

Effect of a short duration feed withdrawal followed by full feeding on marbling fat in beef carcasses [☆]

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Received 11 April 2007; received in revised form 9 August 2007; accepted 12 August 2007

Abstract

The effect of feed withdrawal for 48 h, prior to initiation of the finishing (fattening) period (75 d) on carcass marbling fat was studied in 120 European × British cross-bred heifers with an average weight of 585 ± 39 kg. Heifers were randomized in a 2 × 2 factorial design experiment with two dietary management treatments, where half the heifers were provided the feed components of steam rolled barley and barley silage either free choice or as a total mixed ration (TMR) containing 87% steam rolled barley and 13% barley silage with ad libitum vitamins and minerals via salt blocks for all animals. Within each dietary management treatment, 30 heifers were denied feed (water was available) for 48 h prior to the two week adaptation to the high grain diet preceding the 75 d finishing period. At the end of the 48 h feed denial blood samples were collected from the jugular vein prior to feeding for determination of glucose and insulin concentrations, which indicated that 48 h feed withdrawal consistently decreased ($P=0.0001$) plasma concentrations of both glucose and insulin but the ratios of the concentrations of glucose to insulin were not affected. At slaughter samples of subcutaneous fat from the brisket (BF) and skirt muscle (*pars costalis diaphragmatis*; PCD) were procured for determination of chemical fat content, fat dissected from the muscle and for enumeration of adipocytes, less than 35 µm in diameter and to determine the average cell size in the dissected fat and from the BF by flow-cytometry of adipocytes fixed in osmium tetroxide. The carcass characteristics were also obtained. Although no differences due feed withdrawal for 48 h were evident for carcass weight, percent lean (saleable) meat yield, rib eye area, average fat cover, fat content of PCD or BF, the US marbling score was increased ($P=0.048$) and the amount of dissected fat from the muscle tended to be higher ($P=0.107$), thus 81% of the carcasses graded “US Choice” or “Canada AAA,” or displayed at least a “small” amount of intramuscular fat as compared ($P=0.0807$) to 68% of the heifers not denied feed. Based on more than three years of weekly prices of carcasses that graded “Canada AAA” and “Canada AA,” these experimental results suggested that the expected price of a finished heifer could increase by \$4.61 Canadian if a 48 h feed withdrawal was imposed prior to initiation of the finishing phase. Although significant differences in adipocyte numbers due to a single time 48 h feed withdrawal prior to initiation of the finishing phase were not detected, the carcass quality factors were affected leading to an odds ratio of 1.84 times in favour of cattle carcasses to grade “Canada AAA” than if fed continuously.

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Keywords: Feed withdrawal; Carcass characteristics; Marbling score; Dissected fat; Adipocyte enumeration

[☆] Lethbridge Research Centre Contribution number — 38706038.

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1. Introduction

Restricting the availability of nutrients to growing (backgrounding) beef cattle for predetermined durations, causes a “rebound” effect in growth and is termed “compensatory growth,” which results in increased growth rate on ad libitum intake before slaughter (Yambayamba et al., 1996). Similarly, development of methods for optimizing carcass quality variables such as marbling fat in beef animals is new, but important and warrants exploration. It was observed that the percentage of carcasses that exhibited a high amount of intramuscular or marbling fat, thus warranting a “Canada AAA/US Choice” grade, was greater than industry percentages, among animals that were denied feed for 48 h at yearling age for purposes of the intravenous glucose tolerance test (Mir et al., 2002). The manipulation of the physiology of adipocytes through controlling nutrient availability can be an avenue to increase marbling scores or fat in beef cattle.

Increasing the deposition of marbling fat, to enhance palatability of beef and the value of the carcass, has been a priority for the beef industry (Van Donkersgoed et al., 2001). Marbling fat can be an effective vehicle to provide health enhancing bio-active lipid compounds such as conjugated linoleic acid and ω -3 fatty acids to consumers (Rule et al., 2002; Mir et al., 2002; Scollan et al., 2006). In North America the extent of marbling fat in carcasses affects the price paid to producers and the price of beef at retail.

The objective of this study was to test the hypothesis that a one time feed withdrawal for 48 h prior to initiation of the finishing (fattening) period would improve the marbling score of the carcass. Thus the study was conducted to determine the effect of feed withdrawal for 48 h in yearling beef heifers prior to initiation of the fattening phase on carcass characteristics, including United States (US) marbling score, amount of fat that can be dissected from muscle, muscle fat content, and fasting insulin and glucose concentrations in plasma. In a supportive manner to perhaps explain the differences in marbling score the number of fat cells (less than 35 μ m in diameter) per unit weight of tissue was determined and the approximate possible financial rewards that could accrue to the beef producers was estimated.

