Genetic and non-genetic opportunities for manipulation of marbling

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Abstract. This Symposium provides the opportunity to review recent progress in understanding the biology of intramuscular fat deposition (marbling) in the bovine. Measurement of marbling is still a significant industry issue: subjective assessment based on the degree of visual fat deposition and its distribution is the 'gold standard' for marbling grades. Yet this measurement is subject to operator error and is influenced by chiller temperature. Chemical extraction gives an unequivocal measure of all fat in the muscle (IMF%), but does this mirror exactly what the trade regards as "marbling"? Near Infrared Spectrometry (NIR) may provide an indirect, on line method for estimating IMF%. This needs immediate further research.

Progeny test results from the CRC breeding projects provide improved understanding of breed and genetic effects on IMF% and marble score. EBVs for IMF% have been released to industry for 7 breeds. Heritability estimates confirm that genetic progress will be faster when selection is based on IMF% than marble score. Genetic correlations of IMF% with growth, retail beef yield (RBY%), P8 fat, Residual Feed Intake (RFI) and tenderness are now available to underpin selection indices. A favourable allele for marbling (TG5) on chromosome 14 has been identified by CSIRO/MLA as a direct gene marker for the trait. This is now being marketed as GeneStar Marbling. Other favourable chromosomal regions are under investigation by the CRC.

Nutritional manipulation of marbling remains problematic. It is accepted that high energy grain diets achieve higher marbling than pasture diets. Within grain-based feedlot diets higher marbling is achieved with maize than barley and barley diets in turn are better than sorghum. Steam flaking produces higher marbling than dry rolled grain and this effect is more marked with sorghum than maize. Beyond these principles there are many uncertainties: experiments have examined the effects of diets with high protein; low protein; protected lipid; protected protein; added oil with and without calcium; vitamin A deficiency. None of these manipulations gave consistent improvement in marble score or IMF%. Commercial feedlots supplying Japanese B3/B4 markets may have successful dietary manipulations to enhance marbling but because of its proprietary nature the information is not normally available for scientific scrutiny. It is clear that we do not yet have the ability to induce marbling, in cattle that do not have the genetic propensity for the trait, by smart dietary manipulation.

Japan is the only market for Australian beef where marbling is a significant component of the market specification. There can be no doubt that marbling meets a special consumer preference in that niche market. In other markets scientific evidence for a link between marbling and beef tenderness or eating quality has been difficult to define. (Marbling is a key component of the USA grading scheme for primals but Australia is not a big supplier to that market.) In the domestic MSA market there is a trend for marbling to become more important as a consumer issue in five-star product where higher order sensory attributes of beef come into play. Early meat science investigations concluded that beef flavour elements were water soluble. This would exclude marbling fat as a significant influence on flavour.

Marbling remains the major determinant of carcass value in Australia's most valuable beef market. Research should continue to assist Australian producers to meet the specifications of that market with increased precision and reduced costs.

Introduction

The CRC for Cattle and Beef Quality has been committed to research into marbling in beef cattle since 1992. In fact the original idea to establish a CRC was based in part on the emergence of the Japanese beef market and growth of the Australian feedlot sector to supply the premium B3/B4 grades of marbled beef (Bindon 2001). The marbling phenomenon always creates debate in the Australian beef industry. The debate usually centres around the uncertainty of the link between marbling and beef eating quality, the high cost of achieving high marbling, the merits of grain feeding and the recurring question of which breeds of beef cattle have genetic propensity for marbling. These are all legitimate issues but they seem to overlook the reality that market forces are the main driver of marbling in an Australian beef industry context: if our most valuable export market (Japan) prefers beef with specified levels of marbling, and is prepared to pay premiums for this trait, then why would we not attempt to produce such product?

The biology of marbling presents a substantial scientific challenge. As shown in Figure 1 a successful marbling outcome depends on making progress across an array of disciplines, where genetics, nutrition, biochemistry and meat science all converge. It is clear that an integrated approach is needed; neither genetics alone or nutritional manipulation alone can achieve consistently high marbling specifications.

Marbling is a curious phenomenon. Why would certain cattle breeds, at a particular stage of development, begin to deposit fat in cells distributed through some muscles? What purpose do these fat depots serve? The world's most recognised marbling breed, Japanese Wagyu was developed over centuries as a

