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Effects of dietary energy source and corn oil inclusion on plasma metabolite and lipid profiles and intramuscular adipose and muscle accretion in beef cattle[1]

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SUMMARY

The objectives of this research were to evaluate 1) fluctuations in plasma metabolites and lipid profiles and 2) differences in satellite cell differentiation and proliferation in cattle with differences in marbling relative to total fatness. Dietary starch is thought to optimize accumulation of marbling. In this experiment, readily fermentable fiber or corn oil were substituted for starch from corn in an effort to alter adipose tissue accretion. Crossbred yearling steers (n = 144) were used in a 131 d finishing trial. The trial was designed as a 3 x 2 factorial arrangement of dietary energy source by corn oil inclusion. Dietary energy sources were the high starch diet which contained 8.5% roughage and 81.2% corn or a higher fiber diet in which chopped, high moisture ear corn (43.7%) and dried corn gluten feed (18.2%) were substituted for corn. Corn germ was included in the diet to provide 0, 2, or 6% corn oil in both the starch and fiber diets. Growth performance and carcass characteristics were recorded and blood samples were collected from each animal on d 96 and 131 of the feeding trial. Previously reported carcass data indicated that dietary energy source (starch vs. fiber) did not influence carcass characteristics. However, as the percentage of corn oil increased with additional amounts of corn germ, marbling scores decreased ($P < 0.05$) at a constant subcutaneous fat thickness. Compositional analyses support a tendency ($P \leq 0.10$) for decreased marbling relative to total carcass fat. Feeding germ increases ($P < 0.05$) in all components of serum cholesterol. Earlier in the feeding period, GERM also caused higher ($P < 0.05$) circulating glucose and NEFA concentrations. A sub-population of steers that were highest (n = 12) and lowest (n = 12) for the relationship of marbling to total carcass fatness (M_2Ratio) were scrutinized more closely. Differences in M_2Ratio were not associated with HCW or fatness, but were associated with marbling ($P < 0.001$). Serum collected early in the feeding period from high M_2Ratio steers resulted in higher ($P < 0.05$) satellite cell proliferation and differentiation rates *in vitro* than serum from low M_2Ratio steers. This response diminished with additional days on feed. These results indicate that dietary carbohydrate source has minimal influence on carcass fat distributions, but that dietary oil dramatically alters circulating metabolites and is antagonistic to the production goal of high marbling-high cutability carcasses.

INTRODUCTION

There has been considerable debate within the cattle industry as to whether corn distiller's grains (CDG) causes relatively lower marbling and cutability in fed cattle carcasses. A cursory review of the SDSU data base indicated this may be occurring. Furthermore, Kleinhans et al. (2006) observed that higher

[1] This project was funded by the South Dakota Beef Industry Council and the South Dakota Agricultural Experiment Station.
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inclusion levels of dry milled corn germ caused a substantial decrease in nitrogen retention (NR) by wethers fed high concentrate diets. Reduced NR would be expected to correspond to lower muscle growth, which in turn could be reflected as lower carcass cutability. A trend toward smaller LMA and increased fatness in carcasses of cattle fed comparable diets was observed (unpublished data).

It was hypothesized that the oil fraction of the germ was the causative agent. Corn distiller's grains have relatively lower and more variable corn oil content than corn germ. If corn oil is the causative agent, this would fit the inconsistent observations regarding any relationships between dietary CDG and carcass traits. This experiment was designed to seek insight into mechanisms that may be involved in lowering quality grades and cutability of carcasses when beef cattle consume elevated dietary corn oil via feeding corn processing co-products.

MATERIALS AND METHODS

Cattle Management

Diets for finishing cattle were defined to include no corn germ (CO) or corn germ to provide 2% (LO) or 6% (HI) added corn oil. These levels of germ were fed in high starch (S) or high fiber (F) finishing diets. The higher fiber diets included substitution of chopped high moisture earlage and dried corn gluten feed for rolled high moisture corn and dry whole shelled corn. These diets were used as a 3 x 2 factorial arrangement of treatments with main effects of germ level (GERM, n = 3) and carbohydrate source (CHO, n = 2).

