Effect of Wagyu genetics on marbling, backfat and circulating hormones in cattle

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[2]*Research Centre, Agriculture and Agri-Food Canada, Lacombe, Alberta, Canada T4L 1W1.* *LRC contribution no. 3879950, received 3 December 1999, accepted 12 October 2000.*

Mears, G. J., Mir, P. S., Bailey, D. R. C. and Jones, S. D. M. 2001. **Effect of Wagyu genetics on marbling, backfat and circulating hormones in cattle**. Can. J. Anim. Sci. **81**: 65–73. Backfat depths, marbling scores, and concentrations of plasma insulin, cortisol, triiodothyronine (T3), and thyroxine (T4) were determined in 394 calves with and without Wagyu genetics. Hormone concentrations were determined three to five times for hormones between weaning and slaughter. Calves were weighed and backfat was ultrasonically determined at 28-d intervals, and assigned for slaughter when ultrasonic backfat depths approached 12 mm. Heifers weighed less ($P < 0.001$) and had more backfat ($P < 0.05$) and more marbling ($P < 0.05$) than steers at slaughter. Breed influenced slaughter weight ($P < 0.001$) and average backfat depth ($P < 0.001$), with Wagyu/Angus and Wagyu/Hereford crosses and Angus purebreds generally having greater backfat depths and lower slaughter weights than other crosses and purebreds. Marbling scores were not affected by breed ($P > 0.1$), although Wagyu crosses had more marbling ($P < 0.05$) compared with purebreds when variability in marbling due to differences in backfat depth was removed. Plasma cortisol, T3 and T4 were affected by calf breed, although there were no clear patterns for groups of breeds or breed crosses. There was a slight trend ($P < 0.2$) towards higher insulin levels and lower cortisol, T3 and T4 levels in some groups of calves with more backfat. Higher mean levels ($P < 0.05$) of T3 and T4 were found in all but one group of animals with more marbling. Thus, thyroid hormones may have a role to play in enhancing marbling in cattle.

Key words: Wagyu cattle, marbling, backfat, insulin, cortisol, thyroid hormones

Mears, G. J., Mir, P. S., Bailey, D. R. C. et Jones, S. D. M. 2001. **Effets du génome Wagyu sur le persillé, l'épaisseur du gras dorsal et la concentration des hormones dans le sang chez les bovins**. Can. J. Anim. Sci. **81**: 65–73. On a évalué l'épaisseur du gras dorsal, l'état du persillé et la concentration d'insuline, de cortisol, de tri-iodothyronine (T3) et de thyroxine (T4) dans le sang de 394 veaux avec et sans caractères génétiques Wagyu. La concentration des hormones a été établie trois à cinq fois entre le sevrage et l'abattage. Les veaux ont été pesé et on a déterminé l'épaisseur du gras dorsal à intervalles de 28 jours avant d'envoyer l'animal à l'abattoir quand elle approchait 12 mm, selon la sonde à ultrasons. Les génisses étaient moins lourdes ($P < 0{,}001$) que les bouvillons à l'abattage, mais elles présentaient plus de gras dorsal ($P < 0{,}5$) et un meilleur persillé ($P < 0{,}05$). La race influe sur le poids à l'abattage ($P < 0{,}001$) et une couche de gras dorsal d'épaisseur moyenne ($P < 0{,}001$), les hybrides Wagyu/Angus et Wagyu/Hereford et les Hereford purs se caractérisant généralement par une couche plus épaisse de gras dorsal et un poids plus faible à l'abattage que les autres croisements ou races pures. L'état du persillé n'est pas affecté par la race ($P > 0{,}1$), bien que les hybrides Wagyu présentent un meilleur persillé ($P < 0{,}05$) que les sujets de race pure quand on ne tient pas compte des variations attribuables aux fluctuations de l'épaisseur du gras dorsal. La concentration de cortisol, de T3 et de T4 dans le sang est affectée par la race du veau, même si aucune tendance ne se dessine clairement pour telle ou telle race ou certains croisements. On note une légère tendance ($P < 0{,}2$) à la hausse de la concentration d'insuline et à la baisse de la concentration de cortisol, de T3 et de T4 chez quelques groupes de veaux présentant une couche de gras dorsal plus épaisse. Tous les groupes caractérisés par un meilleur persillé sauf un présentaient une concentration moyenne plus élevée ($P < 0{,}05$) de T3 et de T4. Les hormones de la glande thyroïde pourraient donc jouer un rôle à ce niveau chez les bovins.

Mots clés: Bvins Wagyu, persillé, gras dorsal, insuline, cortisol, hormones thyroïdiennes

Tenderness inconsistency has been cited as the main contributor to the perceived decline in beef quality found in consumer surveys (McDonell 1988). Therefore, improving customer satisfaction with beef tenderness will do much to improve consumer perception of beef. The amount of marbling fat present in beef may (McDonell 1990), or may not (Wheeler et al. 1994), contribute significantly to the tenderness of beef. Regardless of the effect of marbling on tenderness, it positively impacts the eating quality of beef (Jeremiah 1996).