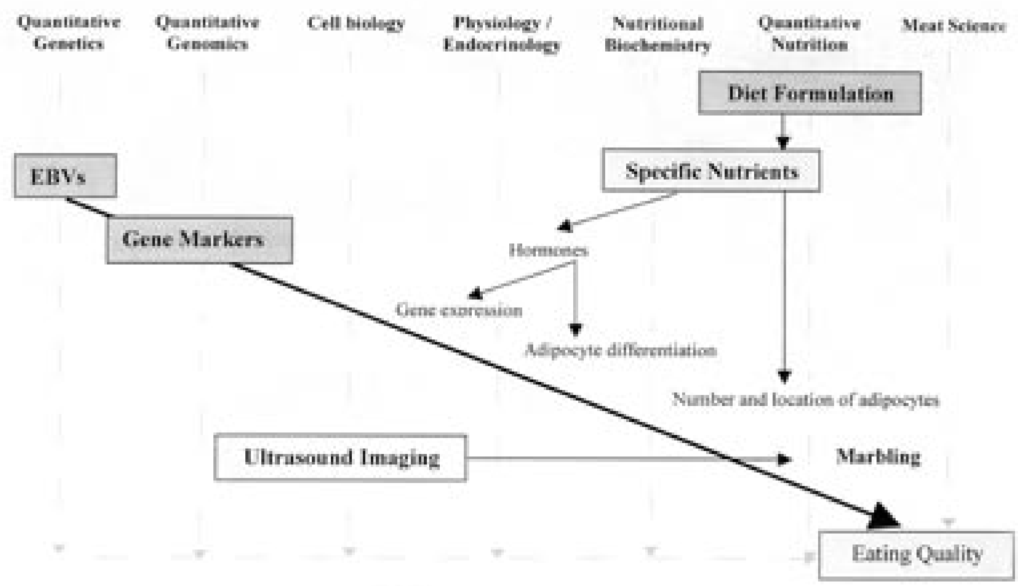

Figure 1. CRC approach to the biology and practical delivery of marbling outcome

draught animal. In Japan consumption of beef from these animals was prohibited by law during AD645 to 1868 so there cannot have been much conscious selection for marbling as an eating quality attribute (Kerr *et al.* 1994). Is marbling fat an energy reserve for muscle tissue in a working animal? Is marbling fat depleted, in preference to subcutaneous or seam fat in a draught cattle beast? If marbling is a feature of draught cattle, why is it not apparent in large European breeds which were also evidently selected initially for draught purposes?

The paper that follows pulls together a variety of CRC studies that contribute to our understanding of marbling. Each area will be covered elsewhere in this Symposium by a scientist specialising in one or other disciplines.

Measurement of marbling

In the AusMeat trading language marbling is subjectively scored in the *M. longissimus* muscle at the 10/11th rib site in the chiller. Marble scores are recorded in unit increments of 1.0 against a set of standard "chips". Since the advent of the Meat Standards Australia (MSA) grading scheme MSA graders use a more detailed subjective marble score system with more discrete increments of 0.1 (i.e. 1.1, 1.2,...1.9 etc.). In the CRC breeding projects marbling was recorded as AusMeat Marble Score in addition to measuring chemically (chloroform) extracted fat from a sample of *L. dorsi* muscle tissue (IMF%) (see Table 1). More detail of the genetic and phenotypic links between marble score and IMF are covered by Johnston (2001). In most studies 30-40% of the observed variation in IMF% was accounted for by AusMeat marble score (Harper *et al.* 2001). In the USA grading scheme for comparison subjective marbling scores range from "Practically devoid" to "Very abundant" as shown in Table 2, matched up against IMF%.

In a sample of 4000 animals from the CRC progeny test

Table 1. Relation between subjective marble score and chemically extracted intramuscular fat (IMF%) in the *L. dorsi* at 12/13 rib site

AusMeat Marble Score	IMF%
0	2.70
1	4.35
2	5.37
3	7.11
4	8.96
5	10.32
6	10.50

Table 2. Marble score matched against chemically extracted intramuscular fat (IMF%) of the *L. dorsi* in the USA Grading

Score	% muscle area covered by fat	IMF%
Practically devoid	0	1.8
Trace	0.5	2.5
Slight	1.5	3.5
Small	2.5	5.2
Modest	7.5	5.8
Moderate	11.0	7.2
Slightly abundant	15.0	8.8
Moderately abundant	18.0	10.5
Abundant	21.0	-
Very abundant	25.0	-

Johnston *et al.* (1999) compared the heritability estimates for marbling based on IMF%, MSA Graders' marble score and AusMeat marble score. The results in Figure 2 show a heritability of more than 40% for IMF% but only 15% based on AusMeat marble score. Heritabilities of MSA scores were closer to the IMF% value but still significantly lower. This means there is significant environmental "noise" in the AusMeat marble score estimate and does not give an accurate picture of the genetic variation for the underlying trait.

Is this a serious industry issue? AusMeat marble score is the 'Gold Standard' for the trade and producers are paid on the basis of this measure. Yet the score could not be used for genetic improvement of the trait (Figure 2 says that only a small proportion of variation observed for AusMeat marble score is due to genes). Since marble score is based on visual assessment of fat in muscle, it depends on temperature in the chiller, to the extent that fat melting point will be temperature dependent. Another argument is that some breeds of cattle are perceived to have different fatty acid composition and this influences the amount of visible marbling. An on line method to measure IMF%, for example a probe capable of positioning a "window" in muscle to enable Near Infrared Spectrometry (NIR) to calculate IMF%, may be a useful alternative. (This technology is used to measure fat content of cakes and biscuits on line.) But does this accurately reflect the visual fat distribution preferred by Japanese consumers? A compromise may be necessary to resolve this dilemma.

