Effects of starch versus fiber-based supplements on adipose tissue gene expression by stocker cattle grazing dormant native range

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STORY IN BRIEF

The objective of this study was to evaluate type of energy supplement on gene expression of adipose tissue in stocker cattle. Fifty-three fall-weaned steers grazed dormant tallgrass native range from December to April. Steers were randomly assigned to 4 treatments: 1) 2.2 lb/hd/d of cottonseed meal supplement (CON; 43% CP, 1.3 Mcal of ME/lb DM), 2) CON plus ground corn supplement fed at 1% of BW (CORN; 24% CP, 1.4 Mcal of ME/lb DM), 3) CON plus soybean hull supplement fed at 1% of BW (SBH; 23% CP, 1.3 Mcal of ME/lb DM), and 4) dried distillers grains plus solubles supplement fed at 1% of BW (DDGS; 29% CP, 1.4 Mcal of ME/lb DM). Three steers from each treatment were harvested in April and samples of subcutaneous and perirenal adipose tissue were collected for analysis of mRNA expression of genes involved in triglyceride synthesis. Treatment did not affect mRNA expression of glucose transporter 4, glucose-6-phosphate isomerase, glucose-6-phosphate dehydrogenase, or ATP-citrate lyase indicating that treatment did not influence glucose utilization by subcutaneous or perirenal adipose tissue. Expression of acetyl-CoA synthetase (ACS) and fatty acid synthase (FAS) mRNA were increased in perirenal adipose tissue of supplemented steers compared with CON steers, but not in subcutaneous adipose tissue. Expression of glycerol-3-phosphate dehydrogenase (GPDH) mRNA was increased in subcutaneous and perirenal adipose tissues of supplemented steers compared with CON steers. No effect of the high-starch CORN versus the two high-fiber SBH or DDGS supplements was observed for any of the genes evaluated. In conclusion, energy supplementation increased triglyceride synthesis in subcutaneous and perirenal fat depots; however, type of energy, starch versus fiber, did not influence substrate for fatty acid synthesis.

Key Words: adipose tissue, gene expression, stocker cattle

INTRODUCTION

Quality grade and marbling score are important traits that influence the value of beef carcasses. However, the percentage of carcasses grading USDA Choice has declined 21% over the last 20 yr (Smith et al., 2006). Approximately 75% of the annual US calf crop enters a backgrounding or stocker program prior to finishing (Peel, 2003). Stocker cattle wintered on low quality forage have reduced fat deposition during this period (Hersom et al., 2004). Intramuscular adipose tissue (marbling) primarily uses glucose from starch digestion as the substrate for fatty acid synthesis, whereas subcutaneous adipose tissue (back fat) primarily uses acetate from fiber digestion (Smith and Crouse, 1984). Calves provided a starch-based creep feed had improved marbling scores compared with calves provided a fiber-based creep feed (Faulkner et al., 1994). Thus, supplementation of stocker cattle grazing low-quality dormant native range with a starch-based supplement may improve marbling fat deposition relative to back fat deposition. The objective of this study was to evaluate starch versus fiber-based supplementation of stocker cattle grazing dormant native range on expression of genes involved in glucose conversion to fatty acids.

MATERIALS AND METHODS

All experimental procedures were approved by the Oklahoma State University Institutional Animal Care and Use Committee. Fall-weaned Angus cross steers (N = 53; 592 ± 48 lb) were randomly assigned to 1 of 4 treatments: 1) 2.2 lb of cottonseed meal based supplement (**CON**; 43% CP, 1.3 Mcal of ME/lb DM), 2) CON plus ground corn-based supplement fed at 1% of BW (**CORN**; 24% CP, 1.4 Mcal of ME/lb DM), 3) CON plus soybean hull-based supplement fed at 1% of BW (**SBH**; 23% CP, 1.3 Mcal of ME/lb DM), and 4) dried distillers grains plus solubles supplement fed at 1% of BW (**DDGS**; 29% CP, 1.4 Mcal of ME/lb DM). All supplements were formulated to meet degradable intake protein requirements. Steers were fed individually once daily using feeding stanchions and supplement was prorated to provide the weekly amount in a 5 d per wk feeding schedule. All steers were allowed to graze dormant tallgrass native range as a single group from December to April. Following the winter grazing period, 3 steers from each treatment were harvested at the Oklahoma State University Food and Agricultural Products Research and Technology Center. Samples of subcutaneous and perirenal (kidney) adipose tissue were collected and frozen in liquid nitrogen, then stored at -80˚C.