Cattle were fed twice daily. Diet ingredients were sampled each week, and nutrient composition was determined. True diet formulations were calculated from actual ingredient inclusion in batches of feed and the weekly compositional values for each ingredient, (Table 1). Energy values reported are based upon tabular (NRC, 1996) values for each ingredient. Diets were fortified to meet or exceed known nutrient requirements (NRC, 2000). Feed records were summarized at 7-d intervals for compiling diet composition data and for calculating DMI.

These diets were evaluated in 144 steers, initial BW 770 ± 33 lb. To allot steers to treatments, 2 d prior to trial initiation, steers were ranked by BW and then sequentially assigned to CHO diets S or F. Steers were then sorted by CHO diet, ranked by BW and randomly assigned to GERM level. The process was repeated to assign steers to pen replicates (n = 3). At each step in the process, data were tested to confirm that mean BW and variance were similar across main effects. The combination criteria of CHO, GERM, and replicate constituted a pen. There were 18 pens housing 8 steers each, and the allotment process resulted in a representative stratification of initial BW in each pen. Growth performance and carcass characteristics and composition were discussed in previously in the 2009 SDSU Beef Report.

The MRatio is a method for comparing marbling with carcass fatness. The following equation was used:

$$\left[\frac{(\text{Obs Var}_1 - \text{Var}_1 \, \bar{X})}{\text{Var}_1 \, S_d} \right] - \left[\frac{(\text{Obs Var}_2 - \text{Var}_2 \, \bar{X})}{\text{Var}_2 \, S_d} \right]$$

Means and Standard deviations used were from the whole population. When both variables deviate normally, the resulting value equals zero. The MRatio reported used marbling and ribfat depth as variables 1 and 2, respectively. For M_2Ratio calculations, percent carcass fat derived from 9-10-11 rib sections was used as variable 2.

Table 1. True diets formulation and composition[1]

	High Starch						Low Starch					
Germ level	Control		Low		High		Control		Low		High	
Item	$\bar{x}$	S_d	$\bar{x}$	S_d	$\bar{x}$	S_d	$\bar{x}$	S_d	$\bar{x}$	S_d	$\bar{x}$	S_d
Sorghum silage, %	8.64	0.75	8.64	0.75	8.64	0.76	8.39	0.83	8.39	0.83	8.39	0.83
Whole shelled corn, %	41.26	0.55	37.08	0.61	28.98	0.68	25.51	0.80	21.09	0.65	12.16	0.37
High moisture corn, %	39.94	0.38	39.94	0.38	39.93	0.37	--	--	--	--	--	--
High moisture earcorn, %	--	--	--	--	--	--	43.69	1.45	43.69	1.46	43.68	1.49
Soybean meal, %	5.84	0.45	5.47	0.60	4.39	0.73	--	--	--	--	--	--
Germ, %	--	--	4.55	0.08	13.75	0.24	--	--	4.42	0.17	13.37	0.50
Corn gluten feed, %	--	--	--	--	--	--	18.21	0.29	18.21	0.28	18.21	0.27
Supplement[2], %	4.32	0.05	4.32	0.05	4.32	0.05	4.20	0.15	4.20	0.15	4.19	0.14
Monensin, g/T	29.4	0.37	29.4	0.36	29.3	0.34	28.5	1.00	28.5	1.00	28.5	0.98
Dry matter, %	71.97	0.98	72.19	0.96	72.65	0.92	69.99	1.07	70.21	1.05	70.66	1.02
Crude protein, %	12.98	0.60	13.05	0.62	13.03	0.59	12.04	0.13	12.25	0.13	12.69	0.13
NDF, %	13.33	0.44	14.00	0.43	15.32	0.42	20.29	1.02	20.95	1.03	22.29	1.04
ADF, %	6.26	0.31	6.55	0.31	7.11	0.30	8.88	0.63	9.19	0.64	9.81	0.64
Ash, %	3.18	0.12	3.16	0.11	3.09	0.11	4.20	0.15	4.20	0.14	4.20	0.14
Germ EE[4], %	0	--	2.09	0.04	6.32	0.11	0	--	2.04	0.08	6.15	0.23
NE_M, Mcal/cwt[3]	93.11	0.28	95.45	0.32	100.18	0.39	88.01	0.27	90.26	0.27	94.83	0.36
NE_G, Mcal/cwt[3]	61.79	0.28	63.73	0.31	67.68	0.37	57.03	0.28	58.89	0.28	62.65	0.33

[1] All values except DM on a dry matter basis.

