Marbling biology - what do we know about getting fat into muscle?

DW Pethick[1], GS Harper[2], JF Hocquette[3], YH Wang[4]

Cooperative Research Centre for Beef Genetic Technologies
[1]Murdoch University, Perth, WA, 6150
[2]Meat and Livestock Australia, 23 Kyabra Street, Newstead, Qld, 4006
[3]INRA, Herbivore Research Unit, 63122 Theix, France
[4]CSIRO Livestock Industries, Qld Biosciences Precinct, 306 Carmody Road, St Lucia, Qld, 4067

Key findings of research

- Intramuscular fat content (% fat) or marbling score is clearly late maturing.
- However fat development within muscle is not late maturing and the expression of marbling is due to maintained fat synthesis in combination with declining muscle growth as animals get older.
- A growth curve for the development of marbling is discussed - key concepts include (i) a period up to about 200kg where intramuscular fat does not increase (ii) a period of linear development as carcass weight increases from 200-450kg and (iii) the suggestion that intramuscular fat content reaches a maximum at mature body size (around 500kg carcass weight depending of course on genotype).
- Expression of intramuscular fat after extended grain finishing is driven by 3 primary genetic loci (i) overall fatness (ii) the degree of muscle development which also interacts with mature body size and (iii) the extent of fat distribution bias toward the intramuscular site. The interaction of these 3 factors requires further modeling to understand the how these genetic factors (including new gene markers) effect final marble score.
- The level of intramuscular fat at the start of finishing is a key determinant of the final level of intramuscular fat after finishing. This raises the feasibility of using ultrasound estimates of intramuscular fat content at the start of finishing as a means of selecting superior marbling animals.
- Recent results from Beef CRC research in collaboration with International partners using modern biochemical and genomic tools suggest that (i) intramuscular fat cells are different to other fat depots (i.e. subcutaneous) and (ii) intramuscular fat cell development is determined relatively early in life (3-8 months of age) which is consistent with the conclusions of our growth and development studies. The possibility for control of intramuscular fat development separately from other depots will be explored in the new CRC.
- The major nutritional and/or management tool for increasing the development of marbling is to maximise the availability of net energy (and glucose) for fat synthesis during finishing.
- Net energy available for fattening is the most likely reason why grain feeding (compared to grass) results in a higher marbling score at equal carcass weights.
- In heavier 'British' type cattle (LW≥540kg, P8=12mm) it is difficult to increase the net energy for fattening by reducing protein supply (that is these cattle have a low protein requirement) and this is clearly an avenue for reducing feed costs.
- Increased processing of the ration (i.e. steam flaking versus dry rolling) will increase the net energy intake and glucose supply and increase marbling. The mechanism is to maximise starch digestion in the rumen (of course within animal health limits) and also the small intestine and so reduce starch loss in the large intestine and faeces.

Introduction

In this review we first discuss the role of intramuscular fat as a factor affecting consumer preferences for meat. The development of intramuscular fat in beef cattle is highlighted and where informative, examples from other species are used to highlight the role of total carcase fatness and muscularity. Our current level of knowledge relating to metabolic and genomic aspects of fat metabolism in intramuscular adipocytes is discussed particularly with reference to glucose metabolism and regulatory genes. Finally the influence of nutrition on the expression of intramuscular fat is discussed. The reader is guided to additional recent reviews of marbling and fat metabolism in cattle (Pethick et al. 2005a; Pethick at al. 2005b; Pethick et al. 2005c; Pethick et al. 2004; Harper and Pethick 2004).

The role of Intramuscular fat in meat palatability

Although marbling assessment is generally an integral part of any beef grading scheme the literature suggests that it has only a minor association with palatability. Dikeman (1987) concluded that marbling accounted for only 10 to 15% of the variance in palatability. The Meat Standards Australia (MSA) research would agree and showed that the contribution of marbling to palatability was significant, but importantly just one of several factors determining final palatability. However, Thompson (2004) concluded that as variations in tenderness are controlled by schemes such as MSA, marbling will become a more important determinant of palatability due to its specific contribution to juiciness and flavour of grilled steaks for Australian consumers.

There is also a concern that very low levels of intramuscular fat will lead to meat that is perceived as dry and less tasty. Such a situation has been found in young highly muscled lean cattle (double-muscled cattle genotypes, young bulls from Belgian Blue or Blonde d'Aquitaine for instance) and in many cuts from modern pig genotypes (Channon et al. 2001). The minimum requirement for ether extractable fat in order to achieve acceptable consumer satisfaction for grilling 'red meat' cuts (beef and lamb) is quoted at 3-4% Savell & Cross (1986) on a fresh uncooked basis.

