The Professional Animal Scientist 30 (2014):502–509; http://dx.doi.org/10.15232/pas.2014-01311

Reproductive performance and serum fatty acid profiles of underdeveloped beef heifers supplemented with saturated or unsaturated rumen bypass fat compared to an isocaloric control

N. M. Long,*[1] T. A. Burns,* S. K. Duckett,* and D. W. Schafer†
*Department of Animal and Veterinary Science, Clemson University, Clemson, SC, 29634; and †Department of Animal Science, University of Arizona, Tucson 85721

ABSTRACT

Research involving fat supplementation to heifers has shown positive reproductive effects, but the effect of lipid composition has had little investigation. Heifers (n = 118) were blocked by age, breed, and BW and pen-fed alfalfa hay. Heifers were individually fed 1 of 3 isocaloric supplements: control (CON) or either half the CON diet with 0.2 kg of a rumen-protected unsaturated fatty acid source (USFA) or a rumen-protected saturated fatty acid source (SFA) fed 5 d/wk. Heifers received treatments for 3 wk before starting the 7-d controlled intravaginal drug release estrus synchronization protocol. Heifers remained on treatment for 28 d after AI. Pregnancy was determined at d 30 post-AI via transrectal ultrasonography and by rectal palpation at 150 d post-AI. Serum progesterone and leptin were measured by RIA and serum triglycerides; cholesterol was measured by colorimetric procedures. Heifers BW gain during supplementation was similar (P = 0.35) between treatment groups (32 ± 2 kg). Percentage of heifers cycling tended to be less (P = 0.08) for USFA heifers compared with CON and SFA treatments (36 vs. 55 and 56%, respectively). Pregnancy rates by AI of heifers detected in estrus were similar (P = 0.35) between treatments. Serum total and specific fatty acids, cholesterol, and triglycerides were greater (P < 0.05) at d 21 and 56 in fat-supplemented heifers compared with CON heifers. At d 56 of treatment, USFA had greater (P < 0.05) plasma leptin compared with SFA, with CON heifers having further reduced serum leptin concentration. Feeding rumen-protected fats to heifers increased circulating lipid and leptin, but did not influence reproduction rates.

[1] Corresponding author: nlong2@clemson.edu

Key words: fat, heifer, reproduction, serum fatty acid

INTRODUCTION

Previous research has been focused on management practices that may improve reproductive efficiency in yearling heifers, which may affect lifetime reproductive and cow performance. Wiltbank et al. (1969) demonstrated that a high level of nutrition decreased the age of puberty onset. Gasser et al. (2006) reported that increasing dietary energy intake in early weaned heifers decreased age at puberty regardless of the diet fed. Conception occurring earlier, during the first breeding season for a heifer, resulted in positive effects on reproductive performance for that cow for the rest of her lifetime (Lesmeister et al., 1973). It has also been shown heifers that calve earlier in their first

calving season weaned heavier calves through 6 parturitions (Cushman et al., 2013).

The use of dietary fats in beef heifers have been thoroughly reviewed by Hess et al. (2008), who indicated that fat feeding of heifers resulted in increased pregnancy rates but raised concerns of differences in response between well developed and underdeveloped heifers. Rumen-protected fat has been used to meet or exceed energy needs of cattle while minimizing DMI reductions and decreasing OM digestion associated with fat supplementation (Spicer et al., 1993; Moallem et al., 1997; Filley et al., 2000). Feeding of heifers a rumen-protected fat source for 60 d before the breeding season resulted in increased pregnancy rates compared with heifers fed only the carrier (Long et al., 2007). Recent research has shifted to feeding fat for a shorter time before breeding and extending supplementation for a period after insemination (Hess et al., 2008). Long-term feeding of diets with different fatty acid profiles starting at 7 mo of age resulted in no difference in the amount of heifers pubertal at 10, 12, or 14 mo of age, and heifers fed a diet high in fat tended to have a lower conception rate compared with control heifers (Shike et al., 2013). Little research has been done utilizing rumen-protected fats that are saturated or unsaturated before and after AI in beef heifers. We hypothesized that feeding a rumen-protected fat source before and after AI will improve beef heifer conception rates and overall reproductive efficiency with possible different effects of fatty acid source.

