m*M*; $P < 0.01$) concentrations were less in LF pigs compared with HF pigs after 10 d of dietary treatments. Pigs consuming the LF diet had a 21% increase in protein accretion (50.5 ± 2.8 vs. 61.2 ± 2.8 g/d; $P < 0.04$) and a 71% reduced lipid accretion rate (28.8 ± 2.0 vs. 8.3 ± 2.3 g/d; $P < 0.001$). Phosphorylation of AMPK was 29% less ($P < 0.03$) in LF pigs compared with HF pigs, whereas mTOR phosphorylation was increased by 37% in LF pigs ($P < 0.01$). We conclude that feeding a LF liquid diet to pigs weaned from the sow at 10 d of age increases feed intake to regulate energy intake while maintaining growth performance. In addition, 10-d-old pigs consuming a liquid LF diet have increased protein deposition by a mechanism mediated through AMPK and mTOR.

Key words: body composition, energy source, protein synthesis, swine

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INTRODUCTION

Dramatic improvements have been made in the postweaning growth performance of swine. However, attempts to improve growth rate during the nursery phase of production have largely been unsuccessful. It is well established that feeding a manufactured liquid diet to neonatal pigs leads to markedly increased growth performance (Harrell et al., 1993; Oliver et al., 2002), and the young pig, at least by 10 d of age, is capable of altering its feed intake to regulate energy intake while maintaining increased growth performance (Oliver et al., 2005). This previous work indirectly indicates that the 10-d-old pig used AA more efficiently when consuming a greater portion of its energy from carbohydrates rather than fat sources. However, it is unclear if protein accretion is affected by energy source in the young pig. Thus, one of the objectives of the current experiment was to determine whether the consumption of a large proportion of dietary energy as carbohydrate

[1]Mention of trade names, proprietary products, or specified equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may be suitable. The authors are grateful to Pat Nuss, Suzy Hassler, Bruce Larsen, and Jeff Waechter (US Meat Animal Research Center, Clay Center, NE) for their assistance in the execution of this experiment and to Donna Griess (US Meat Animal Research Center) for secretarial assistance.

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Table 1. Composition and calculated analysis of the dietary treatments[1]

Item	Diet	
	High fat	Low fat
Ingredient, %		
Nonfat dry milk[2]	45.85	41.92
High fat (80%) source[2]	30.10	1.50
Lactose[3]	0.00	40.90
Whey protein concentrate[4]	6.00	3.30
Na caseinate[3]	10.00	5.50
Arginine	0.30	0.30
Lysine	0.20	0.13
Xanthan gum	1.00	1.00
$CaCO^3$	0.53	0.37
Dicalcium phosphate	3.75	2.81
Vitamin premix[2]	0.13	0.13
Mineral premix[2]	0.50	0.50
NaCl	0.88	0.88
$MgSO_4$	0.20	0.20
KCl	0.56	0.56
Calculated analysis[5]		
ME, kcal/kg	4,804	3,464
CP, %	31.06	22.46
Fat, %	24.99	1.85
Lactose, %	24.02	62.11
Lysine, %	2.78	2.00
Ca, %	1.66	1.32
P, %	1.29	1.02
g of CP/Mcal of ME	64.6	64.8
g of Lysine/Mcal of ME	5.8	5.8
g of Lysine/100 g of CP	8.9	8.9
g of Ca/Mcal of ME	3.5	3.8
g of P/Mcal of ME	2.7	2.9
Ca/P	1.3	1.3

[1]Expressed on a DM basis.
[2]Milk Specialties Corp. (Dundee, IL).
[3]International Ingredient Co. (St. Louis, MO).
[4]Hilmar Ingredients (Hilmar, CA).
[5]Calculated analysis based on analysis provided by companies furnishing product and standard feed tables (NRC, 1998).

vs. fat would increase the accretion of lean BW gain in early weaned pigs.

In the young pig, the increased rate of skeletal muscle growth is generally regulated by an increased rate of protein synthesis, which is affected by a numerous hormones and regulatory factors (Bolster et al., 2004). Adenosine 5′-monophosphate-activated protein kinase (**AMPK**) is a regulator of energy homeostasis in many tissues, including skeletal muscle, and has been termed a nutrient sensor. Generally, activated AMPK downregulates anabolic pathways and upregulates catabolic pathways (Kola et al., 2006), which is true of muscle protein synthesis, where activated AMPK indirectly decreases the activity of mammalian target of rapamycin (**mTOR**), a master regulator of protein synthesis (Inoki et al., 2003). Thus, a second objective of the current experiment was to determine whether a large proportion of dietary energy from lipids vs. carbohydrates would affect the expression and phosphorylation of AMPK and mTOR in early weaned pigs.

