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The effect of different cereal grains on marbling and soft fat

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ABSTRACT

Previous scientific and circumstantial evidence suggests that the amount of marbling can be influenced by the composition of the diet. This appears to be related to grains which can increase the availability of glucose to act as a substrate for marbling fat. The aim of this study was to compare the marbling response and fat texture of steers fed 6 diets based on dry rolled (D/R) and steam flaked (S/F) maize and sorghum and D/R barley ± a chromium supplement. The conclusions of the study were similar for both visual marbling score and intramuscular fat. Diet had a significant effect on marbling with diets based on maize and steam flaking of maize and sorghum giving the highest marbling scores. An organic chromium supplement (chelavite™) resulted in increased insulin action but also a trend for increased muscle growth and reduced fatness with no stimulation of marbling. Thus even in relatively heavy steers chromium tends to stimulate the growth of muscle rather than fat. Only the activity of the glucose pathway for fattening (i.e the enzyme ATP citrate lyase in fat) and total body fatness were correlated with marbling. The activity of the glucose pathway for fattening was best correlated with marbling after 97 days of feeding. This suggests that starter diets should optimise the activity of ATP citrate lyase in adipose tissue. A model was constructed for explaining the marbling response using the activity of the glucose pathway for fattening (i.e the enzyme ATP citrate lyase in fat), total body fatness and dry matter intake and this model explained some 40% of the variation in intramuscular fat. This compares to the known genetic effect which also explains about 40% of the variation. The fat texture in this study was relatively soft for all of the treatments. The feeding of D/R maize and D/R sorghum resulted in softer fat than the steam flaked equivalent suggesting that some of the fat in maize and sorghum was escaping fermentation in the rumen when the grains were fed dry rolled. An additional mechanism was that steam flaking reduced the activity of the enzyme responsible for making soft fat within the animal (Δ9 desaturase).

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EXECUTIVE SUMMARY

The factors controlling the expression of the economically important fat tissue, marbling, are poorly understood and need to be studied to allow Australian producers to compete on export markets in the Asia-Pacific area. For example cattle finished to similar age, weight and fatness specifications in Australian feedlots typically have lower marbling scores than genetically similar cattle in America and Japan. It is suggested that diet may be a factor in this difference.

Typical ruminant diets are heavily fermented in the reticulo-rumen and as a consequence acetate is a major substrate for fat synthesis rather than glucose. Despite this, fat depots show different rates of fat synthesis from glucose versus acetate. Marbling fat shows a preference for glucose while subcutaneous fat uses mainly acetate for fat synthesis. Consequently dietary regimes which increase the availability of glucose are likely to increase the rate of marbling. There are 2 ways of increasing glucose availability in steers (i) to increase the feed intake and (ii) to stimulate starch digestion in the small intestine.

The overall aim of this project was to investigate the effects of 3 cereal grains (barley, maize and sorghum), processing via dry rolling (D/R) versus steam flaking (S/F) and a chromium supplement on the marbling response in steers. It was proposed that diets which promoted increased glucose availability (either via increased feed intake and/or starch digestion in the small intestine) would increase marbling. It was also proposed that in long fed older steers (i.e.initial body weight >400kg) a supplement of chromium would stimulate marbling by activating insulin, the major fattening hormone. Finally it was also proposed that total body fatness would be correlated with marbling.

Major findings

(i) The conclusions of the study were similar for both visual marbling score and intramuscular fat.

(ii) Carcass temperature did effect visual marbling score with colder meat yielding an increased score.

(iii) Diet had a significant effect on marbling with diets based on maize and steam flaking of maize and sorghum giving the highest marbling scores. Below the proposed new category of 5 star beef is used an example where the intramuscular fat must reach at least 6%. The dramatic effects of diet are obvious from the table below.

Dietary treatment	**Intramuscular fat ≥ 6% (% of steers)**
Barley	25
Barley + chromium	31
D/R Maize	63
S/F Maize	75
D/R Sorghum	25
S/F Sorghum	63

The steam flaking of the maize was relatively light while the flaking of the sorghum was comparable to that produced by a major feed mill in Queensland (Ridleys, Toowoomba).

(iv) An organic chromium supplement (chelavite™) resulted in increased insulin action but also a trend for increased growth and reduced fatness with no stimulation of marbling. Thus even in relatively heavy steers chromium still tends to stimulate the growth of muscle rather than fat.

(v) Only the activity of the glucose pathway for fattening (i.e the enzyme ATP citrate lyase in fat) and total body fatness were correlated with marbling. The activity of the glucose pathway for fattening was best correlated with marbling after 97 days of feeding. This suggests that starter diets should optimise the activity of ATP citrate lyase in adipose tissue.

(vi) A model was constructed for explaining the marbling response using the activity of the glucose pathway for fattening (i.e the enzyme ATP citrate lyase in fat), total body fatness and dry matter intake and this model explained some 40% of the variation. This compares to the known genetic effect which explains about 37% of the variation.

(vii) The fat texture in this study was relatively soft for all of the treatments. The feeding of D/R maize and D/R sorghum resulted in softer fat than the steam flaked equivalent suggesting that some of the fat in maize and sorghum was escaping fermentation in the rumen when the grains were fed dry rolled. An additional mechanism was that steam flaking of the diet resulted in a reduced the activity of the enzyme responsible for making soft fat within the animal (Δ^9 desaturase). The mechanism for this reduction is not known but deserves further research.

In addition to cereal grain the only other major grain in the ration was D/R lupins (8-10%) and this strongly implicates cotton seed products as a cause of hard fat on the Eastern sea board of Australia.

(viii) Further work is required to understand the role of feed intake, rumen fermentability and starch digestion in the small intestine as determinants of fat synthesis from glucose and therefore the marbling response. Thus the extent of steam flaking required and whether barley should be flaked is not yet known. This work would suggest processing to optimise feed intake, rumen function AND starch digestion in the small intestine is the target.

When and how can industry benefit

This work has given a clear direction for grain selection and processing to maximise the marbling response.

Who can benefit from the results

Increased marbling has huge financial implications for lot feeding enterprises and exporters with subsequent flow on to other sectors which form the backbone of the Industry. The work will also help the feedlot industry to finish steers to reach ≥6% intramuscualr fat so as carcasses can be graded to the proposed 5 star category.

In addition the powerful effects of grains which allow starch digestion in the small intestine should have significant implications for manipulating glycogen metabolism and so help to reduce dark cutting in beef.

1. BACKGROUND

1.1 Marbling

The pattern of fat accretion is thought to be influenced primarily by the age of the animal and to some extent by genetics (heritability = 0.37, Koots *et al.*. 1994). The economically important depot fat, marbling, is one of the last depot sites to mature and starts to develop as the carcass weight increases above about 400kg in British breed steers. Cattle finished under Australian dietary systems tend to have lower marbling scores than genetically similar cattle in America. It has been speculated that diet may be a factor in this difference.