To evaluate the effect of treatment on substrate utilization for fatty acid synthesis, mRNA expression of several genes within glycolysis and de novo fatty acid synthesis pathways was determined (Figure 1). A brief description of the functions of these genes is provided here, summarized from Nelson and Cox (2000). Glucose transporter-4 is a protein used to regulate glucose entry into the cell. Glucose-6- phosphate isomerase is the first enzymatic reaction in the glycolysis pathway. Glucose-6-phosphate dehydrogenase is the first enzymatic reaction in the pentose cycle. The pentose cycle uses glucose to produce NADPH, which is a necessary co-factor for the fatty acid synthase reaction. Instead of glucose being used to synthesize fatty acids directly, the cell can increase glucose flux through the pentose cycle to increase fatty acid synthesis from acetate by providing increased NADPH. Pyruvate kinase is the last reaction in glycolysis and regulates flux through this pathway. ATP-citrate lyase is the rate limiting reaction in the conversion of glucose to fatty acids in ruminant animals. Acetyl-CoA synthetase is the first enzymatic reaction in the conversion of acetate to fatty acids. Fatty acid synthase is the enzyme responsible for synthesis of fatty acids. Glycerol-3-phosphate dehydrogenase is responsible for synthesis of the glycerol backbone of triglycerides (i.e., fat). Glycerol may be synthesized from glucose, but may also be synthesized from other substrates such as amino acids.

To determine expression of mRNA, total RNA was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's procedure. The integrity of the RNA was determined using gel electrophoresis and quantified using a NanoDrop® ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Total RNA (1.0 μg) from each adipose tissue collected was reverse transcribed into cDNA using dT and random primers with a QuantiTect® reverse transcription kit (Qiagen Inc., Valencia, CA) at 42˚C for 45 min. Quantitative RT-PCR was utilized to determine the expression of genes previously identified as important to triglyceride synthesis: glucose transporter-4 (**GLUT-4**), fatty acid synthase (**FAS**), cytosolic acetyl-CoA synthetase (**ACS**), ATP-citrate lyase (**ACLYS**), glycerol-3-phosphate dehydrogenase (**GPDH**), glucose-6-phosphate dehydrogenase (**G6PDH**), pyruvate kinase (**PK**), and glucose-6-phosphate isomerase (**GPI**). In addition, two genes that promote the progression of adipocyte differentiation (Hausman et al., 2009) were evaluated: sterol regulatory element

binding protein-1c (**SREBP**) and CCAAT/enhancer binding protein beta (**C/EBPβ**). Primers for these genes were designed using Primer3 software package. Real-time PCR reactions contained 4.5 µl of 2X iQ™ SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA), 400 nM forward primer, 400 nM reverse primer, and 100-200 ng of cDNA and were carried out using a MyiQ Real Time PCR detection system (Bio-Rad Laboratories, Hercules, CA). Thermal cycling