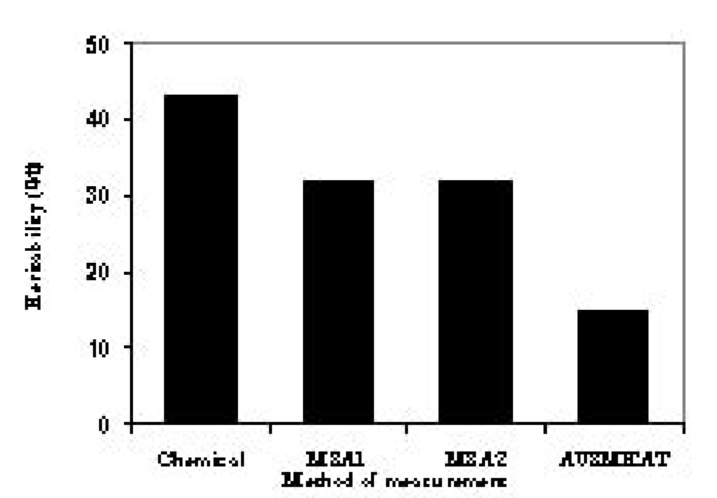

Figure 2. Estimates of heritability of marbling in the same cattle when measured as IMF% or as marble score using AusMeat or Meat Standards Australia (MSA) graders (n=4,000).

Real time Ultrasound (RTUS) is a valuable method for live-animal measurement of marbling. The technology loses accuracy in prediction of IMF when IMF% exceeds 8% (Wilson 1995). Despite this the method provides useful measures of genetic variation in breeding programs (Graser *et al.* 1998) and monitoring marbling in feedlot cattle where up to 36% of variation in marbling can be accounted for by RTUS (Oddy *et al.* 2000).

Breed differences and genetic effects on marbling

Industry progeny tests

In the early 1990s the Meat Research Corporation commissioned a progeny test of 3250 feedlot steers grain fed in southern Australia for 200 days. These were the progeny of 237 sires and drawn from Shorthorn, Angus, Murray Grey, Poll Hereford, Hereford and Euro x British crosses. Note that studies of this type are confounded because the different breeds were sourced from many vendors and different herds of origin. Any differences observed are due to a combination of the cattle genetics and the environmental effects attributed to the herd of origin effects up to the time of feedlot entry. The marbling results are shown in Figure 3, taken from Baud, Goddard and Hygate (1994). Marble score was higher in Shorthorn, Angus and Murray Grey breeds than Hereford, Poll Herefords or Euro x British crosses.

Marbling was also influenced by sire within a breed, as shown in Figure 4. This was the first convincing evidence that genetic variation for marbling within a breed may be as large as variation between breeds.

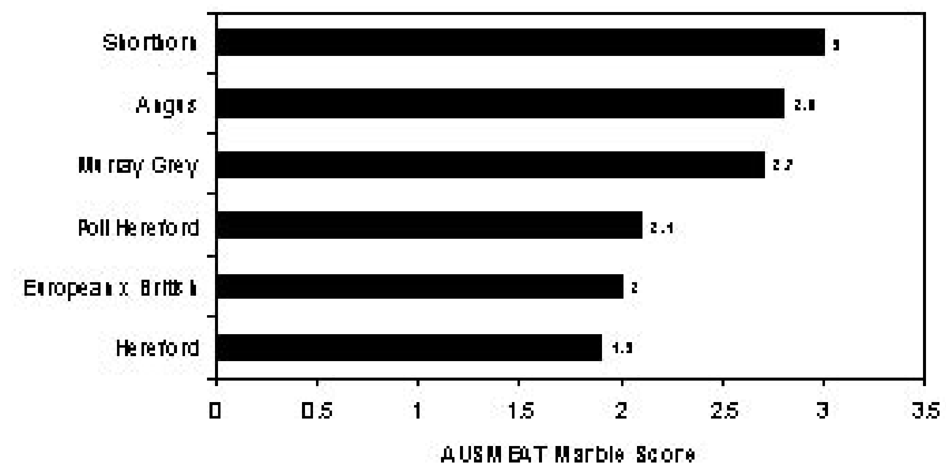

Figure 3. Breed effect on AUSMEAT Marble Score (Southern Australia) Source: Baud *et al.* 1994.

