MEAT & LIVESTOCK AUSTRALIA

On farm

Role of Dietary Protein

as a regulator of the expression of marbling in feedlot cattle (WA)

Project number FLOT.209
Final Report prepared for MLA by:

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Feedlots

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1.0 Acknowledgements

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2.0 Executive Summary/Abstract

2.1 Background/Aims

The primary aim of this project was to test the effects of dietary protein on the expression of marbling.

Two different dietary protein based hypotheses were tested:

- Feeding steers a diet low in rumen degradable protein will increase total body fatness and so the marbling response.
- Feeding steers a diet containing extra rumen degradable and extra rumen undegradable protein will increase the expression of marbling.

The first hypothesis was aimed at constraining muscle growth and therefore encouraging fat deposition both in the carcass and in the muscle. The second hypothesis was based on the proposal that extra amino acids available for degradation within the animal will primarily pass through glucose and so increase glucose availability – this would in turn lead to increased marbling because the intramuscular adipocytes show a preference for glucose as the main lipogenic substrate.

An additional aim was to understand the development of the intramuscular fat depot in relation to the growth of muscle and other fat depots within the carcass. The hypothesis tested was:

- The intramusuclar fat depot develops at the same rate as total body fat (intermuscular + subcutaneous).

2.2 Major findings

The protein supplement - canola meal

All of the protein supplements were highly degraded in the rumen (78-86% degradable) however each protein source had a significantly different degradability with lupin grain > commercial expella canola > formaldehyde treated expella canola. The formaldehyde treated expella canola was significantly less rumen degradable by 5 percentage units when compared to commercial expella canola. This degree of protection is substantially less than attained in other studies where solvent extraction canola meal was the base ingredient.

2.3 Conclusions - the effect of diet on marbling and intramuscular fat

- Marbling score was either unchanged or increased and intramuscular fat was unchanged in the m. longissimus thoracis (LT, eye of the cube role) of steers fed the barley ± urea rations.
- Extra protein in the form of commercial expella canola meal or formaldehyde treated expella canola meal did not influence marbling or intramuscular fat level in the LT.
- The inclusion of formaldehyde treated expella canola meal (10%) reduced muscle growth but did not increase marbling score or intramuscular fat. The reason for this is unknown.
- There is some evidence that during periods of more rapid growth that the addition of expella canola meal to the diet increased growth rate even at high body weights (i.e.>540kg). However during the final 50 days of feeding (690-736kg liveweight) this effect was reversed (i.e. barley

based diets performed better) so that the final liveweight and carcass weight was similar for all diets.

- A very simple diet based on barley and good quality hay (10.7% crude protein) and fed for the last 100-150 days would appear to be the most economic diet for allowing adequate growth and marbling to meet market specifications for Japan. Even the inclusion of urea seemed unnecessary although its relatively low cost and lack of negative effects on marbling would suggest that it should remain part of the ration.

Conclusions about the general growth pattern based on carcass composition at 304 & 427kg HSCW

- The expression of the marbling score (increased % intramuscular fat) relates to reduced muscle growth as the animal reaches maturity.
- fat accretion in the carcass depots (subcutaneous and intermuscular) and within muscle (at least the LT) occur at the same rate through the 304 – 417kg carcass range.
- Since fat accretion within muscle and at the subcutaneous and intermuscular depots occurred at the same rate the increase in the expression of intermuscular fat (marbling) relied upon declining muscle growth. That is, the concentration of fat increased as muscle growth slowed.
- This means that biologically intramuscular fat is not late maturing BUT that commercially the expression of intramuscular fat (i.e. the % fat or marbling score) is late maturing.
- The above conclusions are supported for the 304 – 417 kg HSCW range and the genotype used in this trial but the overall pattern should apply to all genotypes. However the appropriate carcass weight ranges are likely to be different.
- The above conclusions also do not exclude other control factors affecting marbling i.e. genetics and starch digestion in the small intestine.

1.0 MAIN REPORT

1.1 Introduction

The expression of marbling (intramuscular fat) in cattle is a complex phenomena which is poorly understood. The genetic make up of cattle is important with marbling showing a heritability of about 0.3-0.4. However other more general factors such as age and maturity (potential for lean gain) of the animal are important. Thus as the animal reaches maturity it tends to lay down more fat and less protein and it is during this phase that marbling is strongly expressed. Total body fatness and marbling are correlated with an r^2 of about 0.1-0.2 (i.e. 10-20% of the variation explained). Therefore one common production scenario is to take relatively heavy British breed cattle (i.e. 400-500 kg) with preferably optimal genetic propensity to express marbling and feed then a high energy diet for 200-350 days. This is a relatively expensive production system and there is a need to reduce the feeding period so as costs can be reduced but marbling is still expressed.

For the production of marbled beef for the Japanese market to be cost effective it is necessary to hit the marble score ≥3 classification both reliably and also with the least possible feeding cost (a combination of time on feed and unit cost of the feed).

1.2 Background

Can a low protein diet increase the marbling?

This a relatively simple approach that is known to increase the marbling response in pigs (Cisneros et al. 1996). The basis for the response is that the expression of marbling is related to total body fatness especially as the animal reaches maturity. Data from our previous studies (Pethick et al. 1997) has shown that total body fatness can explain about 10-20% of the variation in the expression of marbling – this is a similar figure to that found by the Australian Angus Society (Parnell, personnel communication).

Given the relationship between total body fatness and marbling, a finishing diet low in rumen degradable protein should limit muscle growth and favour fattening. Superficially this might appear a negative response but if the strategy improves the strike rate for marble score 3 it is likely to be economically favourable.

Will the provision of extra rumen undegradable protein increase marbling ?

The metabolism of the intramuscular fat tissue (marbling) is known to be different to other fat tissue. A key difference is the reliance on glucose as a precursor for fat synthesis rather than acetate from rumen fermentation (Smith and Crouse, 1984, Whitehurst et. al 1981). This metabolic difference should allow manipulation of marbling by diets, which maximise glucose availability. There are several ways to increase the availability of glucose:

- increased feed intake;
- grain choice &/or processing methods to allow increased starch digestion in the small intestine (i.e. promoting direct glucose absorption from the small intestine);
- increased by-pass protein levels in the ration to allow greater rates of endogenous glucose synthesis and more efficient intestinal starch digestion due to stimulation of amylase production.

The previous work done by our groups and funded by Meat & Livestock Australia (Projects DAW.053 and UMUR.004) has indeed confirmed that diet can alter the marbling response. Thus diets based on dry rolled maize and steam flaked maize and sorghum resulted in steers showing an increased

expression of marbling. The expression of intramuscular fat was not explained by carcass weight, growth rate or carcass fat depth. Only the activity of the glucose pathway for fattening (i.e. the enzyme ATP citrate lyase in fat tissue) and total body fatness (determined by dissection) were correlated with marbling. This experiment strongly points to diets, which promote an increased availability of starch for digestion in the small intestine as powerful agents for stimulating marbling. An earlier study by Mitsumoto et al. (1993) also found that maize based diets promoted a higher marbling score when compared to barley based diets.