[2] Supplement provided vitamins and trace minerals to meet or exceed nutrient requirements. One common supplement was used in all diets and contained monensin and tylosin.

[3] Based on tabular NE values for ingredients fed.

[4] Ether extract from the germ fraction of the diet.

Plasma and Serum Collection

Blood samples for hormone and metabolite analyses were collected from steers at 96 and 131 d on feed, which corresponded to 41 and 6 d before harvest. At each sampling period, blood samples were collected from the steers beginning 4 h after the morning feeding. For serum samples, blood was collected in 15-mL evacuated tubes with no additives. Whole blood samples were stored refrigerated for 24 h before separating serum. Aliquots of serum were stored at -85^{o} C for satellite cell cultures. Blood samples were collected into a 10–mL glass tube containing 100 mg sodium fluoride and 20 mg potassium oxalate for plasma separation. Blood samples were centrifuged at 4^{o} C for 20 min at 1,100 x g immediately after collection. Two 3-mL aliquots of plasma were stored in glass tubes at -20^{o} C for subsequent quantification of metabolites and hormones.

Plasma Hormones and Metabolites Profiles

Glucose (GLU), non-esterified fatty acids (NEFA), insulin (INS), plasma urea nitrogen (PUN), triglycerides (TG), cholesterol (CHOL), high-density lipoproteins (HDL), and low-density lipoproteins (LDL) were determined in plasma. The remaining lipoprotein fraction, which included very-low density lipoproteins (VLDL), intermediate density lipoproteins, and chylomicrons was calculated by subtracting the HDL and LDL fractions from the total CHOL. Plasma NEFA concentrations were determined with triplicate plasma aliquots using a colorimetric assay according to manufacturer procedures (WAKO Chemicals USA INC., Richmond, VA). Plasma glucose concentrations were assayed in triplicate using a colorimetric glucose oxidase kit (Sigma-Aldrich, St. Louis, MO). Plasma insulin concentrations were determined using an Ultra-sensitive human insulin RIA (Linco-Millipore, St. Charles, MO) with a bovine insulin standard per instruction of the manufacturer. Insulin, NEFA, and GLU assays were performed as described previously by Wertz-Lutz et al. (2008). Plasma urea nitrogen was determined in triplicate using the methodology of Fawcett and Scott (1960) and Chaney and Marbach (1962).

Plasma TG, CHOL, HDL and LDL concentrations were determined with triplicate plasma aliquots using a microtiter plate procedure provided by the manufacturer (WAKO Chemicals USA INC., Richmond, VA). Each assay for a metabolite in the lipid profile was validated in our laboratory by checking linearity of serially-diluted bovine plasma and measurement of recovery by spiking bovine plasma with a known quantity of each metabolite. An R^2 no less than 0.995 was accepted for the standard curve.

M_2Ratio Subset for Plasma Hormones and Metabolites

To evaluate plasma hormone and metabolite concentrations of cattle differing abilities to deposit intramuscular relative to subcutaneous adipose tissue, cattle were separated based on their M_2Ratio. High and low M_2Ratio subsets were established by determining the average and standard error for M_2Ratio within a germ level. An individual animal with an M_2Ratio ≥ 3 standard errors from the average for the respective germ level was designated high M_2Ratio, whereas any individual having an M_2Ratio ≤ 3 standard errors below the mean was designated low M_2Ratio. Individuals within ± 3 standard errors of the mean were considered similar to the average and were not used in the subset of samples to evaluate plasma hormone and metabolite profiles relative to ability to deposit fat.

