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J ANIM SCI published online August 5, 2011

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[1]Funding for this project was provided by Western SARE Grant No.SW07-049. Appreciation is expressed to Dr. Nathan Long (University of Wyoming, Laramie) and Dr. Dennis M. Hallford (New Mexico State University, Las Cruces) for their assistance with hormone assays.

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Published Online First on August 5, 2011 as doi:10.2527/jas.2010-3630

ABSTRACT: Angus × Gelbvieh rotationally crossbred yearling heifers (n = 99, yr 1; n = 105, yr 2) were used in a 2-yr randomized complete block designed experiment with repeated measures to determine the effect of feeding camelina biodiesel co-products (meal and crude glycerin) on serum concentrations of triiodothyronine (T_3), thyroxine (T_4), insulin, β-hydroxybutyrate (BHBA), and glucose, as well as on growth and reproductive performance. Heifers were assigned to 1 of 15 pens and pens were assigned to initially receive 7.03 kg of bromegrass hay plus 0.95 kg of 1 of 3 supplements: control (50% ground corn and 50% soybean meal, as-fed); mechanically extracted camelina meal; crude glycerin (50% soybean meal, 33% ground corn, 15% crude glycerin, 2% corn gluten meal; as-fed) for 60 d before breeding. Preprandial blood samples were collected via the jugular on d 0, 30, and 60 of the feeding period. A 2-injection $PGF_{2\alpha}$ protocol (d 60 and 70 of the study) was used to synchronize estrus. Heifers were artificially inseminated 12 h after estrus was first detected. Heifers not detected in estrus within 66 h received a GnRH injection and were artificially inseminated. Dietary treatment × sampling period interactions were not detected (P = 0.17 to 0.87). Dietary treatment did not affect BW (P = 0.44 to 0.59), or serum concentrations of T_4 (P = 0.96), BHBA (P = 0.46), glucose (P = 0.59), or insulin (P = 0.44). Serum concentrations of T_3 were greater (P = 0.05) in heifers fed camelina meal. Additionally, dietary treatment did not affect the percentage of heifers detected in estrus before timed AI (P = 0.83), first-service pregnancy rates of those heifers detected in estrus (P = 0.97), or overall first-service pregnancy rates (P = 0.58). Heifers fed camelina meal, however, had greater (P = 0.05) first-service pregnancy rates to timed AI than did heifers fed the control and crude glycerin supplements. The cost per pregnancy for heifers fed the crude glycerin or the control supplement was similar, whereas the cost per pregnancy for heifers fed camelina meal was the least. We conclude that camelina co-products can replace conventional corn-soybean meal supplements in diets of developing replacement beef heifers.

Key Words: camelina, biodiesel co-products, replacement beef heifers

INTRODUCTION

Camelina meal is the coproduct resulting from pressing the seeds for oil extraction. It is a good source of protein (Bonjean and Le Goffic, 1999) and PUFA (Hurtaud and Peyraud, 2007). Increasing plasma PUFA status of beef females may be beneficial to reproduction (Hess et al., 2008). However, the oil in camelina meal contains approximately 2 to 5% erucic acid (22:1*n*-9; Putnam et al., 1993). Feeding 22:1*n*-9 could be a concern because 22:1*n*-9 has induced myocardial lipidosis in non-ruminants (Kramer et al., 1990). Camelina seeds also contain glucosinolates, which are compounds also present in rapeseed meal and that can decrease synthesis of thyroxine (T_4) by the thyroid gland (Lardy and Kerley, 1994). The concentration of glucosinolates in camelina (22 μmol/g); however, is less than in rapeseed meal (118 μmol/g) (Lange et al., 1995).

Crude glycerin is a coproduct remaining after the extracted seed oil is used for biodiesel production. Glycerol, the main compound in crude glycerin, has an energy value similar to starch (DeFrain et al., 2004). Glycerol is extensively fermented in the rumen (Kijora et al., 1998), increases molar proportions of propionate and butyrate (Khalili et al., 1997), and can replace up to 30% of dietary forage without affecting ruminal digestibility and fermentation (Nayigihugu et al., 2008).

We hypothesized that biodiesel co-products could replace corn and soybean meal without impairing thyroid gland activity, and feeding crude glycerin could increase serum metabolites generated from metabolism of ruminal VFA. Also, feeding camelina meal could enhance plasma fatty acid status. Our objectives were to evaluate the effects of replacing corn and soybean meal with camelina meal and replacing corn with crude glycerin on serum concentrations of metabolites and metabolic hormones, plasma fatty acids, as well as growth and reproductive performance of developing replacement beef heifers.

MATERIALS AND METHODS

All procedures for the 2-yr experiment were approved by the University of Wyoming Animal Care and Use Committee.

Experimental Design and Animals

The 2-yr randomized complete block designed experiment in which Angus × Gelbvieh rotationally crossed heifers were sorted by initial BW (yr 1, n = 99; 300 ± 9 kg; yr 2, n = 105; 294 ± 8 kg) into BW blocks (blocks 1 to 5 in yr 1; blocks 6 to 10 in yr 2) included randomly assigning 1 of 3 experimental supplements to 1 of 3 pens (6 to 7 heifers/pen) within each BW block.

Body Weights

Heifer BW was recorded as the average pre-feeding live weights taken on 2 consecutive d at the beginning (d 0 and 1), middle (d 30 and 31), and end (d 60 and 61) of the experimental feeding period.