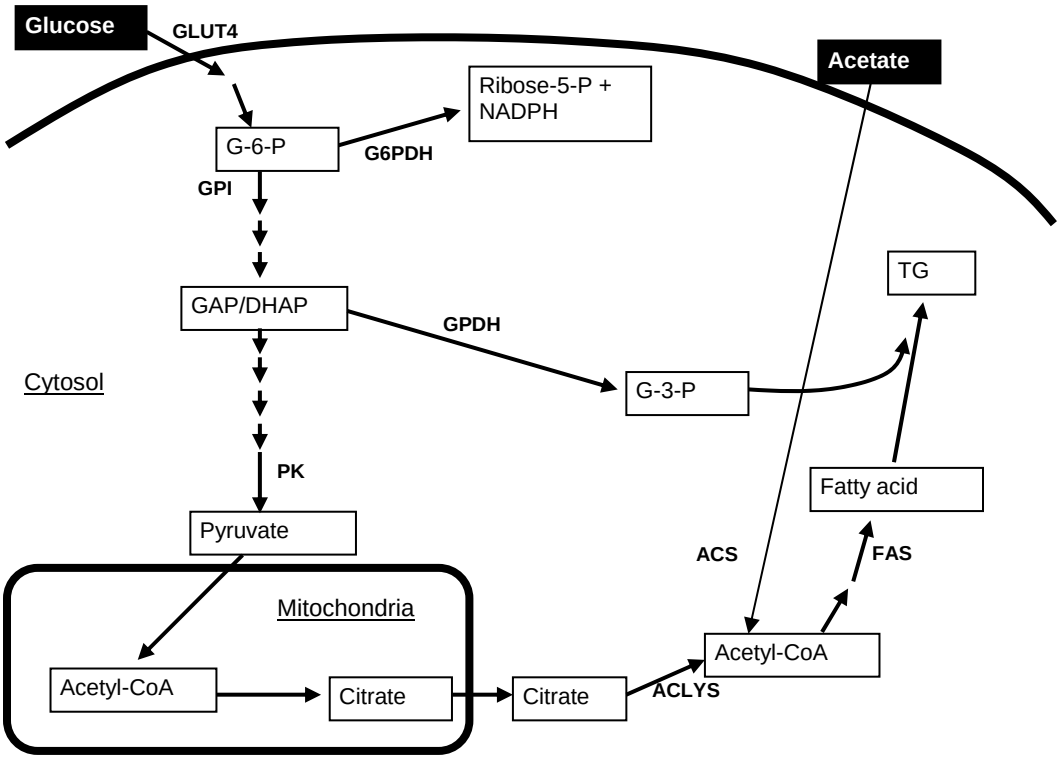

Figure 1. Illustration of genes involved in glucose and acetate conversion to triglycerides. GLUT4 = glucose transporter 4, GPI = glucose phosphate isomerase, G6PDH = glucose-6-phosphate dehydrogenase, GPDH = glycerol-3-phosphate dehydrogenase, PK = pyruvate kinase, ACLYS = ATP citrate lyase, ACS = cytosolic acetyl-CoA synthetase; FAS = fatty acid synthase, TG = triglyceride. Illustration was adapted from Nelson and Cox (2000).

parameters were 95˚C for 5 min, followed by 40 cycles of 95˚C for 10 sec and 60˚C for 30 sec. 18S ribosomal RNA was used as the reference gene for normalization. The relative quantification of mRNA expression was evaluated using the $\Delta\Delta C_T$ method. Fold change in mRNA expression was calculated using the Pfaffl method: Fold Change = $((E_{target}*2)^{\Delta CT,\ target})/((E_{ref}*2)^{\Delta CT,\ ref})$, where E_{target} and E_{ref} are the amplification efficiency of the target and reference gene, respectively, ΔCT target is the difference between CT values of the target gene measured in the treatment of interest and the control, and ΔCT ref is the difference between CT values of the reference gene measured in the treatment of interest and the control.

Data were analyzed as a completely randomized design with treatment, adipose tissue, and the interaction term included in the model. Treatment means were compared using pre-planned orthogonal contrasts with an alpha of 0.05: CON versus average of CORN, SBH, and DDGS treatments (CON vs. supplemented), CORN versus average of SBH and DDGS treatments (starch versus fiber-based supplements), and SBH versus DDGS.

RESULTS AND DISCUSSION

Performance and carcass characteristics are presented in a companion paper (Sharman et al., 2009).