Satellite Cell Isolation and Purification

Satellite cell cultures were used to determine whether growth factors from the plasma of cattle with a greater ability to deposit intramuscular fat resulted in differential growth of satellite cells or increased

lipid accumulation. Satellite cells were isolated previously from the *Longissimus dorsi* muscle of a young growing heifer fed a high-energy diet using the method described by McFarland et al. (1988). These satellite cells were incubated in media and sera from steers in the current experiment that had differences in M_2Ratio. Sera from the 3 or 4 with the highest and lowest M_2Ratios within each level of germ were used. Replicated measures of creatine kinase (CK) and DNA were recorded. Creatine kinase content is a measure of the extent of satellite cell differentiation and the DNA content is an indicator of cell number and proliferation rates. The CK/DNA ratio is a measure of the extent of cell differentiation corrected for cell number. To assess the extent of lipid incorporation into cultured satellite cells, cells were stained with the lipophilic dye Oil Red O as described by Koopman et al. (2001) except that 4% paraformaldehyde was used to fix the cells prior to staining with 3.0 mg/mL Oil Red O and the optical density was determined at 490 nm in a plate reader.

Statistical Analysis

Statistical analysis of cattle performance and carcass variables were conducted using procedures appropriate for a 3 x 2 factorial arrangement of treatments for a completely randomized design. Pen was considered the experimental unit. Serum metabolites of the 54 steer sub-population were evaluated in a similar model, except that each steer represented the experimental unit. The *in vitro* satellite cell cultures were replicated four times and repeated over three runs. The replicates were averaged to produce a single observation. The CHO and GERM tests were made using the nested main effect within run as the error term. Statistical analyses were conducted using the GLM module of SAS (SAS institute, Raleigh, NC). Difference in treatment means that resulted from CHO, GERM, or their interaction were deemed significant at ($P \leq 0.05$). Differences in treatment means that resulted from CHO, GERM, or their interaction were considered a tendency at ($P \leq 0.10$).

RESULTS AND DISCUSSION

Plasma Metabolites and Hormones

Plasma hormone and metabolite data are reported in Table 2. During the first blood sampling (96 d on feed), an interaction of CHO by GERM inclusion level ($P = 0.05$) occurred for plasma GLU concentrations (interaction not reported in table). For the high starch diet, plasma GLU concentrations were highest when germ inclusion rate was LO (69.7 mg/dL), lowest when germ inclusion was HI (63.6 mg/dL), and intermediate (65.2 mg/dL) when no germ was included in the diet. In contrast, plasma GLU concentrations increased as germ inclusion increased from in the high fiber diet (61.0, 67.0, 68.8 mg/dL, respectively). During the second blood sampling period (131 d on feed), there were no significant effects of CHO, GERM, or their interaction on plasma GLU concentrations. Plasma INS concentrations tended ($P = 0.08$) to be higher for cattle on the high starch diet compared with the high fiber diet at 96 d on feed. Plasma INS concentrations did not differ as a result of CHO, GERM level, or their interaction at 131 d on feed. Plasma urea nitrogen concentrations were higher ($P = 0.02$) for cattle on the higher starch diet compared with the higher fiber diet at 96 d on feed. However, PUN concentrations did not differ as a result of CHO, GERM, or their interactions at 131 d on feed.

Table 2. Effects of corn germ inclusion rate and dietary energy source on plasma metabolites and hormones

	Diet		Germ				*P* ≤	
	Starch	Fiber	Control	Low	High	RMSE[1]	Diet	Germ
Number of observations	27	27	18	18	18			
d 96								
Glucose, mg/dL [2]	66.11	65.61	63.12[b]	68.30[a]	66.17[ab]	5.86	0.73	0.04
Non-Esterified fatty acids, uEq/L	152.79	158.92	137.26[b]	160.17[ab]	169.93[a]	38.29	0.51	0.04
Insulin, ng/mL	3.36	2.52	3.08	2.77	2.97	1.68	0.08	0.86
Triglyceride, mg/dL	23.99	23.89	22.81	23.03	25.98	4.48	0.91	0.04
Cholesterol, mg/dL	136.04	131.51	100.69[c]	131.01[b]	169.02[a]	28.18	0.59	0.001
High density lipoprotein, mg/dL	22.24	21.26	19.44[b]	22.26[ab]	23.55[a]	4.42	0.52	0.11
Low density lipoprotein, mg/dL	71.44	68.63	50.97[c]	68.86[b]	90.28[a]	16.54	0.60	0.001
Remaining lipoprotein, mg/dL	42.36	41.62	30.28[b]	40.50[b]	55.19[a]	17.96	0.90	0.01
Plasma urea nitrogen, mg/dL	11.40	10.24	10.55	11.21	10.69	2.17	0.02	0.45
d 131								
Glucose, mg/dL	63.83	64.38	63.86	62.20	66.24	7.63	0.74	0.18
Non-Esterified fatty acids, uEq/L	118.87	117.37	118.41	114.23	121.72	33.49	0.84	0.72
Insulin, ng/mL	3.21	3.02	3.34	2.97	3.05	1.39	0.77	0.89
Triglyceride, mg/dL	25.46	24.35	22.94	25.29	26.50	7.37	0.63	0.39
Cholesterol, mg/dL	133.85	134.72	100.75[c]	125.25[b]	176.85[a]	29.72	0.94	0.001
High density lipoprotein, mg/dL	21.39	21.81	18.34[b]	21.16[b]	25.29[a]	4.42	0.74	0.004
Low density lipoprotein, mg/dL	64.51	68.41	48.39[c]	64.90[b]	86.08[a]	17.63	0.43	0.001
Remaining lipoprotein, mg/dL	47.95	44.50	34.02[b]	39.19[b]	65.47[a]	22.78	0.65	0.01
Plasma urea nitrogen, mg/dL	11.17	11.49	10.68	11.75	11.57	1.79	0.54	0.24