The problem with dry rolled barley based rations is that the starch is heavily fermented in the rumen with less starch available for digestion in the small intestine – this was confirmed by the finding that the glucose pathway for fat biosynthesis in the barley fed steers was low.

There are no reports in the literature on the influence of dietary protein on marbling – however some unpublished information suggests a possible alternative mechanism. Keenans, UK, run a system for alkali treatment of grains which has proved very popular in the Dairy Industry. They claim caustic soda treatment of whole canola causes saponification of the oil and increased by-pass of that oil, and they quote an unpublished study where only 700 gm of "soda canola" fed daily as a supplement in the last 70 days to cattle on various finishing rations (run on 6 farms) increased live weight gain by ~0.2 kg/day and noticeably increased visual marbling and changed flavour (NB; the UK cattle average 300-350kg carcass weight for steers). The marbling response to Keenan's 'soda canola' seems spectacular and being an unpublished series of Industry trials they need repeating. It is worth speculating just what might have caused the responses:

(i) it is possible that the increased lipid intake and so energy consumption of the steers fed the whole 'soda' canola allowed greater fat deposition and more efficient live weight gain (similar to trials using supplemental fat (Brandt 1995). However given the inconsistent response of marbling to supplemental fat (Beef CRC, 1996/97) it is unlikely to fully explain a marbling response.

(ii) an alternative hypothesis is that an increased supply of rumen undegraded protein (for both the canola meal and soda whole canola) would allow for an increased marbling response for both the soda canola and canola meal treatments.

How then could so called "by-pass" protein increase glucose availability and marbling ? Firstly it is possible that starch digestion in the small intestine was stimulated by by-pass protein. Starch utilisation in the small intestine is generally inefficient with digestibility running at about 50% (Owens et. al. 1986). Several authors have postulated that the digestion of starch in the small intestine is limited by the availability of amylase (Ørskov 1986; Owens et al. 1986; Huntington 1997). Thus oligosaccharidase activity and monosaccharide transport across the enteroctye are not thought to be the limiting factor. To date the nutritional manipulation of amylase secretion is not readily understood although there is evidence for a role of high quality protein being digested in the small intestine for stimulating amylase production (Taniguchi et al. 1995; Taniguchi et al. 1993). Even on a ground barley based ration some 0.7-0.8 kg of starch is presented to the small intestine for digestion - any increase in the utilisation within the small intestine (rather than large intestine) will increase efficiency and marbling. Secondly the provision of extra protein delivered to the animal will also increase glucose availability by providing extra substrates for gluconeogenesis. For every 100gm of protein absorbed from the small intestine an extra 28gm of glucose is synthesised by the liver (Judson and Leng, 1973; Lindsay and Williams, 1971).

Given this background a study of the effects of extra dietary rumen escape protein on the marbling response of steers is warranted.

1.3 Project Aims

The primary aim of this project was to test the effects of dietary protein on the expression of marbling.

Two different dietary protein based hypotheses were tested:

- Feeding steers a diet low in rumen degradable protein would increase total body fatness and so the marbling response.
- Feeding steers a diet containing extra rumen degradable and extra rumen undegradable protein would increase the expression of marbling.

An additional aim was to understand the development of the intramuscular fat depot in relation to the growth of muscle and other fat depots within the carcass. The hypothesis tested was:

- The intramusuclar fat depot develops at the same rate as total body fat (intermuscular + subcutaneous).

1.4 Methods & Materials

Animals

The trial was conducted at the Vasse Research Station situated near Busselton, Western Australia. 112 Angus were used for the trial – the steers were the same genotype as the previous cereal grain marbling trial (MRC project UMUR.004). They arrived on the property as 12 month old weaners and were backgrounded on a mixture of hay/grain (during the summer months) and pasture (after the season break).

The steers where then fed a standard feedlot diet typical for Western Australia based on hammermilled hay, lupin and barley for 50 days (see Table 1). After this animals were stratified by weight and groups of 16 animals were randomly allocated to one of 6 dietary treatments which were fed for a further 156-160 days (total days in feedlot = 206 days). During this time animals spent 50 days in the individual feedlot pens for the measurement of individual intake. The remaining 100 days was spent in group pens (Table 2). The age of the steers at slaughter was 26 months (mean ossification score of 170).

Table 1. Composition of the 50 day pre-trial feedlot diet

Ingredient	% inclusion
Hammermilled hay	15.0
Barley grain	67.4
D/R Lupin grain	15.0
Urea	0.5
Limestone	1.0
Gypsum	0.2
Salt	0.5
Potash	0.2
Mineral/vitamin/virginiamycin premix	0.22
Metabolisable energy (MJ/kg DM)	11.5

Crude Protein (% DM)	15

The timeline of activities is shown below in Table 2. 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 2. Time for management and experimental activities

Date	Days relative to main trial	Mean L. weight (kg)/ Scan P8 (mm)	Action
19/2/98	-250	355/4	Steers backgrounded for 200 days
15/9/98	-56	435/8	Pre-trial barley/lupin feedlot diet
4/11/98	0	539/12	Steers switched to treatment diets
24/11/98	20	535/12	Initial kill – 16 steers
1/12/98	27		Steers in individual pens
24/12/98	50		Urea removed from Treatment 2
15/1/98	72		Steers back to group pens
12/2/99	100		Urea removed from Treatment 3
9/4/99	156	736/25	Main slaughter
13/4/99	160		MSA slaughter

Processing

At the start of the 150 day feeding trial 16 steers were randomly allocated to an 'initial' slaughter group so that carcass composition parameters at the start of the trial could be measured. At day 156 of the trial (206 days in feedlot) 86 of the steers were processed at E.G. Greens and Sons. The remaining 10 steers were processed on day 160 (210 days in feedlot) and 6 bodies were selected for eating quality work in the Meat Standards Australia program.

Other methods

Fat samples taken by biopsy were assayed for the activity of ATP citrate lyase activity according to the methodology of Pethick *et al*.(1995). Fat was taken from next to the tail area of the steers using a biopsy drill on day 93 and collected into liquid N_2.

Body composition was estimated using a rib dissection. A rib set (ribs 6-10, Technical Manual of Australian Meat) was obtained at slaughter and then wrapped and frozen. Within 16 weeks each rib set was thawed and a detailed dissection performed. Tissue was dissected into subcutaneous fat, intermuscular fat, muscle (including *m. longissimus thoracis*) bone and connective tissue. Only dissections with a recovery by weight of at least 99% were used for further analysis.