Development of Intramuscular fat

Growth and development

Adipose tissue is deposited in specific depots which are similar for all mammals. The primary depots are within the abdominal cavity (perirenal, mesenteric and omental), intermuscular, subcutaneous and intramuscular. However the proportions differ between the species and are influenced by age. Thus the pig has more subcutaneous fat (70% of total body fat) and less abdominal fat than beef cattle (Wood, 1984). Thompson et al. (1987) measured chemical fat content at all sites in mature Merino sheep and found intramuscular fat was 7% of total body fat. This compared with subcutaneous at 24%, intermuscular 20%, kidney fat 11%, omental fat 16% and mesenteric at 6%. Therefore the marbling depot in ruminants is of moderate to small size.

A common conclusion from animal developmental studies is that intramuscular fat is late developing (Vernon 1981). Indeed the usually quoted developmental order is abdominal, then intermuscular, then subcutaneous, then finally intramuscular. However, because fat is deposited at a greater rate than lean tissues later in life, the concentration of fat in muscle will inevitably increase later in an animal's life. Therefore the commercial trait, marbling, visible intramuscular fat or actual percentage intramuscular fat is late maturing. This does not mean that the rate of fat accretion in intramuscular adipocytes is also late maturing. The study of Johnson et al. (1972), showed that the proportional distribution of fat between carcass pools is found to be constant over a wide range of carcass fat contents (in the range from 5 to over 150 kg total fat) indicating that the major fat depots grow in the same proportion as animals fatten. More recent data from the Beef CRC are consistent with this observation (Pugh et al. 2005).

The development of intramuscular fat in beef cattle is shown in Figure 1. The data suggests a period of minimal change of intramuscular fat content at young ages followed by a linear increase between a carcass weight of about 200 – 400 kg at least for American Angus x Hereford (Duckett et al., 1993), Australian Angus (Pugh et al., 2005) or Japanese Black x Holstein (Aoki et al., 2001) type cattle undergoing prolonged grain feeding. Based on this data we hypothesise three drivers of intramuscular fat development (i) the potential for total carcase fat deposition (ii) the potential for muscle growth and finally (iii) the extent of fat distribution bias for intramuscular fat versus other carcase depots. Mature body size would also be a factor given that it will change the muscle:fat ratio at a given age in growing cattle. Importantly these principles would suggest that genetic selection (via traditional quantitative genetics or via gene marker assisted selection) for increased intramuscular fat alone could come about via alterations in any of the drivers described above and that a more detailed experimental approach is needed to account for this when designing genetic and/or gene marker discovery programs.

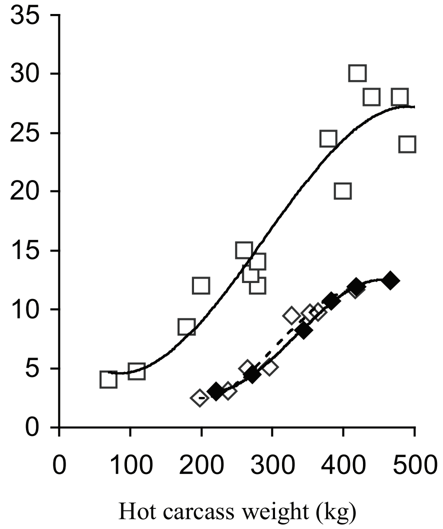

Figure 1. The relationship between carcass weight and intramuscular fat content of the m. longissimus lumborum of American Angus x Hereford (outline diamond = Duckett et al. 1993), Australian Angus (black filled diamond = Pugh et al. 2005) and Japanese Black x Holstein cross cattle (square = Aoki et al. 2001).

Selection for high levels of muscularity is known to reduce both total carcase fatness and intramuscular fat at a given carcase weight. Recent work in sheep has shown that lambs produced from sires with a high estimated breeding value for post weaning eye muscle depth (using the Sheep Genetics Australia system) produce substantially leaner carcases (Heggarty et al. 2006) with reduced intramuscular fat (Hopkins et al. 2005) when compared to lambs from sires with an elevated estimated breeding value for post weaning growth rate. Mutations in the GDF8 gene of beef cattle, which effects the development of the double muscle phenotype, affect muscle development to generally cause increased muscle mass (McPherron et al., 1997). With respect to development of intramuscular fat, Wegner et al. (1998) demonstrated that the GDF8 mutant double muscled animals have a) fewer islands of fat cell development in their LT (longissimus thoracis) muscle, b) a lower rate of growth of these islands and c) smaller adipocytes in marbling islands than conventional (i.e. non GDF8 mutant) cattle. It would seem that the suppression of fat development in animals selected for muscle growth is more than just a shift in allometric development of fat and lean. Certainly cattle breeds with a higher propensity to deposit fat have higher expression of key lipogenic enzyme either within adipose tissue (expressed per mg protein) or muscle (Bonnet et al., 2003) suggesting some level of metabolic down regulation in animals selected for muscle potential.