There is considerable variation in the degree of marbling found in beef carcasses in Canada (Jones et al. 1991), an indication that there is much room for improvement in this area. A number of dietary, genetic and other factors contribute to the differences in marbling that occur in beef carcasses (Jones et al. 1991; Kauffman et al. 1968; Koch et al.

Abbreviations: **RIA**, radioimmunoassay; **T3**, triiodothyronine; **T4**, thyroxine

1976; Lunt et al. 1993; Swortzel et al. 1984). Japanese Wagyu cattle exhibit a high degree of marbling; however, it is not known if this is due to genetics, or feeding and management, or a combination of both (Lunt et al. 1993).

The genotypes of animals are expressed through a number of physiological systems to produce differences in growth and fat deposition. One of these is the endocrine system, which plays a major role in the partitioning of nutrients into either muscle or fat (Trenkle and Marple 1983; Etherton and Kensinger 1984). Insulin, cortisol, and thyroid hormones (T3 and T4) play important roles in determining the amount of total fat found in carcasses of ruminants (Etherton and Kensinger 1984). Little research has been done to determine if these hormones affect the amount of marbling fat deposited in muscle. However, given their role in total fat deposition, it is likely that at least one of these hormones is involved in the deposition of marbling fat.

The objective of this research was to determine the effects of Wagyu genetics on circulating levels of metabolic hormones, marbling and backfat thicknesses of cattle. A better understanding of the factors that control marbling could lead to methods to improve and control marbling in beef cattle, thus enhancing beef tenderness and customer satisfaction.

MATERIALS AND METHODS

Animals

The data reported here were from animals used in a larger experiment to determine growth performance and carcass characteristics of cattle having Wagyu genetics. Hormone data were collected from these cattle and combined with backfat and marbling data from the carcass evaluations for the present study. Calves were obtained from several sources. They were castrated and vaccinated at weaning, prior to the start of this experiment. Dehorning, where required, was done shortly after calving. Care of the animals and procedures used were in accordance with the guidelines of the Canadian Council on Animal Care (1993).

1992–1993

Data from a total of 87 Wagyu crossbred calves that were offspring of Angus, Charolais, Hereford, Holstein, and Simmental cows inseminated with Wagyu semen are reported in this study. In addition, data from 92 Angus, Hereford and Holstein purebred, and Hereford/Charolais and Hereford/Simmental crossbred calves were obtained.

1993–1994

Data from a total of 118 Wagyu crossbred calves that were offspring of Angus, Charolais, Hereford, Holstein, and Simmental cows inseminated with Wagyu semen are reported in this study. In addition, data from 97 Angus, Hereford and Holstein purebreds, and Hereford/Angus, Hereford/Charolais, Hereford/Simmental, and Simmental/Angus crossbred calves were obtained.

In both years the calves were placed in the feedlot trial at weaning, with the majority of them individually fed at the Lethbridge Research Centre. The remainder were group fed at the Lacombe Research Centre (two pens of 25 in 1992–1993 and two pens of 18, plus two pens of 24 in 1993–1994). Calves were assigned to pens based on breed so that numbers of each breed in the pens were balanced. Both locations followed a similar feeding program and data collection schedule, except that daily feed intakes were obtained on an individual basis at Lethbridge and on a pen basis at Lacombe. Calves were weighed at weaning and at 28-d intervals until assigned for slaughter when backfat approached 12 mm based on ultrasound determinations obtained at each 28-d weighing. Animals from Lethbridge were trucked to Lacombe where they were fed the last 2 wk before slaughter.

Table 1. Composition of diet supplements

	Diet[z]		
	80-15-5	40-55-5	60-35-5
Ingredient (%)			
Ground barley	53.6	72.9	64.9
Canola meal	7.25	2.75	4.25
Blood meal	7.50	3.00	4.50
Distillers grains	7.50	3.00	4.50
Molasses	0.75	0.75	0.75
Canola oil	0.25	0.25	0.25
Calcium carbonate	6.00	4.00	5.00
Dicalcium phosphate	2.00	2.00	2.00
Beef mineral mix	10.0	10.0	10.0
Vitamin ADE premix	0.15	0.15	0.15
Perma Pel	0.25	0.25	0.25
Rumensin	4.75	0.95	3.45

[z]Barley, barley silage, supplement percentages, respectively.

Diets

Calves were first placed on a roughage diet for 2–3 wk to get them all to the same starting point since they were assembled from a number of sources both years.

1992–1993

At weaning, calves at Lethbridge were randomly assigned within sex (heifer vs. steer) and breed to either a high-energy or a high-forage diet, whereas at Lacombe steer calves only (heifers not available) were randomly assigned within breed to either diet. The two diets were chosen to evaluate the growth response and carcass composition of Wagyu crosses to two widely different types of diet. Both diets were balanced with respect to protein on a dry matter basis. Calves on the high-energy diet were adapted over a period of 4 wk to a diet of 80% barley, 15% barley silage, and 5% pelleted supplement (Table 1, diet 80-15-5), which they received until slaughter. Calves on the high-forage diet were adapted over a period of 4 wk to a diet of 40% barely, 55% barley silage, and 5% pelleted supplement (Table 1, diet 40-55-5), which they received until they weighed 350–375 kg. They were then switched to a diet of 80% barley, 15% barley silage and 5% pelleted supplement (Table 1, diet 80-15-5) and received this until they were slaughtered.