MATERIALS AND METHODS

All animal procedures were reviewed and approved by the US Meat Animal Research Center Animal Care and Use Committee.

Animal Care and Dietary Treatments

Ninety-six pigs (crossbred population composed of York, maternal Landrace, paternal Landrace, and Duroc breeds; 48 barrows and 48 gilts distributed evenly across treatments) were weaned from the sow at 10 d of age and utilized in a randomized complete block design. Pigs were blocked by BW and assigned to 1 of 6 pens (8 pigs/pen). Each block was then randomly assigned to a high-fat (**HF**, 25%) or low-fat (**LF**, 2%) diet. Diets were reconstituted at 150 g/L of water (approximately 12% DM) and were formulated such that the supply of AA per unit of energy was constant (Table 1). Pigs were allowed to consume the diet ad libitum for 10 d. Pigs were in raised pens, with one-half of each pen containing an enclosed hover maintained at approximately 32°C, as described by Heo et al. (1999). Ambient temperature was maintained at approximately 24°C. Manufactured liquid diets were delivered via a gravity flow feeding system similar to that described by Oliver et al. (2005). Fresh manufactured liquid diet was added twice daily (0800 and 2000 h) to minimize spoilage and to ensure pigs had ad libitum access to the diet. All components of the feeding system were cleaned thoroughly before the first feeding (0800 h) with a liquid chlorinated detergent. The liquid diet was prepared on a daily basis and stored at 4°C.

Sample Collection and Analytical Procedures

Seven millilters of blood was collected via jugular venipuncture into heparinized (20 IU of Li-heparin/mL of blood) syringes on d 10 and 20 of age and immediately placed on ice. After collection, blood samples were centrifuged at 820 × *g* for 20 min at 4°C, with plasma collected and frozen at −20°C until further analysis. Plasma was analyzed in duplicate for urea N (**PUN**), NEFA, and glucose. Plasma urea N was measured (Marsh et al., 1965) using a Technicon Autoanalyzer System (Technicon Autoanalyzer Systems, Tarrytown, NY). Plasma glucose was measured using an immobilized enzyme system (model 2700 YSI, Yellow Springs Instruments, Yellow Springs, OH). Plasma NEFA concentrations were determined by an enzymatic colorimetric method (Wako Pure Chemical Industries Ltd., Richmond, VA). The sample mean for PUN pools was 8.1 ± 0.3 m*M*, and the intraassay CV was 3.6%. The sample mean for NEFA pools was 113 ± 11 μEQ/L, and the intraassay CV was 3.4%. The sample mean for glucose pools was 7.2 ± 0.2 m*M*, and the intra-assay CV was 2.9%.

Table 2. Primer sequences for real-time reverse transcriptase-PCR analysis[1]

mRNA	Primer	Sequence	Fragment size, bp
AMPKα1	F	5′-AAATCGGCCACTACATCCTG	187
	R	5′-GGATGCCTGAAAAGCTTGAG	
AMPKα2	F	5′-AACATGGACGGGTTGAAGAG	193
	R	5′-CGCAGAAACTCACCATCTGA	
mTOR	F	5′-GAAGAGCACGACCTGGAGAG	249
	R	5′-GTCCAGCTTCTCCCCTTTCT	
RPLP2	F	5′-GCTGCAGCAGAGGAGAAGAAAGA	102
	R	5′-TTTGCAGGGAGCAGGACTCTAGT	

[1]The PCR conditions included denaturation (95°C, 2 min) followed by 40 amplification cycles of 95°C for 15 s, 60°C for 15 s, and 70°C for 45 s. F = forward primer; R = reverse primer; AMPK = adenosine 5′-monophosphate-activated protein kinase; mTOR = mammalian target of rapamycin; RPLP2 = ribosomal protein, large, P2.

An initial group of pigs (10 d of age; n = 6 per replicate) were killed for initial body composition analysis (proximate analysis; AOAC, 1997). Dietary treatments were initiated at 10 d of age, and 12 pigs per replicate were killed at d 15 or 20 of age for body composition analysis (n = 12). A small sample of LM was snap frozen in liquid nitrogen, and the contents of the gastrointestinal tract, urinary bladder, and gall bladder were removed before the carcasses were stored at −20°C until further analysis.