A 1 rib thick sample was taken at the 10th rib site from the *m. longissimus thoracis* (LT) after dissection and then frozen for subsequent determination of intramuscular fat. Total fat in the LT was estimated assuming the distribution of fat within the LT was the same throughout. This of course is an incorrect assumption since the level of intramuscular fat increases in the LT over the 6-7th thoracic vertebrae (Mitsumoto et al. 1993) – however this would mean the calculation under estimated the LT fat but the relationship between LT fat and other depots should not be affected. Intramuscular fat was measured on freeze dried portions of the *m. longissimus thoracis* using NIR technology at the CRC for Cattle and Beef Industry (Beef Quality), Armidale (Dr Oddy's laboratory).

Growth rate was estimated using regression analysis – the slope of the relationship between liveweight and time was estimated by using at least 3 points. Given this the weight gains shown in the following tables will not always be identical to those calculated by simple subtraction of one mean weight versus another. Fat depth at both the P8 and 12th rib site were determined be an experienced operator using real time ultra sound.

Carcass assessment was performed by an accredited AUSMEAT assessor (AUSMEAT Marbling, grader 1) and by an accredited Meat Standards Australia (MSA) assessor (USDA Marbling, grader 2). The muscle temperature at the time of assessment was 5-6˚C. The MSA assessor used the USDA system for marbling assessment as shown in Table 3.

Table 3. Relationship between USDA marbling grade and the Meat Standards Australia (MSA) system (Meat Standards Australia, Brisbane).

USDA Marbling Score	**MSA grade**
Practically Devoid	100
Traces	200
Slight	300
Small	400
Modest	500
Moderate	600
Slightly Abundant	700
Moderately Abundant	800
Abundant	900

Treatments

The diets are shown in table 4. Six treatments with 16 animals per treatment :

- Treatment 1: Control urea: barley + urea @ 150 days.
- Treatment 2: Low Protein 1: Treatment 1 50 days; barley (no urea) for 100 days.
- Treatment 3: Low Protein 2: Treatment 1 100 days; barley (no urea) for 50 days.
- Treatment 4: Control canola meal: barley + 10% canola meal @ 150 days.
- Treatment 5: 'Bypass' Protein 1: Control canola meal but replace canola meal with 5% formaldehyde treated canola meal (balance rumen degradable protein with urea).
- Treatment 6: 'Bypass Protein' 2: Control canola meal but replace canola meal with 10% formaldehyde treated canola meal (balance rumen degradable protein with urea).

Table 4. Formulations for the dietary treatments (as fed).

Ingredient	**Control Urea**	**Low Protein**	**Control Canola meal 10%**	**Treated Canola meal 1**	**Treated Canola meal 2**
Hay	0.15	0.15	0.15	0.15	0.15
Barley (9.8% CP)	0.7555	0.767	0.6723	0.669	0.6658
Molasses	0.05	0.05	0.05	0.05	0.05
Canola meal	0	0	0.1	0.05	0
Formaldehyde treated Canola meal	0	0	0	0.05	0.1
Canola oil	0.008	0.008	0	0	0
Urea	0.0115	0	0.0027	0.006	0.0092
Vit/Mins	0.025	0.025	0.025	0.025	0.025
Dry matter (%)	0.89	0.89	0.895	0.89	0.895
Metabolisable energy (MJ/kg DM)*	11	11.2	11.1	11	11
Crude Protein (% DM)*	12.9	10.7	13.6	14.7	16.6

* = measured metabolisable energy protein in final diet

The diets were designed to be isojoulic. Expeller canola meal was sourced from Davidsons. The same meal was also treated using the CSIRO formaldehyde treatment technology (John Ashes at Prospect, NSW.). The degree to which the canola meal protein was protected from rumen fermentation was estimated using the 'nylon bag' technique *in vivo* (SCA, 1990).

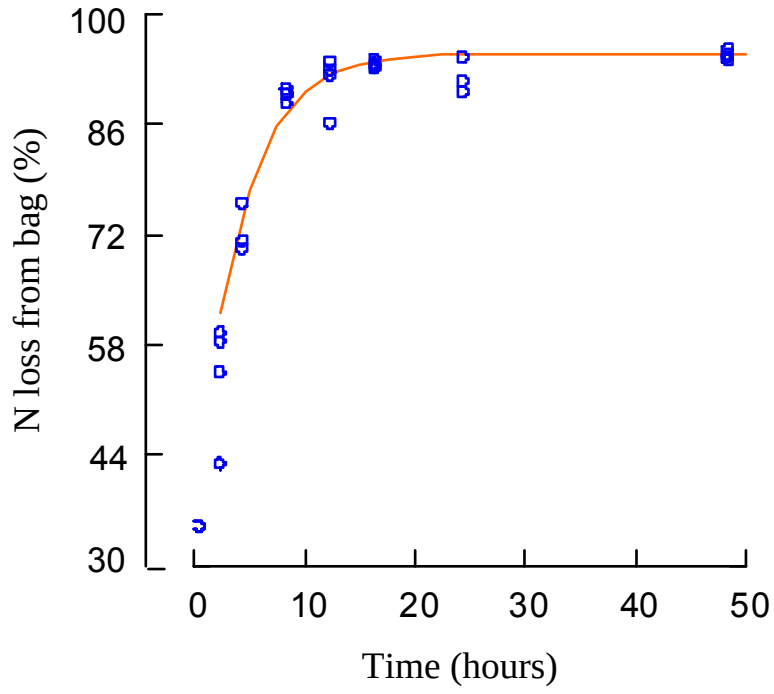
In sacco protein degradability measurements were carried out by CSIRO, Division of Animal Production, Perth (Dr Colin White). It was measured on quadruplicate samples ground in a mill with a 3 mm screen, weighed into dacron bags, washed for one wash and spin cycle in an automatic washing machine (15 minutes) and then suspended in the rumen of four steers for 48 hrs (SCA, 1990). One set of bags was dried and weighed after removal from the washing machine to provide a time zero value, and the remainder were removed from the rumen at intervals of 2, 4, 8, 12, 16, 24 and 48 hours. The residual material was washed in cold water, dried at 70˚C for 24 hrs and analysed for nitrogen. AFRC (1993) degradability parameters (*a*, *b*, *c*; soluble, potentially degradable and rate of degradation) were calculated from the nitrogen loss data using the nonlinear regression function of the microcomputer program Systat (Systat Inc, Evanston Ill). The steers weighed 600 kg and were fed twice daily (0700 and 1600) to maintenance (12 kg air dry). The diet consisted of 15% canola meal, 15% lupins, 15% oats and 55% cereal hay.

1.5 Results & Discussion

1.5.1 Canola meal degradability

The nitrogen degradability curve for commercial expella canola meal is shown in Figure 1. Similar curves were obtained for ground lupin grain and formaldehyde treated canola meal.

Figure 1. The nitrogen loss curve for commercial expella canola using the nylon bag technique for assessing protein degradability in the rumen (Each point on the graph represents one bag at one time in one steer. There were 4 steers sampled over 48 hrs).



An exponential equation to describe N loss from the bags was fitted to the data (Figure 1) using the equation as follows:

Fractional N loss = $a + b(1-EXP^{(-c \times time)})$, where *a* is the N solubility in water, *b* is slowly soluble N fraction and *c* is the rate of solubility (degradability) of *b*.

