Growth, carcass characteristics, muscle conjugated linoleic acid (CLA) content, and response to intravenous glucose challenge in high percentage Wagyu, Wagyu × Limousin, and Limousin steers fed sunflower oil-containing diets^{1,2}

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ABSTRACT: The effect of breed and diet on insulin response to glucose challenge and its relation to intramuscular fat deposition was determined in 36 steers with 12 each of greater than 87% Wagyu (referred to as Wagyu), Wagyu × Limousin, and Limousin breeds. Weaned steers were blocked by weight into heavy, medium, and light calves and placed in six pens with two pens per weight type and with two steers of each breed per pen. Three pens with steers from each weight class were fed backgrounding and finishing diets for 259 d, while the other three pens were fed the same diets where 6% of the barley grain was replaced with sunflower oil. Prior to initiation of the finishing phase of the study the intravenous glucose tolerance test (IVGTT) was conducted in all steers. Once steers were judged as carrying adequate 12th-rib fat, based on weight and days on feed, they were harvested and graded and samples of the longissimus muscle were procured for determination of fat content and fatty acid composition. Dietary oil improved (P = 0.011; 0.06) ADG and feed conversion efficiency of steers during the latter part of backgrounding and only ADG during early part of the finishing period. Generally percent kidney, pelvic, and heart fat was the only adiposity assessment increased (P = 0.003) by dietary oil. The IVGTT results indicated that insulin response to intravenous glucose was lower in Limousin steers than in Wagyu steers. Dietary oil decreased (P = 0.052) fasting plasma insulin concentration in Wagyu steers compared with Limousin steers. The correlation coefficients among the IVGTT measures and intramuscular fat content or marbling score were less than 0.4, and only a negative trend existed between fasting insulin and USDA marbling scores. However, the carcasses of the Wagyu steers graded US Choice, and 66% of the Wagyu carcasses graded US Prime, which were substantially better than the quality grades obtained for the carcasses from the other breed types. Dietary oil did not affect muscle fat content but increased (P = 0.01) conjugated linoleic acid (CLA) concentrations by 339%. Results indicated that IVGTT measures were not appropriate indices of marbling potential in cattle and that dietary oil can enhance CLA content of beef.

Key Words: Carcass Quality, Conjugated Linoleic Acid, Dietary Fat, Glucose, Insulin

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Introduction

A positive price difference exists between Canada AAA and AA, and in the United States among Prime, Choice, and Select beef, because palatability is associated with intramuscular or marbling fat content of meat (May et al., 1993; Jeremiah, 1996). As a result, strategies to increase the proportion of beef carcasses that would grade Canada AAA or USDA Choice are studied (Smith et al., 1995; Van Donkersgoed et al., 1997) along with factors that affect marbling potential of cattle (Brethour, 1994; Dubeski et al., 1997; Middleton et al., 1998).

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Smith and Crouse (1984) showed in vitro that while subcutaneous adipose preferred acetate, glucose was preferred in muscle for fat synthesis, suggesting that increased glucose availability may increase marbling potential. Plasma insulin concentrations in fasted and intravenously glucose-challenged steers with 50 and 75% Wagyu genetics, with high marbling potential (Mir et al., 1997, 1999a), were lower up to 15 min postglucose challenge than in 0% Wagyu cattle (Mir et al., 1998). Furthermore, diet components or their metabolites may influence the physiology of the animal. For example, dietary linoleic acid is converted to conjugated linoleic acids (CLA) in the rumen (Kepler et al., 1966). Conjugated linoleic acid has anticarcinogenic (Ip, 1997), energy partitioning (Dugan et al., 1997; Park et al., 1997) and glucose tolerance normalizing (Ryder et al., 2001) properties. Sheep muscle and goat milk CLA content (Mir et al., 1999b, 2000c) was increased by dietary supplementation with high linoleic acid oils. Thus, the hypotheses of the study were that a delayed or poor response of insulin to glucose challenge would be associated with improved marbling potential and that feeding cattle linoleic acid-rich oils can increase CLA content of beef. Therefore, the objectives of the study were to determine: 1) the relationship between glucose tolerance variables and marbling fat content, and 2) if oil in the feed affected glucose tolerance and CLA content of muscle lipid.