Diets

Diets were formulated to be isonitrogenous and to provide 12.6% CP of dietary DM. Heifers had limited access to bromegrass hay (Table 1), which was offered at 7.03 and 7.34 $kg \cdot heifer^{-1} \cdot d^{-1}$ (as-fed) from d 0 through 30 and d 31 through 60, respectively. Heifers were offered 1 of 3 experimental supplements (Table 1 and 2): a control supplement consisting of 50% finely ground corn and 50% soybean meal (as-fed); 100% mechanically extracted camelina meal; or a crude glycerin supplement consisting of 50% soybean meal, 33% finely ground corn, 15% crude glycerin, and 2% corn gluten meal (as-fed). Supplements were offered daily at 0.33% of average BW for 60 d (as-fed; 0.95 and 0.99 $kg \cdot heifer^{-1} \cdot d^{-1}$ during d 0 through 30 and d 31 through 60, respectively). Supplements were provided at 0800 and completely consumed within 5 min of being offered. Hay was offered immediately after supplements were consumed. On the next morning, any hay remaining in the bunks was removed and weighed before offering the supplements. In yr 1, heifers had free access to water and trace mineralized salt (Ultra Balance Spring & Summer Mineral, Hergert Milling Inc., Scottsbluff, NE; guaranteed analysis, as a percentage of DM: NaCl, 14 to 16; Ca, 18 to 20; P, 8; Mg, 2.5; K, Co, Cu, I, Mn, Zn and Se, < 1) throughout the experiment. Based on average supplement consumption in yr 1, the same mineralized salt was included at 6% (as-fed) of the dietary supplement in yr 2.

Synchronization

On d 60, each heifer received an intramuscular injection containing a 25 mg of $PGF_{2\alpha}$ (Lutalyse, Pfizer Animal Health, New York, NY). Heifers were combined into one large group and had free access to water, trace

mineralized salt (described previously), and bromegrass hay. A second 25 mg intramuscular injection of $PGF_{2\alpha}$ was administered to all heifers on d 70. Heifers were checked twice daily for signs of estrus. Heifers were artificially inseminated 12 h after estrus was first detected. Heifers that were not detected in estrus were given an intramuscular injection containing 100 μg of GnRH (Fertagyl, Intervet, Inc., Millsboro, DE) and artificially inseminated by 66 h after the second $PGF_{2\alpha}$ injection. Any heifer detected in estrus by d 75 was inseminated again 12 h after they were detected in estrus. In yr 1, heifers were observed for estrus 17 d after first service AI. Heifers showing estrus after first service AI were bred via AI 12 h after exhibiting estrus. In yr 2, bulls were placed with heifers 17 d after timed-AI and remained with the heifers for 30 d. Heifers were diagnosed as pregnant if they were not detected in estrus 75 d after AI (yr 1) or with transrectal ultrasonography (variable MHz linear array transducer, MicroMaxx, Sonosite, Bothell, WA) 104 d after AI (yr 2). Pregnancy rates and day of pregnancy were confirmed at calving.

Blood Sampling and Laboratory Analyses

Preprandial blood samples were taken from the jugular vein before treatments were applied (d 0), and on d 30 and 60 of the feeding period. Blood samples were collected into commercial blood collection tubes (Vacutainer, 10 mL; Becton Dickinson and Co., Franklin Lakes, NJ) and EDTA-coated glass tubes (Vacutainer, 10 mL; Becton, Dickson and Co., Franklin Lakes, NJ). Blood samples were placed on ice immediately after collection and then were stored at 4º C for 12 h. Samples were centrifuged at 2,500 x *g* for 20 min, the resulting serum or plasma was decanted and stored at -20ºC until laboratory analyses.

Plasma samples (from EDTA-coated tubes) were lyophilized (Genesis SQ 25 Super ES Freeze Dryer, The Virtis Co., Gardiner, NY) and ground with a mortar and pestle, and fatty acid methyl esters were prepared as described by Lake et al. (2006). Fatty acid methyl esters of hay and supplements were prepared in a single-step direct transesterification using methanolic hydrochloric acid as catalyst (Weston et al., 2008). Separation of fatty acid methyl esters was achieved by GLC (Model 6890 series II, Hewlett-Packard, Avondale, PA) with a 100-m capillary column (SP-2560, Supelco, Bellefonte, PA), with He as the carrier gas at 0.5 mL/min. The oven temperature was maintained at 175°C for 40 min and ramped to 240°C at 10°C/min. Injector and detector

(flame ionization) temperatures were 250°C. Identification of peaks was accomplished with purified standards (Nu-Check Prep, Elysian, MN; Matreya, Pleasant Gap, PA).

Preprandial serum samples were analyzed for glucose (Liquid Glucose Hexokinase kit; Pointe Scientific Inc., Canton, MI; inter- and intraassay CV of 4.6 and 4.1%, respectively), β-hydroxybutyrate (BHBA; Autokit 3-HB, Wako Chemicals, Richmond, VA; inter- and intraassay CV of 5.3 and 2.7%, respectively), and total triiodothyronine (T_3) and T_4 (Hersom et al., 2004; solid-phase ^{125}I RIA; Coat-A-Count kits, Diagnostic Products Corporation , Los Angeles, CA; inter- and intraassay CV of 5.8 and 5.9% for T_3, and 7.3 and 5.5% for T_4, respectively). Insulin assay was performed using components of a commercial kit (Siemens Medical Solutions Diagnostics, Romeoville, IL; intraassay CV of 6.34%).