1.2 Fat Accretion

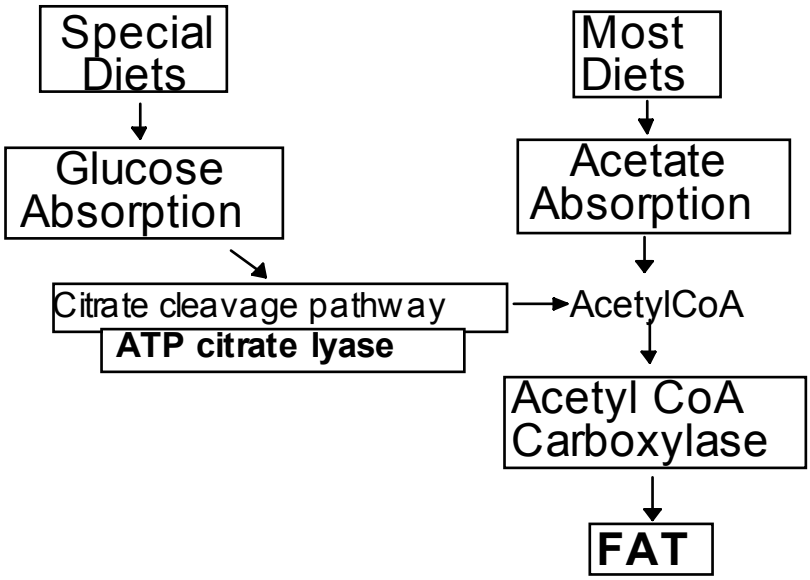
The rate of fat accretion in finishing steers is determined primarily by (i) the metabolic state of the animal (ii) the intake of metabolisable energy and (iii) the age of the animal (Owens *et al*. 1995). Pathways for fat biosynthesis are either accretion of preformed fat in the diet or by synthesis *de novo*. The intake of fat by ruminants is limited since diets containing more than about 4% added fat tend to depress intake and this subject is not the focus of this study. During the fattening phase the synthesis of fats *de novo* is dependent on the rate of supply of carbon, reducing power and glycerol.

The substrates for lipogenesis in ruminants are acetate and glucose. Diets which are extensively fermented in the rumen (i.e. most diets) promote acetate as the major source of carbon and reducing power for lipogenesis with a smaller contribution from glucose for some of the reducing power and all of the glycerol (Vernon,1981). An alternative pathway for lipogenesis with glucose as the primary substrate is typically seen in monogastric animals when glucose is a major end product of digestion. The lipogenic pathway using glucose is quite distinctive and utilises the citrate cleavage pathway as shown schematically in Figure 1.

Key enzymes of the citrate cleavage pathway are ATP citrate lyase and NADP malate dehydrogenase. Both of these enzymes are induced by intravenous glucose infusion and consequently their activity in adipose tissue represents an estimate of the maximal capacity for lipogenesis from glucose (*Pethick et al*. 1995)

Regardless of the substrates used, the overall rate of lipogenesis in controlled by the activity of acetylCoA carboxylase which is under complex substrate, allosteric and hormonal control. Accordingly the activity of acetylCoA carboxylase is an indicator of the total capacity for lipogenesis.

Figure 1. Pathways for lipogenesis *denovo* in ruminant animals

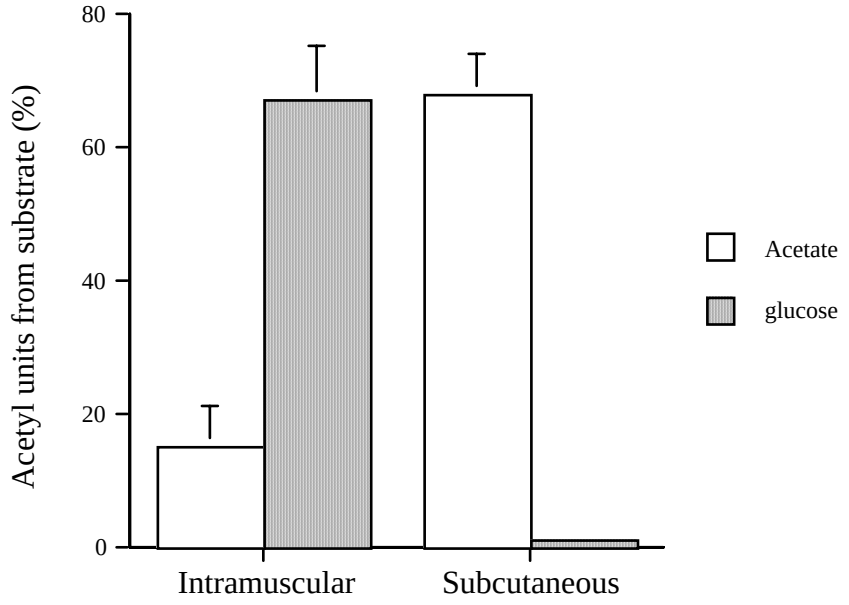


1.3 Glucose availability and lipogenesis

The availability of glucose has long been thought a limiting factor for fat accretion in ruminants. Thus Preston and Leng (1987) speculated that diets high in roughage promote an excess of acetate with respect to glucose and so induce a reduced rate of lipogenesis for a given intake of metabolisable energy. Few studies have critically tested this hypothesis. Two groups (Ballard *et al.* 1972; Prior & Jacobson 1979) have found an increased rate of lipogenesis *in vitro* when glucose was infused into sheep or cattle - the results are equivocal however since glucose was infused in addition to the basal diet and so the experimental design was not isojoulic.

Different adipose tissue sites have been found to have different rates of lipogenesis from glucose versus glucose. Thus marbling adipocytes show a preference for glucose carbon while subcutaneous adipose tissue uses mainly acetate as a source of acetyl units for lipogenesis (Smith & Crouse 1984; Whitehurst *et al.* 1981). This difference in substrate preference is clearly shown in Figure 2.

Figure 2. The contribution of acetate and glucose carbon (glucose + lactate) to lipogenesis in subcutaneous and intramuscilar fat tissue determined *in vitro* (adapted from Smith & Crouse 1984).



The results strongly suggest that the relative availability of glucose might influence the marbling response.

The extent to which different cereal grains can result in the an increased capacity for lipogenesis from glucose was investigated in a previous Meat Research Corporation project (Pethick *et al*. 1995). We showed that the glucose pathway for fat biosynthesis (as measured by the activity of ATP citrate lyase) was stimulated by glucose infusion, diets promoting starch digestion in the small intestine and by increased dry matter intake. The purpose of this project was to translate the previous fundamental work into an Industry relevant trial that would investigate the potential for nutritional regulation of marbling.

2. PROJECT

General Hypothesis:

Diets promoting increased availability of glucose and/or an increased insulin action will promote marbling and soft fat.

In Particular:

(i) Cereal grains allowing for increased starch digestion in the small intestines will promote marbling and soft fat.

(ii) Diets promoting an increased feed intake will promote marbling and soft fat.

(iii) Supplementation with an amino-chromium chelate will increase insulin action/sensitivity and so promote marbling and soft fat.

(iv) Total body fatness will be positively associated with marbling.

Design

6 diets x 16 steers per diet

Dry rolled (D/R) maize
Steam flaked (S/F) maize
D/R sorghum
S/F sorghum
D/R barley
D/R barley + chromium chelate.

Timeline

The timing of management and experimental activities is shown in Table 1. In addition to the procedures shown below the steers were weighed every 2 weeks and ultrasound fat depth was determined at the P8 and 12th rib site every 4 weeks.

Table 1. Time for management and experimental activities

Days on full feedlot	Date	Activity
-189	18th March 96	Purchase steers & background
-27	23rd Sept. 96	96 steers begin acclimating to feedlot diets (group pens)
-	9th Oct. 96	13 steers on backgrounding diet slaughtered
1	9th Oct. 96	96 steers on full feed (group pens)
50	27th Nov. 96	Steers into individual pens
93	9th Jan. 97	Fat biopsy
100	16th Jan. 97	Steers into group pens
127	12th Feb. 97	Muscle biopsy & fecal pH
154	11th March 97	Slaughter

3. METHODOLOGY

Fat samples taken at biopsy and slaughter were assayed for the activity of ATP citrate lyase and acetylCoA carboxylase according to the methodology of Pethick *et al*.(1995). Fat was taken from above the tail area of the steers both at biopsy and slaughter and collected into liquid N_2. The slaughter samples were placed into liquid N_2 approximately 20 minutes post slaughter and all samples were stored at -80°C until analysis.

Determination Δ^9 desaturase activity in subcutaneous fat, fat melting point, fatty acid composition and intramuscular fat was according to the methods of Tume (1997). A 1 rib thick sample was taken from the *m. longissimus dorsi* (cube roll) at the 11/12th rib site and used for determination of intramuscular fat content. The samples were chilled at 5°C for 48 hours and then frozen at -25°C before analysis.