Materials and Methods

Animals and Feeding Management

A total of 36 steers (12 each of 87% Wagyu [referred to as Wagyu], Limousin × Wagyu, and Limousin), born in the spring of 1998 were obtained at weaning (November 30, 1998) and blocked within each breed type by weight into heavy, medium, and light calves. The calves were placed in six pens with two pens per weight type, and two steers of each breed were included in each pen. One pen from each weight class (n = 3) was fed the control diet (Table 1), while the other pens received the sunflower oil (6% of diet DM) supplemented diet. Each pen was 4.9×15.2 m with a roof that covered the feed bunk and 5m of the pen. The experimental diets were introduced on December 18, 1998. Steers were not implanted. All animals were cared for as described by the Washington State University (WSU) Animal Care and Use Committee and experiments were conducted after the approval for the procedures was procured (#2823 and 2722).

Animals were fed the backgrounding diets for 140 d, which included an adjustment period of 4 wk at the initiation of the experiment. After the backgrounding phase, steers were adapted to a high barley diet [80 or 74% on DM basis for control and oil supplemented diets, respectively (Table 1)]. Steers were maintained on the diets from May 28, 1999, until the average weights were 484, 513, and 607 \pm 7.4 kg for the Wagyu, Wagyu × Limousin, and Limousin steers, respectively. The days on the finishing diets were 63 (July 30, 1999), 91 (August 28, 1999), and 118 (September 23, 1999) d for the steers in the heavy, medium, and lightweight blocks, respectively. Animals were fasted for 24 h prior to slaughter, and live weight was determined. One day after slaughter, carcasses were weighed and graded to determine 12th rib backfat thickness, longissimus muscle area, percentage kidney, pelvic, and heart fat (KPH), USDA yield grade, and marbling score (USDA, 1996). A 2.5-cm rib steak from the 12th thoracic rib was obtained from the left side of each carcass for determination of lipid content and the extracted lipid was used for determination of fatty acid composition (Mir et al., 2000c).

Similarly, the fatty acid composition of the diets was also determined. Briefly the fat extracted from either the muscle or the diets was methylated by heating a 10 to 20 mg sample of the fat with 500 μ L of 20% tetramethylguanidine in methanol for 10 min in a boiling water bath. Once the samples were cooled, 5 mL of saturated NaCl and 2 mL of hexane were added and the organic phase was collected, evaporated under a stream of nitrogen and then made up to a volume of 5 mL with hexane (Mir et al., 1999c). The methylated fatty acids were separated by gas chromatography. For gas chromatography the Supelcowax-10, 30 m \times 0.25 mm \times 0.25 µm column was used in a HP5830 gas chromatograph fitted with a 18835B capillary inlet system, 18850A integrator (Hewlett-Packard, Mississauga, Ontario, Canada) and a flame ionization detector. The temperature program and carrier gas flow rates are indicated in Mir et al., 1999c.

Intravenous Glucose Tolerance Test (IVGTT)

When the steers had been on the backgrounding diet for 123 d, the IVGTT was conducted (Mir et al., 1998) on each day of the week of May 5, 1999. The average fasted weights of the steers were 359, 380, and 446 \pm 6.1 kg for the Wagyu, Wagyu × Limousin, and Limousin steers, respectively. Briefly, animals were denied feed for 24 h. Animals were weighed, and a sterile 50% glucose solution in physiological saline at 0.3 g/kg body weight was administered via jugular catheter. Blood samples were taken at 5- and 2-min preglucose administration and 5, 10, 15, 20, 25, 30, 35, 45, 60, 90, and 120 min postglucose administration from the opposite jugular vein. The blood samples were collected in heparinized evacuated tubes and held on ice until they were centrifuged for 15 min at $600 \times g$ to harvest the plasma. Plasma glucose and insulin concentrations were determined by using the glucose oxidase kit (Sigma kit #510 DA, Mississauga, ON) and by radioimmunoassay (Mears, 1993), respectively. The coefficients of variation for inter- and intraassay ranged between 8.63 and 5.46%; and 7.42 and 7.34% for the insulin assay. The plasma glucose and insulin concentrations were plotted