2. Materials and methods

2.1. Animals, housing and diets

One hundred and twenty European X British crossbred, yearling heifers, with an average weight of 585 ± 39 kg were assigned to eight pens with 15 heifers each. Pens were 40.2×27.4 m with centrally located water system and a

2.4×24.5 m concrete apron in front of the bunk. Pens were bedded with straw as required and animals were cared for in accordance with the guidelines of the [Canada Council on Animal Care \(1993\)](#). Post adaptation to the high grain diet over a two week period, all heifers were fed a finishing diet containing steam rolled barley, and whole crop barley silage to provide adequate protein and energy ([National Research Council \(NRC\), 1996](#)). A mineral and vitamin salt block (Beef Cattle Range Mineral Block # 057071, Manufactured by ADM Animal Health and Nutrition St. Hyacinthe, QE, Canada with Product #C1374 and lot # QE19004) was provided ad libitum to the heifers. The composition of the salt block in actual amounts was Calcium — 10.7%, Phosphorus — 10.5%, Magnesium — 4.82%, Sodium — 7.0%, Cobalt — 30 mg/kg, Copper, 2600 mg/kg, Fluorine — 800 mg/kg, Iodine — 80 mg/kg, Iron — 3575 mg/kg, Manganese — 4670 mg/kg, Zinc — 7900 mg/kg, Vitamin A — 525,000 IU/kg, Vitamin D₃ — 52,000 IU/kg, Vitamin E — 625 IU/kg. Heifers were fed daily at 0900, 1300 and 1500 and weighed every two weeks. When heifers were deemed as carrying adequate fat (having more than 4 mm of subcutaneous fat) to grade at least A1, (which occurred 75 d after initiation of the finishing diet) they were shipped to an abattoir for slaughter and processing.

2.2. Experimental design

The effects of dietary management and feed withdrawal were determined in a 2×2 arrangement of treatments. Heifers were equally assigned to either free choice dietary management treatment where the barley silage and steam rolled barley were offered separately or the same diet components were provided as a total mixed ration (TMR) containing 87% barley and 13% silage on DM basis. Within each dietary management treatment, the feed was withdrawn for 48 h (bunks were cleaned in the morning and animals were not fed for 48 h, but water was available) for half the heifers prior to initiation of the two week adaptation to the high grain diet followed by the finishing period which lasted for 75 d.

2.3. Blood chemistry and carcass measurements

Immediately post completion of the feed withdrawal for 48 h, for 30 heifers within each dietary management treatment (total of 60 heifers in the feed denial treatment), blood samples were collected from the jugular vein by vena puncture into heparinized, evacuated tubes from all heifers. Plasma insulin (Mears, 1993) and glucose (Sigma kit # 510 DA, Mississauga, ON) concentrations were determined. The inter and intra assay coefficient variation (CV) for the insulin assays were 8.43 and 7.7% for the control sample with the average concentration of 2.3 ng/ml. The inter assay CV for the glucose determination based on the glucose standard provided in the kit ranged between 3.3 and 9.4%. The heifers were then initiated into the two week adaptation to high grain diets followed by the finishing phase of the study. After the 75 d finishing period and when it was deemed that heifers were carrying adequate

subcutaneous fat for their carcasses to grade A1 for yield they were shipped to an abattoir and processed.

At processing, samples from the *pars costalis diaphragmatis* (PCD; skirt muscle) and the brisket fat (BF), to represent muscle and subcutaneous adipose tissues, respectively, were obtained from each carcass and transported on ice for processing for determination of adipocyte number and fat content. The choice of the PCD muscle relates to the fact that a sample can be procured without destruction of a side of the carcass, which would be required if a sample were to be procured from the Longissimus muscle on day of slaughter (against regulations). From a lipid content perspective Middleton et al. (1998) reported that a significant co-relation exists between PCD and the Longissimus muscle, from which the marbling grade is provided. Similarly, from a fatty acid perspective Basarab et al. (2007) have demonstrated that there is strong relationship among tissues of cattle, and lastly, the lipid content of the PCD is usually higher than in the Longissimus muscle and thus dissection of fat would be more reliable especially when the fat content is low, which can be expected in animals that do not exhibit substantial amount of marbling fat.