[1] Square root of the mean square error.

[2]Germ x diet interaction ($P < 0.05$) resulted for glucose.

[a,b,c] Means within main effect without common superscripts differ ($P < 0.05$).

Plasma Lipid Profile

Plasma NEFA concentrations at 96 d on feed differed as a result of GERM level. When compared with Control, plasma NEFA concentrations tended to be higher ($P = 0.07$) with a LO germ inclusion and were higher ($P = 0.02$) with a HI germ inclusion regardless of CHO source. Plasma NEFA concentrations did not differ as a result of CHO, GERM, or their interaction at 131 d on feed. At 96 d on feed, plasma TG concentrations were ($P = 0.06$) higher with HI germ inclusion regardless of dietary energy source. However, this tendency was not observed at 131 d on feed. The tendency for elevated TG concentrations with increasing germ inclusion at 96 d on feed, likely was the result of greater fatty acids reaching the small intestine for absorption.

Body weight gain throughout the entire study indicated that cattle were in positive energy balance. Most lipids are absorbed from the small intestine bound as TG (Beitz, 1993). Therefore, elevated plasma NEFA concentrations should not result from increasing dietary lipid from germ inclusion or mobilization of body tissue. When lipids are transferred from TG in circulating chylomicrons into tissues for oxidation to ATP (muscle) or re-packaging and storage as TG (adipose tissue), they are first hydrolyzed to NEFA (Beitz, 1993). The majority of NEFA released from circulating chylomicrons are incorporated into the tissues, however, some NEFA will return to circulation (Beitz, 1993). Additionally, insulin is a key hormone that increases incorporation of NEFA by adipose tissue (Beitz, 1993). Because insulin was not concurrently increased with increasing germ (oil) inclusion, the signal for incorporation of NEFA may not have been adequate. Thus, NEFA may have been elevated because the animal was not able to incorporate hydrolyzed NEFA. In the current experiment, plasma NEFA concentrations were elevated with germ inclusion at 96 d but not 131 d on feed. Perhaps, at 96 d on feed, cattle had less capacity to incorporate the NEFA into tissues, whereas by 131 d on feed tissue was more abundant and metabolically capable of incorporating all the liberated NEFA. Lough et al. (1993 and 1994) also observed elevated NEFA concentrations with the addition of palm oil to sheep diets.

Plasma CHOL concentrations as well as the HDL, LDL, and remaining lipoprotein fractions were all increased ($P \leq 0.05$) by increasing GERM at both sampling periods (96 and 131 d on feed). However, neither CHO, nor the interaction of CHO and GERM influenced CHOL or the lipoprotein fractions at either sampling. The results and discussion will therefore focus on the influence of germ inclusion on the plasma lipid profile. At both sampling times, the inclusion of GERM resulted in increased ($P \leq 0.03$) plasma CHOL when compared with the control (0% germ inclusion). Additionally, the HI germ inclusion resulted in increased ($P = 0.001$) plasma CHOL concentrations when compared with the LO germ inclusion. Plasma CHOL concentrations observed in this experiment are similar to serum CHOL concentrations observed previously by Wheeler et al. (1987) in beef cattle and by Lough et al. (1993 and 1994) with sheep. Additionally, Lough et al. (1993 and 1994) observed increased serum CHOL concentrations with the inclusion of palm oil in sheep diets.