The fractional rumen degradability of the feedstuff is then calculated as (Orskov and McDonald, 1979):

Rumen degradability (%) = a + (b x c)/(c + r) x *100*, where *r* is the fractional rate of rumen outflow (%/hr). The fractional rate of rumen outflow is influenced by the level of intake and is usually designated as 0.04 (maintenance fed steer), 0.06 (*ad lib* fed growing steer) or 0.08 (*ad lib* fed dairy cow). This work used *r* = 0.06 for calculating the degradability values shown in Table 5.

Table 5. Nitrogen loss and rumen degradability of nitrogen for the dietary protein supplements

Dietary Ingredient	**N loss parameters†**			**Rumen degradability (%)**
	a	*b*	*c*	
Commercial expella canola meal	0.32^{d}	0.63^{d}	0.25^{d}	82.8^{d}
Formaldehyde treated canola meal	0.25^{e}	0.70^{e}	0.19^{e}	78.2^{e}
Ground lupin seed	0.47^{f}	0.51^{f}	0.20^{de}	86.2^{f}
P for difference between protein sources	<0.001	<0.001	= 0.008	<0.001

† Values with different superscripts are significantly different, $P < 0.05$

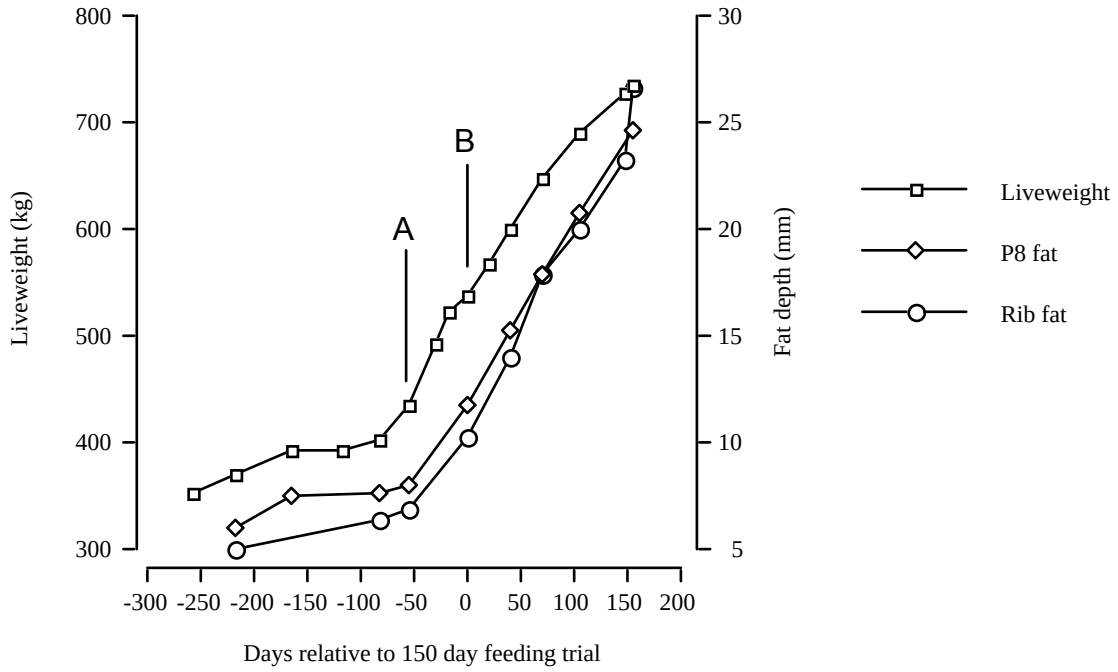
All of the protein supplements were highly degraded in the rumen (78-86% degradable) however each protein source had a significantly different degradability with lupin grain > commercial expella canola > formaldehyde treated expella canola. The formaldehyde treated expella canola was not heavily protected being only 5 percentage units less degradable than commercial expella canola.

This degree of protection is unusually low given that apparently identical technologies used previously for canola meal have resulted in an in sacco protein degradability of 48% (White et al. 2000) with lupin gain in the same study giving a protein degradability in the rumen of 85%. The reasons for the poor protection of canola meal used in this study remain unknown but might be related to the use of expella canola meal. Previous research with formaldehyde treatment has used solvent extracted meal.

1.5.2 Weight gain & feed intake

The overall pattern of weight gain during the backgrounding and feedlotting stage is shown in Figure 2 and table 6. The backgrounding growth rate averaged 0.41kg/day over 198 days and the steers entered the feedlot at 436kg (P8=8mm). During the pre-treatment feedlotting period the steers showed good compensatory growth at 1.93kg/day for 50 days.

Figure 2. Mean change over all treatments in liveweight and fat depth during the backgrounding and feedlotting stages of growth. (A=pre-trial feedlot diet; B= Trial diets)



The first 41 days of the treatment diets saw the mean growth across all treatments decline to 1.52kg/day with no significant effect of treatment. During the next 64 days the growth declined further to 1.37kg/day again with no significant effect of diet. However when growth rate was calculated over the first 105 days of the treatment diets there was a significant effect for the canola based diets to show an improved growth rate. The reason for this change in the data interpretation is almost certainly related to the extra precision of estimating growth by using more points to calculate growth rate using regression analysis (see methods). This result indicates that there was a response to either extra or ‘true’ protein in the diet during the first 105 days of feeding the trial diets.

Table 6. Growth rate data

Time period of trial (days)	**Live Weight (P8fat) (kg; mm)**	**Treat. 1 barley/ urea**	**Treat. 2 barley 100d minus urea**	**Treat. 3 barley 50d minus urea**	**Treat. 4 Canola meal**	**Treat. 5 5% formaldehyde canola meal**	**Treat. 6 10% formaldehyde canola meal**	**All Barley diets**	**All Canola diets**	**SED**	**Significance (P)**	
		Growth rate (kg/day)									Treatments 1-6	Barley vs canola
Backgrounding –254 to –56	353 (5)-436 (8)	0.41±0.011 (one diet only)								-	-	-
Pre feedlotting –56 to –1	436 (8)-538 (9)	1.93±0.038 (one diet only)								-	-	-
-1 to 41	538 (12)-602 (15)	1.36	1.44	1.64	1.46	1.56	1.66	1.478	1.560	0.203	ns	ns
41 to 105	602 (15)-690 (21)	1.34	1.40	1.29	1.44	1.39	1.375	1.346	1.400	0.133	ns	ns
-1 to 105	538 (12)-690 (21)	1.38	1.41	1.46	1.49	1.44	1.55	1.414	1.492	0.111	ns	0.0496
105 to 154	690 (21)-736(25)	1.03	0.91	1.02	0.93	0.84	0.90	0.983	0.890	0.151	ns	0.084
-1 to 154	538 (12)-736 (25)	1.26	1.24	1.30	1.30	1.24	1.32	1.266	1.285	0.086	ns	ns

Table 7. Feed intake and feed/gain data (as fed).