One day post slaughter the carcasses were graded by the abattoir grader and the warm carcass weight, yield grade, percent lean or saleable meat yield and gross marbling score as “Canada A/US standard, Canada AA/US Select or Canada AAA/US Choice” was assigned to the carcasses. A federal grader later, on the same day, provided the detailed US marbling score where “Canada A” received a score of 300, “Canada AA” of 400 and “Canada AAA” received a score ranging from 500–700 based on whether the carcass was deemed to display small (500), modest (600) or moderate (700) amount of marbling fat. The federal grader also provided the grade fat depth and rib eye area. However in the time (about six h) between the assessment of the carcasses by the two graders 33 carcasses had been shipped, thus the replication per treatment as graded by the federal grader was 29 for the feed withdrawn heifers fed free choice, 15 for the heifers fed free choice continuously, 11 for the feed withdrawn heifers fed the TMR and 29 for the heifers fed the TMR continuously.

2.4. Adipocyte enumeration and fat determination

The fat in a known weight of randomly selected muscle (approximately 2 g), was physically dissected, weighed and approximately 80 mg of the dissected fat were processed by fixation with osmium tetroxide for cell enumeration (Cartwright, 1987; Mir et al., 2003). Briefly, three or four pieces (about 80 mg) of the dissected fat were washed with warm saline solution (9 g in 1 L of water at 37 °C) and weighed into vials containing 1 mL of osmium tetroxide (5 g osmium tetroxide in 100 mL of 2 M collidine buffer, Cartwright, 1987). After a week in the buffered osmium tetroxide the cells were washed twice with saline and 5 mL of 8 M urea in saline was added and kept at room temperature to soften the tissue. The fixed cells were rinsed free of the urea and suspended in saline (total volume of 22.2 mL). The cells were filtered through a 35 µm filter (using Falcon cell strainer tubes #352235) and

enumerated using a flow cytometer from Becton Dickinson FacsScan (BD Biosciences, San Jose, Ca.). Flow rate was determined by measuring the weight (mg) of the solution removed from the sample tube by the flow cytometer during a timed interval (min) and the rate calculated as:

$$\text{Flow rate}(\mu\text{L min}^{-1}) = \frac{\text{Change in weight due to sampling (mg)}}{\text{density of sample (mg } \mu\text{L}^{-1}) \times \text{time (min)}}$$

For each sample (the BF and the dissected fat from the PCD) from each animal, the time interval of sampling was recorded and the volume of sample suspension counted was determined from the flow rate. From the forward light scatter of the particles provided by the flow cytometer (WINMDI software from www.flowcyto.cyto.purdue.edu), a gate was set up to enumerate all particles within the gate. This gate was developed by measuring latex beads (Beckman Coulter, Miami Florida) of 2, 5, 10, 15, 20 and 30 µm sizes and all particles that fell within the range from 10 to 30 µm were counted. A standard curve and equation ($0 + 22.24x$, $r^2 = 0.922$, $n = 36$) was developed using the latex beads, which was used to determine the average cell diameter (µm) from the average particle size provided by the flow cytometer for all particles within the gate. The lower extinction limit of the gate was set at 10 µm to include the very small pre-adipocytes and the upper extinction was set at 30 µm because of the limitation of the flow cytometer (could not handle particles larger than 35 µm). Furthermore it was expected that feed withdrawal for 48 h, 89 d (14 d adaptation + 75 d of finishing) prior to slaughter would lead to an increase in smaller rather than larger adipocytes and thus it was expected that measurement of particles under 35 µm would be representative of adipose cellularity that occurred in the animals. From cells in the volume of suspension counted, the cells per mg of BF and PCD were calculated based on the dilution applied to the weight of the sample processed.

The fat in the remaining tissues was extracted (Shah et al., 2006) for determination of fat content of the subcutaneous fat (BF) and the muscle (PCD) samples. Briefly, 15 mL of isopropanol was added to a test tube containing 8.0 ± 0.5 g of finely chopped PCD or 1 ± 0.5 g of BF and was homogenized (Polytron PT 10–35, Kinematic AG, Switzerland) at high speed for about 15 s. After adding 10 mL of hexane to the homogenate, the mixture was again homogenized at high speed for 15 s, and the homogenized mixture was filtered through a Whatman filter paper (No. 1) into a second test tube. Fifteen mL of hexane:isopropanol solution (10:14) was added twice to the first tube, and both times it was filtered into the second tube after vigorous vortexing. Finally, the filtrate (filter paper and residue) was rinsed with 5 mL of hexane:isopropanol (10:14). Then 8 mL of aqueous sodium sulfate ($66.8 \text{ g Na}_2\text{SO}_4/\text{L}$ of distilled water) solution was added to the filtrate and thoroughly mixed by inversion for 30 s before centrifuging at 1100 rpm ($640 \times g$) for 10 min. The hexane layer was collected into a pre-weighed test tube, and evaporated under nitrogen. The residual fat in the pre-weighed test tube was weighed once the tube was completely dried and the

Table 1

Effect of feed withdrawal for 48 h on carcass factors and muscle (*pars costalis diaphragmatis* — PCD) and subcutaneous adipose tissue (brisket fat; BF) of beef heifers