Total cellular RNA was isolated from the LM using RNeasy Mini kits (Qiagen, Valencia, CA), and samples were DNase I treated on the column using DNase I provided by the manufacturer. Total RNA was quantified using a ND-1000 spectrophotometer (Nano-Drop Technologies, Wilmington, DE). Total cellular RNA samples (1 μg) were reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA). Each cDNA sample was assayed in duplicate (25 ng of tcRNA equivalents) for AMPKα1, AMPKα2, and mTOR along with 0.25 μ*M* forward and reverse primers (Table 2) and 12.5 μL of Taq, SYBR green, and ROX supermix (Bio-Rad) in a 25-μL reaction. Ribosomal protein, large, P2 (**RPLP2**) primers (Table 2) were used to measure RPLP2 mRNA concentrations, which was not different between treatments (data not shown). Therefore, RPLP2 was used as the reference control transcript to calculate relative quantities of each target transcript using the comparative CT method (Livak and Schmittgen, 2001). Amplification was performed using a PTC-200 thermocycler fitted with a chromo 4 fluorescence detector (Bio-Rad). The PCR conditions included denaturation (95°C, 2 min) followed by 40 amplification cycles of 95°C for 15 s, 60°C for 15 s, and 70°C for 45 s. Melting curve analysis and gel electrophoresis were used to confirm the amplification of a single product of the predicted size.

Muscle samples were analyzed for total and AA phosphorylation of AMPK and mTOR by western blot analysis. Muscles were powdered at liquid nitrogen temperature and a crude muscle soluble protein extract was prepared in buffer that contained 50 m*M* Tris HCl (pH 7.5); 3 m*M* EDTA; 100 m*M* NaCl; protease inhibitors (Complete Mini (1836153), 1 tab/10 mL, Roche Applied Science, Indianapolis, IN); phosphatase inhibitors [Phosphatase Inhibitor Cocktail 1 (P2850) and 2 (P5726), each at 1:100 vol:vol; Sigma-Aldrich, St. Louis, MO]; and 1% IGEPAL A-630 (USB Corp., Cleveland, OH). The protein concentrations of the extracts were determined using the bicinchoninic acid reagent (Smith et al., 1985). Soluble protein (60 μg) was resolved on 10% SDS-PAGE gels and electroblotted onto polyvinylidene fluoride membranes (Pall Co., Pensacola, FL). The membranes were probed for phosphorylated protein using specific anti-phosphoprotein antibodies (1:2,000, AMPKα- Thr^{172}, 1:2,000, mTOR- Ser^{2448}; Cell Signaling Technology, Danvers, MA). After washing in Tris-buffered saline with 0.1% Tween, the blots were re-probed for total protein with anti-AMPKα (1:2,000, Cell Signaling Technology) and anti-mTOR (1:2,000, Cell Signaling Technology).

Statistical Analyses

Data were subjected to ANOVA using the GLM procedure (Minitab Inc., State College, PA). Data were evaluated for the effects of energy source, day, sex, replication, and all appropriate interactions. No sex differences were observed; thus, sex data have been combined. Energy source and day responses were contrasted using a protected LSD test (Steel et al., 1997). For BW, ADG, blood measurements, body composition, and muscle mRNA and protein measurements, the experimental unit for all statistical procedures was the individual pig. For evaluation of the effects of energy source on ADFI, ME intake, and feed efficiency, the experimental unit was pen of pigs. The significance level for all tests was set at $P < 0.05$.

RESULTS

Pigs gained 347 ± 11 g/d, which resulted in an ending BW of 6,858 ± 135 g (Table 3), regardless of dietary treatment ($P > 0.49$). Pigs consuming the LF diet had 25% greater (2,849 ± 86 g of DM·pen^{-1}·d^{-1}; Table 2) ADFI compared with those consuming the HF

Table 3. Performance by young pigs fed a high- (25%) or low-fat (2%) manufactured liquid diet from d 10 to 20 of age[1]

Variable	Diet		*P*-value for energy source
	High fat	Low fat	
BW, g			
d 10	3,564 ± 133	3,709 ± 108	0.98
d 15	5,342 ± 174	5,305 ± 143	0.99
d 20	6,748 ± 218	6,989 ± 183	0.96
ADG, g/d			
d 10 to 15	346 ± 17	337 ± 21	0.75
d 15 to 20	327 ± 18	366 ± 16	0.17
d 10 to 20	337 ± 13	354 ± 17	0.49
ADFI, g of DM·pen^{-1}·d^{-1}			
d 10 to 15	2,011 ± 64	2,694 ± 76	<0.001
d 15 to 20	2,420 ± 48	3,042 ± 45	<0.001
d 10 to 20	2,269 ± 79	2,849 ± 86	<0.001
ME intake, Mcal·pen^{-1}·d^{-1}			
d 10 to 15	9.3 ± 0.3	9.4 ± 0.1	0.99
d 15 to 20	11.2 ± 0.2	10.7 ± 0.3	0.50
d 10 to 20	10.5 ± 0.2	9.9 ± 0.2	0.29
G:F, g/g of DM			
d 10 to 15	1.27 ± 0.02	1.00 ± 0.07	<0.01
d 15 to 20	1.33 ± 0.03	1.05 ± 0.02	<0.01
d 10 to 20	1.29 ± 0.04	1.04 ± 0.03	<0.01