Visual marbling scores of the *m. longissimus dorsi* were determined at the level of the 12th rib by two certified AUSMEAT assessors at a carcass temperature of 12°C.

Body composition was estimated using a rib dissection of 8 steers from each group. A rib set (ribs 6-10) was obtained at slaughter and then wrapped and frozen. Within 8 weeks each rib set was thawed and a detailed dissection performed. Tissue was dissected into subcutaneous fat, intermuscular fat, muscle, bone and connective tissue with a recovery by weight of 99%.

4. RESULT AND DISCUSSION

Background information on animals and diets

Angus steers (209) were purchased at 324kg liveweight (fat depth:P8=4.6mm, 12th rib=3.4mm) and backgrounded on a 50% D/R lupin grain, 50% hammered milled hay diet for 205 days at a mean daily gain of 0.55kg/day (determined by regression of liveweight change). At an initial body weight of 442kg (fat depth:P8=7.5mm, 12th rib=5.6mm) 96 steers entered the feedlot and the remaining 13 were slaughtered for determination of prefeedloting fat parameters. The feedlot steers were acclimated to their respective diets over 27 days after which they were on full feed for 154 days. The steers were fed (twice daily) one of 6 diets based on either D/R barley, D/R barley plus 1ppm chromium as an amino chelate (chelavite™, Chromium chelavite amino acid chelate, 2.53% chromium, Sureleen-Albion Agra Inc., Arva, Ontario, Canada), D/R maize, S/F maize, D/R sorghum and S/F sorghum. The composition of the diets is shown in Table 2.

Table 2. Composition of the diets (%).

Ingredient	Barley	Barley Chromium	D/R Maize	S/F Maize	D/R Sorghum	S/F Sorghum
Hammermilled hay	15.1	15.1	15.2	15.8	15.2	15.9
Cereal grain	70.3	70.3	67.2	65.9	68.9	65.9
D/R Lupin grain	7.9	7.9	9.9	10.3	8.2	10.3
Urea	0.27	0.27	1.16	1.16	1.16	1.12
Molasses	3.8	3.8	3.84	4.08	3.85	4.1
Limestone	1.56	1.56	1.57	1.63	1.57	1.63
Gypsum	0.2	0.2	0.21	0.21	0.21	0.21
Salt	0.46	0.46	0.46	0.48	0.46	0.48
Potash	0.18	0.18	0.18	0.19	0.18	.019
Mineral/vitamin premix	0.22	0.22	0.22	0.23	0.22	0.23
Chelavite™ (gms/ton)	-	39	-	-	-	-
Metabolisable energy (MJ/kg DM)[a]	11.5	11.5	11.7	11.2	11.1	11.6
Crude Protein (% DM)[a]	13.9	13.9	14.3	14.9	14.3	13.5

a - Values measured *in vitro*

Visual marbling assessment

The frequency distribution of the visual marbling score for *m. longissimus dorsi* is shown in Figure 3. Assessors 1 and 2 assessed all carcasses in the chillers at a carcass temperature of about 11-12°C. The distribution of marbling score is broadly similar for the 2 assessors; however there was some discrepancy at the score 0 end of the scale.

Figure 3. Visual marbling assessment of the *m. longissimus dorsi* at the level of the 12th rib as affected by assessor (Chiller temperature = 12°C).

Figure 3(a) marbling score - assessor 1

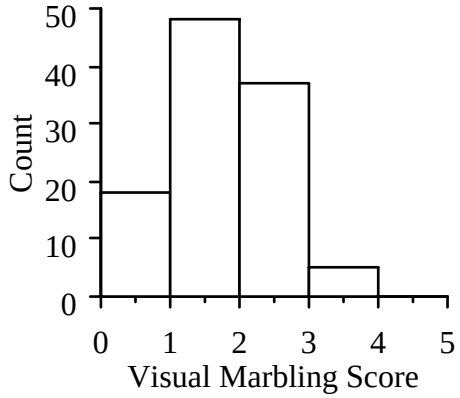
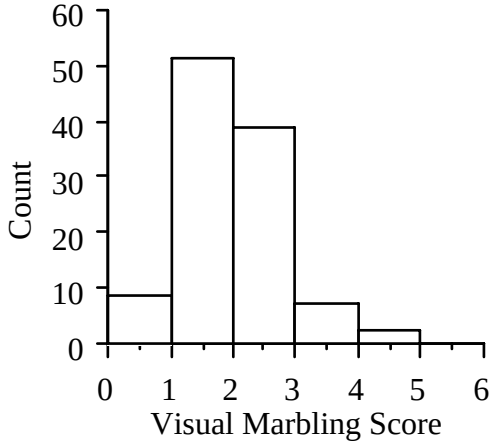


Figure 3(b) marbling score - assessor 2



At the boning table a full cross section of the “cube roll” was sampled at the level of the 12th rib and used for further analysis. The sample was wrapped and chilled to 5°C at Murdoch University and subsequently re-assessed by assessor 1, 24 hours later (Figure 4). This task was not fully reliable since any tendency for the meat sample to be compressed resulted in an obvious visual loss of marbling. Despite the potential problems, re-assessing the samples on the following day at 5°C resulted in an increased marbling score especially in the range of 0-2 (Figure 4). This trend confirms that chiller temperature is critical in the assessment of marbling.

Figure 4. Visual marbling assessment of the *m. longissimus dorsi* at the level of the 12th rib as affected by assessment time.

Figure 4(a) Chiller assessment with whole carcass at 12°C

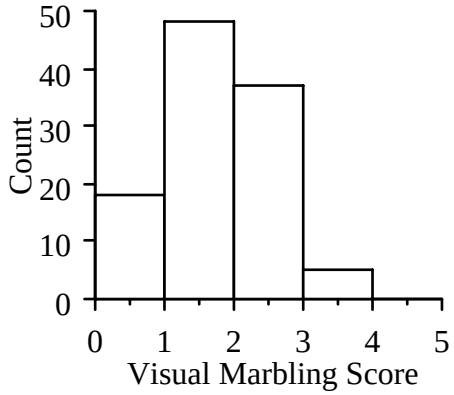
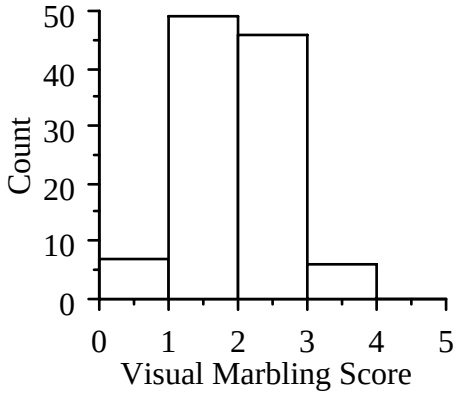


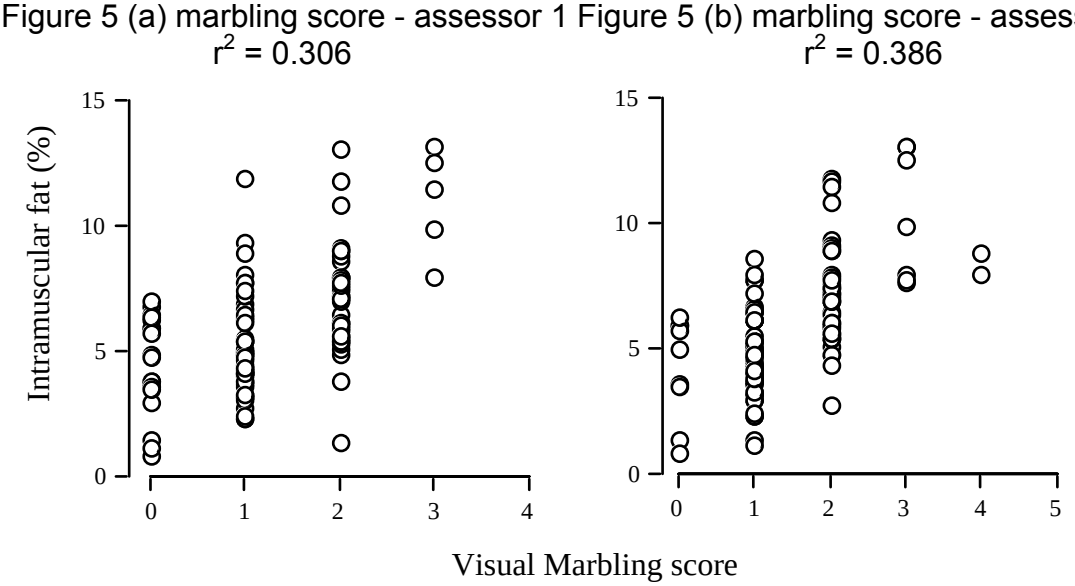
Figure 4(b) Assessment on same surface 24 hours after boning with temperature at 5°C.