Hay and supplements samples were taken weekly for diet analysis. Feed samples were analyzed for DM and ash (AOAC, 1990), N (Leco FP-528 N analyzer, Leco Corporation, Henderson, NV), IVDMD (Ankom Daisy II Incubator, ANKOM Technology, Fairport, NY), and NDF and ADF (Ankom 200 fiber analyzer, Ankom Technology, Fairport, NY).

Statistical Analysis

Data were analyzed as a randomized complete block design using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC, USA, version 9.2). Body weight (d 0, 30, and 60), ADG (d 0 to 30 and d 31 to 60), percentage of heifers detected in estrus, first-service pregnancy rate of heifers inseminated after detection of estrus, first-service pregnancy rate to timed-AI, overall first-service pregnancy rate to AI, and final pregnancy rates were tested with pen as the experimental unit. The model included effects of treatment and block. Serum concentrations of T_3, T_4, glucose, insulin, BHBA and plasma concentrations of fatty acids were analyzed as repeated measures using the MIXED procedure of SAS with pen within block as the subject. There were insufficient df to include year as a second blocking factor because BW blocks in yr 2 were not identical to yr-1 BW blocks. Therefore, yr-2 data were treated as additional blocks (blocks 1 to 5 in yr 1; blocks 6 to 10 in yr 2) and between year sums of squares variation was accounted for in the BW block effect. The model included effects of treatment, period (d 0, 30, and 60), period x treatment, block, and block x period. The criterion for

choosing the covariance structure was the one with the lowest akaike information criterion. The unstructured covariance structure was used for all serum variables (T_3, T_4, insulin, glucose, and BHBA), whereas the Toeplitz structure was used for plasma fatty acids concentrations. Least-squares means were separated using the Tukey multiple comparison test if a significant preliminary F-test was detected ($P < 0.05$).

RESULTS AND DISCUSSION

Growth Performance

Dietary treatment did not affect forage ($P = 0.19$) or total DMI ($P = 0.09$), BW ($P \geq 0.44$) or ADG during the first ($P = 0.59$) or second ($P = 0.63$) 30-d period (Table 3). Supplements were completely consumed within 5 min after being offered. The lack of differences in forage and total DMI and growth performance among heifers fed control, camelina meal, and crude glycerin supplements suggests that, in addition to being formulated to be isonitrogenous, the supplements provided the same amount of energy. In agreement with our results, Price et al. (2009) reported similar DMI and growth performance of lambs fed whole camelina seeds in place of soybean meal in a corn-based diet. Donkin et al. (2009) observed no differences in total diet DMI and milk production when glycerol replaced up to 15% of dietary DM in diets fed to lactating dairy cows. Likewise, Mach et al. (2009) reported similar daily concentrate intake, straw intake, total DMI, ADG, and G:F ratio in feedlot beef cattle fed crude glycerin up to 12% of concentrate DM.

Guidelines have been established suggesting that heifers should achieve 60 to 65% of their expected mature BW at the beginning of the breeding season to maximize the number of heifers in puberty at 13 to 14 mo of age (Patterson et al., 2000). Diets were formulated for a target ADG of 1 kg/d (Whitney et al., 2000) to ensure that heifers would achieve between 60 to 65% of mature BW of the University of Wyoming cattle herd at the beginning of the synchronization period (Brokaw et al., 2002). Heifers fed control, camelina meal, and crude glycerin achieved 62.3, 62.0, and 62.3% of mature BW ($P = 0.41$; SEM = 0.21) on d 60.

Plasma Fatty Acids

Treatment x period interactions were only detected for plasma concentrations of *cis*-9, *trans*-11-CLA ($P = 0.037$), 22:1*n*-9 ($P = 0.001$), 18:1*trans*-11 ($P = 0.001$), 18:1*trans*-12 ($P = 0.005$), 18:1*trans*-13 ($P = 0.001$),

18:1*cis*-10 ($P = 0.023$), 18:1*cis*-11 ($P = 0.001$), and 18:1*cis*-12 ($P < 0.001$). Except for *cis*-9, *trans*-11-CLA and 18:1*trans*-11, treatment x period interactions can be explained by the fact that none of those fatty acids were detected in plasma samples before initiating the experimental feeding period.

The plasma concentrations of 18:1*cis*-9 ($P = 0.025$), 18:2*n*-6 ($P = 0.009$), and 18:3*n*-3 ($P = 0.012$) were greater in heifers fed camelina meal than they were in heifers fed control and crude glycerin rations. This response was expected because heifers fed camelina meal consumed a supplement with greater concentrations of these fatty acids than did control and crude glycerin (Table 2). Fatty acids concentration of the crude glycerin was 0.26% of DM, which resulted in similar fatty acids concentrations between the control and crude glycerin supplements.