In northern Australia another 6340 steers grain fed for 150

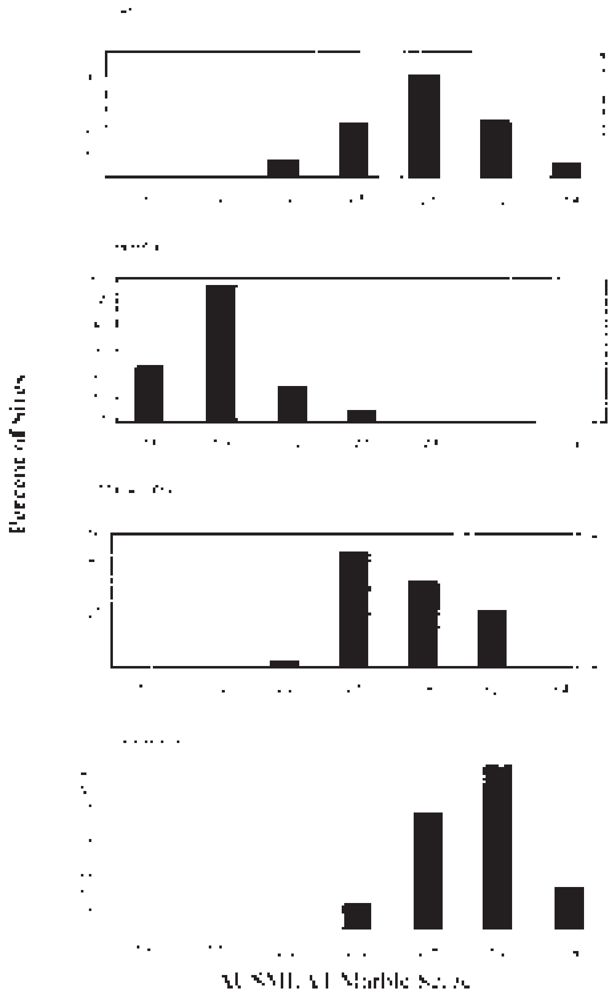

Figure 4. Across and within breed variation in marbling score between sires whose steers were lofted for 200 days (Southern Australia) Source: Baud *et al.* 1994.

days were sampled from various breeds and vendors without knowledge of sire pedigree. It is evident from Figure 5 that Shorthorn cattle, Devon and British crosses had significantly higher marble scores than a variety of crossbred genotypes. High grade Brahmans and Droughtmasters had lowest marble scores.

Progeny tests run by CRC for Cattle and Beef Quality

Since 1993 the CRC has carried out a comprehensive progeny test for beef quality traits and Net Feed Conversion efficiency. The breeds involved were Angus, Hereford, Shorthorn, Murray Grey, Brahman, Santa Gertrudis and Belmont Red. This involved around 8000 progeny. In Queensland the CRC ran another crossbred progeny test involving 1000 Brahman cows joined to 9 sire breeds resulting in another 2000 progeny. All progeny were assessed for growth, live animal measures, Net Feed efficiency, carcass and the meat quality traits including marbling, tenderness, meat colour and retail beef yield. Some 4300 carcasses were assessed for MSA Eating Quality (MQ4) using consumer taste panels. Half the cattle were finished in feedlots and half at pasture. Again the within-breed progeny test was not a strict genetic breed comparison because of confounding due to herd-of-origin differences. (All progeny were bred in seedstock herds and moved to a CRC common property at weaning.)

Most of the genetic results (heritabilities of marbling and genetic and phenotypic correlations with other traits) have been covered by another paper in this Symposium (Johnston 2001). There are clear differences in IMF% between grain- and grass-finished groups (Figures 6 and 7). An interesting feature of marble score and its relation to IMF% is shown in Table 3, which shows IMF% for different breeds and matched up with AusMeat marble score. There is the expected relationship of increased IMF% with increasing marble score. Yet for each marble score the tropically adapted breeds have lower IMF than the temperate (i.e. British) breeds. This says that the tropically adapted breeds are achieving higher marble scores than their IMF would suggest. Is the fat more visible in these breeds? Is this a fat melting point issue? Or, are the differences attributed to operator (grader) variation in northern and southern abattoirs?