MATERIALS AND METHODS

Animals

All animals were handled in accordance with procedures approved by the University of Arizona Animal Care and Use Committee. *Bos taurus* heifers (n = 120) were blocked by age (range 320–430 d), breed (polled Hereford, Hereford cross, or Angus cross), BW (261 ± 3 kg), BCS, and reproductive tract score (LeFever and Odde 1986) into 1 of 3 groups, resulting in 40 heifers/treatment groups. All heifers were pen-fed alfalfa hay (1.37 mCal/kg of NE_m, 26.3 % ADF, and 16.8 % CP on a DM basis, as analyzed by commercial laboratory, Serve-Tech Laboratories, Dodge City, KS) at 4.5 kg/heifer per day at 0700 h each day. Then, heifers were individually fed 1 of 3 isocaloric supplements: a control (CON), which contained 90 % beet pulp (1.21 mCal/kg of NE_m, 19.1% ADF, and 9.5% CP) and 10% molasses at 1.0 kg as fed, or either 0.5 kg of the CON with 200 mg of Strata 113 (**USFA**) or EnerGII (**SFA**; Virtus Nutrition, Corcoran CA) fed 5 d/wk at 1700 h along with 1.4 kg of sudangrass hay (1.21 Mcal/kg of NE_m, 35.8 % ADF, and 8.2 % CP on a DM basis, as analyzed by a commercial laboratory). Nutrient composition and fatty acid composition of the fat sources are provided in Table 1. Heifers received the supplement for 3 wk before the controlled intravaginal drug release (**CIDR**; Pfizer Animal Health, New York, NY) was inserted for 7 d. Blood samples were collected at 0700 h before morning feeding 12 d before CIDR insertion and then every 4 d until CIDR insertion. Blood was collected by tail venipuncture into evacuated blood collection tubes (BD Vacutainer, Franklin Lakes, NJ). Blood was allowed to coagulate for approximately an hour at room temperature and then refrigerated at 4°C overnight before centrifugation at 2,000 × *g* for 20 min. Serum was aliquoted and stored at −20°C pending analysis. Heifers were detected for estrus every 12 h after the prostaglandin $F_{2\alpha}$ (**$PGF_{2\alpha}$**; Lutalyse, Pharmacia and Upjohn Co., Kalamazoo, MI) injection at the time CIDR was removed. Heifers were then artificially inseminated via a single sire for polled Hereford heifers and 2 sires for the crossbreed heifers 12 h after estrus was detected following the AM/PM rule. Estrus detection was aided with the use of Estrotect heat detection patches (Rockway Inc., Spring Valley, WI). Only heifers that were detected in estrus were inseminated. Heifers remained on treatment supplementation for 28 d after insemination. One heifer from both of the fat-supplemented treatment groups was removed from the experiment due to bloat associated with alfalfa hay on the same day. A blood sample before morning feeding was collected at the end of the supplementation period as previously described. Heifers were exposed to a bull that had passed a breeding soundness exam starting at 7 d post-AI for an 80-d natural service breeding period. Pregnancy to AI was determined 30 to 32 d post-AI via transrectal ultrasonography (Aloka 500-V with a 7.5-MHz probe, Corometrics Medical Systems, Wallingford, CT). Overall pregnancy rates were determined at ~150 d post-AI by rectal palpation.

Blood Hormone and Lipid Analysis

From the serum samples collected before CIDR insertion, progesterone concentrations were analyzed by RIA with a sensitivity of 0.10 ng/mL (Siemens Medical Solutions Diagnostics, Los Angeles, CA; Long et al., 2010). Serum progesterone concentrations greater than 1.0 ng/mL in any of the 4 samples were used as the criteria to establish heifers that were pubertal; from these data, the percentage of heifers in each group that are in estrous at start of the synchronization protocol was determined. Serum samples (8 randomly chosen per treatment) at CIDR insertion and at the end of the supplementation were analyzed for plasma lipid profile via gas chromatograph. Briefly, duplicate 1-mL samples of serum were lyophilized for 24 h and then transmethylated according to the method of Park and Goins (1994). An Agilent 6850 gas chromatograph equipped with an Agilent 7673A automatic sampler (Agilent Technologies Inc., Santa Clara, CA) was used for this analysis. Separations were accomplished using a 100-m Supelco SP-2560 (Supelco, Inc., Bellefonte, PA) capillary column (0.25 mm i.d. and 0.20 μm film thickness), according to Duckett et al. (2002). Individual fatty acids were

Table 1. Nutrient analysis and fatty acid (FA) composition of fat sources used to supplement heifers[1]

Item	Unsaturated[2]	Saturated[2]
DM, %	98	98
Calcium, %	9.3	9.2
Ether extract, %	80.6	82.5
Total FA, % DM	80.1	81.8
Total FA, % ether extract	99.3	99.1
C12:0, % total FA DM	2.6	0.4
C14:0, % total FA DM	4.5	1.2
C16:0, % total FA DM	21.4	50.1
C16:1, % total FA DM	8.8	0.2
C18:0, % total FA DM	9.2	4.3
C18:1t, % total FA DM	2.0	0.3
18:1c, % total FA DM	21.3	35.2
18:2, % total FA DM	6.1	8.1
18:3, % total FA DM	2.0	0
20:5, % total FA DM	8.3	0
22:6, % total FA DM	8.6	0
Other FA, % total FA DM	5.2	0.2

[1]Data provided by Kevin Murphy (Virtus Nutrition, Corcoran, CA).

[2]Unsaturated fat source from Strata 113 and saturated fat source from EnergII (Virtus Nutrition).

identified by comparison of retention times with standards (Sigma, St. Louis, MO; Supelco; Matreya, Pleasant Gap, PA). The fatty acids were quantified by incorporating an internal standard, methyl tricosanoate (C23:0) acid, into each sample during methylation and expressed as a percentage of total fatty acids. In addition, 24 heifers per treatment group were randomly chosen based on pregnancy status to AI for triglycerides and cholesterol analysis from serum samples collected at CIDR insertion and the end of the supplementation. Heifers were blocked by treatment to assay plate (n = 3 plates per time period). Plasma triglyceride and cholesterol concentrations were determined by colorimetric procedures using a 96-well plate with standards and liquid reagents (Pointe Scientific, Inc., Canton, MI). Serum samples from 15 heifers/treatment at CIDR insertion and the end of supplementation were analyzed for serum leptin concentrations by RIA (Multispecies leptin RIA, Linco Reseach, St. Charles, MO), previously validated by Long and Schafer (2013).