	Backgrou	inding diets	Finishing diets		
	Control	Sunflower oil	Control	Sunflower oil	
Ingredients (% DM)					
Hay (pea hay)	54	54	16.5	16.5	
Rolled barley	35	29	80	74	
Soybean meal	10	10	2.5	2.5	
Minerals and vitamins	1	1	1.0	1.0	
Oil	—	6	—	6	
Nutrient composition					
Crude protein	15.0	13.2	12.6	11.7	
Ash	9.1	11.0	6.2	6.6	
Neutral detergent fiber	29.7	29.9	22.6	26.2	
Acid detergent fiber	22.2	25.3	12.8	17.0	
Lignin	3.2	4.6	1.2	5.0	
Fat	1.1	7.2	2.2	8.5	
NEm (Mcal·kg)	1.68	1.84	1.94	2.11	
NEg (Mcal·kg)	1.05	1.18	1.29	1.42	
Fatty acid (% DM)					
16:0	0.14	0.45	0.43	0.81	
18:0	0.01	0.03	0.01	0.10	
18:1	0.13	0.90	0.32	2.50	
18:2	0.29	1.78	0.82	3.36	
18:3	0.05	0.07	0.08	0.19	
20:0	0.01	0.02	0.01	0.03	
Conjugated linoleic acids	0.00	0.02	0.00	0.04	

 Table 1. Ingredient, nutritional composition, and fatty acid content of control and sunflower oil (6% of DM) containing diets fed to steers during backgrounding and finishing

to develop the curves and the rate of increase in insulin was calculated for each animal (Mir et al. 1998).

Statistical Analysis

Data obtained for the weights of the animals at different stages of growth were analyzed using the General Linear Models procedure of SAS (SAS Inst. Inc., Cary, NC) as a randomized complete block design with two dietary treatments. The values from the IVGTT and the carcass characteristics were analyzed as a randomized complete block design with 2×3 (diet and breed) factorial arrangement of treatments. Variation due to blocks was extracted in the models employed for the analysis. The protected least significant differences method was used to determine differences among treatment means. Overall correlation coefficients among marbling score, muscle fat content, backfat thickness, percent KPH fat, and IVGTT measurements were obtained.

Results and Discussion

Diet Composition

The composition of the two diets used for backgrounding the steers was comparable (Table 1). Some reduction in protein was observed due to replacement of the barley in the diet with oil. However, compositionally the diets met the requirements of growing steers (NRC, 1996). Similarly, the finishing diets were adequate to meet the requirements of the steers. The fatty acid composition of sunflower oil is comprised of 68.5% 18:2 *cis* 9, *cis* 12, 21.7% mixed 18:1, 5.5% 16:0, 3.5% 18:0, and trace amounts of 18:3 and 20:0 (Palmquist 1988). Since the rolled barley in the control diets was replaced with the sunflower oil at the level of 6% of DM, the fatty acid composition of the oil-containing backgrounding and finishing diets was elevated for all fatty acids (Table 1). The increase in 18:2 *cis* 9, *cis* 12 was 514 and 310% for the backgrounding and finishing diets, respectively.

Steer Growth Characteristics

Body weights for steers fed control and sunflower oilcontaining diets are provided in Table 2, and significant differences due to dietary oil did not exist during the backgrounding phase. The weight of oil-fed steers after the first 4 wk of backgrounding was 4% lower than that of control steers, but by the end of the backgrounding period this deficit had been overcome, and weights of steers in both treatments were comparable. Steer weights did not differ due to dietary oil during finishing.