Concentrations of fatty acids in plasma are directly proportional to the amount of fatty acids absorbed from the small intestine (Noble et al., 1972). Price et al. (2008) reported that lambs fed camelina seeds had a greater percentage of total fatty acids digested in the small intestine than did lambs fed a diet without supplemental fat. In a following trial, Price et al. (2009) reported that weight percentages of 18:3*n*-3 in LM, *semitendinosus* muscle, and tail head adipose tissue were greater for lambs fed camelina seeds. Our results are consistent with Scholljegerdes et al. (2008), who reported that beef cows at 60 d postpartum fed a whole flaxseed supplement (56% 18:3*n*-3) had significantly greater plasma concentrations of 18:2*n*-6, 18:3*n*-3, and 20:4*n*-6 than cows fed a whole soybean supplement. In the present study, heifers fed camelina meal tended ($P = 0.075$) to have greater serum concentrations of 20:4*n*-6 than heifers fed control and glycerin supplements. We anticipated that heifers fed camelina meal would have less 20:4*n*-6 in circulation because diets enriched with 18:3*n*-3 decreased the production of 20:4*n*-6 (Mattos et al., 2000). However, it is also possible that 18:2*n*-6 served as a precursor for the synthesis of 20:4*n*-6 (Staples et al., 1998). Additionally, the amount of 18:3*n*-3 in circulation might not have been sufficient to inhibit production of 20:4*n*-6 due to the extensive biohydrogenation of 18:3*n*-3 (81.1% of intake; Scholljegerdes and Kronberg, 2007).

Ruminal concentrations and biohydrogenation of 18:2*n*-6 increased the extent of biohydrogenation of 18:2*n*-6 (Harfoot et al., 1973). However, as intake of 18:2*n*-6 increases, an inhibition of complete

biohydrogenation and an accumulation of biohydrogenation intermediates occurs (Harfoot et al., 1973). Heifers fed camelina meal had greater concentrations of UFA ($P = 0.012$), *cis*-isomers ($P = 0.010$), and *trans*-isomers ($P = 0.008$) than heifers fed the control and crude glycerin. Scholljegerdes and Kronberg (2008) reported greater concentration of biohydrogenation intermediates in duodenal samples of heifers fed 0.91 kg of whole flaxseed than heifers fed no supplement. Likewise, Hurtaud and Peyraud (2007) observed that feeding camelina seeds or meal increased the concentrations of UFA and all the *trans*-18:1isomers in milk.

Greater intake of 18:2*n*-6 and 18:3*n*-3 for heifers fed camelina meal likely contributed to increased plasma concentrations of 18:1*trans*-11 ($P = 0.003$) and *cis*-9, *trans*-11-CLA ($P = 0.003$) compared with feeding the control and crude glycerin supplements. The *cis*-9, *trans*-11-CLA isomer is the most prevalent CLA isomer in ruminant animals fed forage-based diets (Jenkins et al., 2008; Hess et al., 2008). Hurtaud and Peyraud (2007) reported greater concentrations of 18:1*trans*-11 and *cis*-9, *trans*-11 CLA in milk for lactating cows fed camelina (seeds or meal) vs. cows fed low-fat control diet. Those authors also reported a strong correlation (r = 0.71) between milk concentrations of 18:1*trans*-11 and *cis*-9, *trans*-11 CLA. The 18:1*trans*-11 serves as a precursor for endogenous synthesis of *cis*-9, *trans*-11-CLA via Δ^9-desaturase in small intestine (Archibeque et al., 2005) and other tissues (Griinari et al., 2000; Bauman et al., 2003).

Erucic acid (22:1*n*-9) limits the use of the oil for human consumption (Peiretti and Meineri, 2007) because 22:1*n*-9 may promote myocardial lipidosis (Kramer et al., 1990). Camelina meal used in the present study contained 2.58% of total fatty acid as 22:1*n*-9. Heifers fed camelina meal had greater ($P = 0.001$; 0.01 mg of 22:1*n*-9/g of freeze dried plasma) concentrations of 22:1*n*-9 compared with heifers fed the control or glycerin supplements (0 mg of 22:1*n*-9/g of freeze dried plasma); this was expected because the control and glycerin supplements did not contain any 22:1*n*-9. However, as a percentage of total fatty acids found in plasma of heifers fed camelina meal (23.9 mg of total fatty acids/g of freeze dried plasma), 22:1*n*-9 represented only 0.04%, suggesting that small amounts of 22:1*n*-9 were available for absorption in the small intestine. In agreement with our results, Price et al. (2009) detected only traces of 22:1*n*-9 in KPH after feeding camelina to

feedlot lambs. Those authors also did not detect 22:1*n*-9 in LM and *semitendinosus* muscles or tail head adipose tissue after feeding camelina seeds for 35 d.

Serum Thyroid Hormones

Dietary treatment x sampling period interactions were not detected for serum concentrations of T_4 (P = 0.87) or T_3 (P = 0.17). Serum concentrations of T_3 increased 30 d after feeding (0.86, 0.98, and 0.92 ng/mL for d 0, 30, and 60 respectively; SEM = 0.02), whereas T_4 only increased at d 60 (38.9, 39.0, and 43.8 ng/mL for d 0, 30, and 60, respectively; SEM = 1.1). Although T_4 has little metabolic activity, it is the predominant thyroid hormone in circulation, and can be converted to the more biologically active thyroid hormone, T_3 (Leonard and Visser, 1986). Cassar-Malek et al. (2001) attributed greater T_3 concentrations to the greater conversion of T_4 to T_3 by the hepatic 5'D-deiodinase. The changes in thyroid hormones between sampling periods in the present experiment seem to be reflective of the magnitude of change observed for ADG between the first (1.12 kg/d) and second (0.87 kg/d) 30-d feeding periods. Energy availability affects bovine thyroidal status, with plasma concentrations of thyroid hormones reflecting feed intake and growth rate in growing steers (Blum et al., 1985).