Statistical Analysis

Heifer age, BW, BCS, and reproductive tract scores were analyzed within day of treatment using the GLM procedure of SAS (SAS Institute Inc., Cary, NC) with treatment in the model. Binary reproductive data was analyzed using the GLIMMIX procedure of SAS with treatment in the model and for AI pregnancy rates and AI technician (n = 2); bull was also included in the model statement as a random effect. Serum fatty acids, triglyceride, and cholesterol were analyzed with the GLM procedure of SAS within day of treatment with treatment in the model statement. Serum leptin was analyzed as repeated measurements using the MIXED model of SAS with treatment and day of treatment and their interaction in the model statement. Data are presented as least squares means ± SEM and considered significantly different when $P \leq 0.05$; a tendency was indicated when $P \leq 0.10$.

Table 2. Body weight, BCS, and reproductive tract scores of heifers fed hay and individually fed an isocaloric supplement containing no bypass fat or either a saturated or unsaturated rumen bypass fat source at the beginning, middle, and end of supplementation

Item	Control	Unsaturated[1]	Saturated[1]	*P*-value
n	40	39	39	
Age at start of experiment, d	377 ± 3	380 ± 3	379 ± 3	0.787
BW at start of experiment, kg	260 ± 5	261 ± 5	261 ± 5	0.979
BCS at start of the experiment	5.3 ± 0.1	5.5 ± 0.1	5.4 ± 0.1	0.573
Reproductive tract score at start of experiment	2.8 ± 0.1	3.0 ± 0.1	2.9 ± 0.1	0.584
BW at CIDR insertion (d 21 of treatment), kg	280 ± 5	282 ± 5	281 ± 5	0.962
BW at end of experiment (d 56 of treatment), kg	293 ± 5	291 ± 5	295 ± 5	0.854
BCS at end of experiment	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	0.982
BW gain during experiment, kg	33 ± 2	30 ± 2	34 ± 2	0.354

[1]Individually fed 0.2 kg of either an unsaturated fat source from Strata 113 or a saturated fat source from EnergII (Virtus Nutrition, Corcoran, CA).

Table 3. Percentage of heifers cycling at controlled intravaginal drug release (CIDR) insertion and AI and total breeding season conception rates in heifers fed hay and individually fed an isocaloric supplement containing no bypass fat or either a saturated or unsaturated rumen bypass fat source

Item	Control	Unsaturated[1]	Saturated[1]	*P*-value
n	40	39	39	
Exhibiting estrous at CIDR insertion, %	55	36	56	0.08
AI conception rate in heifers detected in estrus, % of AI heifers	81	77	63	0.58
AI conception rate total, % of exposed heifers	43	53	49	0.63
Pregnancy rate at palpation, % of exposed heifers	84	84	87	0.89

[1]Individually fed 0.2 kg of either an unsaturated fat source from Strata 113 or a saturated fat source from EnergII (Virtus Nutrition, Corcoran, CA).

RESULTS AND DISCUSSION

Heifer age, BW, BCS, and reproductive tract score at the beginning and BW and BCS throughout the experiment are presented in Table 2. Age at the start of the experiment was not different between treatments ($P = 0.79$) and averaged 379 ± 3 d. The range of age at the start of the experiment was 320 to 430 d; this was a result of almost a 100-d breeding season that produced the heifers used in the current experiment. Heifer BW at the start of the experiment averaged 261 kg and was not different between treatments ($P = 0.98$). This BW is ~52% of a 500-kg mature cow weight, which is the goal at the university ranch. Heifer BW gain during treatment was similar ($P = 0.35$) between treatments. This should be expected given the individual isocaloric supplementation and the hay being pen fed at a similar amount per heifer per day. Heifer BCS was similar at the beginning and end of the supplementation period ($P = 0.57$ and 0.98 respectively).

Ours is the first report of supplementation of 2 different rumen bypass fats that differ in lipid composition in beef heifers that investigated reproductive performance associated with lipid type. The use of underdeveloped heifers, heifers that are only ~52 % of mature BW, is a unique, unplanned factor in this experiment and represents a common scenario in the US beef industry. This level of heifer development is within a range of recent research reports that developed heifers to between 50 and 55% of mature BW and reduced development cost without reductions in total breeding pregnancy rates (Martin et al., 2008; Roberts et al., 2009). The lack of differences in final BW and BCS and overall BW gain during supplementation indicated the supplements were isocaloric as formulated.

Even though heifers were allotted to treatment based on age, BW, and reproductive tract scores to prevent differences between treatments, the heifers in the USFA treatment had a tendency for a reduced estrous cycle rate at the time of CIDR insertion compared with the other treatment groups ($P = 0.08$; Table 3). Reproductive performance of heifers was similar between treatment groups (Table 3). A difference was noted in the estrous behavior in the heifers, with more heifers in the 2 fat-supplemented treatments in estrus at 49 to 56 h after CIDR removal ($P = 0.04$, Table 4), as well as a trend for total heifers detected in estrus ($P = 0.09$, Table 4). There is limited information on the effects of supplemental fats on estrous activity in cattle. It has

Table 4. Number of heifers detected in estrus in hours after a prostaglandin $F_{2\alpha}$ injection at controlled intravaginal drug release removal in heifers fed hay and individually fed an isocaloric supplement containing no bypass fat or either a saturated or unsaturated rumen bypass fat source

Treatment	<48 h	49–56 h	57–68 h	69–80 h	81–92 h	≥93 h	Total
Control	8	4[a]	4	3	1	1	21[A]
Unsaturated[1]	5	12[b]	3	3	2	2	27[AB]
Saturated[1]	6	11[b]	3	4	3	3	30[A]

[a,b]Means within a time period between treatments with different lowercase superscripts differed ($P = 0.04$).