1993–1994

All calves were assigned to the same diet at weaning. Calves were adapted over a period of 4 wk to a diet of 60% barley,

35% barley silage and 5% pelleted supplement (Table 1, diet 60-35-5), which they received until they weighed 340 kg. At 340 kg, they were adapted over a 2-wk period to a diet of 80% barley, 15% barley silage and 5% pelleted supplement (Table 1, diet 80-15-5), which they received until slaughter.

Backfat and Marbling

Calves were slaughtered at the Lacombe Research Centre where blue tag carcass data were collected by certified Agriculture and Agri-Food Canada graders. Carcass backfat was the average of three measurements: medial, middle, and lateral loin backfat at the 12th–13th rib site. Carcass marbling was scored on an inverse 10-point scale that specified 1 for maximum (very abundant) and 10 for devoid of marbling (Newman et al. 1994).

Blood Sampling

Blood samples were collected from non-fasted calves in 10 mL EDTA vacutainer tubes via jugular venipuncture after calves were secured in a squeeze headgate. Samples were stored on ice until the completion of sample collection each day. Plasma was separated by centrifugation and stored at –40°C until assayed for hormones.

1992–1993

Blood samples were collected three times during the first year of the experiment, November 1992, and February and March 1993. All calves were on the same roughage diet prior to the first bleeding while they were being adapted to their new surroundings. By the second bleeding, one-third of the calves from the high forage group had been switched to the high energy diet. All calves were on the high energy diet when the third bleeding occurred. At that time the first of the calves went to slaughter.

1993–1994

During the second year, blood samples were collected five times, November 1993, and January, March, May and June 1994. As in the previous year, calves were on a roughage diet prior to the first bleeding. The first animals were slaughtered before the fourth (May) bleeding. In instances where fewer than five blood samples were collected, results from the last sample obtained were used for calculating final sample hormone concentrations.

Hormone Radioimmunoassay Procedures

Insulin Assays

Plasma samples were assayed for insulin in triplicate using the double antibody homologous bovine RIA of Mears (1993). Intra-assay CV ranged from 1.1 to 11.1% for a bovine plasma pool with a low insulin concentration (1.7 ng mL^{-1}) and 2.8 to 9.6% for a bovine plasma pool with a high insulin concentration (9.1 ng mL^{-1}). Inter-assay CV was 14.0% for the low- and 10.8% for the high-insulin bovine plasma pool.

Cortisol Assays

Plasma samples were assayed in triplicate using Cortisol Gammacoat Clinical RIA kits (CA-1549) from Incstar (Stillwater, MN) as outlined by Mears and Brown (1997). The assay kit was validated for bovine use by establishing that dilutions of bovine plasma resulted in a curve identical to that obtained with the human standards supplied with the kit. Intra-assay CV ranged from 3.6 to 12.4% for a bovine plasma pool with a low cortisol concentration (0.8 μg dL^{-1}) and 3.3 to 9.5% for the pool with a high cortisol concentration (2.4 μg dL^{-1}). Inter-assay CV was 14.9% for the low- and 5.6% for the high-cortisol bovine plasma pool.

T3 and T4 Assays

Plasma T3 and T4 were assayed in duplicate using Gammacoat T3 I-125 Clinical RIA kits (CA-1561) and Gammacoat Total T4 I-125 Clinical RIA kits (CA-1555M) from Incstar (Stillwater, MN) as outlined by Mears and Brown (1997). The assay kits were validated for bovine use by establishing that dilutions of bovine plasma resulted in a curves identical to those obtained with the human standards supplied with the kits. Intra-assay CV for T3 ranged from 1.0 to 9.2% for a bovine plasma pool with a T3 concentration of 1.8 ng mL^{-1}. Inter-assay CV for T3 was 8.4% for the T3 bovine plasma pool. Intra-assay CV for T4 ranged from 1.2 to 10.4% for a bovine plasma pool with a T4 concentration of 6.0 μg dL^{-1}. Inter-assay CV for T4 was 10.9% for the T4 bovine plasma pool.

Statistical Analysis

Analyses of variance (Snedecor and Cochran 1980) were carried out to study effects of breed, sex, diet (1992–1993) and location (plus interaction of these main effects) on slaughter liveweight, backfat, marbling and concentrations of plasma insulin, cortisol, T3 and T4. For each hormone, the mean concentration for the samples taken each year and the concentration for the last blood sample before slaughter were analysed. Since there were insufficient animals for some breeds in a year to have animals represented in all combinations of levels of the above factors, missing subclasses (Milliken and Johnson 1984) were present and, therefore, the analysis of subsets of levels of the above factors were carried out. Sources of variation due to factor main effects and interactions were included in the analyses of variance and single degree of contrasts calculated in order to compare breed types. As a result of insufficient numbers in some groups, the number of animals reported for each of the main effects varies.

Analyses of variance were also carried out with the covariate slaughter liveweight included for the analysis of average backfat. For marbling score data analyses, average backfat and slaughter liveweight were used as covariates (data not shown in tabular form).