Glucosinolates are polar compounds present in camelina (Schuster and Friedt, 1998). The derivative products of glucosinolates (thiocyanate and isothiocyantes) are released after breakdown by ruminal microflora activity (Duncan and Milne, 1992). Guyton (1986) demonstrated that thiocyanate ions prevented the iodination of thyroid hormones, resulting in inactive hormones when released into the blood stream. Lardy and Kerley (1994) observed a decrease in serum concentrations of T_4, but no effect on T_3 concentrations after 28 d of feeding rapeseed meal to steers. In the present study, dietary treatment did not affect (P = 0.956) serum concentrations of T_4 (Table 5), which may be explained by the lower concentrations of glucosinolates in camelina (22 μmol/g; Lange et al., 1995) compared with the rapeseed meal fed by Lardy and Kerley (1994).

Heifers fed camelina meal had greater (P = 0.045) average concentrations of T_3 in serum compared with heifers fed either the control or glycerin supplement; serum concentrations of T_3 did not differ (P = 0.990) between the control and glycerin treatments (Table 5). Likewise, Bunting et al. (1996) observed no differences in preprandial T_4 concentrations but greater T_3 concentrations in calves supplemented with fat. Unsaturated

fatty acids, including *cis*- and *trans*-isomers, decrease the binding of T_3 to its nuclear receptor (Wiersinga et al., 1988; Romo et al., 1997), which in turn, might increase circulating concentrations of T_3. As discussed previously, heifers fed camelina meal had greater concentrations of UFA, and *cis*- and *trans*-isomers in plasma compared with heifers fed the control and glycerin supplements.

Serum Glucose, Insulin, and β-hydroxybutyrate (BHBA)

Neither dietary treatment x sampling period ($P = 0.606$, 0.356 and 0.395; data not shown) nor dietary treatment effects ($P = 0.585$, 0.440 and 0.461; Table 5) were detected for serum concentrations of glucose, insulin, or BHBA. A period effect was detected for serum concentrations of glucose ($P < 0.001$), insulin ($P < 0.001$), and BHBA ($P < 0.001$), respectively. Serum glucose concentrations on d 30 were less than d 0 (63.8 ± 0.55 vs. 67.8 ± 0.75 mg/dL, respectively; $P < 0.001$) and d 60 (68.1 ± 0.57 mg/dL; $P < 0.001$), but did not differ ($P = 0.782$) between d 0 and 60. Serum concentrations of insulin were greater on d 60 (0.15 ± 0.01 ng/mL) than d 0 (0.08 ± 0.004 ng/mL; $P < 0.001$) and d 30 (0.10 ± 0.008 ng/mL; $P < 0.001$), but did not differ ($P = 0.171$) between d 0 and 30. Serum concentrations of BHBA on d 30 were less than on d 0 (0.13 ± 0.003 vs. 0.16 ± 0.006 μmol/L, respectively; $P = 0.001$) and d 60 (0.14 ± 0.004 μmol/L; $P = 0.030$); however, serum concentrations of BHBA were similar ($P = 0.117$) between d 0 and 60 of the feeding period.

Glycerol is extensively fermented to propionate (Rémond et al., 1993) and butyrate by ruminal bacteria (Czerkawski and Breckenridge, 1972) at the expense of acetate (Rémond et al., 1993; Khalili et al., 1997). Glycerol is a potent glucose precursor after phosphorylation to glycerol-3-phosphate by glycerol kinase in the liver (Mourot et al., 1994). However, the amount and method of feeding glycerol may influence the percentage of glycerol reaching the liver (Kijora et al., 1998; DeFrain et al., 2004). A slight increase in blood glucose was reported when glycerol was delivered as a drench (Linke et al., 2004). In the present study, crude glycerin accounted for 1.93% of total dietary DM (145 $g \cdot heifer^{-1} \cdot d^{-1}$) and did not increase serum concentrations of glucose. DeFrain et al. (2004) observed that the inclusion of glycerol at approximately 2.5 to 7.2% of dietary DM (up to 840 $g \cdot d^{-1}$) did not increase plasma concentrations of glucose or insulin. Likewise, Mach et al. (2009)

did not detect differences in plasma concentrations of glucose and insulin in Holstein bulls fed increasing concentrations of glycerin (0, 4, and 12% of concentrate DM).

A lower acetate:propionate ratio after fat supplementation has been reported (Jenkins et al., 1989; Whitney et al., 2000) because of decreased ruminal NDF digestibility (Scholljegerdes et al., 2004) or ruminal fermentation of glycerol (Chalupa et al., 1986; Hess et al., 2008). Brokaw et al. (2002) reported similar forage intake, total VFA, but greater ruminal molar proportions of propionate in heifers supplemented soybean oil at 1.75% of dietary DM compared with heifers fed cracked corn (0.345% of BW). Whitney et al. (2000) reported greater concentration of glucose in serum of heifers fed 3% added supplemental soybean oil compared with heifers fed a corn-soybean meal supplement. Supplemental fatty acid concentration from camelina meal was 1.33% of total dietary DM and might have not been enough to elicit the effects noted by Whitney et al. (2000) and Brokaw et al. (2002).

Insulin increased when energy status of the animals was improved with supplemental fat, but decreased when the energy intake was depressed (Staples et al., 1998). Hess et al. (2008) reported no effects of fat supplementation on DMI when supplemental fat was included at 3% of dietary DM. In our study, heifers fed camelina meal had similar BW gain, forage and total DMI compared with heifers fed the control and glycerin supplements. This reflects similar energy intake among dietary treatments. In agreement with our results, Whitney et al. (2000) reported similar concentrations of insulin in serum of heifers fed 3% added supplemental soybean oil and heifers fed a corn-soybean meal supplement.