Therefore we propose that the developmental curve for intramuscular fat will be shifted to the right in animals with a high propensity to grow muscle or with a greater mature body size (and in these cases with the same propensity to marble at maturity, Figure 2). This 'right shift' would also occur in response to metabolic modifiers such as hormonal growth promotants, ß agonists and organic chromium supplementation, all of which can increase muscle growth. In very highly muscled animals it might be that intramuscular fat does not reach the 'linear accumulation' phase discussed in Figure 1 within normal commercial slaughter weights (that is the right shift described in Figure 2 is profound). This would appear to be the case for the modern pig genotypes where intramuscular fat % does not increase over a wide range of commercial carcass weights (Dunshea & D'Souza, 2003).

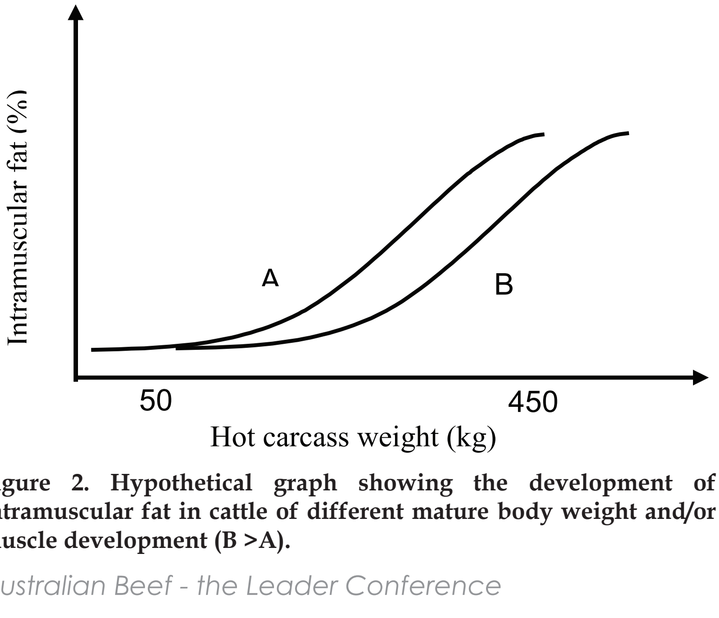

Figure 2. Hypothetical graph showing the development of intramuscular fat in cattle of different mature body weight and/or muscle development (B >A).

The initial or 'starting' intramuscular fat content at ≤200kg carcass weight is likely driven by the genetic predisposition for development of adipocytes at the intramuscular site relative to other depots (Figure 3). Importantly there is a proportional developmental difference that is maintained when the American or Australian cattle are compared to the Japanese Black cross cattle such that the starting (2 vs 4%) and final (13 vs 27%) intramuscular fat contents are proportionally different at about 2 fold. Cellular studies in rabbits (Gondret et al., 1998) have shown that intramuscular fat develops due to both an increased number and size of clustered adipocytes with associated increases in lipogenic enzyme activity (Gondret et al., 1997). The cattle data would suggest that the potential for cellular development of adipocytes is fixed relatively early in life and there after changes in either size and or number of cells occurs in proportion to the initial cell number and/or lipogenic proteins. This would clearly indicate that a variety of 'fat' measurements taken on muscle tissue in early life would hold great potential for predicting subsequent intramuscular fat development. Examples would include intramuscular fat content (perhaps by non invasive methods such as ultrasound), markers of adipocytes such fatty acid carrier proteins and/or functional lipogenic enzymes involved in fatty acid biosynthesis.