Parameter	Fed free choice		Fed the total mixed ration		F/W	Probability of difference	
	Feed	Fed	Feed	Fed		Feed	Interaction
	Withdrawn	Continuously	Withdrawn	Continuously			
Glucose mM	4.23±0.16b	5.39±0.33a	4.17±0.11b	5.45±0.28a	0.0001	ns	ns
Insulin ng ml ⁻¹	1.09±0.14b	1.61±0.16a	0.87±0.07b	1.36±0.21a	0.0001	ns	ns
Glucose /insulin	0.96±0.11	0.76±0.07	1.01±0.09	0.96±0.09	ns	ns	ns
<i>Carcass</i>							
Characteristics (n)	30	29	28	30			
Warm carcass wt (kg)	395±3.8	400±5.3	407±4.8	396±4.7	ns	ns	0.09
Average fat (mm)	14.5±0.7	13.1±0.9	15.0±1.3	14.9±0.9	ns	ns	ns
Fat content (%)	61.4±1.3	61.2±1.9	62.9±1.9	60.9±2.1	ns	ns	ns
Rib eye area (cm ²)	93.9±1.7	98.1±2.9	95.7±3.9	94.2±1.8	ns	ns	ns
Saleable meat (%)	56.0±0.7	58.5±1.2	55.9±1.1	55.9±0.9	ns	ns	ns
US Marbling Score (n) (Fed. Grader)	545±17(29)	487±22(15)	545±31(11)	517±19(29)	0.04	ns	ns
US Marbling Score (n) (Abattoir Grader)	527±14(30)	479±13(29)	504±13(28)	503±15(30)	ns	ns	0.08
Fat content (%)	10.2±0.5	10.0±0.8	9.9±0.7	8.4±0.7	ns	ns	ns
Dissected fat (%)	11.6±1.0	10.7±1.2	13.8±1.5	10.7±1.1	0.107	ns	ns
<i>BF factors</i>							
Cell size (µm)	20.3±0.13ab	20.2±0.15ab	20.0±0.14b	20.4±0.14a	ns	ns	0.05
Cell number mg ⁻¹ fat	1466±218a	2130±292a	1361±228b	1248±191b	ns	0.04	ns
<i>PCD factors</i>							
Cell size (µm)	19.6±0.19b	20.1±0.14a	20.2±0.17a	19.6±0.15b	ns	ns	0.0002
Cell number mg ⁻¹ fat	88715±25842	67974±9927	75579±16950	77961±8725	ns	ns	ns
Cell number mg ⁻¹ muscle	8372±1645	7124±1410	11501±3809	8466±1737	ns	ns	ns

F/W = fed or feed withdrawn for 48 h prior to initiation of finishing phase.

Feed = free choice or as TMR.

Interaction = interaction among the above two factor.

a,b=means within a row followed by the same letter are not significantly different ($P>0.05$).

fat content of the samples was calculated as percentage of tissue on an as is basis.

2.5. Statistical analysis

The objective of the study was to determine if a short duration feed withdrawal prior to initiation of the finishing period would affect the carcass characteristics of the heifers, thus the data were analysed using each carcass as the experimental unit. The US marbling scores were categorized as 400, 500 or 600 and greater (for the 33 carcasses that were not graded by the federal grader the abattoir graders assignment was used with the score of 400 or 500 given to carcasses that graded “Canada AA” or “Canada AAA” respectively) and a cumulative logit model was fitted using the LOGISTIC procedure from SAS (SAS Institute Inc., 2005) with the dietary management treatment, the feed withdrawal treatment, and their interaction in the initial model. The interaction term was not significant for marbling score and was removed when fitting the final model with the DESCENDING option. This analysis provided the odds of obtaining a higher marbling score for one level of treatment versus the other level. The LOGISTIC procedure was also used to relate the US

marbling scores individually to dissected fat, insulin, glucose or the glucose to insulin ratio. The other dependent variables were statistically analyzed with the MIXED procedure from SAS (SAS Institute Inc., 2005) using a completely randomized design with the dietary management treatment, the feed withdrawal treatment, and their interaction in the model as fixed effects. Least squares means were generated for significant effects and Fisher’s protected LSD test was used to compare differences among means that were of interest. The UNIVARIATE procedure from SAS (SAS Institute Inc., 2005) was used to produce normal probability plots to check the residuals for normality and for outliers. A few obvious outliers were removed before rerunning the analysis.