The ADG through the first 4 wk of the experiment tended to be lower for steers fed the oil-containing diet than those fed the control diet (Table 3), which may be related to the ability of the ruminal bacteria in the animals to adapt to the oil in the diet. However, after 140 d of backgrounding, dietary sunflower oil added at 6% of the diet tended to increase (P = 0.07) ADG by 8%

	Dietary t	reatment	
Response	Control	Oil	SEM
Backgrounding phase			
Initial weight (kg) $(n = 18)$	248	245	5.9
d 28 weight (kg) $(n = 18)$	273	262	6.7
d 140 weight (kg) (n = 18)	391	399	10.6
Finishing phase			
Initial weight (kg) $(n = 18)$	421	427	11.6
d 63 weight (kg) $(n = 18)$	496	510	13.2
Final weight ^b (kg) $(n = 17 \text{ or } 18)$	$531~\pm~15.4$	539 ± 16.7	

Table 2. Weights of steers from high percentage (>87%) Wagyu, Wagyu × Limousin,and Limousin breeds at different stages prior to slaughter fed dietswithout (Control) or with (Oil) 6% sunflower oil^a

^aData are pooled across breed types.

^bStandard errors of means for final weight are means \pm SEM.

(Table 3). These results concur with observations in cattle fed sunflower seed (Gibb et al., 2001). If the first 4 wk during backgrounding were eliminated, a 16% improvement (P = 0.011) in ADG of steers fed the oil treatment was observed relative to that of control steers (29 to 140 d; Table 3). Similar improvement in ADG was observed in sheep fed alfalfa diets containing acidulated fatty acids at 5% of the diet (Mir, 1988). Through the first 63 d of finishing, dietary oil tended to improve (P = 0.06) the ADG of steers by 10%, which is comparable to the average benefit of implanting (Duckett et al., 1997). The rates of gain noted in the present study for the steers fed the control diet during the backgrounding or finishing period compared well with growth rates

observed in earlier studies, where the animals were neither implanted nor provided with ionophores (Mir et al., 1997, 1999a).

Dietary oil decreased feed consumption by 14% during the backgrounding phase, leading to feed:gain (F:G) ratios of 7.45 and 5.93 in the control and oil-fed steers, respectively. This resulted in a 20% improvement (P =0.05) in steers fed the oil-containing diet. During the first 63 d of the finishing phase, the intake of the control and oil-containing diets was 8.4 and 8.6 kg/(head·d), respectively, and the respective feed:gain ratios were 7.1 and 6.5 and not different due to diet. The efficiencies observed due to dietary oil, especially during the backgrounding phase, may have been related to the higher

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Table 3. ADG, dry matter intake (DMI), and feed conversion efficiency (F:G) during
backgrounding and finishing of steers from high percentage (>87%) Wagyu,
Wagyu × Limousin, and Limousin breeds at different stages prior to
slaughter fed diets without (Control) or with (Oil) 6% sunflower oil ^x

	Dietary t		
Response	Control	Oil	SEM
Backgrounding phase			
ADG (kg/d)			
d $0-28 (n = 18)$	0.85	0.64	0.10
d 0–140 (n = 18)	1.02	1.10	0.04
d 29–140 (n = 18)	1.06^{b}	1.23^{a}	0.04
DMI (kg/d)			
$d \ 0 - 140 \ (n = 18)$	7.60	6.53	0.42
F:G			
$d \ 0-140 \ (n = 18)$	7.45^{a}	$5.93^{ m b}$	0.33
Finishing phase			
ADG (kg/d)			
$d \ 0-63 \ (n = 18)$	1.20	1.33	0.05
d 0-final weight (n = $17 \text{ or } 18$)	$1.25~\pm~0.05$	$1.26 ~\pm~ 0.05$	
DMI (kg/d)			
d $0-63$ (n = 18)	8.40	8.60	0.44
F:G			
d $0-63 (n = 18)$	7.10	6.50	0.46

 $^{\rm a,b,c}\!{\rm Within}$ a row means without a common superscript letter differ (P < 0.05).

^xData are pooled across breed types.

^yStandard errors of means for \hat{ADG} for d 0—final weight are means \pm SEM.