Intramuscular chemical fat and visual marbling

The relationship between visual marbling score and intramuscular chemical fat is shown in Figure 5. Some 30-40% of the variation in intramuscular fat content was explained by the visual marbling score. The remaining variation could be accounted for by factors such as variation in fat distribution through the muscle e.g. the visual assessment measures the fat distribution in a very narrow strip of muscle. Variations in fat melting point between diets is not a reason for variation in marbling score since the fatty acid composition of the marbling fat was similar for all dietary treatments (Table 4).

Figure 5. The relationship between visual marbling score and intramuscular chemical fat.



Animal Health

Five steers died during the course of the feedloting programe. One died of acidosis at the beginning of feedloting and one died of bloat. The remaining 3 deaths occurred during a record hot period associated with high humidity some 3 weeks before slaughter. One animal was excluded from analysis in the D/R sorghum group since it managed to sift much of the sorghum out of the diet (observed during the period in individual pens).

Time course of performance during feedloting

The liveweight and ultra sound fat depth at the P8 and 12 rib site during the entire feedloting period is shown in Figures 8 and 9 in Appendix 1. The feed intake versus days on feed between days 50 -100 of feedloting is shown in Figure 10 of Appendix 1.

The estimate of liveweight gain discussed below was determined as the linear correlation of 9 liveweight determinations during feedloting. The fat depth the P8 site increased slowly after 80 days of feeding while the 12th rib fat depth increased at a more linear rate throughout the 150 days of feedloting.

The feed intake during the period when steers were in individual pens reached a stable value within 7 days of entry into the pens.

Marbling

There were similar and significant effects of diet on visual marbling score and intramuscular fat level. The feeding of maize and steam flaking of maize and sorghum was associated with higher marbling score and intramuscular fat (Table 4).

Fat Depth and Dissectable Fat

The error associated with the ultra sound fat depth measurement was 50% lower than for the similar measurement using the ruler on the carcass (Table 3). This indicates that measurement of fat depth by ultra sound is a more reliable estimate of fat depth.

There were no significant effects of any treatment on fat depth at the P8 site measured on the carcass (Table 3). However the fat depth determined by ultra sound scanning of the live animal was higher for D/R maize and D/R barley when compared to D/R sorghum. Steam flaking did not influence subcutaneous fat depth (Table 3). There was a trend for chromium to reduce the ultra sound fat depth at the P8 site (Table 3).

The amount of carcass fat is better determined by a dissection of a rib section (Owens *et al*. 1995). The total dissectable fat determined from a rib dissection of 8 steers per treatment is shown in Table 4. The scan fat depth at either the P8 or 12th rib site measured close to slaughter (142 days) accounted for about 30% of the variation in total dissectable fat. Diet had no effect on dissectable fat when carcass weight was used as a covariate in the analysis.

Subcutaneous fat depth at either site was not correlated with visual marbling score (Table 6). However the ultra sound scan fat depth at the 12th rib site was correlated with intramuscular fat content. Total dissectable fat was correlated with both visual marbling score and with intramuscular fat. This indicates that total body fatness is associated with marbling.

Liveweight gain and carcass weight

Daily live weight gain tended to be higher for the D/R maize and D/R barley when compared to the D/R sorghum based diet. Liveweight gain being significantly increased by steam flaking of sorghum and maize (Table 3). There was a trend (P=0.057, repeated measures ANOVA) for chromium supplementation to increase liveweight in the last 90 days of feedloting. The effects of diet on carcass weight showed a similar trend to the liveweight gain. Neither carcass weight, or liveweight gain was correlated with marbling score (Table 5) or intramuscular fat (Table 6).

Feed intake

Feed intake, measured from day 57-100 of feedloting, was not effected by cereal grain type; however steam flaking of maize and sorghum resulted in a significant increase of dry matter intake (Table 3). Feed intake during this period of feedloting did not correlate with marbling score (Table 5) or intramuscular fat (Table 6).

Feed efficiency

Feed efficiency was calculated from the growth determined over the 150 days of feedloting and dry matter intake measured during days 50-100. Liveweight gain was significantly associated with dry matter intake with about 50% of the variation accounted for (r^2 = 0.50; Figure 6). However the cereal grain and processing of the grain significantly affected the feed:gain ratio (dry matter intake:live weight gain ratio; Table 3; Figure 4). For the dry rolled grains barley>maize>sorghum with respect to feed conversion efficiency. Steam flaking of maize and sorghum improved the feed conversion efficiency. The feed:gain ratio was not associated with visual marbling or intramuscular fat content (Table 5 & 6).

Table 3. Performance and commercial fat parameters of steers fed different cereal grains and a chromium supplement

Parameter	Barley		Maize		Sorghum		Standard error of difference	Significance of effect (*P*)			
	Dry rolled	Dry rolled Chromium	Dry rolled	Steam flaked	Dry rolled	Steam flaked		Cereal grain[h]	maize vs sorghum[i]	Processing[i]	Chromium[j]
Final Liveweight (kg)[a]	632	643	606	653	593	612	18.0	NS	NS	NS	NS
Carcass weight (kg)[a]	358	367	354	381	338	348	12	0.055	0.055	NS	NS
Dressing %[b]	55.9	56.4	57.2	57.0	56.0	56.2		NS	0.03	0.005	NS
Scan fat P8 (mm)[c]	17.3	15.7	16.3	16.7	14.9	15.3	1.7	NS	0.021	NS	0.076
Scan fat 12th rib (mm)[c]	16.5	14.8	15.7	14.9	13.1	14.1	1.7	0.011	0.033	NS	NS
Carcass fat P8(mm)	19.5	18.9	20.6	18.4	19.8	19.2	3.4	NS	NS	NS	NS
Live weight gain (kg/day)[d]	1.33	1.41	1.16	1.53	1.13	1.37	0.16	0.08	0.075	<0.0001	NS
Dry matter intake (kg)e	12.9	13.5	13.1	14.3	12.7	13.6	1.0	NS	NS	0.032	NS
Feed/Gain[f]	9.9	9.6	11.1	9.1	13.1	10.5	1.1	0.009	0.003	0.0002	NS
Fecal pH[g]	6.60	6.68	6.03	6.57	6.25	6.40	0.23	0.002	NS	0.005 Interaction	NS

a Body weight upon entry into the feedlot was used as a covariate for statistical analysis.
b Hot carcass weight was used as a covariate for statistical analysis.
c Ultrasound fat depth determined on day 142 of feedloting; Body weight at 142 days used as covariate for statistical analysis.
d Calculated from the linear regression of 9 body weight determinations over days 19-149 of the feedlot period.
e Measured while steers were in individual pens from days 50 -100. Values are the mean of 43 days from day 57-100 of feedloting.
f Calculated from live weight gain from Day 19-149 and feed intake from day 57-100.
g Measured on Day 127 of feedloting.
h One way ANOVA on dry rolled grains.
i Two way ANOVA on maize/sorghum & dry rolled/steam flaking.
j Students 't' test on barley ± chromium suplementation as Chelavite™.