3. Results and discussion

3.1. Carcass characteristics

The major objective of this study was to determine if pre-finishing feed withdrawal resulted in altered variables of adiposity in beef cattle. In the current study, feed withdrawal for 48 h led to decreases in plasma glucose

and insulin concentrations, as expected, and confirmed that nutrient availability was markedly reduced, but no differences ($P > 0.05$, Table 1) in the ratio of glucose to insulin concentrations were observed. The earlier observation that cattle with Wagyu genetics exhibited higher marbling scores along with delayed insulin response to intravenous glucose during the intravenous glucose tolerance tests (IVGTT) led to the concept that marbling fat deposition may be in response to elevated glucose availability (Mir et al., 1998). This observation concurred with the report of Smith and Crouse (1984) that muscle utilised glucose for lipogenesis while acetate was the principal substrate for lipid synthesis in subcutaneous tissue. However, Mir et al. (2002) found that elevated glucose concentrations during the IVGTT and ultimate marbling score may not be related. Induction of increases in the ratio of glucose to insulin concentrations by alloxan treatment indicated a r^2 of only 0.51 for the equation Longissimus muscle fat content = $14.245 + 0.757$ ratio of glucose to insulin concentrations post 48 h feed withdrawal ($n = 11$, $P = 0.013$; Mir et al., 2001). Further it was noted that the feed withdrawal regimen imposed on the cattle for purposes of the IVGTT led to an increased proportion of cattle receiving elevated marbling scores and was hypothesized that feed withdrawal itself affected marbling fat deposition. In concurrence, Cassar-Malek et al. (2004) cite publications where intramuscular fat deposition has been observed to be increased in cattle that have undergone feed restriction followed by re-feeding. As a result it was important to determine if a cost-free intervention of feed withdrawal for 48 h hours prior to initiation of the fattening phase would lead to improvements in marbling score and net value of the carcass.

A trend for interaction ($P = 0.086$) was observed for warm carcass weight, but the differences among the treatments were very small, and might not be of any biological value from the perspective of the feed withdrawal treatment. Differences in average grade fat, rib eye area and percent lean or saleable meat were not significant ($P > 0.05$). The rib eye areas were on average greater than 90 cm^2 , which compare well with reported areas for the rib eye in the Canadian Beef Audit (Van Donkersgoed et al., 2001). The average marbling score assigned by the federal grader, where a distinction was made among the amounts of intramuscular fat of “small-500,” “modest-600” and “moderate-700” within the “Canada AAA,” indicated that feed withdrawal for 48 h at initiation of the finishing period increased ($P = 0.04$) the marbling score. However, when the abattoir grader’s score was considered, where a distinction was not made among small, modest or moderate amount of marbling (all considered as “Canada AAA or as having a score of 500” for purposes of economic

value of the carcass), a trend ($P = 0.08$) was observed where the feed withdrawal for 48 h increased marbling scores, largely among the carcasses of animals fed the diet components free choice. Concurrently, although, the fat content of the PCD muscle was not affected significantly by the 48 h feed withdrawal treatment, a consistent non-significant increase in fat content of PCD and BF and average grade fat depth was observed in heifers that underwent 48 h feed withdrawal. The dissected fat from the muscle tended to be greater ($P = 0.107$) for heifers that had undergone feed withdrawal prior to initiation of the finishing phase, and the increase in the dissected fat was observed for animals in both dietary management treatments. Based on the abattoir grader’s report 81 %, or 47 out of 58 carcasses from the heifers whose feed had been withdrawn for 48 h, prior to initiation of the finishing phase received the “Canada AAA,” which tended ($P = 0.08$) to be higher than for carcasses of heifers fed continuously where only 68 % or 40 out of 59 carcasses were awarded the “Canada AAA” grade, whereas the rest of the carcasses graded “Canada AA”. The “Canada AAA” grade is equivalent to “US Choice” or a US marbling score between 500 and 700. In the beef audit it is reported that the percentage of heifer carcasses that graded as having a “small to moderate” amount of marbling or receiving the “Canada AAA” grade was 41% of all heifers slaughtered (Van Donkersgoed et al., 2001). In general all heifer carcasses from the present study performed better than industry expectations for marbling score and those that underwent the 48 h feed withdrawal treatment tended to have higher ($P = 0.0807$ from Chi square analyses) percentage of carcasses with the “Canada AAA” grade. Furthermore, the odds of finding a higher marbling score among animals that were in the 48 h feed withdrawal treatment was 1.84 times greater in comparison to heifers that were in the continuous feeding treatment.