	Wagyu		Wagyu × Limousin		Limousin			
Response	Control	Oil	Control	Oil	Control	Oil	SEM	
Slaughter weight (kg) Cold carcass weight (kg) Longissimus muscle area (cm ²) USDA Yield Grade	$493^{ m bc}\ 310^{ m c}\ 85^{ m bc}\ 2.33^{ m a}$	459° 290° 79° 2.07ª	472° 313° 83° 2.22ª	$521^{ m b}\ 335^{ m b}\ 89^{ m b}\ 2.28^{ m a}$	$587^{ m a}\ 382^{ m a}\ 106^{ m a}\ 1.50^{ m b}$	$egin{array}{c} 603^{ m a} \\ 396^{ m a} \\ 107^{ m a} \\ 1.60^{ m b} \end{array}$	$10.5 \\ 6.1 \\ 2.9 \\ 0.07$	

 Table 4. Slaughter weight and carcass characteristics of steers from high percentage

 (>87%) Wagyu, Wagyu × Limousin, and Limousin breeds fed diets

 without (Control) or with (Oil) 6% sunflower oil

 $^{\rm a,b,c}\!{\rm Within}$ a row means without a common superscript letter differ (P < 0.05).

energy content of the diet (Table1), but also to the effect of dietary oil on ruminal protozoa numbers (Ivan et al., 2001), or on small intestinal digesta characteristics, such as viscosity and enzyme activity (Mir et al., 2002). These factors in tandem can lead to elevation of nutrient concentrations in the portal vein (Mir et al., 2000a) and improved production efficiency.

Carcass Characteristics

As expected, the carcass weights of Limousin steers were greater than those of the crossbred and Wagyu steers (Table 4). The longissimus muscle area was least in oil-fed Wagyu steers and greatest in Limousin steers receiving either diet. The yield grade was greatest in Wagyu steers fed the control diet, followed by the oilfed crossbred steers, and least in Limousin steers fed the control diet. Similarly, 12th rib fat and percent KPH fat was greatest in oil-fed crossbred steers and Wagyu steers, respectively (Table 5).

Feeding the oil-containing diet to the steers did not affect marbling scores and this concurs with the response observed to dietary high oil corn (Duckett et al., 2000). Breed of cattle influenced marbling score. The carcasses from Wagyu steers had the highest marbling score, followed by those from crossbred steers, and carcasses from Limousin steers had the lowest marbling scores (Table 5). As a result, 34% of the Wagyu carcasses graded Choice, and 66% of these carcasses graded Prime, while 8% of the carcasses from the crossbred steers graded Select and 92% of these carcasses graded Choice. This compares with the reports of Mir et al. (1999a) for Wagyu \times Angus steers. For carcasses from Limousin steers, 75% were Select and only 25% graded Choice, which was as expected. The lipid content of the longissimus muscle corresponded with the marbling scores of the carcasses of the steers in the three breed groups.

Fatty Acid Composition

The fatty acid composition of fat extracted from the longissimus muscle indicated that dietary oil decreased percentage of 16:0 and 16:1 (Table 6). Decreases of endogenously synthesized fatty acids have been observed when exogenous fatty acids were provided to adipose tissue in vitro (Vernon, 1981) and have been noted in fat from steers fed sunflower seeds (Gibb et al., 2001) and in sheep fed sunflower oil (Ivan et al., 2001). These decreases in the C16 fatty acids indicate a negative feedback inhibition of fatty acid synthesis by the exogenous fatty acids. Since C18:2 was provided in the diet as sunflower oil, which is 68.5% 18:2 cis 9; cis 12, a trend towards an increase in the fatty acid content of 18:2 in the muscle was observed and corroborates previous observations in sheep (Mir et al., 2000c; Ivan et al., 2001). The CLA content of the muscle lipid was increased from 0.28 to 1.23%, an increase of 339%, due to feeding sunflower oil-containing diets to the steers. The observed increase in CLA is comparable to observations of Mir et al. (2000c) in sheep muscle. Failure to observe an increase in CLA to dietary extruded soybean has been reported (Madron et al. 2000). The diets used by these authors were comprised of addition of soybean

Table 5. Carcass characteristics of steers from high percentage (>87%) Wagyu, Wagyu × Limousin,and Limousin breeds fed diets without (Control) or with (Oil) 6% sunflower oil