Covariance analyses were carried out to relate average backfat and marbling score to each of the mean and final hormone concentrations for the hormone variables. The data were pooled over the breeds, sexes, diets and locations to obtain average within subclass linear regressions. For these analyses the slopes of the linear regressions are presented only for those hormone variables where a significant effect or trend towards one was evident. Similar analyses were also carried for each sex over locations, diets and breeds and also for each breed separately.

Table 2. Effect of diet on liveweight at slaughter, backfat, marbling, and plasma insulin, cortisol, T3 and T4 concentrations in 1992–1993 steers and heifers from Lethbridge ands steers from Lacombe

	n	Livewt at slaughter (kg)	Backfat depth (mm)	Marbling score	Final[z] insulin (ng mL^{-1})	Mean[z] insulin (ng mL^{-1})	Final cortisol (μg dL^{-1})	Mean cortisol (μg dL^{-1})	Final T3 (ng mL^{-1})	Mean T3 (ng mL^{-1})	Final T4 (μg dL^{-1})	Mean T4 (μg dL^{-1})
High Energy	76	475	13.2	8.0	1.64	1.44	9.16	8.07	2.54	2.22	11.81	10.02
High Forage	78	473	12.9	8.3	2.11	1.65	9.87	8.99	2.33	2.05	10.53	8.82
SE_{max}[y]		4	0.4	0.1	0.18	0.11	0.54	0.38	0.05	0.04	0.25	0.19
P		NS[x]	NS	NS	NS	NS	NS	< 0.10	< 0.01	< 0.01	< 0.001	< 0.001

[z]Final = hormone concentration in final blood sample; Mean = average hormone concentration for all blood samples.
[y]Maximum standard error of mean.
[x]Not significant ($P > 0.10$).

Statistical calculations were carried out using SAS (SAS Institute, Inc. 1989) software.

RESULTS AND DISCUSSION

Slaughter Liveweights, Backfats and Marbling Scores

Diet Effects

The different diets that the calves were fed at the beginning of the 1992–1993 trial had no effect ($P > 0.10$) on slaughter liveweight, average backfat depth at the 12th–13th rib site, and marbling score (Table 2), most likely due to the short time the calves were on different diets. Calves on the high-forage diet were only on it from the time they entered the feedlot at weaning until they weighed 350 to 375 kg, usually less than 50 d. Thereafter, all calves were fed the high-energy diet. Using liveweight at slaughter as a covariate in the backfat data analysis and slaughter weight and backfat depth as covariates in the marbling data analysis did not alter this lack of dietary effect.

Sex Effects

Sex of the calves significantly affected slaughter liveweight, average backfat depth at the 12th–13th rib site, and marbling score in both years of the study (Table 2). Heifers weighed less ($P < 0.001$, both years) and had more backfat ($P < 0.05$, 1992–1993; $P < 0.001$, 1993–1994) and more marbling (i.e., lower marbling scores) ($P < 0.05$, 1992–1993; $P < 0.01$, 1993–1994) than steers. Adjusting the data for slaughter liveweight (backfat depth) and slaughter liveweight and backfat depth (marbling score) reduced the extent of the sex effects, but all remained significant ($P < 0.05$).

The sex differences in slaughter weight and backfat depth were impacted by the method used to determine when the calves were to be slaughtered. Attempts were made to slaughter the calves at a common ultrasonic-backfat depth of 12 mm, which was not always possible as steers from some breed crosses kept growing without depositing much backfat. These steers were eventually slaughtered with less backfat than desired, resulting in a lower average backfat for steers compared with heifers. Heifers were generally slaughtered at a lower body weight in this study since they reach physiological maturity at a lower body weight than steers and deposit more backfat at a lower body weight (Suess et al. 1966).

The greater marbling for heifers compared with steers was similar to that reported by others (Hedrick et al. 1969; Wilson et al. 1969; Martin et al. 1971; Jones et al. 1991). Bulls have less marbling fat than either heifers or steers (Hedrick et al. 1969; Wilson et al. 1969; Martin et al. 1971; Jones et al. 1991). Gender differences are most likely the result of the different reproductive hormones present in bulls and heifers, with testosterone causing the production of lean muscle and estrogen resulting in more fat deposition in muscle. Steers, with little of either hormone, tend to grow slower and deposit less intramuscular fat.

Breed Effects

Calf breed affected liveweight at slaughter ($P < 0.001$) and average backfat ($P < 0.001$), but not marbling scores ($P > 0.10$) (Table 4). The variation in liveweight at slaughter was expected because the calves were slaughtered based on backfat depth, not body weight. Because some breeds and breed crosses reach physiological maturity earlier than others (Koch et al. 1976), these breeds/crosses acquired the desired backfat layer at a lower body weight and were slaughtered at a lower weight. Thus, the Angus, Wagyu/Angus and the Wagyu/Hereford calves were significantly lighter than the Hereford/Charolais and Hereford/Simmental at slaughter in 1992–1993. Similarly, the Angus, Wagyu/Angus and the Hereford calves were significantly lighter than the Wagyu/Charolais, Wagyu/Simmental and Hereford/Simmental at slaughter in 1993–1994. The breed variation in backfat reflects the problem in obtaining the desired backfat depth in some of the breed crosses in this study. This was particularly evident for the Wagyu/Charolais cross in 1992–1993 where mean slaughter weight was intermediate, but mean backfat depth was much less compared with other breeds and breed crosses, with the exception of the Hereford/Charolais cross.

