Livestock Science 131 (2010) 65–72

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Body condition score and day of lactation regulate fatty acid metabolism in milk somatic cells and adipose tissue of beef cows

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ARTICLE INFO

Article history:
Received 11 September 2009
Received in revised form 12 February 2010
Accepted 28 February 2010

Keywords:
Adipose tissue
Mammary gland
Lactation
Somatic cells
Lipogenesis
Messenger RNA
Transcription regulators
Fatty acid transporters

ABSTRACT

We hypothesized that body condition score (*BCS*) at parturition and day of lactation will alter transcript abundance of mammary and adipose tissue lipogenic enzymes, as well as adipose tissue protein concentrations of lipogenic and transcription factors during early lactation in beef cows fed supplemental fat. Beginning 3 d postpartum three-year-old Angus×Gelbvieh beef cows nutritionally managed to achieve a BCS of 4±0.07 (BW=479±36 kg; $n=18$) or 6±0.07 (BW=579±53 kg; $n=18$) at parturition were assigned to a hay diet plus low-fat control supplement or high-fat supplements (isonitrogenous and isocaloric with 5% of DMI as fat) until day 60 of lactation. At day 30 and day 60 of lactation, somatic cells from 300 mL of milk were collected for ribonuclease protection assay as a measure mammary transcript abundance for lipoprotein lipase (*LPL*), acetyl-CoA carboxylase (*ACC*), fatty acid synthase (*FAS*), and stearoyl-CoA desaturase (*SCD*). Abundance of mRNA was greater for FAS ($P=0.02$) and SCD ($P=0.04$) in cows of BCS 4 compared with 6. By using RT-PCR to quantify mRNA in biopsies of adipose tissue we determined that BCS 4 cows had less mRNA for LPL ($P<0.001$) and hormone sensitive lipase (*HSL*) ($P=0.05$) compared with BCS 6 cows. Abundance of LPL mRNA was lower ($P=0.001$) at day 30 postpartum compared to day 60; whereas, HSL mRNA was greater at day 30 ($P<0.001$). Concentration of CD36 protein was greater ($P=0.03$) in the BCS 6 cow adipose tissue. Both signal transducer and activator of transcription-subtype 5 (STAT-5) ($P<0.001$) and peroxisome-proliferator activated receptor-subtype gamma (PPAR-λ) ($P=0.05$) were greater at day 30 compared to day 60 postpartum. We conclude that abundance of mammary gland lipogenic enzyme mRNA was mainly affected by BCS at parturition; whereas, in adipose tissue, mRNA for enzymes for fatty acid uptake and mobilization, as well as fatty acid transport were affected by both BCS and day of lactation. Also, data suggest there was a shift in nutrient partitioning away from the mammary gland to subcutaneous adipose tissue at 60 d postpartum in beef cows.

1. Introduction

The metabolic goal during lactation is to support mammary gland nutrient production through coordinated genetic regulation between mammary and adipose tissues (Frayn et al., 1995). Thus, repression of adipose tissue lipoprotein lipase (*LPL*) and acetyl-CoA carboxylase (*ACC*) genes (Travers et al., 1997), with simultaneous up-regulation of the hormone sensitive lipase (*HSL*) gene (Martin-Hidalgo et al., 1994) would provide the mammary gland with endogenous energy supplies to meet the metabolic demands of lactation. Our laboratory has examined the effects of dietary supplemental fat on body condition score (*BCS*; 1=emaciated and 9=obese; Wagner et al., 1988) and energy partitioning during early lactation in beef cows (Bottger et al., 2002; Lake et al., 2005, 2006a,b; Scholljegerdes et al., 2009). Generally, dietary oil supplementation affected tissue fatty acid profile, but effects on biochemical events were not always evident.

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doi:10.1016/j.livsci.2010.02.028

On the other hand BCS at parturition and/or day of lactation affected adipose tissue lipolysis. For example, greater adiposity of BCS 6 cows supported greater fatty acid mobilization from adipose tissue than BCS 4 cows. Several biochemical and molecular events have been identified that are likely involved in these observations in the beef cow during lactation. The signal transducer and activator of transcription-subtype 5 (*STAT-5*) and peroxisome-proliferator activated receptor-subtype gamma (*PPAR-γ*) are involved in transcription regulation of specific adipose tissue lipogenic enzymes, for example ACC, LPL, and HSL (Schoonjans et al., 1996; Mao et al., 2002; Deng et al., 2006). Moreover, CD36, a plasma membrane fatty acid transport protein, is highly expressed in adipose tissue and directly affects fatty acid storage in adipose tissue (Hajri and Abumrad, 2002). Therefore, quantifying mammary gland mRNA and specific adipose tissue protein concentrations of lipogenic enzymes and regulatory factors should provide deeper insights into regulation of lipid metabolism during lactation in the beef cow. The objectives of the present study were twofold: to quantify mammary gland mRNA of lipogenic enzymes and to quantify adipose tissue transcript abundance of ACC, HSL, and LPL, as well as adipose tissue protein abundance of phosphorylated ACC (ACC-P), CD36, STAT-5, and PPAR-γ in beef cows of BCS 4 and BCS 6 during early lactation.