Plasma concentrations of β-hydroxybutyrate (BHBA) can be influenced by the omasal and ruminal epithelium conversion of butyrate to BHBA (DeFrain et al., 2004). Kristensen et al. (2000) demonstrated that ruminal infusions of butyrate increased arterial concentrations of BHBA. Linke et al. (2004) observed an increase in molar percentages of ruminal butyrate and plasma BHBA after providing 800 g of glycerol in the diet of dairy cows. Mach et al. (2009) reported that rumen molar proportions of butyrate were not affected in Holstein bulls receiving increasing concentrations of glycerin (0, 4, 8, and 12% of concentrate DM), and this is

consistent with the lack of differences on serum concentrations of BHBA between heifers fed the control and crude glycerin supplements in the present study.

Scholljegerdes et al. (2004) observed greater ruminal molar proportions of butyrate in heifers fed a corn/soybean meal-based supplement (low-fat) compared with heifers fed a high-linoleate or oleate safflower seeds to provide fatty acids at 5% of dietary DM. Feeding the same diets used in the study of Scholljegerdes et al. (2004), Lake et al. (2006) observed that lactating postpartum beef cows fed the low-fat control supplement had greater plasma concentrations of BHBA than cows fed the high-linoleate or high-oleate safflower seeds supplement. However, providing soybean oil at 1.75% of dietary DM did not affect ruminal molar proportion of butyrate (Brokaw et al., 2002). Thus, the lack of differences observed for serum concentrations of BHBA between heifers fed control and camelina meal supplements was expected because the camelina meal only provided 1.33% of DM as fatty acids.

The ruminal epithelium is the major source of circulating ketone bodies (such as BHBA and acetoacetate) in fed ruminants (Beck et al., 1984; Heitmann et al., 1987), although, it is not the only source for ketone bodies formation. Therefore, ketone bodies are always present in the blood (Laffel, 1999). Insulin controls formation of ketone bodies (Laffel, 1999). Although insulin concentrations in the present study only increased at d 60, it is possible that insulin activity increased due to supplementation, which might have stimulated cellular uptake of glucose and inhibited hepatic production of BHBA on d 30. This rationale is supported by greater ADG for heifers during the first 30 d vs. the second 30 d. Also, insulin resistance by peripheral tissues of beef steers increased as BW, age, and body fat content increased (Eisemann et al., 1997). As suggested by León et al. (2004), perhaps heifers developed some insulin resistance as they accumulated fat, which may explain the increase in serum concentrations of insulin, glucose, and BHBA on d 60 and the decreased ADG for the second 30 d of the feeding period.

Reproductive Performance

Data presented in Table 6 illustrate that dietary treatment did not affect heifers detected in estrus before timed AI ($P = 0.825$), pregnancy rate of those bred by heat ($P = 0.965$), overall pregnancy rate to AI ($P =$

0.577), and final pregnancy rate ($P = 0.376$). In agreement with our results, Funston et al. (2002) reported no improvement in estrous response or pregnancy rates of beef heifers supplemented with sunflower seeds at 0.91kg/d for 30 or 60 d before breeding. In an extensive review of the literature, Hess et al. (2008) concluded that overall pregnancy rates for heifers fed supplemental fat increased by 15% compared with heifers fed supplements without fat. Although not statistically significant, the 17% improvement in final pregnancy rate observed for heifers fed camelina meal vs. heifers fed the control supplement was consistent with literature results summarized by Hess et al. (2008). In our study, the magnitude of difference in overall pregnancy rates between heifers fed supplements with or without fat can be attributed to the greater ($P = 0.046$) pregnancy rates to timed-AI of heifers fed camelina meal

Summary of Experimental Supplement input Costs

The CP content of crude glycerin is nearly zero. Thus, whenever glycerol replaces corn grain, additional protein should be added to balance the CP content of the diet. Inclusion of crude glycerin and corn gluten meal at 15 and 2% (as fed basis) of the supplements, respectively, resulted in equal daily cost between the control and glycerin treatments (Table 7). This occurred despite crude glycerin being purchased at approximately $0.03/kg more than it should have been worth according to estimates of Hess (2007). Using the actual cost of the camelina meal purchased for this experiment, daily cost was $0.05/heifer less for camelina meal than the control and glycerin treatments.

Heifers fed crude glycerin had similar cost per pregnant heifer ($25.1/pregnant heifer) compared with heifers fed the control supplement ($25.6/pregnant heifer). Heifers fed camelina meal had the least cost per pregnant heifer ($16.0/pregnant heifer). Therefore, using prices actually paid for supplemental ingredients in this experiment, camelina biodiesel co-products are economically feasible when compared with feeding supplements containing corn and soybean meal.

Conclusion

In conclusion, feeding camelina meal to developing replacement beef heifers did not decrease the synthesis of thyroid hormones, and it increased concentrations of fatty acids in plasma. Feeding camelina meal

at 0.33% of average BW or an average of 145 g/d crude glycerin did not affect growth or overall reproductive performance of peripubertal beef heifers. Therefore, camelina coproducts (meal and crude glycerin) are suitable replacements for conventional corn-soybean meal supplements when offered to replacement beef heifers for 60 d before estrus synchronization. Including crude glycerin at 15% of the supplement, did not have an advantage on a daily cost per heifer basis because additional protein must be added when corn is replaced with crude glycerin. Using prices actually paid for the experimental ingredients, the camelina meal supplement cost less per heifer daily and per pregnant heifer. Therefore, camelina biodiesel co-products should be economically feasible supplemental ingredients for developing replacement heifers.