The lack of a breed effect on marbling score was unexpected since others have reported greater marbling in Wagyu crosses (Lunt et al. 1993; Mir et al. 1997). Two differences between the present experiment and the above two experiments could explain this discrepancy. First, the

Table 3. Effect of sex on liveweight at slaughter, backfat, marbling, and plasma insulin, cortisol, T3 and T4 concentrations in 1992–1993 and 1993–1994 steers and heifers at Lethbridge

	n	Livewt at slaughter (kg)	Backfat depth (mm)	Marbling score	Final[z] insulin (ng mL^{-1})	Mean[z] insulin (ng mL^{-1})	Final cortisol (μg dL^{-1})	Mean cortisol (μg dL^{-1})	Final T3 (ng mL^{-1})	Mean T3 (ng mL^{-1})	Final T4 (μg dL^{-1})	Mean T4 (μg dL^{-1})
1992–1993												
Steers	57	477	12.8	8.4	1.50	1.33	7.48	7.45	2.39	2.11	11.25	9.53
Heifers	52	438	14.3	7.8	1.41	1.19	9.60	8.65	2.36	2.09	10.34	9.28
SE_{max}[y]		5	0.5	0.2	0.23	0.13	0.68	0.47	0.06	0.05	0.31	0.23
P		< 0.001	< 0.05	< 0.05	NS[x]	NS	< 0.05	< 0.10	NS	NS	< 0.05	NS
1993–1994												
Steers	63	459	12.3	8.6	3.30	2.03	8.11	7.59	2.57	2.30	10.12	9.49
Heifers	44	418	14.4	8.0	3.52	2.00	9.60	9.13	2.54	2.36	9.95	9.43
SE_{max}		6	0.4	0.1	0.35	0.14	0.51	0.34	0.06	0.04	0.29	0.22
P		< 0.001	< 0.001	< 0.01	NS	NS	< 0.05	< 0.01	NS	NS	NS	NS

[z]Final = hormone concentration in final blood sample; Mean = average hormone concentration for all blood samples.
[y]Maximum standard error of mean.
[x]Not significant ($P > 0.10$).

Wagyu genetic influence was different in that calves in the present study were one-half Wagyu whereas those of Mir et al. (1997) and Lunt et al. (1993) were three-quarters and seven-eighths Wagyu, respectively. Second, Lunt et al. (1993) fed their animals on a high-grain diet for a longer period of time to a heavier slaughter weight, which may have allowed any propensity of Wagyu calves for enhanced marbling to be expressed to a greater degree. In the present experiment, the Wagyu crosses had, for the most part, the lowest body weights at slaughter. The lack of a breed effect may also be the result of small sample sizes for some of the breeds, which led to large SE. In addition, the SE may be larger due to unexplained variation resulting from things such as unequal slaughter weights and ages, although the results were unaffected when slaughter weight was included as a covariate in the analysis.

Breed Type Effects

When the results were grouped according to breed type, the Wagyu cross, domestic purebred and domestic crossbred steers had similar ($P > 0.10$) backfat depths and marbling scores, although the Wagyu crosses had numerically lower marbling scores than purebred calves both years (Table 5). However, including backfat as a covariate in the analysis resulted in Wagyu crosses having more marbling compared to purebreds ($P < 0.05$), findings similar to those of Lunt et al. (1993) and Mir et al. (1997). Also, for all animals in 1992–1993, marbling increased as backfat depth increased ($P < 0.05$), indicating some sort of a relationship between backfat depth and marbling.

The domestic crossbreds weighed the most at slaughter both years while the Wagyu crosses weighed the least ($P < 0.01$) in 1992–1993 and intermediate to purebred and crossbred calves in 1993–1994. The lower weights in Wagyu crosses compared with other crosses is similar to that reported by Mir et al. (1997). Purebred calves had the highest backfat depths and the lowest weights at slaughter ($P < 0.05$) in 1993–1994. This contrasts with the data from 1992–1993 wherein the purebred calves weighed more than the Wagyu crosses at slaughter. The reason for this is unclear. One possibility is that the diet fed the 1993–1994 calves was lower in grain than the primary diet of the year before. The Wagyu crosses may not have deposited backfat as rapidly with this diet and, therefore, were taken to a higher weight to achieve the desired backfat. Another possibility is that sex of calf influenced these results since only steer data were reported in 1992–1993, whereas heifers were included in the analysis for 1993–1994.

Plasma Hormone Results

Diet Effects

Calves on a high-energy diet were previously found to have elevated plasma insulin concentrations (Mears 1993). In the present experiment, however, the diets that the calves were assigned to in 1992/1993 had no effect on plasma insulin concentrations (Table 2), presumably because of the short time that the calves were fed the two different diets. In addition, less than half of the insulin measurements were obtained while the calves were on the different diets. Similarly, plasma cortisol was not affected by diet, although there was a trend ($P < 0.10$) for the mean cortisol concentrations to be higher in calves on the high forage diet.