	Wa	Wagyu		Wagyu $ imes$ Limousin		Limousin	
Response	Control	Oil	Control	Oil	Control	Oil	SEM
12th rib fat depth (mm)	9.80 ^{xy}	8.30^{y}	8.25^{y}	12.50 ^x	7.30^{y}	9.25^{xy}	1.52
Kidney, pelvic, and heart fat (%)	3.0^{y}	3.8^{x}	2.8^{yz}	3.3^{xy}	2.2^{z}	2.6^{yz}	0.22
Marbling score ^a	882 ^x	852^{x}	585^{y}	581^{y}	463^{z}	478^{z}	31
Lipid (% DM)	26.3 ^x	29.8 ^x	18.7^{y}	18.4 ^y	12.1^{z}	13.8^{z}	2.7

 a U.S. marbling score: Slight = 400 to 499; Small = 500 to 599; Modest = 600 to 699; Moderate = 700 to 799; and Slightly Abundant = 800 to 899.

^{x,y,z}Within a row means without a common superscript letter differ (P < 0.05).

without (Control) or with (Oil) 6% sunflower oil								
	Wagyu		Wagyu $ imes$ Limousin		Limousin			
Fatty acids (weight %)	Control	Oil	Control	Oil	Control	Oil	SEM	
14:0	4.39	4.35	4.94	4.86	4.44	4.81	0.23	
14:1	1.36^{a}	$1.10^{ m bc}$	1.44^{a}	$1.17^{ m bc}$	$1.10^{ m bc}$	1.00^{c}	0.10	
16:0	31.96^{a}	28.86^{b}	32.84^{a}	29.08^{b}	31.25^{a}	28.18^{b}	0.61	
16:1	5.50^{a}	4.01^{b}	$5.93^{\rm a}$	4.22^{b}	5.18^{a}	3.82^{b}	0.30	
18:0	9.91^{a}	12.22^{b}	10.19°	12.84^{b}	11.92^{b}	14.08^{a}	0.40	
18:1	44.16^{ab}	45.25^{a}	41.67°	43.70^{ab}	42.25^{bc}	43.37^{abc}	0.73	
18.2	1.18^{bc}	1.91^{ab}	$1.52^{\rm bc}$	1.95^{ab}	1.66^{bc}	2.23^{a}	0.21	
18:3	$0.11^{ m b}$	$0.05^{\rm c}$	0.17^{a}	$0.08^{ m bc}$	0.21^{a}	0.17^{a}	0.04	
Conjugated linoleic acids	0.27^{b}	1.29^{a}	0.28^{b}	1.19^{a}	0.29^{b}	1.22^{a}	0.13	

Table 6. Fatty acid composition of longissimus muscle from steers from highpercentage (>87%) Wagyu, Wagyu × Limousin, and Limousin breeds fed dietswithout (Control) or with (Oil) 6% sunflower oil

^{a,b,c}Within a row means without a common superscript letter differ (P < 0.05).

to silage containing diets, while in the present study, the oil was provided in diets containing pea hay. Beaulieu et al. (2000) and McGuire et al. (1998) report that inclusion of soybean oil to 80% corn diets or the feeding of high oil corn diets, respectively, did not increase the CLA content of the tissues substantially, even though one of the CLA isomer (C18:2 trans 10; cis 12) was increased in rumen contents. However the values in tissues that these authors reported were higher than that observed in the present study for steers fed control diets and is related to the grain in the diet. In the present study barley was used, and it has half the oil content of corn (Palmquist, 1988); thus, the lower CLA content in steers fed the control diets relative to reported values (Beaulieu et al., 2000). The observed levels of CLA are comparable to values reported by Mir et al. (2000b). French et al. (2000) determined that inclusion of ensiled forage or high proportions of grain in the diet of cattle did not support deposition of CLA in tissues, while pasture or hay diets were conducive to deposition of CLA. Similarly, Ivan et al. (2001) found that sheep fed sunflower oil-supplemented and 65% barley silage-containing diets did not have a substantial increase in CLA content in muscle, adipose tissue, or organs. On the contrary Mir et al. (2000c) found a greater than threefold increase in muscle CLA content when ruminating sheep were fed a pelleted diet comprised of alfalfa and barley (1:1) that was supplemented with 6% safflower oil. In the present study the supplementation of diets containing pea hay with sunflower oil, which is high in 18:2 cis 9; cis 12, resulted in the substantial increases in CLA content of the longissimus muscle. The oil-containing diets were fed both through backgrounding and finishing, and it is suspected that the duration of feeding also contributed to the observed increase in CLA.