Table 4. Total dissectable fat and subcutaneous and marbling fat parameters of steers fed different cereal grains and a chromium supplement.

Parameter	Barley		Maize		Sorghum		Standard error of difference	Significance of effect (*P*)			
	Dry rolled	Dry rolled Chromium	Dry rolled	Steam flaked	Dry rolled	Steam flaked		Cereal grain[c]	maize vs sorghum[d]	Processing[d]	Chromium[e]
Rib Dissection total fat (%)[a]	39.9	37.4	39.5	42.2	39.5	36.1	2.4	NS	NS	NS	NS
Intramuscular fat (%)	5.4	5.5	6.9	7.3	4.7	6.9	1.3	0.005	0.043	0.04	NS
Visual marbling assessor 1	1.1	0.9	1.6	1.7	0.9	1.7	0.4	0.027	0.004	NS	NS
Visual marbling assessor 2	1.3	1.3	1.6	2.1	0.9	1.5	0.4	0.031	0.004	0.006	NS
Fat melting point (°C)	35.0	34.4	32.4	35.1	32.8	34.0	1.5	0.02	NS	0.02	NS
Slaughter Subcutaneous Fat (mono+poly/sat)[b]	1.13	1.23	1.29	1.15	1.26	1.22	0.15	0.068	NS	0.09	NS
Biopsy Subcutaneous Fat (mono+poly/sat)[b]	1.21	1.21	1.20	1.21	1.31	1.21	0.1	NS	NS	NS	NS
marbling Fat (mono+poly/sat)[b]	0.97	0.98	0.97	1.01	0.95	0.96	0.06	NS	NS	NS	NS

a Analysed using hot carcass weight as a covariate.
b Mono+polyunsaturated/saturated fatty acid ratio
c One way ANOVA on dry rolled grains.
d Two way ANOVA on maize/sorghum & dry rolled/steam flaking.
e Students 't' test on barley ± chromium suplementation as Chelavite™.

Table 5. Correlation's between feedlot and carcass parameters with visual marbling score

Parameter	Correlation (R^2)		Significance (*P*)	
	Assessor 1	Assessor 2	Assessor 1	Assessor 2
Carcass weight	NS	NS	NS	NS
Live weight gain	NS	NS	NS	NS
Feed intake	NS	NS	NS	NS
Feed/Gain	NS	NS	NS	NS
Scan P8 fat depth	NS	NS	NS	NS
Scan 12th rib fat depth	NS	NS	NS	NS
Carcass P8 fat depth	NS	NS	NS	NS
Total dissectable fat	0.122	0.176	0.016	0.003
Day 97 ATP citrate lyase - all data[a]	0.083	0.173	0.006	<0.0001
Day 97 ATP citrate lyase - excluding chromium data[b]	0.131	0.265	0.002	<0.0001

See text and Tables 3,4 & 5 for further details of parameters.
a Includes all data; *b* excludes barley plus chromium treatment

Figure 6. The relationship between dry matter intake (measured from Day 57-100) and live weight gain (measured from Day 19-149).

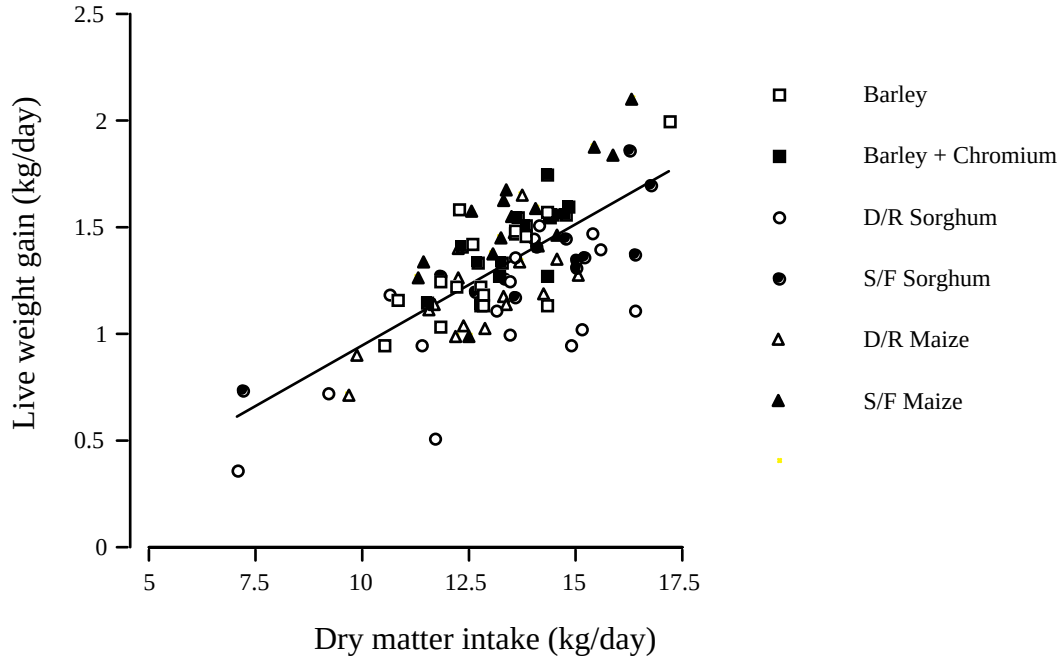


Table 6. Linear correlation between feedlot and carcass parameters with intramuscular fat, day 97 ATP citrate lyase and acetyl CoA carboxylase and total dissectable rib fat.

Parameter	Intramuscular fat		Day 97 ATP citrate lyase (all data)		Total Dissectable fat		Day 97 AcetylCoA carboxylase	
	r^2	*P*	r^2	*P*	r^2	*P*	r^2	*P*
Carcass weight	NS	NS	0.12	0.001	0.23	0.001	0.067	0.036
Live weight gain	NS	NS	0.27	0.0001	0.14	0.01	0.163	0.0001
Feed intake (DMI)	NS	NS	0.16	0.0001	0.25	0.0003	0.086	0.017
Feed/Gain	NS	NS	0.11	0.0013	NS	NS	0.11	0.007
Scan P8 fat depth	NS	NS	NS	NS	0.32	0.0001	NS	NS
Scan 12th rib fat depth	0.082	0.007	NS	NS	0.29	0.0001	NS	NS
Carcass P8 fat depth	NS	NS	NS	NS	0.15	0.007	NS	NS
Total dissectable fat	0.095	0.035	NS	NS	-	-	NS	NS
Day 97 ATP citrate lyase - all data[a]	0.072	0.012	-	-	NS	NS	0.28	0.0001
Day 97 ATP citrate lyase - excluding chromium data[b]	0.12	0.003	-	-	NS	NS	0.32	0.0001
Day 97 AcetylCoA carboxylase	NS	NS	0.28	0.0001	NS	NS	-	-

Table 7. Enzyme levels in subcutaneous fat of steers fed different cereal grains and a chromium supplement

Parameter	Barley		Maize		Sorghum		Standard error of difference	Significance of effect (*P*)			
	Dry rolled	Dry rolled Chromium	Dry rolled	Steam flaked	Dry rolled	Steam flaked		Cereal grain[b]	maize vs sorghum[c]	Processing[c]	Chromium[d]
Biopsy ATP citrate lyase (nmol/min/gm)[g]	193	372	211	409	161	372	85	NS	NS	<0.0001	<0.0001
Biopsy AcetylCoA carboxylase (nmol/min/gm)[g]	133	202	154	243	111	259	36	NS	NS	<0.0001	0.029
Slaughter ATP citrate lyase (nmol/min/gm)[g]	46	50	35	46	32	51	15	0.08	NS	0.049	NS
Desaturase (nmol/min/mg protein)	0.73	0.69	0.80	0.62	0.80	0.65	0.12	NS	NS	0.002	NS

a Measured on samples taken by biopsy at day 97 of feedloting.
b One way ANOVA on dry rolled grains.
c Two way ANOVA on maize/sorghum & dry rolled/steam flaking.
d Students 't' test on barley ± chromium suplementation as Chelavite™.