The grade differentials of the heifer carcasses that resulted from feed withdrawal can have significant economic impacts on beef feeding operations. Weekly Canadian price data on Canadian AAA and AA grades over a period from 17 May 2003 to 1 July 2006 revealed a mean difference of \$0.1969/kg of carcass weight (Canfax, 2006 <http://www.albertabeef.org/canfaxfullreport.asp>). The price difference varied widely over time (from a minimum of \$0.0555 to a maximum of \$0.5057 kg^{-1}) and was always positive. For a single animal (with a 400 kg carcass), the improved grade could result in a financial gain of \$78.76. The experimental results of 81% of the carcasses receiving the “Canada AAA” grade in the feed withdrawal treatment versus only 68% in the continuous feeding treatment results in an expected financial gain of \$4.61 per animal (400 kg carcass).

3.2. Adipocyte number and average cell size

The cellularity data indicated an interaction for adipocyte size for cells from both tissues indicating that both feed withdrawal and dietary management affected this factor, while cell numbers in the BF were affected by dietary management treatments rather than the feed withdrawal treatments. In the BF, the adipocyte numbers of cells less than 35 μm in diameter were higher in heifers fed free choice than those provided the TMR. Furthermore, cell counts were lower in BF than in the PCD possibly because only cells less than 35 μm were counted, however, Smith et al. (2007), enumerated total cells in both subcutaneous and intramuscular tissue, cells, they report lower cell numbers, but of larger volume, in subcutaneous than in muscle of steers in agreement with our observations. In the PCD, adipocyte numbers were not affected ($P=0.393$) by treatment when determined per mg of dissected fat or mg tissue, but feed withdrawal within both dietary management treatments, although non-significantly ($P=0.96$), consistently increased the number of cells per mg of PCD. It is possible that if we were able to count all the cells, differences could have achieved significance. Marques et al. (2000) reported that, in rat adipose, the total number of cells in the inguinal and epididymal adipose tissues was related to weight of each tissue, which varied with fat content of the diet. The percentage of cells of different diameters varied between the two adipose tissues. Marques et al. (2000) observed a three fold increase in cells up to 30 μm in diameter and among the larger cells in rats fed the high fat diets, but the extent of increase in cell numbers among the cells ranging in size from 50 to 80 μm was substantially less. Thus, in the present study it was expected that although only the cells less than 35 μm in diameter were counted, it is probable that the cells where the most increase occurred due to the feed withdrawal treatment may have been counted.

Yang et al. (2006) report average cell diameters ranging from 65 to 80 μm for adipocytes in intramuscular fat of cattle, which are larger than were counted in the present study, due to technological limitations, but these authors also found that in smaller flecks of marbling fat the smaller adipocytes were more numerous than in larger flecks of fat. However, these cattle had been provided a high concentrate diet from weaning and were older than our cattle at slaughter, which could have affected the ultimate adipocyte sizes. Similarly, Smith et al. (2007) report larger cell volumes than we were able to count, but found cells with substantially lower cell volumes (575 pL) for intramuscular adipocytes from cattle that were fed finishing diets after they were

yearlings as opposed to those fed the same diets from weaning (1157 pL). In the present study the finishing diets were initiated after the heifers were of yearling age and the duration of the finishing diet lasted only 75 d as opposed to the 93 d employed by Smith et al. (2007) thus it is expected that, although only cells less than 35 μm in diameter were enumerated, those that would be affected by the feed withdrawal intervention were counted. However, since all the cells were not enumerated the possibility of establishing a significant increase in the cells per mg of tissue was not possible for the two adipose tissues, but in the PCD the feed withdrawal treatment resulted in consistent increase in cell numbers.

A case has been made that bovine adipocytes may not be terminally differentiated (Dodson et al., 2005), in which case feed withdrawal might induce either pre-adipocyte proliferation, or for mature adipocytes to de-differentiate (Femyhough et al., 2004, 2005) and multiply in order to supply additional cells which can accumulate fat when nutrient flux is re-established (Adebonjo, 1975). The probable factors underlying the initiation of adipocyte proliferation may be the induction of growth hormone secretion during feed withdrawal, which has been observed in humans even after day 1 of a 5 d fast, where both amplitude and frequency of the episodic release of growth hormone was increased (Ho et al., 1988). Although, the specific association between adipocyte proliferation and elevated growth hormone has not been documented in cattle, an association between nutrient restriction and increased plasma growth hormone concentrations has been observed in beef heifers (Yambayamba et al., 1996). In the present study, the trend towards an increase in the amount of dissected fat from the muscle in heifers that were denied feed for 48 h prior to initiation of the finishing period, suggests the possible involvement of growth hormone during feed withdrawal followed by lipogenesis during the finishing period.