At both samplings (96 and 131 d on feed), the plasma HDL fraction tended ($P \leq 0.10$) to be increased for LO diets and was increased ($P = 0.001$) for the HI diet compared with control. Plasma HDL concentrations were similar for LO and HI germ inclusion at 96 d on feed, but HDL was higher ($P \leq 0.01$) for HI germ inclusion compared with LO germ inclusion at 131 d on feed. Serum HDL concentrations also were reported to increase with the addition of palm oil to sheep diets (Lough et al., 1993 and 1994). At both sampling periods (96 and 131 d on feed), the plasma LDL fraction was increased ($P \leq 0.01$) with germ inclusion compared with control. Additionally, HI germ inclusion resulted in increased ($P \leq 0.001$) plasma LDL fractions when compared to LO germ inclusion. Plasma LDL concentrations, as determined

by enzymatic reaction in this experiment have not previously been reported for growing-finishing beef cattle. Much work has been done with dairy cattle, mostly in early- and mid-lactation when cows are in a negative energy balance and prone to metabolic diseases. The research with dairy cattle has focused on quantifying the amounts of CHOL, protein, and TG in various lipoprotein fractions that have been separated by ultracentrifugation and does not provide an estimate of the LDL fraction in mg/dL of serum or plasma. The remaining lipoprotein fraction was calculated by subtracting HDL and LDL fractions from total CHOL. This fraction includes primarily chylomicrons and VLDL, however, because it is calculated by difference it also may contain intermediate density lipoproteins that result during the metabolism of VLDL to LDL. The inclusion of LO germ increased ($P \leq 0.01$) the remaining lipoprotein fraction when compared with the control at 96 and 131 d on feed. At both sampling times, HI germ inclusion increased the remaining lipoprotein fraction when compared with control ($P \leq 0.01$) and LO germ inclusion ($P \leq 0.01$). These data indicate that increasing germ inclusion increases CHOL and the lipoprotein fractions regardless of dietary carbohydrate source. This finding was highly significant and consistent at both sampling periods. Whereas altered circulating lipid profile did not influence the amount of intramuscular fat deposition or lean tissue accretion, it may influence the lipid profile of these depots thus influencing shelf-life or the healthfulness of the product.

Hormone and metabolite profiles for cattle sorted by M_2Ratio are reported in Table 3. Plasma TG and CHOL concentrations were lower ($P \leq 0.05$) in cattle with a high M_2Ratio at 96 d on feed. At 131 d on feed, plasma CHOL concentrations were lower ($P \leq 0.05$) for cattle with a high M_2Ratio. Although numerical trends similar to those observed at 96 d on feed were observed for plasma TG concentrations at 131 d on feed, statistical differences in plasma TG concentrations were not detectable between M_2Ratio groups. For both sampling periods (96 and 131 d on feed), an interaction ($P \leq 0.05$) of M_2Ratio and germ inclusion amount occurred for the lipoprotein fraction that remained after accounting for HDL, LDL, and total CHOL. For both sampling periods, as germ inclusion increased, the remaining lipoprotein fraction was less ($P \leq 0.05$) for cattle with a high M_2Ratio compared with cattle with a low M_2Ratio. This remaining lipoprotein fraction contains primarily VLDL and chylomicrons. Chylomicrons transport lipids from the intestine to other body tissues, whereas VLDL transports lipids from the liver to other body tissues. As germ inclusion in the diet increases, circulating chylomicrons and VLDLs also would be expected to increase as a means of transporting lipid in the body. The finding that circulating chylomicrons and VLDLs increased to a lesser extent in high M_2Ratio cattle indicates that the ability of cattle to incorporate lipids into available depots differs among cattle and is related to an individual's capacity to deposit intramuscular fat.