[1]Values are least squares means ± SEM; for BW and ADG, n = 12 (d 20) to 24 (d 10 and 15); for ADFI, ME intake, and G:F, n = 6.

diet (2,269 ± 79 g of DM·pen^{-1}·d^{-1}; $P < 0.001$). This resulted in calculated ME intakes that did not differ between dietary treatment ($P > 0.5$). Due to the changes in feed intake along with similar rates of BW gain, G:F was increased by 24% in pigs fed the HF compared with the LF diets ($P < 0.01$).

Dietary treatment had no effect on circulating glucose concentrations (Table 4; $P > 0.79$), but circulating glucose was greater in both treatment groups at d 20, compared with d 10 ($P < 0.03$). The PUN and NEFA concentrations did not differ between dietary treatment groups at the initiation of the experiment (Table 4; $P > 0.09$). By 20 d of age, PUN concentrations increased in pigs consuming the HF diet, resulting in 81% lesser PUN concentrations (17.7 ± 0.8 vs. 3.0 ± 0.6 m*M*; $P < 0.001$) in pigs consuming the LF diet compared with pigs consuming the HF diet. At d 20 of age, NEFA concentrations were increased in pigs consuming the HF diet ($P < 0.001$), resulting in 71% lesser concentrations of circulating NEFA in pigs consuming the LF diet.

Regardless of dietary treatment, pigs had less lipid and ash and greater water, as a percentage of BW, at d 15 and 20 of age compared with the initial reference group (Table 5; $P < 0.05$). Protein percentage of both dietary treatment groups was similar to the initial group ($P > 0.23$), except at d 20 of age, when pigs consuming the LF diet had a greater proportion (16.3 vs. 14.9; $P < 0.03$) of protein in the body compared with the initial group. Pigs consuming the LF diet had similar body composition of ash and water compared with pigs consuming the HF diet ($P > 0.5$). Pigs consuming the LF diet had 8% greater protein in the body at d 20

Table 4. Effect of dietary energy source on plasma variables of young pigs fed a high- (25%) or low-fat (2%) manufactured liquid diet from d 10 to 20 of age[1]

Variable	Diet		*P*-value for energy source
	High fat	Low fat	
PUN, m*M*			
d 10	3.5 ± 0.4	2.9 ± 0.2	0.31
d 20	17.7 ± 0.8*	3.0 ± 0.6	<0.001
NEFA, μEq/L			
d 10	118 ± 27	128 ± 23	0.47
d 20	138 ± 21	40 ± 14*	<0.001
Glucose, m*M*			
d 10	6.5 ± 0.2	6.9 ± 0.2	0.79
d 20	7.5 ± 0.3*	7.7 ± 0.5*	0.99

[1]Values are least squares means ± SEM; n = 12 (d 20) to 24 (d 10). PUN = plasma urea nitrogen.
*Mean differs from d 10 within treatment ($P < 0.05$).

Table 5. Effect of dietary energy source on the composition of the empty body and tissue accretion rates of young pigs fed a high- (25%) or low-fat (2%) manufactured liquid diet from d 10 to 20 of age[1]