2. Materials and methods

2.1. Animals, treatments, and diets

The University of Wyoming Institutional Animal Care and Use Committee approved all procedures for these studies. Cows were nutritionally managed and assigned to treatments as described by Lake et al. (2005). Thirty-six three-year-old Angus×Gelbvieh beef cows were nutritionally managed to achieve either a BCS of 4 (BCS 4 ± 0.07, initial BW = 479.3 ± 36.3 kg; $n = 18$) or 6 (BCS 6 ± 0.07, initial BW 579.6 ± 53.1 kg; $n = 18$) at parturition. Briefly, cows assigned to a BCS of 4 at parturition were managed to be in an energy deficit state during the second trimester and then were fed to meet maintenance requirements throughout the third trimester of gestation to ensure that postpartum cow and calf performance was not affected. During their second trimester, cows assigned to BCS 4 had a BCS of 4.8. To accomplish a BCS of 4 these cows were allowed to graze pasture containing low forage availability and consumed an estimated 8.78 kg/d of 6.9% CP and 56.5% TDN. During their third trimester these cows were maintained in a dry lot on bromegrass hay and alfalfa pellets. Details of feeding management were previously reported (Lake et al., 2005).

Cows assigned to a BCS of 6 at parturition were managed to meet energy requirements throughout gestation according to the NRC (1996). These cows had an initial BCS of 5.4, and were fed to gain weight and condition throughout the second and third trimesters by grazing pastures with adequate forage availability (dry matter intake estimated at 10.9 kg pasture/d). During the third trimester the BCS 6 group of cows was housed in dry lot and fed 9.4 kg of bromegrass hay and 3.0 kg of dehydrated beet pulp pellets daily.

Cows were randomly assigned within BCS group to postpartum dietary treatment as they calved. Beginning 3 d postpartum, cows were placed into one of six pens (six animals per pen) with individual feeding stanchions. Each treatment (BCS 4 or 6 at parturition and dietary treatment) was represented in every pen with an average calving interval not greater than 7 d within each pen. Cows were fed twice daily a hay diet plus low-fat control supplement or supplements with either cracked high-linoleate safflower seeds or cracked high-oleate safflower seeds. Diets, described in detail by Lake et al. (2005), were formulated to meet the energy requirements of a 544 kg beef cow producing 9 kg/d of milk at peak lactation. Diets were formulated to provide equal quantities of N and TDN, and safflower seed supplemented diets were formulated to be isolipidic, providing 5% DMI as fat.

2.2. Milk/mammary sampling and mRNA quantification

Lactating mammary gland epithelial cells obtained from the milk were used as a source of mRNA for specific lipogenic enzymes (Murrieta et al., 2006). At 30 and 60 d of lactation, following oxytocin administration (20 USP; Vedco, Inc., St. Joseph, MO), cows were milked using a sterilized mechanical milking device. Milk was thoroughly mixed before a 300 mL subsample was placed into six, sterile 50-mL tubes and centrifuged at 2500×*g* for 20 min at 4 °C. After decanting the supernatant, the somatic cell pellet from each tube was washed in sterile PBS (pH 7.4, 4 °C) to remove any remaining infranate and centrifuged again. The total somatic cell pellet was resuspended in 0.75 mL of Tri Reagent LS (Molecular Research, Inc., Cincinnati, OH) for total RNA extraction according to manufacturer's instructions, and then 50 μL of sterile water containing 0.1% diethyl pyrocarbonate was added before storage at −80 °C. Total RNA integrity was verified by using a denaturing 6% acrylamide gel containing 7 M urea, adjusted to pH 8.3. The RNA concentration was determined by UV–Vis spectrophotometry (λ = 260 nm). Ten micrograms of total RNA was used for ribonuclease protection assay to quantify mRNA transcript abundance for ACC, FAS, SCD, LPL, and using 28s ribosomal RNA as the reference transcript (Murrieta et al., 2006). Probes for the assay were generated as described by Lee et al. (2002) from bovine mammary RNA.

2.3. Quantitative PCR analysis of bovine adipose tissue

Subsequent to ribonuclease protection assay evaluation of milk somatic cell mRNA, quantitative RT-PCR methods were employed to evaluate adipose tissue mRNA. Variation of gene expression of the 18s and 28s RNA reference standards with BCS and day of lactation was not compared. Because the 18s reference standard was available for RT-PCR its use allowed for multiplex analysis of the mRNA. For the RPA, we have used the 28s reference standard successfully in previous studies where multiple mRNAs were evaluated simultaneously (Murrieta et al., 2006). The RPA was used in the initial study where milk somatic cell mRNA was evaluated. Use of RT-PCR was employed for the adipose tissue analyses as this method became available subsequently, and offered a more advanced technique to quantify the mRNA. Data on 18s RNA gene transcription with day of lactation or BCS in beef cow adipose tissue have not been reported; however, as more options for reference standards occur, this comparison should be undertaken. To obtain adipose tissue at 30 and 60 d of lactation, each cow was injected with a