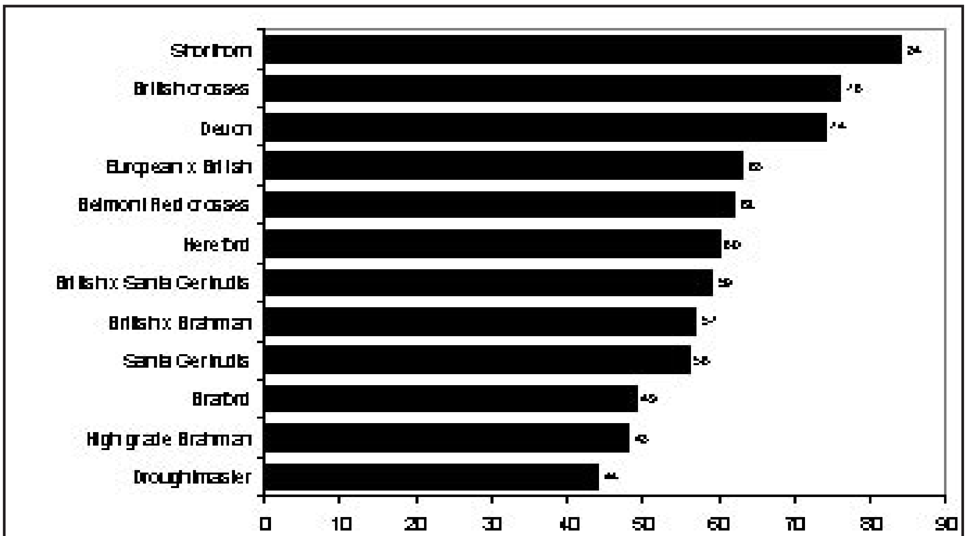

Figure 5. Percentage of northern feedlot cattle (150 days) achieving marble score 2 or higher (Baud, Goddard and Hygate 1994).

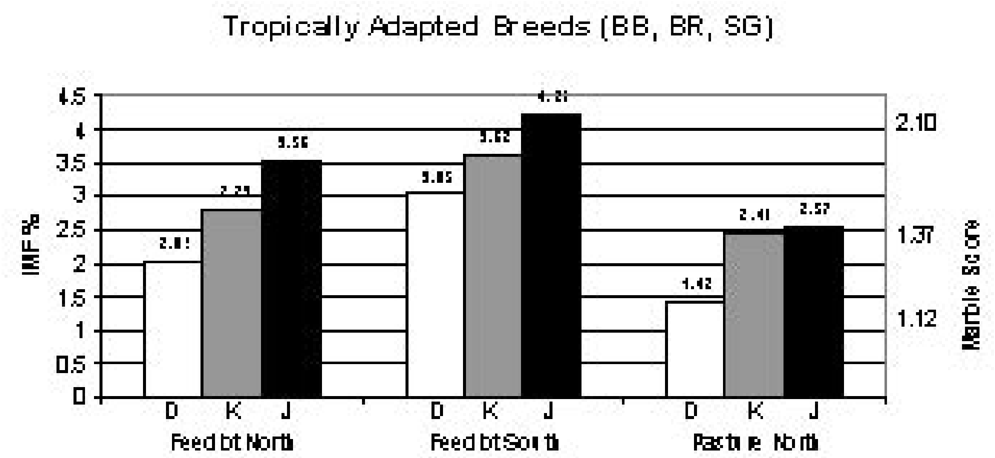

Figure 6. Tropically adapted breeds (BB, BR, SG)

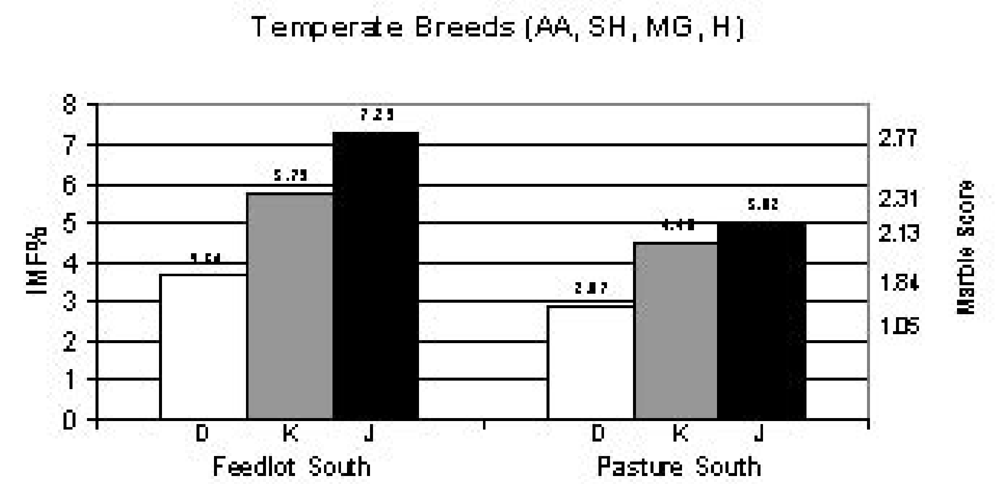

Figure 7. Temperate Breeds (AA, SH, MG, H)

Table 3. Least square means for Intramuscular fat (IMF%) according to breed and marble score. Data from CRC straightbreeding project.