Plasma thyroid hormone concentrations were affected by the diets, even though the calves were on the high forage diet for only a short time (Table 2). Calves fed the high-energy diet had higher plasma T3 ($P < 0.001$) and T4 ($P < 0.01$) concentrations and than those fed the high-forage diet. The reasons for this are unclear although Yambayamba et al. (1996) reported lower plasma T3 and T4 concentrations during restricted feeding, indicating that energy intake may affect plasma concentrations.

Sex Effects

Final cortisol levels were higher ($P < 0.05$) and final T4 levels were lower ($P < 0.05$) in heifers than in steers in 1992–1993 (Table 3), with significant ($P < 0.05$) breed × sex interactions for these two parameters. In 1993–1994, the final cortisol ($P < 0.05$) and the mean cortisol ($P < 0.01$)

Table 4. Effect of breed on liveweight at slaughter, backfat, marbling, and plasma insulin, cortisol, T3 and T4 concentrations in 1992–1993 and 1993–1994 calves from both sites

Breed[z]	*n*	Livewt at slaughter (kg)	Backfat depth (mm)	Marbling score	Final[y] insulin (ng mL^{-1})	Mean[y] insulin (ng mL^{-1})	Final cortisol (μg dL^{-1})	Mean cortisol (μg dL^{-1})	Final T3 (ng mL^{-1})	Mean T3 (ng mL^{-1})	Final T4 (μg dL^{-1})	Mean T4 (μg dL^{-1})
1992–1993												
WA	31	452*abc*	13.8*bc*	7.9	1.88	1.58	11.30*cd*	9.55*b*	2.36	2.02*a*	11.28	9.86*c*
WH	23	446*ab*	12.5*b*	8.1	1.68	1.33	9.79*abc*	8.07*ab*	2.40	2.03*ab*	11.54	9.56*bc*
WC	9	475*c*	9.7*a*	8.0	1.84	1.56	10.84*bc*	9.88*b*	2.55	2.32*c*	11.69	9.88*bc*
AA	41	439*a*	15.8*d*	7.9	2.03	1.64	11.30*cd*	9.57*b*	2.48	2.08*ab*	10.54	9.05*ab*
HH	24	462*bc*	15.2*cd*	8.5	1.71	1.60	7.51*a*	6.53*a*	2.32	1.99*a*	10.26	8.63*a*
HC	13	522*d*	11.9*ab*	8.3	1.70	1.39	8.18*ab*	8.23*ab*	2.48	2.23*bc*	11.53	9.68*bc*
HS	13	524*d*	12.6*b*	8.1	2.33	1.70	7.67*ab*	7.85*ab*	2.45	2.27*c*	10.74	9.25*abc*
SE_{min}[x]		5	0.5	0.1	0.22	0.13	0.67	0.46	0.06	0.05	0.31	0.23
Se_{max}[x]		10	1.0	0.3	0.46	0.27	1.38	0.95	0.13	0.10	0.64	0.47
P		< 0.001	< 0.001	NS[w]	NS	NS	< 0.01	< 0.01	NS	< 0.01	NS	< 0.05
1993–1994												
WA	33	409*a*	14.1*bc*	8.1	2.42	1.64	10.67	9.48	2.59*bc*	2.30*bc*	9.81*b*	9.76*b*
WC	34	472*b*	11.3*a*	8.2	2.81	1.80	10.15	9.03	2.43*b*	2.23*b*	9.95*b*	9.61*b*
WS	18	461*b*	12.5*ab*	8.1	2.85	1.74	9.36	8.36	2.56*bc*	2.28*bc*	9.78*ab*	9.69*b*
AA	40	412*a*	15.0*c*	8.0	2.66	1.64	11.65	9.77	2.71*c*	2.38*c*	10.39*b*	9.99*b*
HH	17	409*a*	14.4*bc*	8.4	2.63	1.46	10.35	8.51	1.96*a*	1.94*a*	8.69*a*	8.26*a*
HS	12	462*b*	13.0*abc*	8.7	2.26	1.73	9.68	8.78	2.59*bc*	2.40*bc*	9.30*ab*	8.52*a*
SE_{min}		7	0.5	0.2	0.32	0.13	0.61	0.41	0.08	0.05	0.29	0.24
SE_{max}		14	1.0	0.3	0.62	0.25	1.20	0.80	0.15	0.10	0.57	0.46
P		< 0.001	< 0.001	NS	NS	NS	NS	NS	< 0.001	< 0.001	< 0.05	< 0.001

[z]Breeds: W = Wagyu, A = Angus, H = Hereford, C = Charolais, S = Simmental.
[y]Final = hormone concentration in final blood sample; Mean = average hormone concentration for all blood samples.
[x]SE_{min}, SE_{max} = Minimum, maximum standard error of mean for breed means.
[w]Not significant ($P > 0.10$).
a–d Means with different letters within year and column differ ($P < 0.05$).

concentrations were higher in heifers (Table 3), with no breed × sex interaction. The sex of the calves had no effect on the other plasma hormones measured in this study.