Fecal pH

Fecal pH was sensitive to diet with dry rolled barley having a higher pH than either maize or sorghum implying that the later 2 grains resulted in starch being fermented in the large intestine (Table 3). Steam flaking of both maize and sorghum increased fecal pH (particularly for maize), indicating that digestion in the rumen and/or small intestine was stimulated.

The activity of ATP citrate lyase and Acetyl CoA carboxylase in subcutaneous adipose tissue

Variation of activity with time: The activity of ATP citrate lyase was measured in the subcutaneous fat of the steers immediately before feedloting and at day 97 and finally at slaughter. The activity of the enzyme per gram of fat declined as the steers aged with the activity at slaughter being only 15% that at day 97 of feedloting (Table 7). About half of this decline was due to a reduced protein and increased fat content of the adipose tissue as the steers became fatter. Thus the initial kill protein level was 8.7 mg/gm fat compared to the final slaughter value of 4.5 mg/gm fat. The protein level of the fat samples taken by biopsy (about twice the initial kill values) was inaccurate due to unavoidable contamination with blood.

Clearly the potential significance of glucose as a substrate for lipogenesis declined as the steers became fatter. This may have also been related to a declining intake as the steers became fatter and older.

Dietary effects on enzyme activity: The activity of ATP citrate lyase and acetylCoA carboxylase at day 97 of feedloting was significantly increased by both the addition of chromium to the diet and by steam flaking of maize and sorghum. The effects were similar but smaller at slaughter, except for the chromium supplementation group where there was no longer a response.

The mechanism for the increase in the activity of ATP citrate lyase and acetylCoA carboxylase is likely to be different for the chromium treatment. Chromium's primary known mode of action is to increase insulin sensitivity which will increased the expression of ATP citrate lyase and acetylCoA carboxylase. This change in enzyme activity was associated with a trend toward decreased fat thickness at the P8 site (a similar response to our work in sheep; Gardner *et al.* 1997) and no change in visual marbling score. This suggests that an increased insulin sensitivity alone did not result in an increased lipogenesis at the marbling site. The conclusions for the chromium supplement are confounded however since there was a trend for chromium to increase liveweight during the last 90 days of feedloting indicating a greater rate of protein accretion. Consequently it is possible that extra energy was required for protein accretion, thus drawing substrate away from lipogenesis.

The increases in the activity of ATP citrate lyase and acetlyCoA carboxylase due to the steam flaking of maize and sorghum must have arisen due to changes in both insulin and glucose availability. This can be explained by 2 mechanisms. Firstly our previous work has shown that feed intake is a strong stimulus for both the lipogenic enzymes (Pethick *et al*. 1995) and so the significant increase in dry matter intake due to steam flaking would have resulted in a stimulation of the lipogenic pathways. An additional effect would be due to the higher ME of the steam flaked maize and sorghum. In addition we suggest that steam flaking may have allowed for increased digestion of starch and associated absorption of glucose in the small intestine. Steam flaking of maize and sorghum is known to increase the digestibility of starch both in the rumen and small intestine (Huntington, 1994). Janes *et al.* (1985b) compared insulin level in the blood, and insulin responsiveness and sensitivity in

sheep fed dried grass and maize based diets. The maize based diet resulted in an increased glucose turnover and more importantly 61% of the glucose turnover in the blood was derived from glucose absorption in the small intestine (Janes *et al.* 1985a). These changes in glucose metabolism were associated with no increase in blood insulin concentration, no change in insulin sensitivity and a slight increase in insulin responsiveness. Despite these lack of changes in insulin metabolism (at least in sheep), the metabolic pathways within adipose tissue are known to respond to the altered source of glucose (intestinal versus hepatic) within the animal suggesting precise regulation at a level which is not yet understood.

Associated with the increased activity of ATP citrate lyase in subcutaneous adipose tissue due to steam flaking was a similar increase in the visual marbling score. Indeed there was a significant correlation between the activity of ATP citrate lyase in subcutaneous adipose tissue and visual the marbling score and intramuscular chemical fat. On the basis that the dietary regulation of ATP citrate lyase activity in adipose tissue was via a different mechanism for the chromium supplemented treatment, separate correlation's were calculated with this treatment excluded. The correlation was further improved when the chromium supplemented treatment was not included (Table 5 and 6). This points to factors which increase both insulin action AND glucose availability as key contributors to the marbling response.

There is something special about increasing glucose availability (through whatever mechanism) and we might hypothesise differences in the effectiveness of hormonal axes or possibly substrate (glucose or a metabolite) induced gene regulation. There is evidence in rats that several hormones are involved in the communication of nutritional state to adipose tissue and these include insulin, glucagon, thyriod hormones (T_3) and glucocorticciods (Hillgartner *et al.* 1995). Alternative and interacting regulatory candidates might be some of the hormonal or neural activities of the gut (Uvgus Moberg, 1992). There is also strong evidence, again in laboratory rodents, that glucose and/or a metabolite (glucose-6-phosphate) primarily controls expression of lipogenic enzymes and that insulin (and other hormones) have only a potentiating or indirect role (Foufelle *et al.* 1996). It is not obvious just how this proposition can apply to ruminants, since peripheral blood glucose does not undergo large changes in the post prandial period even when diets which encourage the digestion of glucose in the small intestine are fed. However given the long term nature of fat deposition at the marbling site (i.e. 150 days of feeding), small increases in blood glucose that may be difficult to detect in acute experiments could still have regulatory effects.

Correlation's with marbling

In this study only 2 parameters correlated with both visual marbling score and intramuscular fat; they were the activity of ATP citrate lyase at day 97 of feedloting and total body fatness measured by rib dissection (the 12th rib scan fat was also correlated with intramuscular fat). Interestingly the activity of ATP citrate lyase at slaughter was not correlated with marbling suggesting that the first 100 days of feeding is critical for setting cattle up to marble. It is also remarkable that the activity of acetylCoA carboxylase measured at day 97 of feedloting was not correlated with marbling. AcetylCoA carboxylase is the rate limiting step for lipogenesis and this indicated that the rate of lipogenesis in subcutaneous fat is not closely linked to the marbling response. This further highlights the importance of the pathways of fat biosynthesis (i.e. glucose versus acetate) and providing ATP citrate lyase is stimulated in a manner that allows more substrate (glucose) to be made available then it will lead to increased marbling.

Given these correlation's a simple multiple linear regression model has been constructed to show the combined association between the important 'animal' parameters and visual marbling score or intramuscular fat content. The models were based on predicting intramuscular fat (%) using day 97 ATP citrate lyase (w, nmol/min/gm fat), slaughter ATP citrate lyase (x, nmol/min/gm fat), total dissectable fat (y, %) and dry matter intake (z, kg/day) using the following linear equation:

% Intramuscular fat = a + bw + cx + dy + ez

No other parameters measured in this experiment increased the accuracy of the model.