Marble Score	Temperate Breeds				Tropically Adapted Breeds		
	1	2	3	4	1	2	3
1	3.95	3.20	4.30	4.09	2.13	2.58	2.26
2	4.51	3.70	5.14	4.58	2.69	3.36	2.91
3	5.64	4.55	5.76	5.08	3.95	4.06	3.80
4	6.88	6.24	7.88	6.11	-	5.18	4.71

The CRC's Northern Crossbreeding Project provides the only unconfounded Australian data on sire breed effects on marbling. These results are dealt with in this Symposium by Burrow (2001). The genes for marbling, perhaps as predicted from other research, reside predominantly in the Angus, Shorthorn and Belmont Red breeds.

Estimated breeding values (EBVs)

The CRC has now released EBV tables for carcass and meat quality information, including IMF%, for some 370 sires, across 7 breeds. The top 4 sires for IMF% EBV are presented in Table 4. (Note that comparisons across breeds are not valid.)

Table 4. Estimated breeding values (EBVs) for carcass traits, including IMF%, from CRC Sire Progeny Tests latest Group Breedplan (2001) (Note: EBVs cannot be contrasted across different breeds)

[illegible]

For completeness, in Table 4, the EBVs for the other carcass traits (carcass weight, eye muscle area; rib fat; P8 fat; Retail Beef Yield %) are listed for each of the 4 sires. There are some outstanding marbling sires in each breed. In the Angus, Hereford and Murray Grey breeds the top 4 sires were often above average for carcass weight EBV. This was not always the case in the tropically adapted breeds. Across all breeds the top marbling sires were generally below average for RBY%, as expected from the negative correlation between marbling and yield.

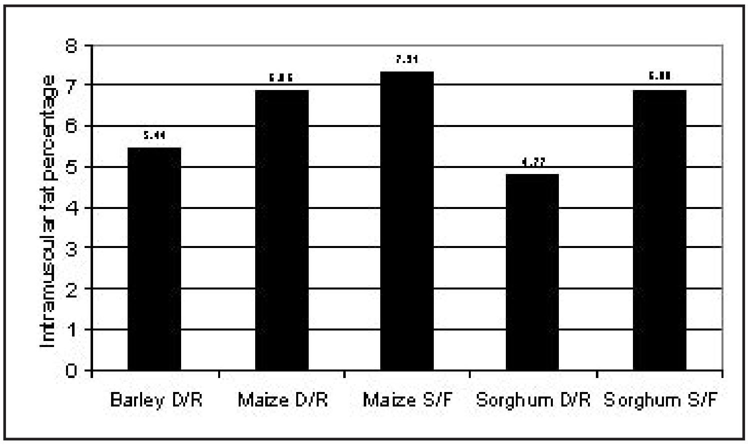

Figure 8. Effects of grain type and steam flaking on IMF% of Angus cattle fed 150 days. Redrawn from Pethick *et al.* (1997).

Nutritional manipulation of marbling

When the CRC began in 1992 we imagined that by 1999 it would be possible to induce increased marbling, by nutritional manipulation, even in cattle that do not have genetic propensity for the trait. This goal was not achieved. The task is far more complex than anticipated, as illustrated in Figure 1.

In Western Australia the effects of different grains and of steam flaking on marbling of Angus steers grain fed for 150 days were investigated. The results in Figure 8 taken from Pethick *et al.* (1997) illustrate two principles that relate to nutritional effects on marbling. The first is that maize is more effective than barley which is, in turn, more effective than sorghum in enhancing IMF%. The second is that steam flaking further enhances marbling when applied to maize and sorghum, but the effect is more pronounced in the case of sorghum. The interpretation of these results (Pethick *et al.* 1997) is that marbling fat is derived from glucose absorbed in the small intestine. Maize diets evidently result in enhanced glucose digestion in the small intestine and steam flaking results in increased digestibility in the rumen and the small intestine. In that study the differences in IMF% were not correlated with subcutaneous fat depth. The theory here is that this fat depot relies on acetate as a substrate whereas marbling depends on glucose as substrate, as explained above.

In other investigations carried out by the CRC marbling was studied in British breed cattle fed a barley based diet (122 days) modified by addition of canola oil (5.6%), with and without added calcium to protect the fat from microbial attack. Another strategy was to replace 19% of the grain in the diet with a "protected canola" product (Rumentek, Moree NSW). The results in Table 5 show that none of the treatments caused significant changes in marble score or IMF%, with most steers achieving marble score 2. The results with the Rumentek product fail to confirm previously reported (Oddy 1995) improvements in marbling in cattle fed this diet.

Table 5. Effects of canola oil modifications to a barley based feedlot diet on marble score and IMF (Source: Oddy, Bird and Walker 2001)

	Dietary Treatments				
	Control	Ca^{2+}	Canola oil[A]	Ca^{2+} + Canola oil	Rumentek[B]
Animals in groups	29	30	30	30	29
Marble Score					
1	1	3	2	3	6
2	23	20	22	23	17
3	4	6	5	2	3
4	1	1	1	2	3
IMF%	4.5	4.0	4.6	4.4	4.8

[A]5.6% of diet.

[B]Protected canola oil substituted for 19% of barley in diet.