Breed Effects

Plasma insulin concentrations were similar ($P > 0.10$) for all the breeds and breed crosses studied both years (Table 4). Final cortisol ($P < 0.01$), mean cortisol ($P < 0.01$), mean T3 ($P < 0.01$), and mean T4 ($P < 0.05$) plasma concentrations were affected by the breed and breed cross in 1992–1993, with significant breed × sex interactions for these parameters. Cortisol concentrations were not affected by breed in 1993–1994 ($P > 0.10$), whereas final T3 ($P < 0.001$), mean T3 ($P < 0.001$), final T4 ($P < 0.05$), and mean T4 ($P < 0.001$) plasma concentrations were affected by breed and breed cross. Breed × sex interactions were not found for any of the parameters in 1993–1994.

There was no clear pattern as to which group of breeds and crosses had the highest or lowest hormone levels, although the Hereford purebreds generally had lower levels of the adrenal and thyroid hormones. Similarly, O'Kelly and Spiers (1994) found that Herefords had lower plasma T3 and T4 concentrations than Brahmans.

Breed Type Effects

When the hormone results were grouped according to breed type, the Wagyu cross, domestic purebred and domestic crossbred steers had similar ($P > 0.10$) plasma insulin and cortisol concentrations (Table 5). Plasma T3 concentrations were higher ($P < 0.05$) in the domestic crossbreds than in the purebreds, with the Wagyu crosses having intermediate levels of plasma T3 both years. In 1992–1993, mean plasma T4 concentrations were similar ($P > 0.10$) for the Wagyu cross, purebred and crossbred steers. However, in 1993–1994 the Wagyu crosses had higher mean plasma T4 concentrations than the other breed types. The difference between years could be due to the inclusion of heifers in 1993–1994. The breed type differences in hormone concentrations did not clearly indicate a pattern for the breed types, and were most likely simply a reflection of the breed to breed variation in concentrations shown above.

Relationship of Plasma Hormones to Backfat Depth

None of the plasma hormone concentrations was related to backfat depth ($P > 0.10$) when all of the animals were considered (Table 6). However, when the sexes were analysed separately, some of the hormone concentrations were found to be related to backfat depth. Steers with higher final plasma cortisol concentrations had greater backfat depth ($P < 0.05$), and heifers with lower mean plasma T4 concentrations tended to have more backfat ($P < 0.10$). Also, there was a slight trend for heifers with higher insulin concentrations and lower mean cortisol and T3 concentrations to have greater backfat depths, although these were not significant ($P < 0.2$) (Table 6).

Table 5. Effect of breed type on liveweight at slaughter, backfat, marbling, and plasma insulin, cortisol, T3 and T4 concentrations in 1992–1993 steers at Lethbridge and 1993–1994 steers and heifers from both sites

Breed type[z]	*n*	Livewt at slaughter (kg)	Backfat depth (mm)	Marling score	Final[y] insulin (ng mL^{-1})	Mean[y] insulin (ng mL^{-1})	Final cortisol (μg dL^{-1})	Mean cortisol (μg dL^{-1})	Final T3 (ng mL^{-1})	Mean T3 (ng mL^{-1})	Final T4 (μg dL^{-1})	Mean T4 (μg dL^{-1})
1992–1993												
WX	36	466*a*	11.7	8.4	1.37	1.26	6.91	7.16	2.38	2.12*bc*	11.00*ab*	9.66
Pbred	32	481*b*	12.1	8.7	1.35	1.28	7.80	7.13	2.40	2.03*ab*	10.25*a*	9.11
Xbred	6	524*c*	12.0	8.3	1.71	1.52	5.12	5.83	2.52	2.33*c*	12.55*b*	10.40
SE_{min}[x]		6	0.5	0.2	0.19	0.10	0.69	0.51	0.07	0.05	0.35	0.27
SE_{max}[x]		14	1.0	0.4	0.44	0.23	1.48	1.13	0.16	0.11	0.77	0.61
1993–1994												
WX	85	447*b*	12.6*a*	8.1	2.69	1.73	10.06	8.96	2.53*b*	2.27*ab*	9.85	9.69*b*
Pbred	57	410*a*	14.7*b*	8.2	2.64	1.55	11.00	9.14	2.34*a*	2.16*a*	9.54	9.13*a*
Xbred	12	462*b*	13.0*ab*	8.7	2.26	1.73	9.68	8.78	2.59*b*	2.40*b*	9.30	8.52*a*
SE_{min}		5	0.3	0.1	0.21	0.09	0.27	0.41	0.05	0.03	0.19	0.19
SE_{max}		14	1.0	0.3	0.62	0.25	0.80	1.20	0.15	0.10	0.57	0.46

[z]Breed types: WX = Wagyu crossbreds, Pbred = domestic purebreds, Xbred = domestic crossbreds.
[y]Final = hormone concentration in final blood sample; Mean = average hormone concentration for all blood samples.
[x]SE_{min}, SE_{max} = Minimum, maximum standard error of mean for breed type means.
a–c Means with different letters within year and column differ ($P < 0.05$).