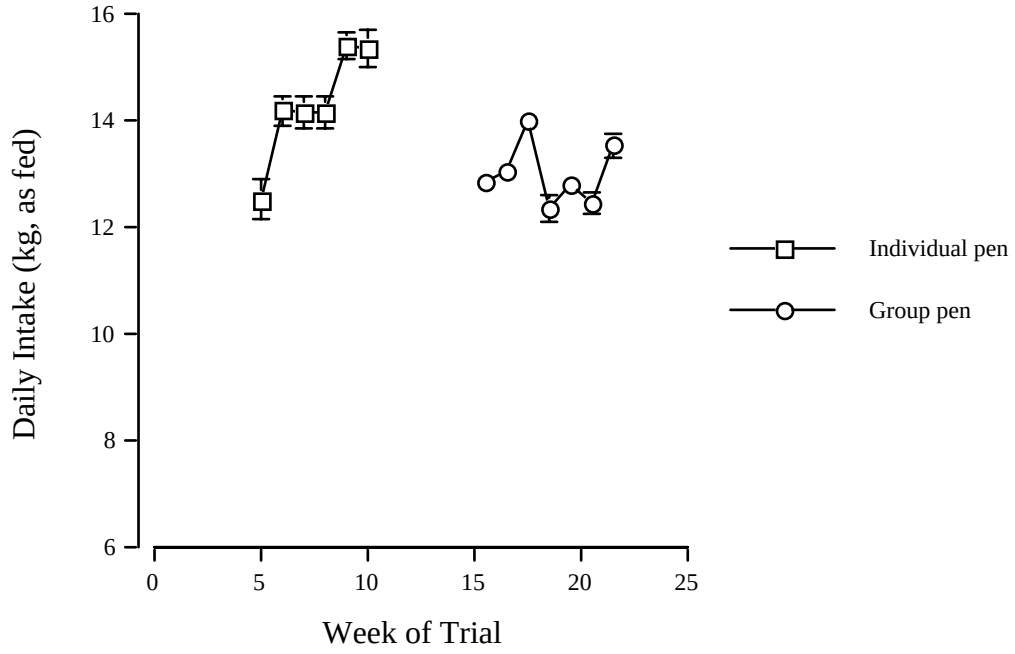
Item & Time period	Treat. 1 Barley/ urea	Treat. 2 Barley 100d minus urea	Treat. 3 barley 50d minus urea	Treat. 4 Canola meal	Treat. 5 5% formaldehyde canola meal	Treat. 6 10% formaldehyde canola meal	All Barley diets	All Canola diets	SED	Significance (P)	
										Treatments 1-6	Barley vs canola
Individual feed intake (kg/hd/day) 27-71days	14.4	14.4	14.1	14.4	13.6	14.8	14.3	14.3	0.93	ns	ns
Feed/gain 27-71 days	10.7	10.1	9.6	9.9	9.2	9.8	10.1	9.7	1.5	ns	ns
Group Feed intake (kg/hd/day) 108-154 days[A]	12.7	12.9	13.2	12.9	12.9	13.4	13.0	13.1	-	-	-
Feed/gain 108-154 days[A]	12.3	14.2	12.9	13.9	15.4	14.9	13.2	14.7	-	-	-

[A] statistical analysis was not possible as steers were in group pens with one pen per treatment

During the last 50 days of feeding the mean growth rate was 0.94kg/day and there a trend for the barley based diets to sustain greater growth rates during this phase. This may have been related to the higher starch content of the barley based diets allowing for more efficient synthesis of fat during the final 'fattening' stage. Alternatively it might be related to a 'growth path' effect.

The was no effect of treatment on the feed intake of steers measured in individual pens during days 27-71 of the trial (Table 7). Feed intake averaged 14.3 kg/hd/day on an 'as fed' basis during this period which was equivalent to 12.7kg/hd/day of dry matter intake. Feed:gain (as fed) during this period was 9.9. Later in the feeding period (days 108-154) the intake dropped to 13kg/hd/day on an 'as fed' basis and the feed:gain declined to 14 (as fed) as the steers gained less liveweight per day (Figure 3. Table 7.).

Figure 3. Mean feed intake (mean ± sem, 'as fed') of steers during the period in individual and group pens.



1.5.3 Initial versus final slaughter carcass data

The carcass traits and ribset dissection data for the initial and final kill groups are shown in Table 8. There was a substantial increase in total dissectable fat as the animals went from 304 to 417 kg HCW – 29% increase in % total fat and a 58% increase in fat/bone ratio. During this period the subcutaneous fat increased relative to intermuscular fat. Similarly the total chemical fat in the *m. longissimus thorasis* (LT) increased by 88% and the % intramuscular fat increased by 61%. However the increase in muscle mass was far smaller as indicated by only a 15% increase in eye muscle area and an 8% increase in the total muscle or LT/bone ratio.

Given the above what is the mechanism for the increase in the % intramuscular fat (marbling) between the initial and final slaughter ? Based on the ratio LT fat/total fat it would appear that the rate of fat deposition within the LT was similar at both the lighter and heavier weight. However during this time the muscle grew relatively slowly and therefore the expression of the intramuscular fat (i.e. %) increased.

1.6 Conclusions about the general growth pattern

- The expression of the marbling score (increased % intramuscular fat) relates to reduced muscle growth as the animal reaches maturity.
- fat accretion in the carcass depots (subcutaneous and intermuscular) and within muscle (at least the LT) occur at the same rate through the 304 – 417kg carcass range.
- Since fat accretion within muscle and at the subcutaneous and intermuscular depots occurred at the same rate the increase in the expression of intermuscular fat (marbling) relied upon declining muscle growth. That is, fat concentration increases as muscle growth slows.
- This means that biologically intramuscular fat is not late maturing BUT that commercially the expression of intramuscular fat (i.e. the % fat or marbling score) is late maturing.
- The above conclusions are supported for the 304 – 417 kg HSCW range and the genotype used in this trial but the overall pattern should apply to all genotypes. However the appropriate carcass weight ranges are likely to be different.
- The above conclusions also do not exclude other control factors affecting marbling i.e. genetics and starch digestion in the small intestine.

These conclusions have important implications for backgrounding and feedlotting. In general the sooner an animal reaches its near maximal potential for muscle growth the sooner it would begin to commercially express intramuscular fat. A very long feeding period allows the cattle to obtain a high level of intramuscular fat since there is time for muscle maturity to be reached followed by time for the muscle to 'fill up' with fat. Shorter feeding periods will have a higher risk of failure particularly if there is a relatively short period of fattening after muscle maturity is reached. For the shorter feeding scenario a heavier live weight entry rate would help to secure the required muscle growth.