In another strategy CRC scientists manipulated the protein content of a feedlot diet to test the theories that low- or high-protein diets can cause increased IMF deposition (Oddy *et al.* 2000). Angus and Shorthorn steers of known high genetic merit for marbling were fed these diets for various periods during a 184 day feeding schedule. The results in Table 6 show that none of these dietary manipulations caused significant alteration of IMF%.

Table 6. Effects of low and high protein diets on IMF in Angus and Shorthorn cattle fed 184 days (Oddy *et al.* 2000)

Dietary treatment	Crude protein g/kg	N	Carcase weight (kg)	P8 fat (mm)	IMF% post slaughter
1. Dry rolled barley feedlot control throughout 184 days	115	34	389	23.8	9.05±0.34
2. Low protein diet throughout 70-184 days	86	34	383	23.4	9.33±0.52
3. High protein diet throughout 70-184 days	157	34	387	23.3	8.78±0.35
4. Low protein diet during days 105-184	86	34	382	22.4	9.34±0.46
5. Low protein diet during days 140-184	86	34	391	23.1	9.56±0.34

In the light of the nutritional research described above the CRC and MLA decided to go back to the drawing board to review our knowledge of fat metabolism in cattle. Work in the CRC now concentrates on the developmental aspects of adipocytes in muscle as well as the genes that control this phenomenon. The hope is that these strategies may lead to an understanding of the nutritional "triggers" that allow marbling genes to be expressed.

These remarks should not be taken to mean that grain finishing does not have consistently beneficial effects on marbling: this is shown clearly in Figure 9, taken from many thousands of carcasses in the CRC breeding projects.

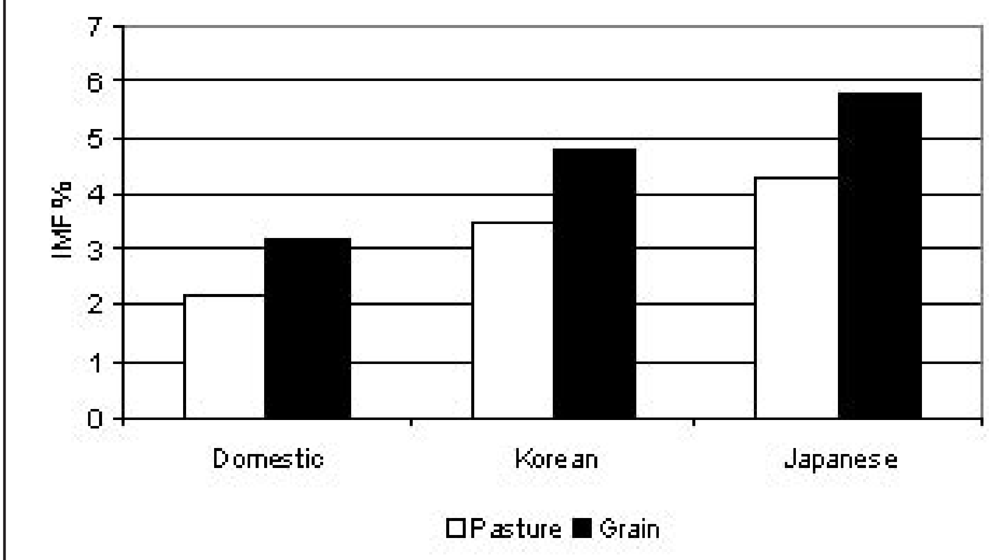

Figure 9. Intramuscular fat % (IMF%) in domestic, Korean and Japanese weight carcasses finished on grain or pasture in the CRC's straightbreeding project.

Marbling in the (beef) market place

The only markets for Australian beef where marbling is an unambiguous component of the market specification are the Japanese grainfed B3, B2, B1 and Japanese Grainfed Yearling grades. These are summarised in Table 7, compiled from the Meat Research Corporation Report (1995) "Input Requirements for Cattle Feedlot Industry. Vol. 1 Summary and Strategies". Table 7 shows marble score and IMF% specifications for each market, grain feeding periods, age at slaughter, fat cover and saleable yield %. Table 7 also attempts to describe the major cattle breed types that are used for each market. This picture may have changed somewhat since 1995 and a new census is needed to bring this up to date. Current industry practice seems to confirm that the top marbling grades (Japanese B3/B4) are achieved by long-feeding Shorthorn, Angus, Murray Grey and Wagyu breeds and some British breed crosses with these breeds. As the marbling specification declines in other Japanese grades (B2, B1 etc) the diversity of breed types suited to the grade increases. Detailed information about the level of success in achieving marbling specifications by exporters is difficult to obtain because of its commercial sensitivity.