[A,B]Means within a time period between treatments with different uppercase superscripts differed ($P = 0.09$).

[1]Individually fed 0.2 kg of either an unsaturated fat source from Strata 113 or a saturated fat source from EnergII (Virtus Nutrition, Corcoran, CA).

been shown in postpartum cows that the duration of estrus or number of mounts received was not influenced by BCS at calving or postpartum protein supplementation (Lents et al., 2008). However, it has been reported that BCS tended to influence estrus length in postpartum cows treated with exogenous estrogen (Long et al., 2009). In postpartum lactating beef cows, oilseed supplementation did not influence the number of mounts received or percent of cows that were nonresponders after the 7-d CIDR synchronization protocol (Scholljegerdes et al., 2011).

The tendency for reduced cycling rates at the start of synchronization in USFA-supplemented heifers coupled with similar AI pregnancy rates could indicate some positive reproductive effects of USFA supplementation. It has been reported that feeding supplement before the breeding season is probably the most effective time to supplement fat to heifers (Hess et al., 2008). This has been confirmed by Long et al. (2007), who showed that feeding rumen-protected saturated fat to heifers for 60 d before breeding resulted in greater reproductive rates in heifers. The use of unsaturated fats in cows and heifers has been recently investigated, with supplementation beginning before breeding and being maintained after breeding (Moriel et al., 2012). The period of supplementation before AI should allow the difference in plasma lipids to develop and influence the ovary, and possibly the uterus. Post-AI supplementation should allow for fatty acids to possibly affect pregnancy loss. In dairy cattle, feeding flaxseed results in improved embryo quality (Thangavelu et al., 2007), decreased pregnancy loss (Ambrose et al., 2006), and increased conception rate (Petit et al., 2002; Ambrose et al., 2006). In heifers, feeding oilseeds from 9 d before timed AI until 18 d post-AI resulted in no difference in conception rate to AI (Scholljegerdes et al., 2011). Using rumen-protected unsaturated fat sources in lactating postpartum beef cows resulted in no difference in reproduction when supplemented from 10 d before

Table 5. Total and specific fatty acids in serum of heifers individually fed an isocaloric supplement containing no bypass fat or either a saturated or unsaturated rumen bypass fat source

	d 21 of feeding					d 56 of feeding				
Item, μg/mL (unless otherwise noted)	Control	Unsaturated[1]	Saturated[1]	SEM	*P*-value	Control	Unsaturated[1]	Saturated[1]	SEM	*P*-value
Serum (n)	8	8	8			8	8	8		
14:0	7.44	7.08	7.13	0.64	0.909	6.86	12.40	6.99	1.21	0.005
15:0	12.99	15.75	12.82	1.18	0.167	11.21	14.20	10.55	1.12	0.069
16:0	131.28	158.28	172.59	9.29	0.016	87.29	149.62	181.34	11.16	<0.001
16:1	10.66	23.05	9.67	1.43	<0.001	8.82	29.05	10.48	2.27	<0.001
17:0	18.92	16.95	17.49	1.25	0.293	9.79	13.49	11.53	1.85	0.383
18:0	167.62	154.92	194.96	10.2	0.033	119.71	119.21	182.54	10.03	<0.001
18:1 *cis*-9	125.37	95.81	145.39	9.32	0.004	76.97	71.43	178.75	7.19	<0.001
18:2	202.73	250.67	423.82	26.48	<0.001	159.15	205.31	455.40	24.59	<0.001
18:3	108.70	126.97	113.00	9.97	0.415	82.70	88.19	93.16	8.33	0.679
20:4	28.75	57.94	38.37	4.02	<0.001	24.75	55.86	40.15	4.84	<0.001
20:5	22.24	141.92	21.17	7.83	<0.001	18.25	186.43	19.71	17.42	<0.001
22:5	19.32	21.68	16.85	1.42	0.077	13.99	21.38	16.56	1.71	0.020
22:6	5.28	19.52	4.86	0.76	<0.001	3.53	19.09	3.63	1.68	<0.001
Total FA	852.98	1,194.21	1,295.62	78.64	0.002	714.12	1,091.89	1,306.91	87.73	<0.001

[1]Individually fed 0.2 kg of either an unsaturated fat source from Strata 113 or a saturated fat source from EnergII (Virtus Nutrition, Corcoran, CA).