There was no effect of treatment on expression of GLUT4, GPI, G6PDH, PK, or ACLYS mRNA in subcutaneous or perirenal adipose tissues (Table 1). This indicates that there were no differences in glucose utilization. Smith and Crouse (1984) found similar G6PDH enzyme activities in subcutaneous adipose tissue between concentrate and corn silage-based diets, but the concentrate-based diet had greater ACLYS enzyme activities. Similarly, a concentrate-based diet increased ACLYS enzyme activity in subcutaneous adipose tissue compared to an alfalfa

Table 1. Effect of treatment and adipose tissue on gene expression (Fold Change) in stocker cattle

	Treatment Effect[1]				Tissue Effect[2]		*P*-value[3]		
Gene[4]	CON	CORN	SBH	DDGS	SC	PR	TRT	Tissue	T*T
GLUT4	1.0	2.0	2.8	1.8	1.0	3.0	0.12	0.01	0.31
GPI	1.0	0.8	1.1	1.0	1.0	1.3	0.73	0.20	0.37
G6PDH	1.0	1.4	1.7	1.9	1.0	3.3	0.39	0.01	0.34
GPDH[a]	1.0	2.2	3.2	3.1	1.0	13.9	0.01	0.01	0.53
PK	1.0	0.7	0.9	0.7	1.0	0.5	0.91	0.08	0.21
ACLYS	1.0	1.5	1.7	1.6	1.0	2.9	0.45	0.01	0.38
SREBP	1.0	1.0	1.4	1.3	1.0	7.0	0.85	0.01	0.44
CEBPβ	1.0	1.3	1.2	1.5	1.0	3.5	0.73	0.01	0.16

[1]CON = cottonseed meal supplement, CORN = ground corn supplement, SBH = soybean hull supplement, DDGS = dried distillers grains plus solubles supplement.
[2]SC = subcutaneous adipose tissue, PR = perirenal adipose tissue.
[3]TRT = treatment main effect, Tissue = adipose tissue depot main effect, T*T = treatment by tissue interaction. Pre-planned treatment contrasts were used to compare supplement treatments using an alpha level of 0.05.
[4]GLUT4 = glucose transporter 4, GPI = glucose phosphate isomerase, G6PDH = glucose-6-phosphate dehydrogenase, GPDH = glycerol-3-phosphate dehydrogenase, PK = pyruvate kinase, ACLYS = ATP citrate lyase, SREBP = sterol regulatory element binding protein, CEBPβ = CCAAT/enhancer binding protein beta.
[a]Contrast of CON versus average of CORN, SBH, and DDGS is significant ($P \leq 0.05$).
[b]Contrast of CORN versus average of SBH and DDGS is significant ($P \leq 0.05$).
[c]Contrast of SBH versus DDGS is significant ($P \leq 0.05$).

hay-based diet, but there was no effect on PK enzyme activity (Smith et al., 1984). Similar to our results with GLUT4, Smith et al. (1984) found no effect of diet on hexokinase enzyme activity indicating similar glucose entry into the cell. In contrast to our study, Scott and Prior (1980) found increased G6PDH enzyme activity in subcutaneous and perirenal adipose tissue for a concentrate-based diet compared to a corn silage-based diet. In these studies (Scott and Prior, 1980; Smith and Crouse, 1984; Smith et al., 1984), concentrate-based diets provided greater starch and energy compared with the roughage-based diets, which is similar to the CORN supplement compared with the SBH and DDGS supplements, and supplemented treatments compared with CON in our study.

Energy supplementation increased GPDH mRNA expression compared with CON indicating that triglyceride synthesis was increased. However, the type of energy supplement (starch versus fiber) did not influence GPDH mRNA expression. There was a significant treatment by adipose tissue interaction for ACS and FAS mRNA (Table 2). Supplemented steers had greater

expression of ACS and FAS mRNA compared with CON steers in perirenal adipose tissue, but not subcutaneous adipose tissue. Furthermore, SBH steers had greater expression of FAS mRNA in perirenal, but tended (P = 0.10) to have lower expression of ACS in subcutaneous adipose tissue compared with DDGS steers. These data indicate that DDGS affected de novo fatty acid synthesis differently in perirenal compared with subcutaneous, possibly due to dietary fat contained in DDGS. Previous studies (Smith and Crouse, 1984; Smith et al., 1984) found that concentrate-based diets increased FAS enzyme activity in subcutaneous adipose tissue compared with roughage-based diets. A concentrate-based diet increased FAS enzyme activity in subcutaneous and perirenal adipose tissue compared with a corn silage-based diet (Scott and Prior, 1980).