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Table 1. Composition of the hay and supplements[1] fed to developing replacement heifers for 60 d before the breeding season.

		Supplements[1]		
Ingredients	Hay	Control	Camelina	Glycerin
DM, %	93.17	90.20	91.15	86.73
IVDMD, %	61.21	92.55	70.57	93.78
	----------*% of DM*----------			
OM	91.64	93.11	94.74	94.59
NDF	66.62	10.75	34.75	8.95
ADF	39.30	5.85	15.95	5.22
CP	8.21	27.83	23.46	24.54

[1]Supplements (as-fed) consisted of 50% finely ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% finely ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).

Table 2. Average fatty acid composition of the hay and supplements[1] offered to developing replacement beef heifers for 60 d before the breeding season.

Item	Hay	Supplements[1]		
		Control	Camelina	Glycerin[2]
	----------% of DM----------			
Total fatty acid	1.30	2.70	11.40	2.54
	----------*% of total fatty acids*----------			
16:0	19.61	16.24	7.51	16.22
18:0	0.37	2.95	2.44	2.90
18:1*cis*-9	3.70	18.16	16.83	19.97
18:2*n*-6	16.24	51.69	22.75	54.73
18:3*n*-3	36.91	5.31	23.75	5.17
20:1	0.00	0.00	16.00	0.00
22:1*n*-9	0.00	0.00	2.58	0.00
Other fatty acids[3]	23.16	5.65	8.15	1.01

[1]Supplements (as-fed) consisted of 50% finely ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% finely ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).

[2]The fatty acids concentrations of the crude glycerin included in the glycerin supplement was 0.26% of DM (36.9% 16:0, 8.1% 18:0, 34.9% 18:1*cis*-9, and 20.0% 18:2*n*-6).

[3]Fatty acids not identified with purified standards.

Table 3. Dry matter intake and growth performance of developing replacement beef heifers fed supplements[1] for 60 d before the breeding season.

	Control	Camelina	Glycerin	SE[2]	*P*-value
DMI, kg					
Forage	6.67	6.64	6.67	0.01	0.187
Total	7.60	7.57	7.56	0.01	0.089
BW, kg					
d 0	297.4	296.7	296.5	0.6	0.585
d 30	330.5	328.2	329.0	1.3	0.440
d 60	356.2	354.6	356.3	1.1	0.495
60-d gain, kg	59.3	57.3	58.9	1.1	0.221
ADG, kg/d					
d 0 to 30	1.14	1.09	1.12	0.04	0.589
d 31 to 60	0.85	0.87	0.88	0.04	0.630

[1]Supplements (as-fed) consisted of 50% finely ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% finely ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin). Supplements were provided at 0.95 and 0.99 kg·heifer^{-1}·d^{-1} (as-fed) during d 0 through 30 and d 31 through 60, respectively. Hay was offered immediately after supplements were consumed. On the next morning, any hay remaining in the bunks was removed before offering the supplements, weighed and recorded for forage DMI estimate.

[2]n = 10/treatment.

Table 4. Mean concentrations of fatty acids in plasma (mg of fatty acid/g of freeze dried plasma) of developing replacement beef heifers fed supplements[1] for 60 d before the breeding season.

Fatty acids	Control	Camelina	Glycerin	SE[2]	*P*-value
14:0	0.20	0.16	0.17	0.018	0.540
14:1	0.134[b]	0.117[a]	0.124[ab]	0.004	0.014
15:0	0.17	0.18	0.17	0.007	0.502
15:1	0.22	0.23	0.22	0.006	0.409
16:0	2.59[a]	2.76[b]	2.59[a]	0.051	0.047
16:1*trans*-9	0.41[a]	0.51[b]	0.44[ab]	0.025	0.042
16:1*cis*-9	0.25	0.27	0.25	0.008	0.096
17:0	0.17	0.15	0.16	0.005	0.304
17:1	0.06	0.06	0.06	0.006	0.944
18:0	3.36	3.85	3.48	0.151	0.070
18:1*trans*-11	0.18[a]	0.35[b]	0.21[a]	0.037	0.003
18:*1trans*-12	0.001[a]	0.04[b]	0.01[ab]	0.010	0.067
18:1*trans*-13	0.01[a]	0.15[b]	0.03[b]	0.025	0.002
18:1*cis*-9	2.26[a]	3.00[b]	2.39[a]	0.194	0.025
18:1*cis*-10	0.11[a]	0.12[b]	0.11[a]	0.005	0.032
18:1*cis*-11	0.01[a]	0.10[b]	0.03[a]	0.032	0.027
18:1*cis*-12	0.00[a]	0.04[b]	0.003[a]	0.007	0.002
18:2*n*-6	4.67[a]	5.18[b]	4.60[a]	0.129	0.009
20:1*n*-9	0.14	0.15	0.14	0.009	0.615
18:3*n*-3	1.85[a]	2.47[b]	1.99[a]	0.137	0.012
CLA[3]	0.02[a]	0.03[b]	0.02[a]	0.004	0.003
20:3*n*-6	0.30	0.29	0.29	0.008	0.595

22:1*n*-9	0.00[a]	0.01[b]	0.00[a]	0.002	0.001
20:4*n*-6	0.60	0.65	0.61	0.016	0.071
20:5*n*-3	0.37	0.43	0.38	0.019	0.121
22:5*n*-3	0.27	0.26	0.27	0.008	0.415
22:6*n*-6	0.10	0.10	0.11	0.009	0.695
Unidentified[4]	1.84	2.20	2.08	0.305	0.583
Trans-isomers[5]	0.60[a]	1.07[b]	0.71[a]	0.103	0.008
Cis-isomers[6]	9.14[a]	11.19[b]	9.38[a]	0.473	0.010
UFA[7]	11.95[a]	14.60[b]	12.31[a]	0.627	0.012
Total	20.29[a]	23.90[b]	20.96[a]	1.069	0.033

[1]Supplements (as-fed) consisted of 50% finely ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% finely ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).