Table 6. Relationship between backfat depth and hormone concentration over all locations, diets, breeds and years

	Final[z] insulin (ng mL^{-1})	Mean[z] insulin (ng mL^{-1})	Final cortisol (μg dL^{-1})	Mean cortisol (μg dL^{-1})	Final T3 (ng mL^{-1})	Mean T3 (ng mL^{-1})	Final T4 (μg dL^{-1})	Mean T4 (μg dL^{-1})
*Both sexes (*n = *394)*								
b[y]	0.0881	0.2014	0.0497	–0.0478	0.0767	0.0318	–0.0386	–0.1035
SE	0.0907	0.1981	0.0407	0.0602	0.3668	0.5408	0.0853	0.1110
P	NS[x]	NS	NS	NS	NS	NS	NS	NS
*Steers (*n = *250)*								
b			0.1098					
SE			0.0487					
P			< 0.05					
*Heifers (*n = *144)*								
b	0.2582	0.6088		–0.1617		–1.5103		–0.3739
SE	0.1621	0.4306		0.1056		0.9744		0.2106
P	NS	NS		NS		NS		< 0.10

[z]Final = hormone concentration in final blood sample; Mean = average hormone concentration for all blood samples.
[y]Slope of regression line.
[x]Not significant ($P > 0.10$).

When the breeds were analysed separately (not shown in tabular form), Holsteins with higher final insulin and mean insulin concentrations had greater backfat depth ($P < 0.05$, < 0.10, respectively) than Holsteins with lower insulin concentrations. Likewise, Wagyu/Charolais and Hereford/Simmental crosses with lower final plasma T4 concentrations had more backfat ($P < 0.05$, < 0.10, respectively). Similarly, Wagyu/Charolais crosses and Angus purebreds with lower mean plasma T4 concentrations had more backfat ($P < 0.05$, < 0.10, respectively). Within breeds, these relationships generally fit with the concept that insulin is involved in total fat deposition, whereas cortisol and thyroid hormones are involved in muscle growth (Trenkle and Marple 1983).

Relationship of Plasma Hormones to Marbling Score

Most plasma hormone concentrations were negatively related to marbling scores (Table 7), although this relationship was not always significant. Thus, animals with higher final plasma T3 ($P < 0.10$), mean plasma T3 ($P < 0.001$), and mean plasma T4 ($P < 0.10$) concentrations tended to have, or had more marbling (i.e., lower marbling scores). When the sexes were analysed separately, steers with higher final cortisol tended ($P < 0.1$) to have, and steers with higher mean T3 and mean T4 plasma concentrations had more marbling ($P < 0.01$ and < 0.05, respectively). Heifers with higher mean plasma T3 concentrations had more marbling ($P < 0.05$).

When the breeds were analysed separately (not shown in tabular form), Herefords with higher final and mean plasma

Table 7. Relationship between marbling score[z] and hormone concentration over all locations, diets, breeds and years

	Final[y] Insulin (ng mL^{-1})	Mean[y] Insulin (ng mL^{-1})	Final Cortisol (μg dL^{-1})	Mean Cortisol (μg dL^{-1})	Final T3 (ng mL^{-1})	Mean T3 (ng mL^{-1})	Final T4 (μg dL^{-1})	Mean T4 (μg dL^{-1})
*Both sexes (*n = *394)*								
b[x]	–0.0227	–0.0668	–0.0094	–0.0142	–0.2392	–0.7007	–0.0374	–0.0767
SE	0.0332	0.0723	0.0150	0.0221	0.1338	0.1944	0.0313	0.0404
P	NS[w]	NS	NS	NS	< 0.10	< 0.001	NS	< 0.10
*Steers (*n = *250)*								
b			-0.0326		-0.2570	-0.6965		-0.1073
SE			0.0186		0.1629	0.2402		0.0485
P			< 0.10		NS	< 0.01		< 0.05
*Heifers (*n = *144)*								
b			0.0389			-0.7102		
SE			0.0249			0.3325		
P			NS			< 0.05		

[z]Inverse marbling score.
[y]Final = hormone concentration in final blood sample; Mean = average hormone concentration for all blood samples.
[x]Slope of regression line.
[w]Not significant ($P > 0.10$).

T3 concentrations had more marbling ($P < 0.05$) than Herefords with lower T3 concentrations. Similarly, Hereford/Charolais crosses with higher final and mean plasma T3 concentrations, also had superior marbling ($P < 0.01$). Also, Wagyu/Charolais crosses with higher mean plasma T3 concentrations had better marbling ($P < 0.10$) . Likewise, Hereford/Simmental crosses with higher final and mean plasma T4 concentrations had greater marbling ($P < 0.10$, $P < 0.05$, respectively). Furthermore, Angus purebreds with greater mean plasma T4 concentrations had superior marbling ($P < 0.05$) than Angus with lower mean plasma concentrations.

CONCLUSIONS

The effects of Wagyu genetics on marbling and metabolic hormones were not as clearly expressed in this study as anticipated. Wagyu crossbred cattle had significantly more marbling at slaughter than domestic purebred and crossbred cattle, but only after the data were adjusted for backfat depth variability. Plasma insulin, cortisol, T3 and T4 were generally not influenced by Wagyu genetics. However, some relationships between marbling and cortisol and thyroid hormones became evident when data were examined for all animals across all breeds. Steers with higher final cortisol and mean T3 and T4 concentrations had more marbling. Heifers with higher mean T3 concentrations also had more marbling. Thus, there is some indication that cortisol, T3 and T4 may have a role to play in determining the amount of marbling in beef cattle. Further research is needed to determine the nature of this role before hormone manipulation can be used as a tool to enhance marbling in cattle.

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