MODEL I - including all treatments

r^2 = 0.37, *P*=0.0014

Item	Coefficient	*P*
constant	4.09 (a)	0.11
Day 97 ATP citrate lyase	0.007 (b)	0.0002
Slaughter ATP citrate lyase	-0.028 (c)	0.016
Total dissectable fat	0.128 (d)	0.049
Dry matter intake	-0.305 (e)	0.093

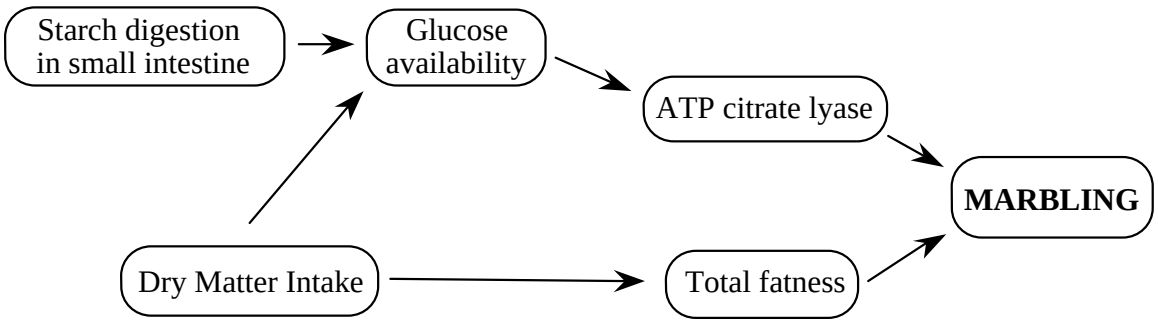
MODEL II - excluding the chromium supplemented group.
r^2 = 0.44, *P*=0.0009

Item	Coefficient	*P*
constant	4.807 (a)	0.057
Day 97 ATP citrate lyase	0.008 (b)	0.0002
Slaughter ATP citrate lyase	-0.028 (c)	0.016
Total dissectable fat	0.134 (d)	0.039
Dry matter intake	-0.359 (e)	0.045

Daily Liveweight gain significantly improved the model as it was correlated with both the activity of ATP citrate lyase in subcutaneous adipose tissue and with total dissectable fat at slaughter.

Similar models were also constructed to explain the visual marbling score and when data from all diets were used the r2 was 0.22-0.35 (range of the 2 assessors) and with the chromium treatment excluded the r2 was 0.27-0.39.

Summary of Model



Fat Texture

Key enzymes of the citrate cleavage pathway are ATP citrate lyase and NADP malate dehydrogenase. Both of these enzymes are induced by intravenous glucose infusion and consequently their activity in adipose tissue represents an estimate of the maximal capacity for lipogenesis from glucose (*Pethick et al*. 1995)

Regardless of the substrates used, the overall rate of lipogenesis in controlled by the activity of acetylCoA carboxylase which is under complex substrate, allosteric and hormonal control. Accordingly the activity of acetylCoA carboxylase is an indicator of the total capacity for lipogenesis.

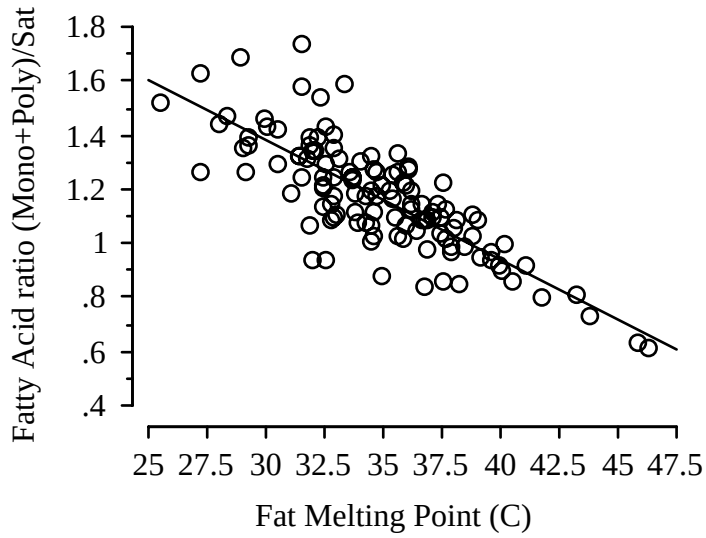
Table 8. Live & carcass weight plus subcutaneous fat parameters for the steers killed immediately prior to feedloting

Parameter	Mean±SEM
Liveweight (kg)	449±8
Carcass weight (kg)	251±5
Scan Rib fat depth (mm)	6.9±0.6
Scan P8 fat depth (mm)	7.3±0.9
Carcass P8 fat depth (mm)	10.3±1.4
Fat melting point (°C)	40±1.1
Fat composition (mono+poly/sat)[a]	0.93±0.06

a (Mono+polyunsaturated)/saturated fatty acid ratio

The fatty acid profile (mon+poly unsaturated/saturated fatty acid ratio) results supported the fat melting point measurements since there is a close relationship between the two parameters (r2=0.63, Figure 7). In addition they showed that the fat had become relatively softer by day 97 of feedloting (Table 4), however significant differences between the dietary treatments were not detected until slaughter (154 days of feeding).

Figure 7. The relationship between fatty acid ration (mono+polyunsaturated/saturated fatty acids) and fat melting point of subcutaneous fat (r2=0.63).



The mechanism for the softer subcutaneous fat found when steers consumed the DR sorghum and maize diets might be (i) steam flaking may have allowed for a greater release of dietary fat into the rumen and so more microbial hydrogenation could have taken place (ii) an alternative possibility is that S/F allowed for a significant reduction of the ∆9 desaturase activity (Table 5). The mechanism for the reduction in the activity of ∆9 desaturase is unknown but deserves further research.

5 CONCLUSIONS

(i) The conclusions of the study were similar for both visual marbling score and intramuscular fat.

(ii) Carcass temperature did affect visual marbling score with colder meat yielding an increased score.

(iii) Diet had a significant effect on marbling with diets based on maize and steam flaking of maize and sorghum giving the highest marbling scores. Below the proposed new category of 5 star beef is used an example where the intramuscular fat must reach at least 6%. The dramatic effects of diet are obvious from the table below.

Dietary treatment	**Intramuscular fat ≥ 6% (% of steers)**
Barley	25
Barley + chromium	31
D/R Maize	63
S/F Maize	75
D/R Sorghum	25
S/F Sorghum	63

The steam flaking of the maize was relatively light while the flaking of the sorghum was comparable to that produced by a major feed mill in Queensland (Ridleys, Toowoomba).

(iv) An organic chromium supplement (chelavite™) resulted in increased insulin action but also a trend for increased growth and reduced fatness with no stimulation of marbling. Thus even in relatively heavy steers chromium still tends to stimulate the growth of muscle rather than fat.

(v) Only the activity of the glucose pathway for fattening (i.e the enzyme ATP citrate lyase in fat) and total body fatness were correlated with marbling. The activity of the glucose pathway for fattening was best correlated with marbling after 97 days of feeding. This suggests that starter diets should optimise the activity of ATP citrate lyase in adipose tissue.

(vi) A model was constructed for explaining the marbling response using the activity of the glucose pathway for fattening (i.e the enzyme ATP citrate lyase in fat), total body fatness and dry matter intake and this model explained some 40% of the variation. This compares to the known genetic effect which explains about 37% of the variation.

(vii) The fat texture in this study was relatively soft for all of the treatments. The feeding of D/R maize and D/R sorghum resulted in softer fat than the steam flaked equivalent suggesting that some of the fat in maize and sorghum was escaping fermentation in the rumen when the grains were fed dry rolled. An additional mechanism was that steam flaking of the diet resulted in a reduced the activity of the enzyme responsible for making soft fat within the animal (Δ^9 desaturase). The mechanism for this reduction is not known but deserves further research.

In addition to cereal grain the only other major grain in the ration was D/R lupins (8-10%) and this strongly implicates cotton seed products as a cause of hard fat on the Eastern sea board of Australia.

(viii) Further work is required to understand the role of feed intake, rumen fermentability and starch digestion in the small intestine as determinants of fat synthesis from glucose and therefore the marbling response. Thus the extent of steam flaking required and whether barley should be flaked is not yet known. This work would suggest processing to optimise feed intake, rumen function AND starch digestion in the small intestine is the target.