Importantly these conclusions have been based on an estimate of body composition only at 2 carcass weights. Work by Aoki *et al*. (1999) has shown that carcass fat accumulation does not necessarily increase for ever. Aoki and colleagues found that both the % total body fat and the % intramuscular fat reached a maximum as the carcass weight of Japanese Black x Holstein cross steers reached 450kg. These steers were still increasing in liveweight (~0.5kg/day) but carcass composition was not changing indicating that fat must have been deposited elsewhere (i.e. abdominal area ?). It would therefor seem of crucial importance to perform a serial slaughter experiment right out to 500kg HSCW (350 days feeding) so that the pattern of body composition change over a wider range of carcass weights can be determined. Such an approach would help the commercial feedlot optimise their feeding periods.

Table 8. Whole carcass and ribset composition data for the initial and final slaughter steers (mean ± sem).

Carcass attribute	**Initial slaughter**	**Final slaughter**	**Ratio of final/initial**	**Significance *P***
Hot Carcass wt. (kg)	304 ± 5.0	417 ± 2.6	1.37	<0.001
Ossification score	na	170 ± 1.1	-	-
Eye muscle area (cm^2)	67.1 ± 1.6	77.3 ± 0.7	1.15	<0.001
P8-hot (mm)	14.8 ± 1.0	26.7 ± 0.7	1.80	<0.001
AUSMEAT Marbling	1.3 ± 0.2	3.2 ± 0.1	2.46	<0.001
Intramuscular fat (%)	7.01 ± 0.47	11.3± 0.28	1.61	<0.001
Total dissectable fat (%)†	34.5 ± 1.1	44.4 ± 0.5	1.29	<0.001
Total Fat:Bone ratio†	2.65 ± 0.14	4.19 ± 0.08	1.58	<0.001
Total dissectable muscle (%)†	50.2 ± 1.0	43.8 ± 0.4	0.87	<0.001
Total muscle:bone ratio†	3.8 ± 0.08	4.1 ± 0.04	1.08	0.012
LT muscle/bone ratio†	0.72 ± 0.03	0.78 ± 0.01	1.08	0.082
gm fat in LT†	107 ± 7	201 ± 6	1.88	<0.001
LT fat/total fat ratio†	0.021±0.001	0.021±0.001	1	ns
LT fat/bone ratio†	0.05±0.004	0.09±0.003	1.80	<.001
Subcutaneous fat/total fat†	0.40±0.02	0.46±0.01	1.15	<0.001
Intermuscular fat/total fat†	0.60±0.02	0.54±0.01	0.90	<0.001

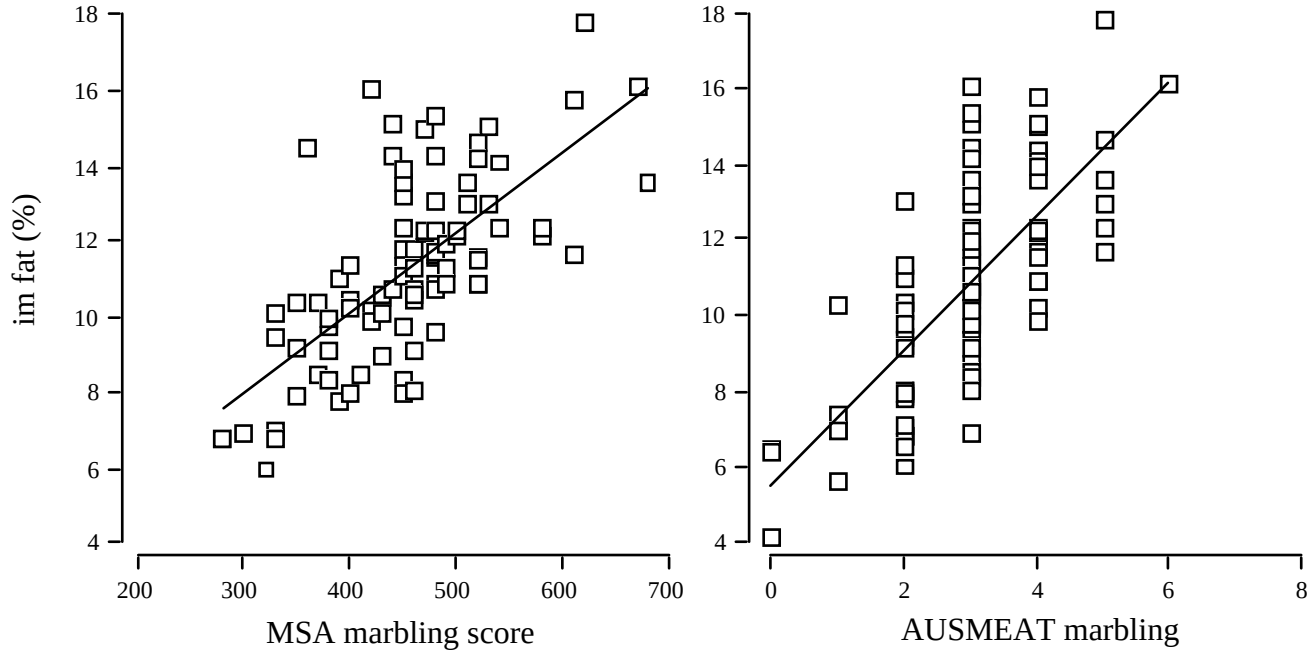
† - Measured from the ribset dissection
LT = *m.longissimus thoracis*
na = not measured

1.6.1 Visual marbling

Both the MSA and AUSMEAT graders produced marbling scores that were significantly related to intramuscular fat. The visual marbling grade accounted for about 50% of the variation shown in intramuscular fat (Figure 4).

Figure 4. Relationship between marbling score, MSA and AUSMEAT, and intramuscular fat

Figure 4(a) MSA marbling score r^2=0.46	Figure 4(b) AUSMEAT marbling score r^2=0.52



There was no effect of individual treatment on visual marbling score (Table 9). However when a comparison between barley versus barley + canola diets was made there was a significantly higher marbling score for the barley diets as assessed by the AUSMEAT system (Figure 5)

Figure 5. The frequency distribution of AUSMEAT marbling scores (assessed by an AUSMEAT grader) for the barley alone and canola meal based diets.

Figure 5(a) barley alone diets Left side

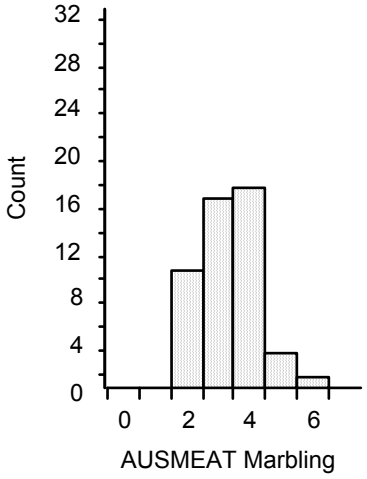
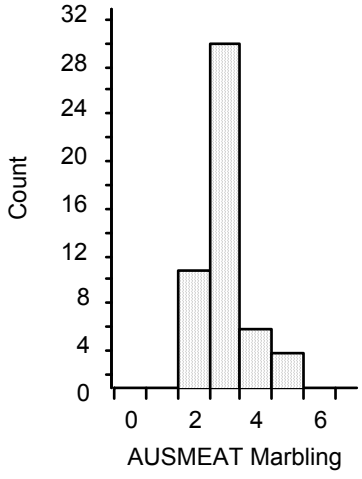


Figure 5(b) Canola based diets Left side



The frequency distribution of the AUSMEAT grades (assessed by the AUSMEAT grader) is shown in Figure 5. The distributions show that there were more marbling scores in the AUSMEAT 4 range when steers were fed the barley alone based diets. There was no difference in visual marbling when a similar analysis was made of the MSA marbling scores. This indicates differences in the interpretation of results dependant on the grader used.