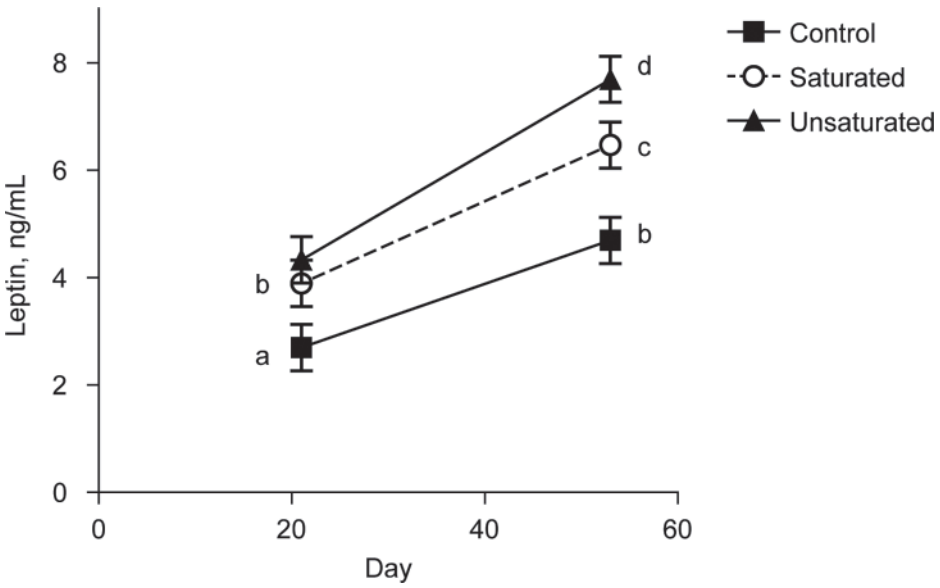

Figure 1. Serum leptin concentrations in heifers fed an isocaloric supplement given 5 d/wk (control; n = 15), or a supplement containing 200 g of saturated (n = 15) or unsaturated (n = 15) rumen-protected fat sources at d 21 and 56 of treatment. Letters that differ (a–d) show means differ ($P < 0.05$).

to 30 d after timed AI (Moriel et al., 2012). Other reports have shown that rumen-protected PUFA supplemented before and for 28 d after timed AI improved pregnancy rates in *Bos indicus* cows (Lopes et al., 2009). Feeding of fish meal, a naturally high source of n-3 fatty acids, to beef cows has been shown to be able to change endometrial n-3 fatty acid composition (Burns et al., 2003). In addition, fish meal supplementation has been shown to decrease uterine $PGF_{2\alpha}$ synthesis in heifers that have low serum progesterone (Wamsley et al., 2005).

The serum analyses, unlike the reproductive results, show a clear difference between treatments. In Table 5, total and specific serum fatty acids are given at d 21 and 56 of feeding. At d 21 of feeding, USFA increased ($P < 0.01$) serum 16:1, 20:4, 20:5 (eicosapentaenoic acid), and 20:6 (docahexaenoic acid) concentrations compared with CON and SFA heifers. At d 21 of treatment, USFA-treated heifers had reduced ($P < 0.01$) 18:1c9 and increased ($P < 0.05$) 16:0 serum fatty acids concentrations compared with control heifers. Treatment of heifers with SFA resulted in increased ($P < 0.05$) serum concentrations of 16:0, 18:0, 18:1c9, and 18:2 fatty acids compared with CON and USFA-treated heifers. Heifers supplemented with SFA had increased ($P < 0.05$) serum 20:4 fatty acids compared with CON heifers. At d 21 of treatment, total serum fatty acids were increased ($P < 0.01$) in both fat-supplemented treatment groups compared with CON heifers. At d 56 of feeding, USFA-treated heifers had increased ($P < 0.02$) serum concentrations of 16:1, 20:4, 20:5 (eicosapentaenoic acid), 22:5 (docasapentaenoic acid), and 22:6 (docahexaenoic acid) compared with CON and SFA-treated heifers. At d 56 of treatment, USFA-treated heifers had increased ($P < 0.01$) serum 16:0 and total fatty acids concentrations compared with CON heifers. Heifers supplemented with SFA had increased ($P < 0.01$) serum 16:0, 18:0, 18:1c9, 18:2, and total fatty acids compared with CON and USFA-treated heifers. Serum leptin was increased in both of the fat supplement treatments at d 21 of treatment ($P < 0.05$; Figure 1). However at d 56 of treatment, the USFA heifers had greater plasma leptin compared with SFA-treated heifers, with CON heifers having a lower serum leptin concentration ($P < 0.05$). Serum triglycerides and cholesterol at d 21 (CIDR insertion) and d 56 are shown in Figure 2. Heifers that were supplemented with rumen bypass lipid, regardless of lipid type, had elevated serum triglycerides and cholesterol concentrations compared with control heifers ($P < 0.05$) at d 21 of supplementation. This difference in serum triglycerides and cholesterol concentrations remained ($P < 0.05$) at d 56 of supplementation.

The current experiment is also the first, to our knowledge, demonstrating that supplementing unsaturated fats can increase serum leptin to a greater extent than supplementation of an isocaloric amount of saturated fats. Leptin is an adipose-derived hormone that acts on the hypothalamus as an indicator of energy homeostasis and regulates food intake (Chilliard et al., 2005). The role of nutritional intake or specific nutrients on plasma leptin is still not completely known. Plasma insulin has been shown to be increased along with leptin in beef cows fed a diet high in CP and energy (Ciccioli et al., 2003). When supplementation ended, both plasma leptin and insulin decreased as soon as 1 wk after diet change, indicating that insulin may influence leptin secretion. This conclusion is supported by the observation that insulin has been shown to increase the expression and secretion of leptin from bovine adipose tissue (Houseknecht et al., 2000). In the current experiment, insulin was not measured, thus the role of insulin cannot be determined. Propionate has been shown to not be an important regulator of plasma leptin in dairy cows (Bradford et al., 2006). Supplementation of dietary lipids in the form of calcium salts have been shown to increase serum leptin in beef heifers (Long et al., 2007). This experiment confirms the findings of the previous research and expands on them and shows that lipid composition further influences serum leptin.