Treatment	Protein	Lipid	Ash	Water
Body composition, %				
Initial				
d 10	14.9 ± 0.3	12.4 ± 0.4	5.2 ± 0.19	69.2 ± 0.6
High fat				
d 15	15.2 ± 0.15	8.6 ± 0.41‡	4.6 ± 0.08‡	72.5 ± 0.39‡
d 20	15.1 ± 0.26	8.8 ± 0.28‡	4.3 ± 0.08‡	72.4 ± 0.40‡
Low fat				
d 15	15.8 ± 0.19	6.7 ± 0.26‡†	4.1 ± 0.07‡	73.8 ± 0.44‡
d 20	16.3 ± 0.26‡†	5.8 ± 0.25‡†	4.0 ± 0.07‡	74.7 ± 0.28‡
Accretion rate, g/d				
High fat				
d 10 to 15	48.5 ± 4.3	21.6 ± 3.8	9.3 ± 1.1	247.3 ± 16.2
d 15 to 20	52.8 ± 4.1	31.8 ± 4.7	12.9 ± 1.2	257.7 ± 10.0
d 10 to 20	50.5 ± 2.8	28.8 ± 2.0	11.1 ± 0.9	252.3 ± 9.4
Low fat				
d 10 to 15	57.6 ± 4.4	6.5 ± 2.1†	8.1 ± 1.4	248.3 ± 17.9
d 15 to 20	66.6 ± 4.2†	16.5 ± 3.8‡†	13.9 ± 1.2‡	385.9 ± 12.7
d 10 to 20	61.2 ± 2.8†	8.3 ± 2.3†	10.1 ± 0.9	264.7 ± 10.4

[1]Values are least squares means ± SEM; n = 10 to 12.

For body composition: ‡Mean differs from d 0 ($P < 0.05$), and †low fat differs from high fat, within day ($P < 0.05$). For accretion rates: ‡Mean differs from d 10 to 15 within treatment ($P < 0.05$), and †low fat differs from high fat, within day ($P < 0.05$).

of age ($P < 0.05$) and had 22 and 23% less lipid in the body at d 15 and 20 of age, respectively ($P < 0.05$), compared with pigs consuming the HF diet.

No differences ($P > 0.14$) were observed in ash or water accretion rates from d 10 to 15, d 15 to 20, or d 10 to 20 of age, regardless of dietary treatment (Table 5). Pigs consuming the LF diet had similar ($P > 0.19$) protein accretion rates from d 10 to 15 of age compared with pigs consuming the HF diet. However, pigs consuming the LF diet accrued 26% more protein (66.6 ± 4.2 vs. 52.8 ± 4.1 g/d; $P < 0.04$) from d 15 to 20 of age. This resulted in a 21% increase in protein accretion for pigs consuming the LF diet for the entire 10-d study ($P < 0.04$). Pigs consuming the LF diet accrued less lipid from d 10 to 15 and d 15 to 20 of age compared with pigs consuming the HF diet ($P < 0.001$), which resulted in a 71% reduction in lipid accretion rate (28.8 ± 2.0 vs. 8.3 ± 2.3 g/d; $P < 0.001$) in pigs consuming the LF diet.

No differences in mRNA abundance in LM of AMPKα1, AMPKα2, or mTOR were observed between dietary treatments ($P > 0.15$). The AMPKα1 and mTOR mRNA abundance in LM did not change over the course of the study (Figure 1; $P > 0.53$). However, AMPKα2 mRNA abundance in LM increased from d 10 to 15 and from d 15 to 20 of age, regardless of dietary treatment ($P < 0.001$). Total protein abundance in LM of AMPKα (Figure 2) and mTOR (Figure 3) did not differ between dietary treatments ($P > 0.56$). Protein phosphorylation of AMPKα in LM did not differ at d 10 of age between dietary treatment groups (Figure 2; $P > 0.71$). However, AMPKα phosphorylation in LM was decreased 26 and 29% on d 15 and 20 of age ($P < 0.02$), respectively, in pigs consuming the LF diet compared with pigs consuming the HF diet. No differences in mTOR phosphorylation in LM were observed at d 10 or 15 of age (Figure 3; $P > 0.23$). By d 20 of age, mTOR phosphorylation in LM was 37% greater ($P < 0.01$) in pigs consuming the LF diet.

DISCUSSION

In the current experiment, we confirmed that pigs weaned from the sow at 10 d of age respond to a LF diet with increased feed intake, while maintaining growth rate. Pigs consuming the LF diet in the current experiment consumed 25% more feed than pigs consuming the HF diet, which resulted in similar ME intakes between dietary treatment groups. In addition, the growth rates in the current experiment (347 ± 11 g/d) are greater than those typically reported from pigs suckling the sow (250 g/d; NRC, 1998) and are similar to previous studies utilizing manufactured liquid diets (Harrell et al., 1993; Oliver et al., 2002, 2005). Similar to our previous work (Oliver et al., 2005), feed conversion (G:F) was 24% greater in HF-fed compared with LF-fed pigs due to the differences in feed intake with no change in growth rate. These data indicate that pigs weaned from the sow at 10 d of age are capable of using fat or carbohydrate equally well as the primary energy source for growth.