Since the fat content of the muscle in Wagyu steers was greater than that in the other animals, the available CLA would be substantially enhanced. Both bioactive CLA isomers (18:2; *cis* 9, *trans* 11 and 18:2; *trans* 10, *cis* 12) are included in the values presented (Table 6) because it is reported that the predominant CLA is 18:2; *cis* 9 *trans* 11 in ruminant products Bauman et al. (2000) and at the time of the study chromatography columns and individual standards to quantify these two isomers were not available. Fat from muscle of Wagyu cattle has been found to contain higher percentages of 18:1 (Sturdivant et al., 1992; Xie et al., 1996; Mir et al., 2000b). However, substantially greater percentages of 18:1 in samples from Wagyu steers were not observed in the present study.

Intravenous Glucose Tolerance Test

The IVGTT was conducted in yearling steers to determine the prevalence of associations between marbling potential and their potential to handle glucose. As indicated previously the in vitro work of Smith and Crouse (1984) suggests that muscle requires glucose for adipogenesis unlike subcutaneous fat. The timing of intramuscular fat deposition occurs mostly when high grain diets are fed to cattle. It follows, therefore, that as grain in the diet increases the production of propionate is increased (Sutton, 1980), this propionate, along with gluconeogenic amino acids, has been concluded to be a significant source of the glucose (Reynolds, 1995). This glucose increase has resulted in increased insulin concentrations (Mir et al. 2000a), but can also be the resource for the increased intramuscular fat deposition observed during the finishing phase. Although plasma insulin concentration and its association to marbling fat has been investigated previously (Trenkle and Topel, 1978; Matsuzaki et al., 1997), insulin response to glucose challenge and the association of the response to marbling fat in cattle has not been thoroughly evaluated. It was hypothesized that the glucose management potential prevalent at the yearling stage might influence the ultimate marbling fat deposition in the animals, and as a result, the IVGTT were conducted prior to initiation of the finishing period. The IVGTT has been used previously to identify and develop lines of sheep with varying adiposity (Francis et al., 1994). Previously, Mir et al. (1998) found significant associations



Figure 1. Jugular plasma glucose concentrations prior to and during the intravenous glucose tolerance in test steers of high percentage (> 87%) Wagyu (W), Wagyu × Limousin (LW) and Limousin (L) breeds fed diets without (A) and with (B) 6% sunflower oil (n = 6).

to exist especially between fat content of muscle and glucose and insulin concentrations during the IVGTT, which needed confirmation.

In the present study, the curves for plasma glucose and insulin during the IVGTT (Figures 1 and 2) were different for the steers from the three breed types, and dietary oil affected the insulin curves in the three breeds of steers differently. In steers fed either diet, the peak plasma glucose concentration occurred 5 min postglucose administration, similar to previous observations (Mir et al., 1998). In steers fed either diet, the range of these peak values was between 18 and 22.6 mM, and similar to values reported in Holstein cows (Bigner et al., 1996), but higher than values noted previously in beef cattle (Mir et al., 1998). The glucose concentration was highest for the control crossbred and oil-fed Limousin steers (Figure 1). In an experiment conducted with 0, 50, and 75% Wagyu steers (Mir et al., 1998), differences in plasma glucose concentrations were not observed during the IVGTT, unlike the results obtained in the present study. In general, the plasma glucose concentrations tended to be higher (P = 0.1) in Limousin and crossbred steers fed the control diet, 45 min postglucose administration, while these concentrations were elevated only in Limousin steers when the oil-containing diet was fed.