Table 3. Effects of M_2Ratio and germ inclusion on plasma metabolite and hormone concentrations

	M_2Ratio[1]							*P ≤*	
	Low			High			RMSE[2]	M_2Ratio	Germ x M_2Ratio
	Germ								
	Control	Low	High	Control	Low	High			
Number of observations	6	5	5	6	5	6			
d 96									
Glucose, mg/dL	63.65	70.37	63.84	61.80	70.17	66.85	5.44	0.87	0.56
Non-Esterified fatty acids, uEq/L	128.12	147.30	160.27	154.96	159.29	179.63	47.18	0.27	0.94
Insulin, ng/mL	3.13	2.32	4.37	3.07	3.13	2.26	1.88	0.51	0.22
Triglyceride, mg/dL	24.06	23.00	28.29	21.64	18.47	23.33	4.40	0.02	0.77
Cholesterol, mg/dL	105.60	128.89	203.94	101.57	108.40	163.99	22.44	0.01	0.18
High density lipoprotein, mg/dL	19.45	20.04	24.93	20.38	20.95	25.97	4.35	0.55	1.00
Low density lipoprotein, mg/dL	53.70	60.53	97.39	52.62	65.47	100.33	17.75	0.73	0.92
Remaining lipoprotein, mg/dL	32.45^{cd}	48.32^{b}	81.61^{a}	28.57^{cd}	21.98^{d}	37.69^{bc}	10.11	0.001	0.001
Plasma urea nitrogen, mg/dL	10.51	10.07	10.28	10.02	10.08	10.44	1.97	0.88	0.92
d 131									
Glucose, mg/dL	66.53^{ab}	59.59^{d}	59.51^{d}	61.07^{bc}	64.90^{abc}	67.46^{a}	5.02	0.15	0.01
Non-Esterified fatty acids, uEq/L	117.96	102.67	124.15	126.28	96.25	132.12	31.43	0.77	0.83
Insulin, ng/mL	3.46^{ab}	2.16^{b}	3.03^{ab}	2.45^{ab}	3.87^{a}	2.30^{ab}	1.31	0.99	0.05
Triglyceride, mg/dL	19.92	22.04	30.57	22.78	22.19	22.76	6.27	0.48	0.16
Cholesterol, mg/dL	109.63	124.22	213.89	98.06	107.26	161.92	31.73	0.02	0.28
High density lipoprotein, mg/dL	19.63	20.07	25.64	19.40	19.24	27.67	4.50	0.84	0.74
Low density lipoprotein, mg/dL	51.12	54.04	89.37	51.66	65.96	93.83	18.69	0.39	0.78
Remaining lipoprotein, mg/dL	38.87^{bc}	50.09^{bx}	98.85^{a}	27.00^{bcy}	22.07^{c}	40.42^{bc}	19.31	0.001	0.03
Plasma urea nitrogen, mg/dL	11.58	10.89	11.80	9.88	11.60	11.43	2.11	0.54	0.42

[1] Proportionality of marbling to carcass fatness.

[2] Square root of the mean square error.

[a,b,c,d] Means within main effect without common superscripts differ as a result of Germ x M_2Ratio ($P < 0.05$).

[x, y] Means within main effect without common superscripts differ Germ x M_2Ratio ($P < 0.10$).

Satellite Cell Cultures

Carcass data results suggested that there may be minimal information to be gained by comparing GERM levels in the cell culture experiments. Since regulation of intramuscular adipose is the root concern, we elected to focus on steers producing carcasses with the highest and lowest M_2Ratio. Twenty four serum donors were selected, representing the highest and lowest M_2Ratio within each GERM treatment. Carcass traits for this sub-population are shown in Table 4.

Table 4. The M_2Ratio and range of carcass traits for sera donors for satellite cell cultures[1]

	M_2Ratio								
	High				Low				
	$\bar{x}$	Range			$\bar{x}$	Range			P^4
n	12				12				
M_2Ratio[2]	1.32	0.88	to	1.92	-1.40	-2.45	to	-0.93	0.001
Ribfat, in	0.43	0.15	to	0.80	0.53	0.30	to	0.80	0.089
Marbling[3]	6.61	4.50	to	8.50	5.18	4.50	to	6.10	0.001
Carcass fat, %	30.0	22.1	to	38.6	33.4	30.7	to	36.5	0.202
Carcass wt, lb	814	765	to	880	796	734	to	853	0.293

[1]Least squares means.
[2] Proportionality of marbling to carcass fatness.
[3] 4.0 = Select^o; 5.0 = Small^o.
[4] Probability that High and Low M_2Ratio category means differ.