Plasma urea nitrogen is an indirect measure of the extent of AA oxidation. The young growing animal is actively accreting skeletal muscle, and therefore, PUN is a relatively good measure of the oxidation of dietary AA. Similarly, circulating NEFA is an indirect measure

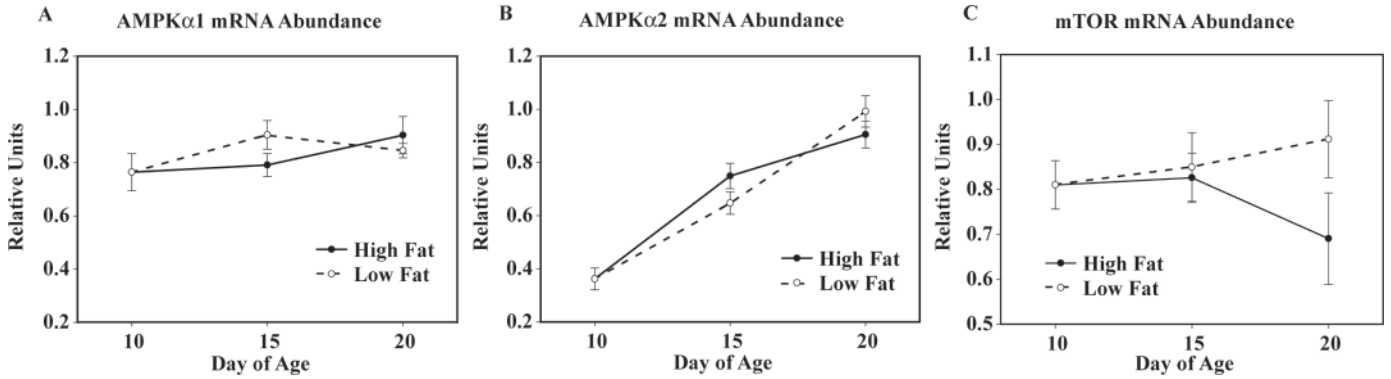

Figure 1. Effect of dietary energy source on the mRNA abundance of adenosine 5′-monophosphate-activated protein kinase (AMPK)-α1 (A), AMPKα2 (B), and mammalian target of rapamycin (mTOR; C) in young pigs fed a high- (25%) or low-fat (2%) manufactured liquid diet from d 10 to 20 of age. Values shown are means ± SEM: n = 12 (d 20) to 24 (d 10 and 15). Ribosomal protein, large, P2 mRNA abundance was used as a correction factor for variations in RNA isolation and sample aliquoting. Age effect for AMPKα2, $P < 0.001$; all other comparisons, no difference.

of lipolysis, fatty acids available for uptake into tissues, or both, and lipolysis rates should be very small in pigs weaned from the sow at 10 d of age. In a previous, similar experiment, Oliver et al. (2005) observed that pigs weaned from the sow at 10 d of age and fed a HF diet had greater PUN and NEFA compared with pigs consuming a LF diet, indicating that pigs consuming the HF diet were accruing less muscle and more lipid compared with pigs consuming the LF diet. However, the effects of energy source on body composition were not measured. In the current experiment, we observed similar changes in PUN and NEFA in response to feeding an HF and LF diet for 10 d. At the initiation of the experiment, PUN was less in both treatment groups due to the increased rate of protein synthesis in the young pig (Davis et al., 1996) and that the fact that the sow limits pig growth, presumably due to a limiting amount of milk yield (Boyd et al., 1995; Azain et al., 1996). When allowed to consume HF manufactured liquid diets ad libitum in the current study, PUN increased more than 3-fold by 20 d of age. In contrast, PUN in pigs consuming the LF diet remained decreased for the duration of the study, even with increased overall feed intake and similar intakes of AA. Similar to previous results (Gentz et al., 1970), circulating NEFA was greater in the current experiment at 10 d of age because fat provides approximately 50% of the total calories in the milk of sows (Klobasa et al., 1987). By 20 d of age, NEFA concentrations were 71% less in pigs consuming the LF, which is in agreement with our previous work (Oliver et al., 2005) after 10 d of feeding HF vs. LF diets. Therefore, it seems that pigs consuming the HF diet were accruing less muscle and more lipid compared with pigs consuming the LF diet.