[2]n = 5 pens/treatment with 3 sampling times (d 0, 30, and 60) in both yrs = 30/treatment.

[3]CLA = *cis*-9, *trans*-11-CLA.

[4]Fatty acids not identified with purified standards.

[5]*Trans*-isomers = 16:1*trans*-9 + 18:1*trans*-10 + 18:1*trans*-11 + 18:1*trans*-12 + 18:1*trans*-13.

[6]*Cis*-isomers = 16:1*cis*-9 + 18:1*cis*-9 + 18:1*cis*-10 + 18:1*cis*-11 + 18:1*cis*-12 + 18:2*n*-6 + 18:3*n*-3.

[7]UFA = unsaturated fatty acids = 14:1 + 15:1 + 17:1 + 20:1*n*-9 + CLA + 20:3*n*-6 + 22:1*n*-9 + 20:4*n*-6 + 20:5*n*-3 + 22:5*n*-3 + 22:6*n*-6 + *trans*-isomers + *cis*-isomers.

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

Table 5. Mean concentrations of thyroid hormones (T_4 and T_3), glucose, insulin, and β-hydroxybutyrate (BHBA) in serum of developing replacement beef heifers fed supplements[1] for 60 d before the breeding season.

	Control	Camelina	Glycerin	SE[2]	*P*-value
T_4, ng/mL	40.4	40.4	40.9	1.5	0.956
T_3, ng/mL	0.89[a]	0.97[b]	0.90[a]	0.02	0.045
Glucose, mg/dL	66.6	67.0	66.0	0.7	0.585
Insulin, ng/mL	0.12	0.11	0.10	0.01	0.440
BHBA, µmol/L	139.5	140.3	148.2	5.3	0.461

[1]Supplements (as-fed) consisted of 50% finely ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% finely ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).

[2]n = 5 pens/treatment with 3 sampling times (d 0, 30, and 60) in both yrs = 30/treatment.

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

Table 6. Reproductive performance of developing replacement beef heifers fed supplements[1] for 60 d before being synchronized for estrus[2].

	Control	Camelina	Glycerin	SE[3]	*P*-value
Detected in estrus, %	42.7	41.5	48.2	8.3	0.825
Pregnancy rates to AI, %					
By heat[4]	54.2	53.3	49.3	13.6	0.965
Timed-AI[5]	24.2[a,b]	43.2[b]	17.5[a]	6.2	0.046
Overall[6]	37.1	45.2	34.0	7.5	0.577
Final pregnancy rate[7], %	61.0	71.2	62.1	5.3	0.376

[1]Supplements (as-fed) consisted of 50% finely ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% finely ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).

[2]On d 60 and 70, each heifer received an intramuscular injection containing a 25 mg of $PGF_{2\alpha}$. Heifers were artificially inseminated 12 h after estrus was first detected. Heifers that were not detected in estrus were given an intramuscular injection containing 100 µg of GnRH (Fertagyl, Intervet, Inc., Millsboro, DE) at 0800 and artificially inseminated by 66 h after the second $PGF_{2\alpha}$ injection. Any heifer detected in estrus by d 75 was inseminated again 12 h after they were detected in estrus.

[3]n = 10/treatment.

[4]First-service pregnancy rate of heifers bred 12 h after being detected in estrus.

[5]First-service pregnancy rates of heifers bred via timed-AI on d 74 after a 2 mL injection of GnRH.

[6]Overall first-service pregnancy rates.

[7]Final pregnancy rates after first-service AI, second-service AI (yr 1) and bull exposure (yr 2).

[a,b]Within a row, means without a common superscript differ ($P < 0.05$).

Table 7. Daily cost of supplementing developing replacement beef heifers fed the control, camelina meal, and crude glycerin supplements[1] for 60 d before the breeding season.

	%	kg·heifer^{-1}·d^{-1}	\$/kg[2]	Cost \$·heifer^{-1}·d^{-1}
Control				
Soybean meal	50	0.49	0.33	0.16
Finely ground corn	50	0.49	0.20	0.10
Total	100	0.97		0.26
Glycerin				
Soybean meal	50	0.49	0.33	0.16
Finely ground corn	33	0.32	0.20	0.06
Crude glycerin	15	0.14	0.11	0.02
Corn gluten meal	2	0.02	1.11	0.02
Total	100	0.97		0.26
Camelina meal	100	0.97	0.20	0.19

[1]Supplements (as-fed) consisted of 50% finely ground corn and 50% soybean meal (Control), mechanically extracted camelina meal (Camelina), and 50% soybean meal, 33% finely ground corn, 15% crude glycerin, and 2% corn gluten meal (Glycerin).

[2]Total cost of camelina meal was equal to the cost of camelina seeds (\$0.12/kg) plus the cost (\$0.08/kg) of transporting seed to and meal from the processing plant.