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7 PUBLICATIONS ARISING FROM THE WORK

Pethick, D.W. and Dunshea, F.R. (1996). The partitioning of fat in farm animals. *Proceedings of the Nutrition Society of Australia* **20**: 3-13.

Pethick, D.W., McIntyre, B.L., Tudor, G., and Rowe, J.B. (1997). The partitioning of fat in ruminants - can nutrition be used as a tool to regulate marbling. *Recent Advances in Animal Nutrition in Australia 1997*, 151-158 (Publ. Dept. Animal Science, Uni. New England)

Gardner, G.E., Pethick, D.W. and Smith, G.M. (1997) The effect of chromium supplementation on the metabolism of glycogen and lipid in adult Merino sheep. *Australian Journal of Agricultural Research* Accepted for publication.

8 COMMUNICATION TO INDUSTRY

Interview about research into dietary control of marbling: ABC Country Hour, 23rd February, 1997

Vasse Research Station Field Day, Vasse, Western Australia, February, 1997.

9 Appendix 1

Figure 8. The effects of diet on liveweight

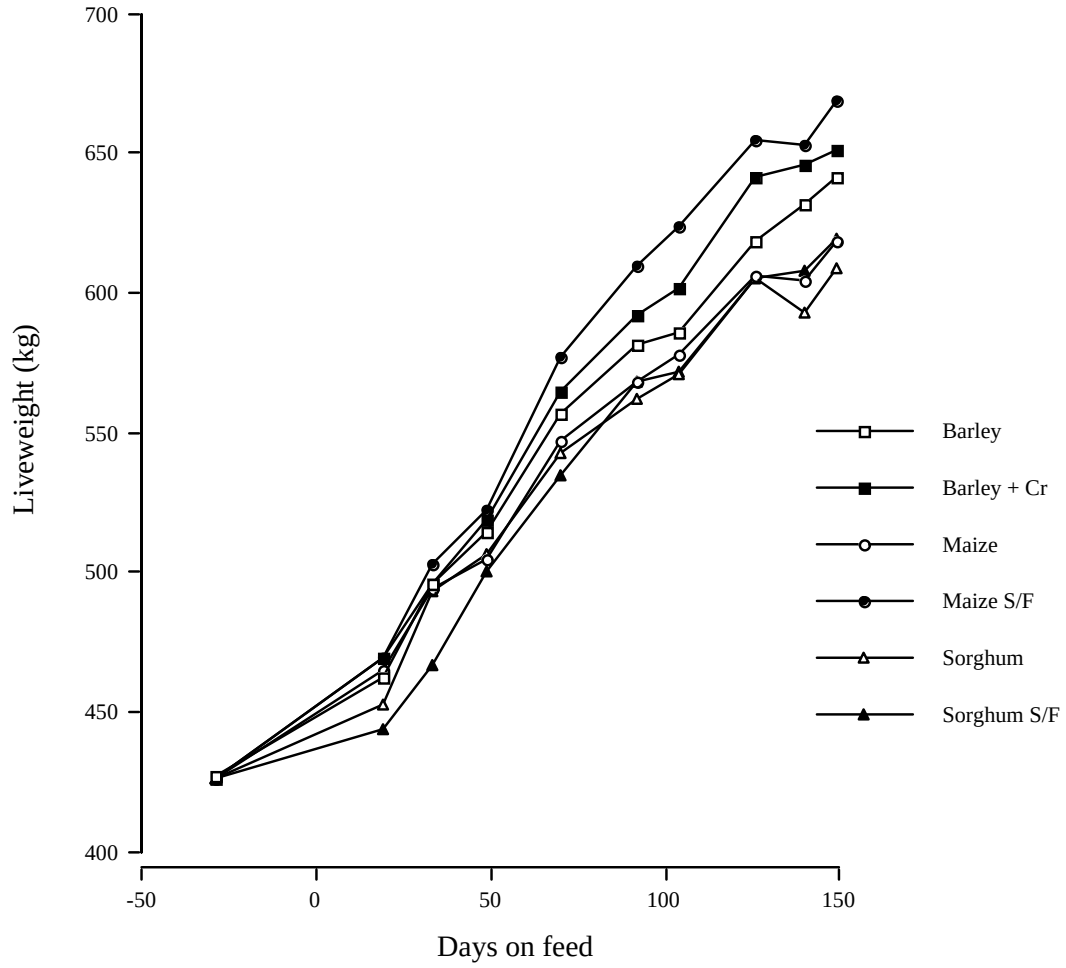
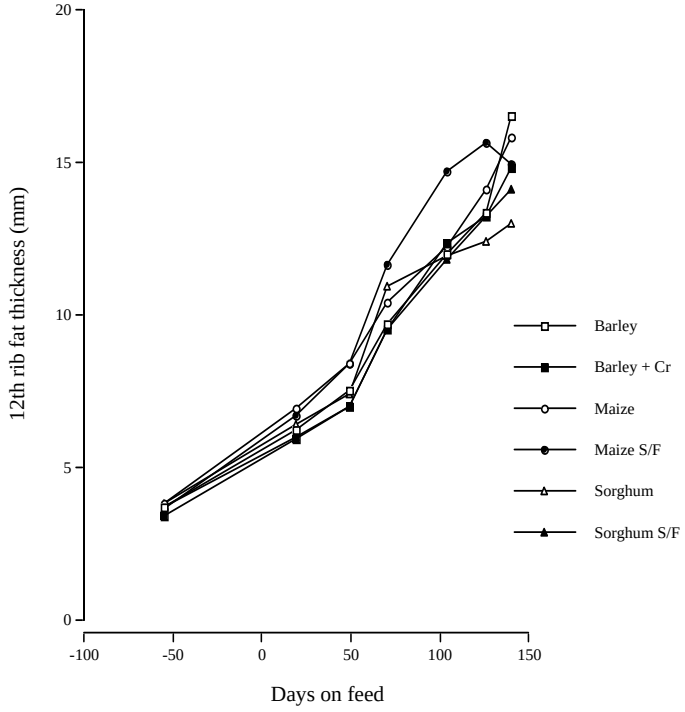


Figure 9. The effects of diet on the fat depth at the P8 and 12th rib site as determined by ulta sound.



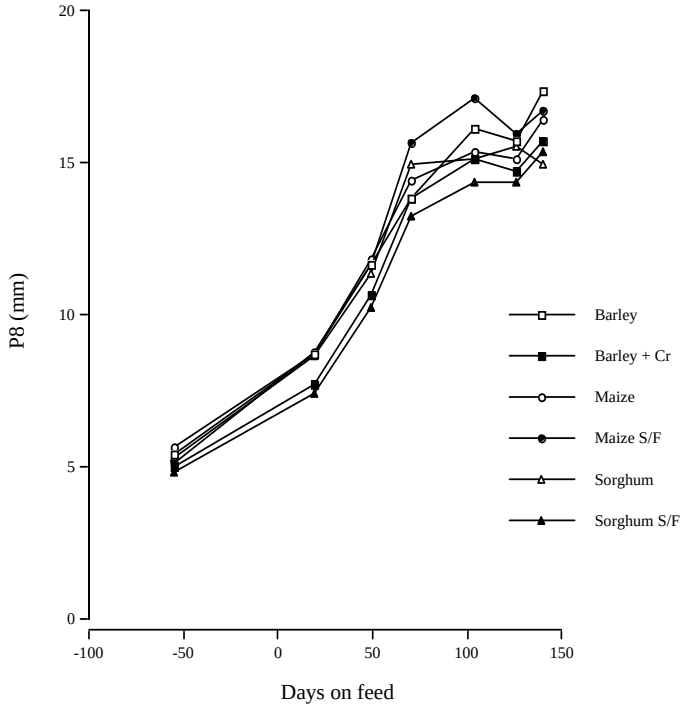


Figure 10. Dietary intake during the 50 days in individual pens.