The plasma insulin concentrations during the IVGTT are provided in Figure 2. Plasma insulin concentrations in steers fed oil were greater (P = 0.05) than in steers fed the control diet at fasting and up to 60 min postglucose administration. In general, the Limousin steers had lower concentrations of plasma insulin than the Wagyu steers after glucose administration, which was unexpected, based on the original premise of the experiment. In previous experiments (Mir et al., 1998), the 0% Wagyu steers had higher insulin concentrations than steers with 50 or 75% Wagyu genetics at fasting, and up to 15 min postglucose administration. In the present study, the peak plasma insulin concentrations for the Wagyu steers were similar to those observed by Mir et al. (1998), but the values for the Limousin steers were much lower than that observed for the 0% Wagyu steers. Although the forage used was pea hay and not barley silage as in the study by Mir et al. (1998), it was not expected that this difference in dietary composition would affect the plasma insulin concentrations to the extent observed in the crossbred and Limousin steers.



Figure 2. Jugular plasma insulin concentrations prior to and during the intravenous glucose tolerance test in steers of high percentage (> 87%) Wagyu (W), Wagyu × Limousin (LW) and Limousin (L) breeds fed diets without (A) and with (B) 6% sunflower oil (n = 6).

Among steers fed the oil-containing diets, the Wagyu steers had the lowest (P = 0.05) fasting insulin concentration. Although differences among plasma insulin concentrations after glucose administration did not exist, the peak plasma insulin concentrations for sunflower oil-fed steers were higher than those observed in steers fed the control diet. These relatively higher insulin concentrations indicate that the steers fed oil may be less reactive to circulating insulin, owing to higher adiposity, which was perhaps reflected in the percent KPH fat at slaughter, but not in other adiposity indices, such as backfat depth or muscle fat content.

The correlation coefficients among IVGTT traits, marbling scores, muscle fat content (DM basis), backfat thickness, and KPH were less than values observed previously (Mir et al., 1998). Marbling scores tended to be negatively correlated to postglucose administration plasma glucose concentrations at 60 min (r = -0.31, P = 0.07, n = 36), 90 min (r = -0.33, P = 0.05, n = 36), and 120 min (r = -0.30, P = 0.08, n = 36), contrary to expectation. Furthermore, a negative correlation existed between muscle fat content and plasma glucose concentration at 90 min postglucose administration (r = -0.29, P = 0.09, n = 36).

Only a small negative trend (r = -0.27, P = 0.12, n = 36) was observed between fasting insulin concentrations and marbling scores. No other plasma insulin concentrations during the IVGTT were correlated to either marbling score or muscle fat content, which was contrary to expectations. Significant correlation coefficients were not observed between backfat thickness and plasma concentration of glucose and insulin, pre- or postglucose administration. A small trend existed between plasma glucose concentration at 10 min postglucose administration and backfat thickness. The absence of response in backfat depth to dietary oil may be related to the energy partitioning effect of the CLA formed and the timing of the maturation of this depot. The subcutaneous adipose tissue is a later maturing depot than KPH fat (Jones et al., 1985) and may have been affected by the CLA, which was bioformed and deposited in this tissue.

Correlation coefficients ranging from -0.28 to -0.42 (P = 0.10 to P = 0.012) were observed between KPH and plasma glucose and insulin concentrations in samples collected 25 to 120 min after glucose administration. Since all the correlation coefficients were small, regression equations were not developed for prediction of intramuscular fat deposition from IVGTT parameters.

The major deviations from the previous study (Mir et al., 1998) observed with regard to response to glucose administration may be due to innate differences in breed types selected for the present study, especially with regard to the Limousin steers. These steers were different from the 0% Wagyu steers employed previously, which were European and British crossbred steers.

Implications

Many strategies have been adopted to improve the number of carcasses that grade Canada AAA or USDA Choice. The expected association between glucose availability to the animal and marbling did not exist and the insulin response to glucose challenge observed in steers fed control and oil-containing diets may be related to insulin insensitivity, rather than as a predictor of marbling potential. Dietary oil can be employed to alter fatty acid composition of adipose in cattle but not to alter content of intramuscular fat. Dietary oil can be used to substantially increase CLA content of muscle fat, but the relative increase may be affected by other components of the diet.

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