Table 2. Relative gene expression (Fold Change[1]) of subcutaneous and perirenal adipose tissue in stocker cattle

	Subcutaneous				Perirenal				*P*-value[2]		
Gene[3]	CON[4]	CORN	SBH	DDGS	CON	CORN	SBH	DDGS	TRT	Tissue	T*T
ACS[bd]	1.0	1.0	1.9	6.2	9.6	61.9	84.3	50.4	0.01	0.01	0.10
FAS[df]	1.0	1.4	1.8	3.0	2.6	11.3	34.5	8.6	0.02	0.01	0.09

[1]The CON treatment for subcutaneous adipose tissue was used as the reference treatment for Fold Change calculations.
[2]TRT = treatment main effect, Tissue = adipose tissue main effect, T*T = treatment by tissue interaction. Pre-planned treatment contrasts were used to compare supplement treatments using an alpha level of 0.05.
[3]ACS = cytosolic acetyl-CoA synthetase; FAS = fatty acid synthase.
[4]CON = cottonseed meal supplement, CORN = ground corn supplement, SBH = soybean hull supplement, DDGS = dried distillers grains plus solubles supplement.
[a]Contrast of CON versus average of CORN, SBH, and DDGS for subcutaneous adipose tissue is significant ($P \leq 0.05$).
[b]Contrast of CORN versus average of SBH and DDGS for subcutaneous adipose tissue is significant ($P \leq 0.05$).
[c]Contrast of SBH versus DDGS for subcutaneous adipose tissue is significant ($P \leq 0.05$).
[d]Contrast of CON versus average of CORN, SBH, and DDGS for perirenal adipose tissue is significant ($P \leq 0.05$).
[e]Contrast of CORN versus average of SBH and DDGS for perirenal adipose tissue is significant ($P \leq 0.05$).
[f]Contrast of SBH versus DDGS for perirenal adipose tissue is significant ($P \leq 0.05$).

Expression of SREBP and C/EBPβ mRNA were not affected by supplementation treatment indicating that treatment did not affect recruitment and differentiation of new adipocytes. Thus, the treatment difference in growth of these fat depots is presumably due to hypertrophy not hyperplasia at this stage of development.

The mRNA expression of the genes examined, excluding GPI and PK, were greater in perirenal adipose tissue compared to subcutaneous adipose tissue suggesting greater metabolic activity of perirenal adipose tissue. In contrast, Scott and Prior (1980) found that subcutaneous adipose tissue had greater enzyme activity of G6PDH and FAS compared with perirenal adipose tissue.

In conclusion, energy supplementation increased mRNA expression of genes involved in triglyceride synthesis from acetate (ACS, FAS, and GPDH) compared with CON steers. However, type of energy, starch versus fiber, did not influence substrate for fatty acid synthesis. Subcutaneous fat thickness at the end of the winter grazing period was 0.02, 0.05, 0.06, and 0.06 inches for CON, CORN, SBH, and DDGS steers, respectively. There was a trend (P = 0.13) for supplemented steers to have increased fat thickness compared with CON steers, but no difference between energy sources, which is similar to the increased mRNA expression in supplemented compared with CON steers. The lack of a significant difference in fat thickness was probably due to the small number of steers harvested. In our study, samples of intramuscular fat were

unable to be collected. Due to the differences in development and metabolism of intramuscular fat compared with other fat depots, it is difficult to determine the effect of our treatments on mRNA expression in intramuscular adipose tissue. Marbling scores at the end of the winter grazing period were 120, 137, 150, and 157 for CON, CORN, SBH, and DDGS steers, respectively. These treatment means were not different, possibly due to the small number of steers harvested. However, the trend is similar to fat thickness measurements suggesting that energy supplementation, but not energy source, may have influenced mRNA expression of intramuscular adipose tissue. Therefore, type of energy supplement may not influence subcutaneous and perirenal adipose tissue development.

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