Some discussion of the biohydrogenation or rumen degradation of calcium salts of fatty acids has occurred, particularly regarding the calcium salts containing unsaturated fatty acids. Work in dairy cows indicated that calcium salts of fish oil at 2 different

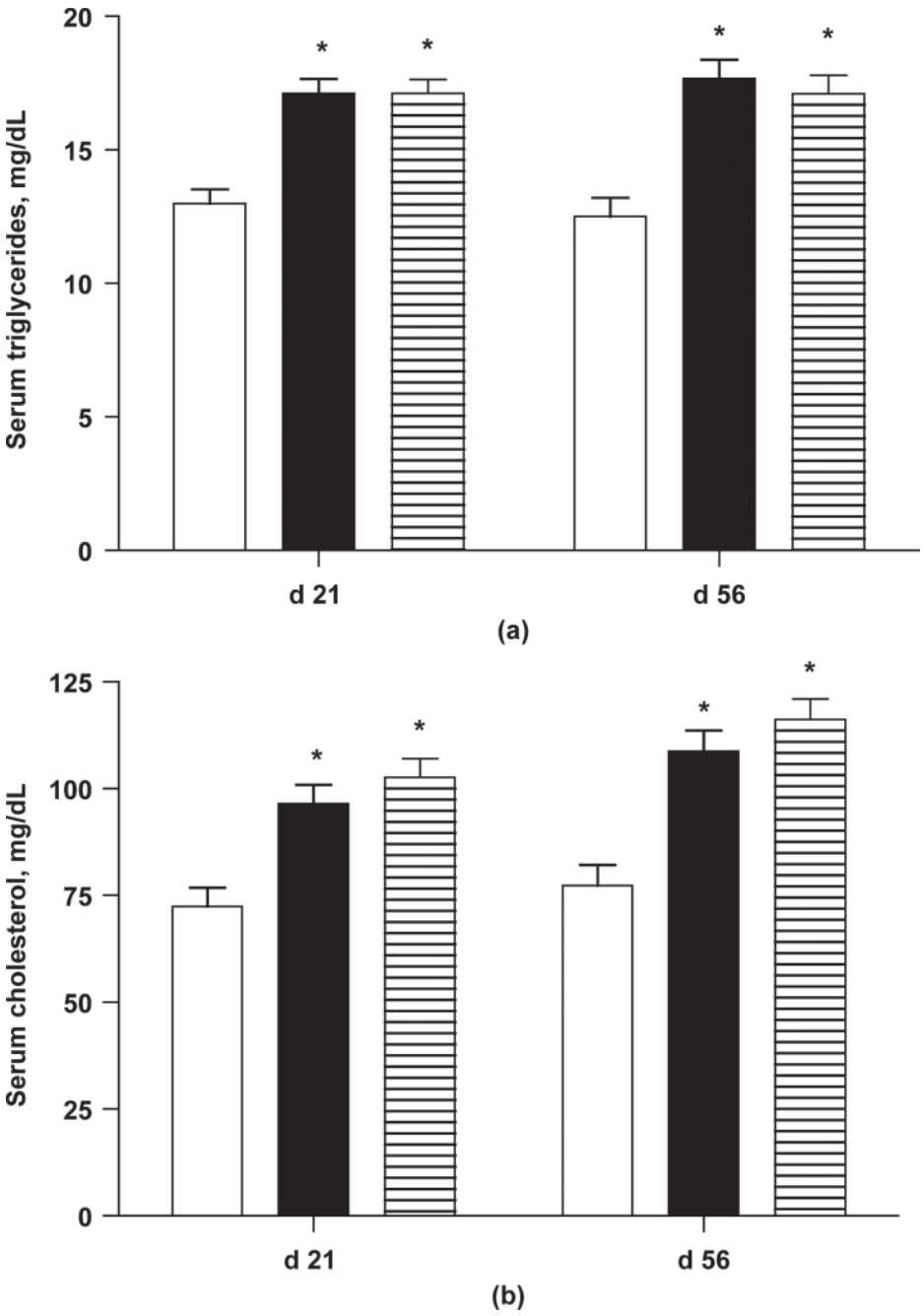

Figure 2. Plasma triglycerides (A) and cholesterol (B) concentration of heifers fed an isocaloric supplement given 5 d/wk (control; open bar; n = 24), or a supplement containing 200 g of saturated (solid bar; n = 24) or unsaturated (hatched bar; n = 24) rumen-protected fat sources at d 21 and 56 of treatment. An asterisk (*) represents differing ($P < 0.001$) means compared with control within a day.

doses resulted in extensive rumen biohydrogenation (Castañeda-Gutierrez et al., 2007). This fact may not be true for beef cows. Ashes et al. (1992) found that unprotected fish meal was able to escape rumen biohydrogenation and result in an increased plasma n-3 fatty acid composition. Burns et al. (2003) showed that supplementing fish meal to beef cows was able to increase plasma and endometrial n-3 fatty acids. The specific fatty acid sources used in the current experiment have been shown to increase n-3 fatty acids in muscle and liver of forage finished steers (Rule et al., 2011). The plasma fatty acid composition in our experiment showed the supplementation strategy resulted in a drastic increase in long chain unsaturated fatty acids, such as eicosapentaenoic acid and docahexaenoic acid.