Higher ($P < 0.05$) CK/DNA levels in satellite cell cultures indicate that cell differentiation was greater when satellite cells were grown in serum collected on d 96 of the feeding period compared with d 131 of the feeding period (Table 5). Rate of satellite cell growth, indicated by DNA content, tended ($P \leq 0.10$) to be grater in cultures administered sera from the 96-d blood collection compared with the 131-d blood collection. The CK/DNA ratios did not differ ($P \geq 0.05$) between different dietary germ levels regardless of blood sampling time (Table 6). However, DNA levels within a bleed time tended to differ ($P \leq 0.10$). With 96-d, serum from LO germ animals resulted in greater satellite cell growth than did serum form HI germ animals. The reverse was observed with samples taken at 131 d on feed.

Table 5. Influence of sera collected at different time points in growth on satellite cell differentiation and proliferation [1]

	Days on Feed			
	96	131	SEM	$P \leq$
CK/DNA, mU/μg[1]	1.93	1.74	0.02	0.001
DNA, μg[2]	0.49	0.47	0.003	0.07

[1] M_2Ratio proportionality of marbling to carcass fatness.
[2] Indicator of extent of cell differentiation corrected for cell number.
[3] Indicator of cell number and proliferation rate.

Table 6. Influence of sera from the high and low M_2Ratio sub-population cattle fed different germ levels on satellite cell differentiation and proliferation [1]

	d 96 Germ Level				
	Control	Low	High	SEM	$P \leq$
CK/DNA, mU/μg^2	1.90	1.92	1.96	0.040	0.60
DNA, μg^3	0.50	0.50	0.47	0.006	0.10
	d 131 Germ Level				
	Control	Low	High	SEM	$P \leq$
CK/DNA, mU/μg^2	1.70	1.81	1.72	0.030	0.19
DNA, μg^3	0.48	0.44	0.47	0.006	0.09

[1] M_2Ratio proportionality of marbling to carcass fatness.
[2] Indicator of extent of cell differentiation corrected for cell number.
[3] Indicator of cell number and proliferation rate.

Table 7 describes the relationship between cattle M_2Ratios and sera effects on DNA and CK/DNA ratios. At d 96, sera from high MRatio cattle resulted in greater proliferation and differentiation activity when compared with sera from low MRatio cattle ($P \leq 0.05$). Sera samples from the d 131 resulted in similar satellite cell growth and differentiation regardless of MRatio.

Table 7. Influence of sera from cattle with different M_2Ratios satellite cell growth and differentiation[1]

	d 96 M_2Ratio			
	Low	High	SEM	$P \leq$
CK/DNA, mU/μg^2	1.87	1.99	0.007	0.05
DNA, μg^3	0.48	0.50	0.001	0.03
	d 131 M_2Ratio			
	Low	High	SEM	$P \leq$
CK/DNA, mU/μg^2	1.79	1.70	0.018	0.16
DNA, μg^3	0.46	0.47	0.006	0.84

[1] M_2Ratio proportionality of marbling to carcass fatness.
[2] Indicator of extent of cell differentiation corrected for cell number.
[3] Indicator of cell number and proliferation rate.

Although microscopic examination detected minimal lipid inclusions in oil red O stained cells, the levels were too low to quantify using plate reader spectroscopy (data not reported). It is possible that with higher levels of serum in the media (> 10%), lipid inclusions may increase and be measurable.

Bovine satellite cells proliferated at greater rates when administered serum from the first bleed (96 d on feed). Serum from CO, LO, and HI germ-fed animals on d 96 also contained greater levels of NEFA. Higher levels of NEFA may be supportive or responsible for the greater rates of satellite cell proliferation and subsequent differentiation. Additionally, serum from animals with higher M_2Ratios stimulated greater proliferation and subsequent differentiation rates. These latter findings suggest that local tissue growth factors may be such that conditions for both muscle differentiation and marbling fat accumulation may be increased.

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