The correlation coefficients among US marbling score, dissected fat, insulin, glucose or glucose to insulin ratio were not significant ($P>0.05$) and concur with results obtained previously (Mir et al., 2002). However, a correlation coefficient of $r=0.33$ ($P=0.007$, $n=66$, Mir et al., 2004) between the glucose to insulin ratio and adipocyte number in muscle tissue has been observed previously, indicating that the relative levels of circulating glucose may influence fat deposition in muscle. Smith and Crouse (1984) found through tissue culture that glucose is the favoured substrate for lipogenesis in muscle unlike subcutaneous adipose, where acetate is the favoured substrate. Further it appears that in mammals elevated circulating glucose results in steatosis of insulin resistance, which is in

agreement with the hypothesis of Frayn (2001) who reported that lipid deposition is increased in muscle when there is insulin resistance in adipose tissue and that energy bearing carbon is redirected to other tissues for deposition as fat. A significant relation between the ratios of glucose to insulin concentrations to marbling score would have provided a tool for prediction of cattle that could preferentially deposit marbling fat and could have been used as a selection tool, but the significance was not evident and the utility of this factor still needs to be established. It is not known if higher fasting insulin concentrations would be a signal for insulin resistance and an indication of steatocis and increased marbling deposition.

4. Conclusions

The results of the current study appear to suggest that minimal dietary restriction such as a single 48 h feed withdrawal prior to initiation of the finishing period increased marbling scores. Ad libitum feeding post restriction may increase the potential for fat deposition in muscle, which enhances consumer appeal of beef and can be a successful avenue to transfer health benefits of biologically active fatty acids to consumers. The single 48 h feed withdrawal intervention that was applied in this study tended to result in an increase in the percentage of carcasses that graded “Canada AAA” and can be expected to lead to an economically significant increase in returns of \$4.61 per finished heifer that is brought to slaughter, without affecting other carcass characteristics of the beef heifers.

Acknowledgements

The authors thank Charmaine Ross, Fiona Brown, Bobby Yip and Kristen Lotwin for their participation in collecting and processing of the samples. The authors are also obliged to Ms. Laurie Robertson of University of Calgary for conducting the flow cytometry of the fixed adipocytes.

References

Adebonojo, F.O., 1975. Studies in human adipose cells in culture: relation of cell size and cell multiplication to donor age. *Yale J. Biol. Med.* 48, 9–16.

Basarab, J.A., Mir, P.S., Aalhus, J.L., Shah, M.A., Baron, V.S., Okine, E.K., Robertson, W.M., 2007. Effect of sunflower seed supplementation on the fatty acid composition of muscle and adipose tissue of pasture-fed and feedlot finished beef. *Can. J. Anim. Sci.* 87, 71–86.

Canada Council on Animal Care. 1993. Guide to the care and use of experimental animals. Vol. 1. Olfert, E.D., Cross, B.M. and McWilliam, A.A. (eds) CCAC, Ottawa ON.

Cartwright, A.L., 1987. Determination of adipose tissue cellularity. In: Hansan, Martin (Eds.), *The Biology of the Adipocyte*, pp. 229–254.

Cassar-Malek, I., Hocquette, J.F., Jurie, C., Lustrat, A., Jailler, R., Bauchart, D., Briand, Y., Picard, B., 2004. Muscle-specific metabolic, histochemical and biochemical responses to a nutritionally induced discontinuous growth path. *Anim. Sci.* 79, 49–59.

Dodson, M.V., Fernyhough, M.E., Vierck, J.L., Hausman, G.J., 2005. Adipocytes may not be a terminally differentiated cell type: implications for animal production. *Anim. Sci.* 80, 239–240.

Fernyhough, M.E., Vierck, J.L., Hausman, G.J., Mir, P.S., Okine, E.K., Dodson, M.V., 2004. Primary adipocyte culture: adipocyte purification methods may lead to a new understanding of adipose tissue growth and development. *Cytotechnology* 46, 163–172.

Fernyhough, M.E., Helderline, D.L., Vierck, J.L., Hausman, G.J., Hill, R.A., Dodson, M.V., 2005. Dedifferentiation of mature adipocytes to form adipofibroblasts: more than a possibility. *Adipocytes* 1, 17–24.

Frayn, K.N., 2001. Adipose tissue and the insulin resistance syndrome. *Proc. Nutr. Soc.* 60, 375–380.

Ho, K.Y., Veldhuis, J.D., Johnson, M.L., Furtanetto, R., Evans, W.S., Alberti, K.G.M.M., Thorner, M.O., 1988. Fasting enhances growth hormone secretion and amplifies the complex rhythms of growth hormone secretion in man. *J. Clin. Invest.* 81, 968–975.

Marques, B.G., Hausman, D.B., Latimer, A.M., Kras, K.M., Grossman, B.M., Martin, R.J., 2000. Insulin-like growth factor 1 mediates high-fat diet induced adipogenesis in Osborne Mendel rats. *Am. J. Physiol., Regul. Integr. Comp. Physiol.* 278, R654–R662.