In the current experiment, we measured accretion rates using the slaughter balance technique. We observed that pigs consuming a large proportion of dietary energy as carbohydrate vs. fat (LF diet) had increased protein accretion rates and a decreased rate of lipid accretion, which is in agreement with the interpretation of circulating concentrations of PUN and NEFA. Extensive research has been conducted to optimize the supply of nutrients to maximize lean tissue gain during the grow-finish phases of growth, but little

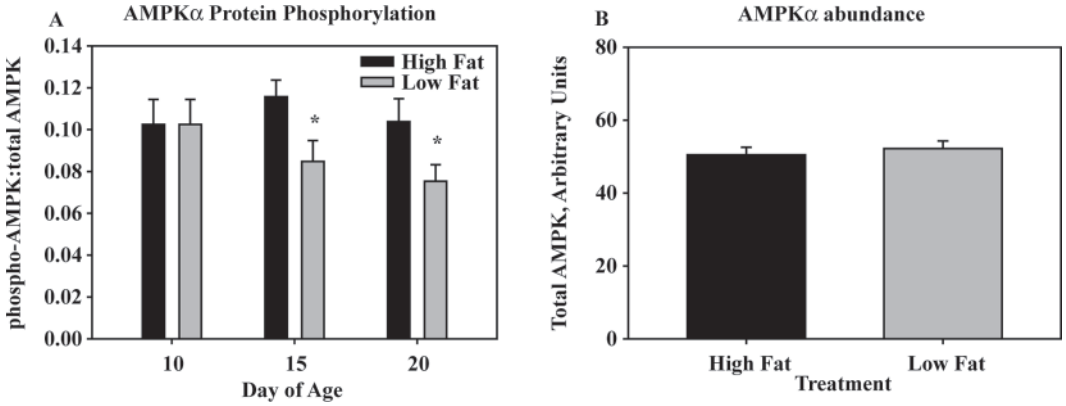

Figure 2. Threonine phosphorylation (A) and total (B) abundance of adenosine 5′-monophosphate-activated protein kinase-α (AMPKα) in young pigs fed a high- (25%) or low-fat (2%) manufactured liquid diet from d 10 to 20 of age. Phosphorylation values are expressed as the ratio of threonine phosphorylation to total AMPKα abundance. Values shown are means ± SEM: n = 12 (d 20) to 24 (d 10 and 15). *Effect of energy source, within day, $P < 0.02$. All other comparisons, no difference.

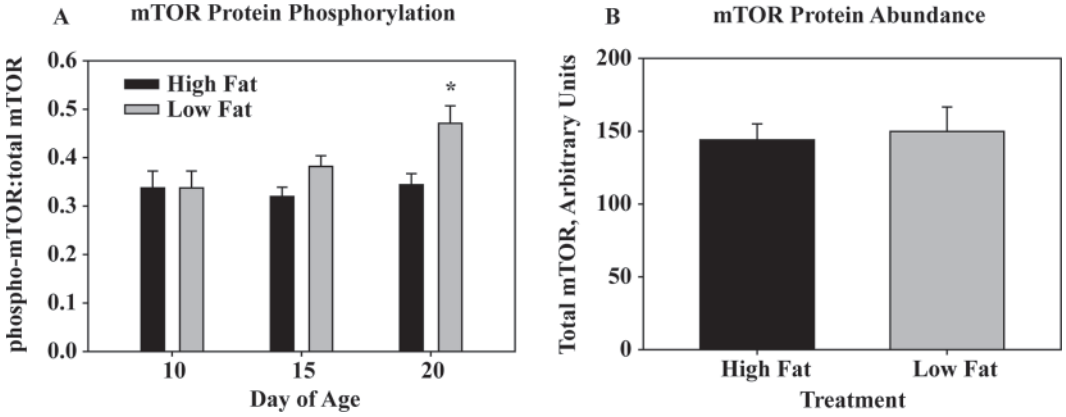

Figure 3. Serine phosphorylation (A) and total (B) abundance of mammalian target of rapamycin (mTOR) in young pigs fed a high- (25%) or low-fat (2%) manufactured liquid diet from d 10 to 20 of age. Phosphorylation values are expressed as the ratio of serine phosphorylation to total mTOR abundance. Values shown are means ± SEM: n = 12 (d 20) to 24 (d 10 and 15). *Effect of energy source, within day, $P < 0.02$. All other comparisons, no difference.