1.6.2 Intramuscular chemical fat

There was no effect of dietary treatment on the level of intramuscular fat in the *m. longissimus thoracis*.

1.6.3 Carcass composition - final slaughter

The carcass data is shown in Tables 9 and 10. There was no significant effect of treatment on HSCW although treatment 6 (10% formaldehyde treated expella canola) did produce the heaviest carcasses. Consistent with this was a significant increase in the rib fat depth for treatment 6 and the same trend was found for P8 fat depth although this was not significant. When the data was analysed with carcass weight as a covariate the increase in rib fat thickness for treatment 6 was no longer significant. Total dissectable fat was not affected by treatment although again treatment 6 was higher.

There were several muscle growth parameters that were influenced by the addition of the formaldehyde treated expella canola. Namely a significant reduction in the muscle/bone and LT muscle/bone ratio. Despite the reduced muscle growth and maintained or increased total body fatness the absolute amount of fat in the LT and the LT fat/bone ration were also reduced especially in the formaldehyde treated 10% expella canola ration. The final outcome was no change in intramuscular fat. This was a surprising finding given that formaldehyde treated canola meal was included so as to assure an optimum supply of amino acids for both rumen fermentation and intestinal digestion. The authors have no ready explanation for this response ?

1.6.4 ATP citrate lyase

The expression of ATP citrate lyase was not influenced by diet suggesting that the inclusion of extra protein in the diet as expella canola meal (± formaldehyde treatment) did not increase the availability of glucose to the tissues of the steers. That is the extra amino acids did not influence total glucose turnover or the digestion of starch in the small intestine. Previous work has shown the ATP citrate lyase is a good marker for glucose turnover and site of starch digestion (Pethick et al. 1995).

1.7 *Conclusions - the effect of diet on marbling and intramuscular fat*

- Marbling score was either unchanged or increased and intramuscular fat was unchanged in the LT of steers fed the barley ± urea rations.
- Extra protein in the form of commercial expella canola meal or formaldehyde treated expella canola meal did not influence marbling or intramuscular fat level in the LT.
- The formaldehyde treated canola meal used in this experiment was unexpectedly not highly protected.
- The inclusion of formaldehyde treated expella canola meal (10%) reduced muscle growth but did not increase marbling score or intramuscular fat.
- There is some evidence that during periods of more rapid growth that the addition of expella canola meal to the diet increased growth rate even at high body weights (i.e.>540kg). However during the final 50 days of feeding (690-736kg liveweight) this effect was reversed (i.e. barley based diets performed better) so that the final liveweight and carcass weight was not effected by diet.
- A very simple diet based on barley and good quality hay (10.7% CP) and fed for the last 100-150 days would appear to be the most economic diet for allowing adequate growth and marbling to meet market specifications for Japan. Even the inclusion of urea seemed unnecessary although its relatively low cost and lack of negative effects on marbling would suggest that it should remain part of the ration.
- This work suggests that finishing type rations (high energy, lower protein) can be fed earlier in the life of long fed cattle with no loss in live weight gain and either improved or similar marbling responses.

Table 9. Carcass data

Parameter	Treat. 1 barley/ urea	Treat 2. barley 100d minus urea	Treat. 3 barley 50d minus urea	Treat. 4 Canola meal	Treat. 5 5% formaldehyde canola meal	Treat. 6 10% formaldehyde canola meal	All Barley diets	All Canola diets	SED	Significance (P)	
										Treatments 1-6	Barley vs canola
HCW (kg)	415.0	418.2	414.6	415.8	414.9	424.2	415.9	418.4	14.9	ns	ns
EMA (cm^2)	78.6	79.0	75.7	79.7	75.7	74.6	77.8	76.6	3.6	ns	ns
Rib fat (mm)	25.4	23.6	23.2	23.6	22.9	28.9a	24.1	25.1	3.1	0.024	0.079
P8–cold (mm)	27.0	28.9	27.9	26.4	26.6	30.1	27.9	27.7	3.5	ns	ns
P8–hot (mm)	26.7	28.3	28.3	26.1	22.3	28.2	27.8	25.6	3.8	ns	ns
Marbling L AUSMEAT	3.44	3.25	3.33	3.06	3.27	2.75	3.34	3.02	0.50	ns	0.078
Marbling R AUSMEAT	3.56	3.44	3.33	3.06	3.33	2.87	3.45	3.08	0.051	ns	0.049
Marbling USA	420	384	405	445	461	402	404	435	62	ns	ns
% intramuscular fat	11.8	11.4	11.3	10.9	11.1	11.0	11.5	11.0	1.4	ns	ns

HCW= hot carcass weight; EMA = eye muscle area. Marbling AUSMEAT – measured using the AUSMEAT scale; R = right side, L = left side. Marbling USA – Grader 2 – measured using the USA grading system as operated by Meat Standards Australia.

Table 10. Ribset dissection and ATP citrate lyase data

Parameter	Treat. 1 barley/ urea	Treat 2. barley 100d minus urea	Treat. 3 barley 50d minus urea	Treat. 4 10% Canola meal	Treat. 5 5% formaldehyde canola meal	Treat. 6 10% formaldehyde canola meal	All Barley diets	All Canola diets	SED	Significance (P)	
										Treatments 1-6	Barley vs canola
Total dissectable fat (%)	43.6	44.7	44.5	43.5	44.5	46.8	44.3	44.9	2.3	ns	ns
Total dissectable fat/bone ratio	4.1	4.3	4.2	4.1	4.1	4.3	4.2	4.2	0.2	ns	ns
LT fat (gm)	217	215	201	202	185	174	211	187	29	ns	0.035
LT fat/total dissectable fat ratio	0.023	0.021	0.022	0.021	0.020	0.018	0.022	0.02	0.003	ns	0.11
LT fat/bone ratio	0.095	0.095	0.090	0.087	0.082	0.078	0.094	0.083	0.018	ns	0.047
Total muscle (%)	44.7	43.9	43.5	44.7	43.3	41.1	44.1	43.1	1.9	ns	ns
Total muscle/Bone ratio	4.2^{a}	4.2^{a}	4.1^{a}	4.2^{a}	4.0^{ab}	3.8^{b}	4.2	4.0	0.2	0.048	0.018
LT muscle/bone ratio	0.80	0.81	0.79	0.78	0.75	0.72	0.80	0.75	0.06	ns	0.045
ATP citrate lyase (nmol/h/gm fat)	124	-	-	141	120	131	124	131	31	ns	ns