IMPLICATIONS

Isocaloric supplementation of calcium salts of fatty acids increased plasma triglycerides and cholesterol and increased the number or heifers in estrous earlier after $PGF_{2\alpha}$ administration. After 28 d of supplementation, serum leptin was increased in heifers treated with dietary lipid. Heifers supplemented with USFA had greater serum leptin at the end of supplementation compared with heifers supplemented with SFA. There appeared to be possible positive reproductive effects associated with USFA supplementation, but a need for further research exists to confirm this fact. This conclusion is in addition to increased insulin sensitivity that has been shown in growing steers to be associated with feeding of USFA (Cartiff et al., 2013).

ACKNOWLEDGMENTS

The authors acknowledge support from Virtus Nutrition (Corcoran, CA) and Zoetis (Florham Park, NJ) in the form of product. The authors thank K. G. Cannon and K. O. Cannon (Department of Animal Science, University of Arizona) for animal care and assistance with collection of samples. The authors thank S. Loughlin and A. Manley (Department of Animal and Veterinary Science, Clemson University) for assistance with laboratory work

LITERATURE CITED

Ambrose, D. J., J. P. Kastelic, R. Corbett, P. A. Pitney, H. V. Petit, J. A. Small, and P. Zalkovic. 2006. Lower pregnancy losses in lactating dairy cows fed a diet enriched in alpha-linolenic acid. J. Dairy Sci. 89:3066–3074.

Ashes, J. R., B. D. Siebert, S. K. Gulati, A. Z. Cuthbertson, and T. W. Scott. 1992. Incorporation of n-3 fatty acids of fish oil into tissue and serum lipids of ruminants. Lipids 27:629–631.

Bradford, B. J., M. Oba, R. E. Ehrhardt, Y. R. Boisclair, and M. S. Allen. 2006. Propionate is not an important regulator of plasma leptin concentrations in dairy cattle. Domest. Anim. Endocrinol. 30:65–75.

Burns, P. D., T. E. Engle, M. A. Harris, R. M. Enns, and J. C. Whittier. 2003. Effect of fish meal supplementation on plasma and endometrial fatty acid composition in nonlactating beef cows. J. Anim. Sci. 81:2840–2846.

Cartiff, S. E., V. Fellner, and J. H. Eisemann. 2013. Eicosapentaenoic and docasahexaenoic acids increase insulin sensitivity in growing steers. J. Anim. Sci. 91:2332–2342.

Castañeda-Gutierrez, E., M. J. de Veth, A. L. Lock, D. A. Dwyer, K. D. Murphy, and D. E. Bauman. 2007. Effects of supplementation with calcium salts of fish oil on n-3 fatty acids in milk fat. J. Dairy Sci. 90:4149–4156.

Chilliard, Y., C. Devavaud, and M. Bonnet. 2005. Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. Domest. Anim. Endocrinol. 29:3–22.

Ciccioli, N. H., R. P. Wettemann, L. J. Spicer, C. A. Lents, F. J. White, and D. H. Keisler. 2003. Influence of body condition at calving and postpartum nutrition on endocrine function and reproductive performance of primiparous beef cows. J. Anim. Sci. 81:3107–3120.

Cushman, R. A., L. K. Kill, R. N. Funston, E. M. Mousel, and G. A. Perry. 2013. Heifer calving date positively influence calf weaning weights through six parturitions. J. Anim. Sci. 91:4486–4491.

Duckett, S. K., J. G. Andrae, and F. N. Owens. 2002. Effect of high oil corn or added corn oil on ruminal biohydrogenation of fatty acids and conjugated linoleic acid formation in beef steers fed finishing diets. J. Anim. Sci. 80:3353–3360.

Filley, S. J., H. A. Turner, and F. Stormshak. 2000. Plasma fatty acids, prostaglandin $F_{2\alpha}$ metabolite, and reproductive response in postpartum heifers fed rumen bypass fat. J. Anim. Sci. 78:139–144.

Gasser, C. L., E. J. Behlke, D. E. Grum, and M. L. Day. 2006. Effect of timing of feeding a high-concentrate diet on growth and attainment of puberty in early weaned heifers. J. Anim. Sci. 84:3118–3122.

Hess, B. W., G. E. Moss, and D. C. Rule. 2008. A decade of developments in the area of fat supplementation research with beef cattle and sheep. J. Anim. Sci. 86(E. Suppl.):188–204.

Houseknecht, K. L., C. P. Portocarrero, S. Ji, R. Lemenager, and M. E. Spurlock. 2000. Growth hormone regulates leptin gene expression in bovine adipose tissue: correlation with adipose IGF-1 expression. J. Endocrinol. 164:51–57.

LeFever, D. G., and K. G. Odde. 1986. Predicting reproductive performance in beef heifers by reproductive tract evaluation before breeding. Pages 13–15 in CSU Beef Program Report. Colorado State Univ., Fort Collins.