The Japanese predilection for highly marbled beef seems to derive from the Japanese culinary tradition of cooking wafer-thin slices of beef in boiling water or oil. Evidently this requires a high degree of marbling which melts around the muscle tissue, sealing in natural juices and keeping the meat tender (Kerr *et al.* 1994). This type of consumer preference has no equivalent in "western" beef markets such as USA or Australia so it is unwise to attempt to translate Japanese market forces into an Australian context.

Does marbling affect eating quality of beef cooked "Australian style"? Our early meat scientists were adamant that it does not. Yeates, Edey and Hill (1975) reviewed 1940s research showing a zero correlation between degree of marbling and (shear force) tenderness. The authors also presented evidence that flavour elements in muscle were largely water soluble, ruling out IMF as a contributor to flavour. They also quoted USA evidence from the 1960s showing no link between marble score and beef juiciness.

CRC studies covered by John Thompson at this Symposium (Thompson 2001) show that in the comprehensive palatability tests in the MSA scheme marbling does have an influence on juiciness and flavour, especially in MSA 4 star and 5 star grades (Perry *et al.* 1999). Based on the size of the database contributing to this conclusion the Australian industry should now agree that marbling does affect beef eating quality.

Table 7. Markets for Australian beef where marbling is a significant specification (Source: compiled from Meat Research Corporation

[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
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Discussion

Marbling is a complex phenomenon requiring an integrated research program involving genetics, nutrition, biochemistry and meat science. The CRC for Cattle and Beef Quality is committed to such research, particularly in partnership with MLA and ALFA.

There has been spectacular recent progress in understanding the genetic control of marbling. We now know that the trait is moderately to highly heritable in both temperate and tropically adapted breeds. The genetic associations (correlations) between marbling and most other growth, carcass, meat quality and feed efficiency traits have now been delivered to industry. The positive genetic correlation between marbling and tenderness (and eating quality), and the negative association between marbling and Retail Beef Yield (RBY%) are probably the most significant from an industry perspective. If IMF and RBY are important in a particular breeding objective then this negative genetic correlation must be taken into account. In all breeds studied by the CRC some outstanding marbling sires have been identified and EBVs released to industry. These are recorded here. Some sires have high EBVs for both marbling and RBY% even though these traits are generally antagonistic, as discussed above.

Molecular genetic progress has also been a highlight of marbling research. The work of Dr Bill Barendse in the CSIRO/ MLA marbling project has resulted in a gene marker test (TG5, GeneStar Marbling) for marbling now commercialised in Australia. This test is being used in selection of seedstock for herds producing steers for the Japanese grainfed market. Current CRC/MLA/CSIRO studies are evaluating other potential gene markers for marbling.

Measurement of marbling needs further research to eliminate anomalies that arise between subjective marble score and IMF%. In the CRC data carcasses from tropically adapted breeds were scored higher than their IMF suggests. Is this an operator/abattoir effect or a genuine issue arising from the enhanced visibility of fat in tropically adapted cattle?

Because the CRC data represent the largest set of records where AusMeat Marble Score, MSA Marble Score and IMF% were all measured on the same carcasses, there is an opportunity to refine the equation relating marble score and IMF%. This work is under way at AGBU and should lead to more accurate IMF data in Breedplan, derived (e.g.) from MSA Marble Scores. There is a hope that a direct, on line measure of IMF% might be developed from NIR technology, but we need to ask if IMF exactly equates with visual marbling, currently the industry “gold standard” on which payment is based.

Nutritional manipulation of marbling has been more disappointing. Many dietary modifications have been evaluated since 1994 but it seems clear that we do not yet have a full understanding of the nutritional “trigger” that stimulates the growth of adipocytes (fat cells) in muscle. Research confirms increased marbling from maize-based diets than barley or sorghum-based diets. Steam flaking further improved marbling and this was more pronounced with sorghum than maize-based diets. The work of Pethick *et al.* (1997) favours the theory that glucose absorption from the small intestine is a key substrate for marbling fat deposition. CRC research failed to confirm the idea that canola oil or protected canola oil product added to feedlot diets will increase IMF deposition. Manipulation of dietary protein levels both well above and well below normal levels did not alter marbling. It is clear both genetics and nutrition are important in achieving high marble scores.

This paper summarised the markets for Australian beef where marbling is a key specification. There is some confusion as to whether Japanese consumer attitudes to marbling have relevance for the Australian domestic beef market preferences. CRC research in the MSA taste panel database now confirms that marbling is a contributor to overall beef eating quality, probably acting via effects on juiciness and flavour, rather than tenderness. Despite the uncertainty about (phenotypic) links between marbling and eating quality the paper by Johnstone (2001) at this Symposium now confirms that marbling is positively genetically correlated with both tenderness and MQ4 (eating quality) score. This could be a valuable new influence in breeding programs directed at improved tenderness of Australian beef.

The conclusion of this author is that marbling will remain an important issue in relation to beef breeding research, beef exports and beef consumption by Australian consumers.

Acknowledgements

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