has been done in the nursing phase or with very early weaned pigs. In the growing pig, energy source affects overall lipid deposition (Mersmann et al., 1984), in that 25-kg pigs consuming a HF diet for 20 wk deposited more fat, as measured by ultrasonic backfat measurements and carcass measurements. However, Halas et al. (2010) observed no differences in fat deposition when feeding 48-kg pigs nonstarch polysaccharides, starch, or unsaturated fat. These data likely differ from Mersmann et al. (1984) due to the use of more refined ingredients in the experimental diets. The decreased rate of fat deposition in the current experiment is likely due to the greater rate of growth and protein deposition in the young pig. Protein accretion rates were 21% greater in pigs consuming the LF diet in the current study. It is well known that energy intake in growing pigs affects protein deposition (Campbell and Taverner, 1988; Bikker et al., 1995), but much less is known about the effect of energy source on protein deposition the young animal. Kashyap et al. (2001) observed that carbohydrate was more effective than fat in enhancing growth and protein accretion in enterally fed low-birth-weight infants, but they also observed increases in fat deposition, likely due to a limiting amount of AA available for protein synthesis. Thus, excess energy was likely partitioned to lipid reserves. In the current experiment, due to constant AA:ME, pigs consumed similar amounts of ME and AA, allowing for the increase in protein deposition and the decrease in lipid deposition in pigs consuming the LF diets.

Adenosine 5′-monophosphate-activated protein kinase is a regulator of energy homeostasis in many tissues, including skeletal muscle, and has been termed a nutrient sensor. Generally, activated AMPK downregulates anabolic pathways and upregulates catabolic pathways (Kola et al., 2006). This is true of muscle protein synthesis, where activated AMPK indirectly decreases the activity of mTOR (Inoki et al., 2003). Mammalian target of rapamycin is considered a master protein kinase that is regulated independently by insulin, AA, and energy sufficiency, and it contributes to the control of protein synthesis by regulating components of cellular signaling that directly affect protein synthesis (Avruch et al., 2005). In the current experiment, we found that the AMPK activation was decreased and mTOR activation was increased, as measured by AA phosphorylation, in pigs consuming the LF diet. This is consistent with our observation that protein accretion was greater in LF-fed pigs. Most of the work on dietary effects on AMPK has been conducted in longevity studies, and it appears that AMPK plays a central role in the effect of caloric restriction on longevity (Greer et al., 2007) due to the role of ATP:AMP in regulating AMPK activity (Hardie, 2007).

Although not measured in the current experiment, ATP:AMP likely contributed to the decreased AMPK activation. Liu et al. (2006) observed that rats consuming a high-fat diet had impaired RNA expression and activities of AMPK. However, that study was conducted after 5 mo of feeding a high-fat diet, and the AA consumption and growth rates are not known. In contrast, Suchankova et al. (2005) observed increased hepatic AMPK activity in rats fed PUFA, and hepatic AMPK phosphorylation was decreased in rats fed a fat-free diet. In a pancreatic-substrate clamp study, Jeyapalan et al. (2007) reported no changes in AMPK or mTOR phosphorylation in pigs chronically infused with glucose alone. Similar to the current study, mTOR phosphorylation, but not AMPK phosphorylation, was increased in pigs receiving the majority of their energy from carbohydrate, but only when given with AA and insulin, thereby simulating a complete meal. A lipid energy source was not used by Jeyapalan et al. (2007) for comparison with the carbohydrate energy source, but it is clear that an energy source, AA, and insulin are required to stimulate protein synthesis via mTOR. Adenosine 5′-monophosphate-activated protein kinase activity is regulated by diet, particularly dietary energy levels, and in the current experiment, feeding a LF diet resulted in decreased AMPK phosphorylation. Protein

synthesis signaling is regulated by AMPK by at least 2 mechanisms. First, AMPK phosphorylates tuberous sclerosis complex 2, which indirectly downregulates mTOR (Inoki et al., 2003). The second is the phosphorylation of mTOR-associated protein raptor (Gwinn et al., 2008), which decreases mTOR activity. Mammalian target of rapamycin activity in the current study was increased in pigs consuming the LF diet, which is consistent with the decreased activity of AMPK in those pigs. It is well established that branched-chain AA, particularly leucine, stimulate translation initiation through a pathway involving mTOR (Hara et al., 1998; Anthony et al., 2000), but to our knowledge, this is the first example of dietary energy source regulating the activity of mTOR in early weaned pigs.

This study confirms earlier work that the young pig responds to a decreased energy-density liquid diet with increased feed intake, without altering growth performance. In addition, circulating PUN and NEFA were decreased in LF-fed pigs, confirming our earlier results. Novel to the current experiment, we observed that pigs consuming a LF diet, compared with a HF diet, accrued more protein and less fat over the course of the study. In addition, skeletal muscle AMPK activation was decreased and mTOR activation was increased, as measured by AA phosphorylation, in pigs consuming the LF diet. We conclude that feeding a LF liquid diet to pigs weaned from the sow at 10 d of age increases feed intake to regulate energy intake and increases protein deposition by a mechanism mediated through AMPK and mTOR.

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