LT – m. longissimus thoracis

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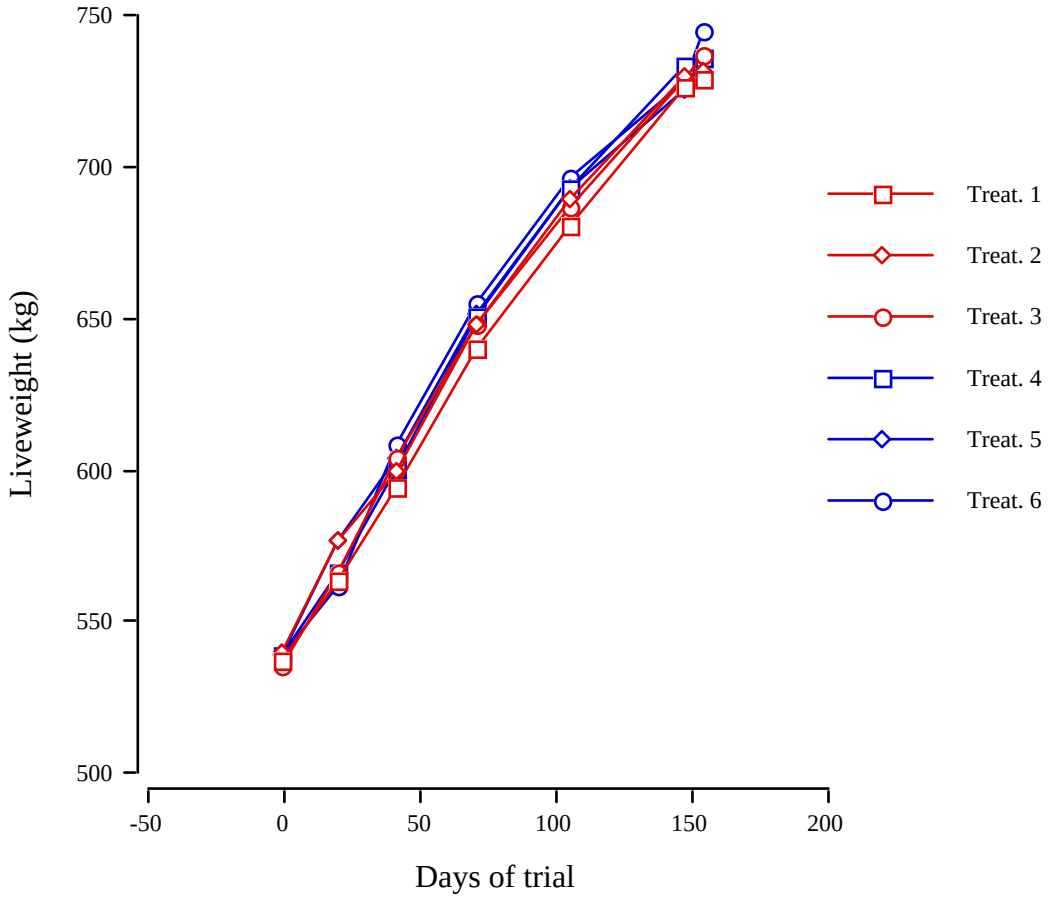
Appendix

Figure 1. Change in liveweight during the feeding of the trial diets



- Treatment 1: Control urea: barley + urea @ 150 days
- Treatment 2: Low Protein 1: Treatment 1 50 days; barley (no urea) for 100 days
- Treatment 3: Low Protein 2: Treatment 1 100 days; barley (no urea) for 50 days
- Treatment 4: Control canola meal: barley + 10% canola meal @ 150 days
- Treatment 5: 'Bypass' Protein 1: Control canola meal but replace canola meal with 5% formaldehyde treated canola meal (balance rumen degradable protein with urea)
- Treatment 6: 'Bypass' Protein 2: Control canola meal but replace canola meal with 10% formaldehyde treated canola meal (balance rumen degradable protein with urea)

Figure 2. Change in scan P8 fat depth with time

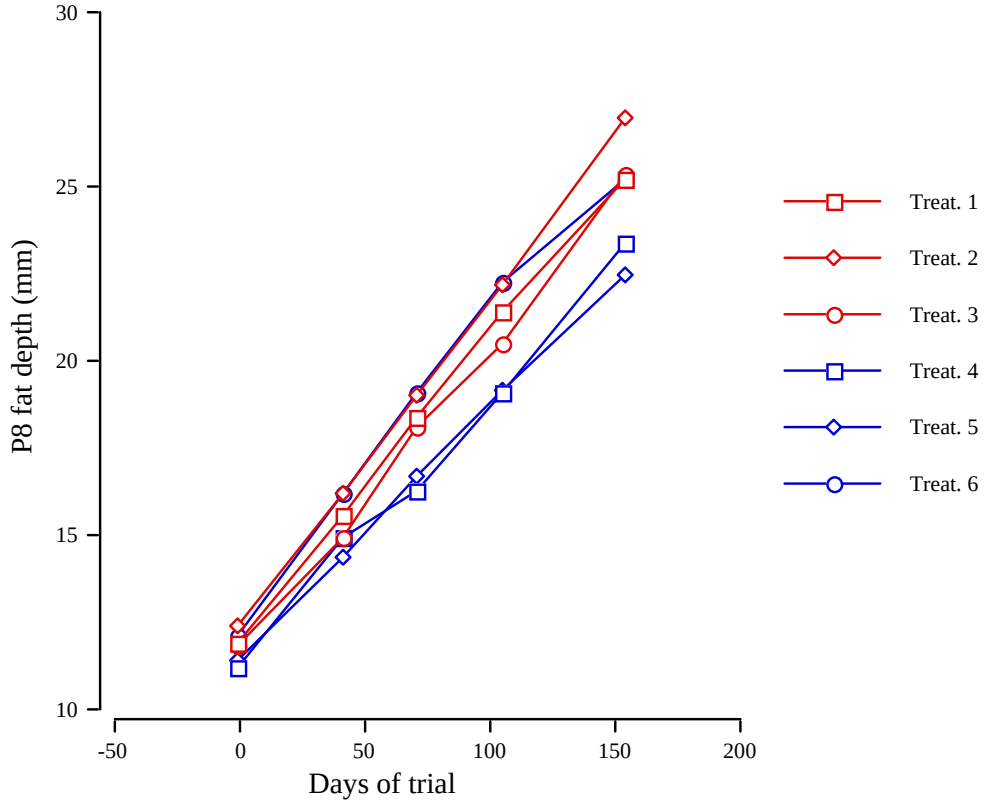


Figure 3. Change in scan rib fat depth with time