Mears, G.J., 1993. Influence of feeding and diet on diurnal patterns of plasma growth hormone and insulin levels in calves. *Can. J. Anim. Sci.* 73, 987–991.

Middleton, C.K., Kazala, E.C., Lozeman, F.J., Hurlly, T.A., Mir, P.S., Bailey, D.R.C., Jones, S.D.M., Weselake, R.J., 1998. Evaluation of diacylglycerol acyltransferase as an indicator of intramuscular fat content in beef cattle. *Can. J. Anim. Sci.* 78, 265–270.

Mir, P.S., Mears, G.J., Ross, C.M., Husar, S.D., Robertson, W.M., Jones, S.D.M., Mir, Z., 1998. Insulin response during glucose tolerance test in cattle with increasing Wagyu genetic influence. *Can. J. Anim. Sci.* 78, 233–235.

Mir, P.S., Mears, G.J., Ross, C.M., Husar, S.D., Dodson, M.V., Okine, E.K., Kennelly, J.J., Christopherson, R.J., 2001. Effect of alloxan treatments on growth performance, carcass characteristics, insulin response to glucose and intramuscular fat deposition in cattle. *Ann. Nutr. Metab.* 45, 383 (suppl).

Mir, P.S., Mir, Z., Kuber, P.S., Gaskins, C.T., Martin, E.L., Dodson, M.V., Elias Calles, J.A., Johnson, K.A., Busboom, J.R., Wood, A.J., Pittenger, G.P., Reeves, J.J., 2002. Growth, carcass characteristics, muscle conjugated linoleic acid (CLA) content, and response to intravenous glucose challenge in high percentage Wagyu, WagyuX limousin, and Limousin steers fed sunflower oil-containing diets. *J. Anim. Sci.* 80, 2996–3004.

Mir, P.S., Okine, E.K., Goonewardene, L., He, M.L., Mir, Z., 2003. Effects of synthetic conjugated linoleic acid (CLA) or bioformed CLA as high CLA beef on rat growth and adipose tissue development. *Can. J. Anim. Sci.* 83, 583–592.

Mir, P.S., Ross, C.M., Okine, E.K., 2004. Effect of a short duration feed withdrawal on adipogenesis in cattle. *Proceedings of the 10th International College of Clinical Nutrition in Pukhet, Thailand, From November 30th to December 3rd 2004*, p. 136. abstract #s7.17.

National Research Council (NRC), 1996. *Nutrient Requirements of Beef Cattle*, 7th ed. National Academic Press, Washington, DC.

Rule, D.C., Broughton, K.S., Shellito, S.M., Maiorano, G., 2002. Comparison of muscle fatty acid profiles and cholesterol

- concentrations of bison, beef cattle, elk and chicken. *J. Anim. Sci.* 80, 1202–1211.
- SAS Institute Inc., 2005. SAS OnlineDoc® 9.1.3. SAS Institute Inc, Cary, NC.
- Scollan, N., Hocuette, J.-F., Nuernberg, K., Dannenberger, D., Richardson, I., Moloney, A., 2006. Innovations in beef production systems that enhance the nutritional and health value of beef lipids and their relationship with meat quality. *Meat Sci.* 74, 17–33.
- Shah, M.A., Mir, P.S., Aalhus, J.L., Basarab, J., Okine, E.K., 2006. Effects of sunflower seed inclusion in finishing diets for steers on performance, carcass characteristics, muscle and adipose fatty acid composition and meat quality. *Can. J. Anim. Sci.* 86, 37–48.
- Smith, S.B., Chapman, A.A., Lunt, D.K. Harris, J.J., Savell, J.W. 2007. Adiposity of calf-and yearling-fed brangus steers raised to constant age and constant -BW endpoints. *J. Anim. Sci.* (in Press on line on Jan 15 2007).
- Smith, S.B., Crouse, J.D., 1984. Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. *J. Nutr.* 114, 792–800.
- Van Donkersgoed, J., Jewison, G., Bygrove, S., Gillis, K., Malchow, D., McLeod, G., 2001. Canadian beef quality audit 1998–99. *Can. Vet. J.* 42, 121–126.
- Yambayamba, E.S.K., Price, M.A., Foxcroft, G.R., 1996. Hormonal status, metabolic changes and resting metabolic rate in beef heifers undergoing compensatory growth. *J. Anim. Sci.* 74, 57–69.
- Yang, X.J., Albrecht, E., Ender, K., Zhao, R.Q., Wegner, J., 2006. Computer image analysis on intramuscular adipocytes and marbling in the longissimus muscle of cattle. *J. Anim. Sci.* 84, 3251–3258.