Lents, C. A., F. J. White, N. H. Ciccioli, R. P. Wettemann, L. J. Spicer, and D. L. Lalman. 2008. Effects of body condition score at parturition and postpartum protein supplementation on estrous behavior and size of the dominant follicle in beef cows. J. Anim. Sci. 86:2549–2556.

Lesmeister, J. L., P. J. Burfening, and R. L. Blackwell. 1973. Date of first calving in beef cows and subsequent calf production. J. Anim. Sci. 36:1–6.

Long, N. M., M. P. Davis, M. J. Prado-Cooper, I. Rubio, and R. P. Wettemann. 2009. Estrus and luteal activity of postpartum beef cows after treatment with estradiol. Prof. Anim. Sci. 25:481–486.

Long, N. M., G. M. Hill, J. F. Baker, W. M. Graves, M. A. Froetschel, D. H. Keisler, and B. G. Mullinix Jr.. 2007. Reproductive performance of beef heifers supplemented with corn gluten feed and rumen-protected fat before breeding. Prof. Anim. Sci. 23:316–324.

Long, N. M., M. J. Nijland, P. W. Nathananielsz, and S. P. Ford. 2010. The impact of early to mid-gestational nutrient restriction on female offspring fertility and hypothalamic-pituitary-adrenal axis response to stress. J. Anim. Sci. 88:2029–2037.

Long, N. M., and D. W. Schafer. 2013. Sex effects on plasma leptin concentrations in newborn and postnatal beef calves. Prof. Anim. Sci. 29:601–605.

Lopes, C. N., A. B. Scarpa, B. I. Cappellozza, R. F. Cooke, and J. L. M. Vasconcelos. 2009. Effects of rumen-protected polyunsaturated fatty acid supplementation on reproductive performance of *Bos indicus* beef cows. J. Anim. Sci. 87:3935–3943.

Martin, J. L., K. W. Creighton, J. A. Musgrave, T. J. Klopfenstein, R. T. Clark, D. C. Adams, and R. N. Funston. 2008. Effect of prebreeding body weight or progestin exposure before breeding on beef heifer performance through the second breeding season. J. Anim. Sci. 86:451–459.

Moallem, U., M. Kaim, Y. Folman, and D. Sklan. 1997. Effect of calcium soaps of fatty acids and administration of somatotropin in early lactation on productive and reproductive performance of high producing dairy cows. J. Dairy Sci. 80:2127–2136.

Moriel, P., E. P. Goncalves, B. I. Cappellozza, V. Nayigihugu, E. Block, and B. W. Hess. 2012. Plasma fatty acids and reproductive performance of lactating beef cows fed rumen-protected fat. Prof. Anim. Sci. 28:89–96.

Park, P. W., and R. E. Goins. 1994. In situ preparation of FAME for analysis of fatty acid composition in foods. J. Food Sci. 59:1262–1266.

Petit, H. V., R. J. Dewhurst, N. D. Scollan, J. G. Proulx, M. Khalid, W. Haresign, H. Twagiramungu, and G. E. Mann. 2002. Milk production and composition, ovarian function, and prostaglandin secretion of dairy cows fed omega-3 fats. J. Dairy Sci. 85:889–899.

Roberts, A. J., T. W. Geary, E. E. Grings, R. C. Waterman, and M. D. MacNeil. 2009. Reproductive performance of heifers offered ad libitum or restricted access to feed for a one hundred forty-day period after weaning. J. Anim. Sci. 87:3043–3052.

Rule, D. C., B. W. Rule, S. Paisley, W. J. Means, K. Underwood, and O. Kucuk. 2011. Supplemental rumen-protected fish oil increases concentrations of long-chain n-3 fatty acids in tissue of grass fed beef. Proc. West. Sect. Amer. Soc. Anim. Sci. 62:390–393.

Scholljegerdes, E. J., L. A. Lekatz, and K. A. Vonnahme. 2011. Effects of short-term oilseed supplementation on reproductive performance in beef heifers. Can. J. Anim. Sci. 91:221–229.

Shike, D. W., F. A. Ireland, and D. B. Faulkner. 2013. Influences of supplemental fat, differing in fatty-acid composition, on performance, plasma fatty-acid content, and reproduction of developing beef heifers. Prof. Anim. Sci. 29:580–586.

Spicer, L. J., R. K. Vernon, W. B. Tucker, R. P. Wettemann, J. F. Hogue, and G. D. Adams. 1993. Effects of inert fat on energy balance, plasma concentrations of hormones, and reproduction in dairy cows. J. Dairy Sci. 76:2664–2673.

Thangavelu, G., M. G. Colazo, D. J. Ambrose, M. Oba, E. K. Okine, and M. K. Dyck. 2007. Diets enriched in unsaturated fatty acids enhance early embryonic development in lactating Holstein cows. Theriogenology 68:949–957.

Wamsley, N. E., P. D. Burns, T. E. Engle, and R. M. Enns. 2005. Fish meal supplementation alters uterine prostaglandin $F_{2\alpha}$ synthesis in beef heifers with low luteal-phase progesterone. J. Anim. Sci. 83:1832–1838.

Wiltbank, J. N., C. W. Kasson, and J. E. Ingallis. 1969. Puberty in crossbred and straightbred beef heifers on two levels of feed. J. Anim. Sci. 29:602–605.