Metabolic Understanding

Another feature associated with the development of intramuscular fat is the fibre type or metabolic pattern of energy metabolism expressed by the muscle tissue. Within the one animal genotype the more glycolytic muscle types (e.g. m. semitendinosus) have lower levels of intramuscular fat (rabbits: Gondret et al., 1998; cattle: Hocquette et al., 2003; sheep: Garner et al. 2005). Across genotypes a similar response can be found. Thus in the study by Hocquette et al., (2003) where 2 muscle types were contrasted across 3 breeds of cattle with disparate propensity to accumulate intramuscular fat, there was a strong correlation between intramuscular fat and the aerobic markers cytochrome-c oxidase and isocitrate dehydrogenase as well as the adipose specific fatty

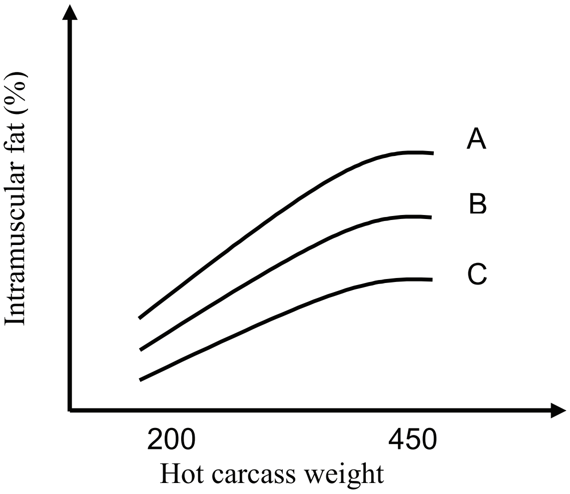

Figure 3. The effect of initial intramuscular fat (%) content (A >B >C) on final value in cattle of similar muscle growth potential.

acid binding protein. Of course studies across genotypes which positively correlate the extent of anaerobic muscle metabolism to the level of intramuscular fat accumulation are confounded by the observation that highly muscled cattle are more glycolytic (Pethick et al. 2005d) and also have less carcass fat. However, these studies as well as others in rabbits (Gondret et al., 2004) and sheep (Gardner et al. 2005) suggest that intramuscular fat content results from a balance between catabolic and anabolic pathways rather than from the regulation of a specific biochemical pathway. It has thus been speculated that a high fat turnover (which is a characteristics of oxidative muscles) would favour fat deposition (Hocquette et al., 2003).

There is evidence that marbling adipocytes show a preference for glucose/lactate carbon while subcutaneous adipose tissue uses mainly acetate as a source of acetyl units for lipogenesis. This was initially proposed by Smith and Crouse (1984) and even inferred by Whitehurst et al. (1981). More recently extensive collaboration between the Beef CRC and French scientists have confirmed that an extensive range of enzymes, proteins and genes associated with glucose metabolism and lipogenesis from glucose are more highly expressed in intramuscular fat that had been purified by dissection (Hocquette et al. 2005). Moreover the lipogenic pathway from glucose is highly responsive to diet in ruminants (Smith et al. 1992, Pethick and Dunshea 1996).

Further gene expression work has been undertaken by the Beef CRC this time in collaboration with Japanese scientists (Wang et al. 2005; Lenhert et al. 2006) and the results confirm that developmental changes occur quite early in life. Transcriptoms of whole m. longissimus tissue from Japanese Black cattle were compared to age matched Holstein animals. The results showed that at 11 months, the genes associated with adipogenesis, monounsaturated fatty acid synthesis and fatty acid accumulation were highly expressed in Japanese Black cattle (Wang et al. 2005). This simply indicated that, by 11 months, Japanese Black cattle have developed significantly more intramuscular fat than the Holstein cattle. In another breed comparison experiment, gene expression was studied in m. longissimus tissue of progeny from Australian Hereford dams joined to either Japanese Black or Piedmontese sires. The results indicated that the genes associated to adipogenesis/ lipogenesis were not differentially expressed between Japanese Black and Piedmontese sires at both fetal and the new born stage (Lehnert et al 2006). When the samples were compared from young animals (3, 8 and 12 months), the expression of various fat related genes (adipocyte fatty acid binding protein, adiponectin, C1Q and collagen containing domain and stearoyl-CoA desaturase) were significantly up regulated in the Japanese Black sires only at 8 month of age (not at 3 or 12 months, Wang unpublished data). This indicates that the intramuscular fat development, at least in the Japanese Black cross animals, is very active between 3 and 8 months of age (before the end of weaning). In the Japanese Black versus Holstein study, thyroid hormone responsive element spot 14 (THRSP) was significantly more highly expressed in Japanese Black cattle. This expression difference between 2 breeds was most pronounced at the youngest age of sampling (11 months) such that THRSP expression was 25 fold higher in Japanese Black animals.

The gene expression studies clearly underpin that fat development is occurring early in life (well before grain finishing) and confirm the conclusions from our growth and developmental work cited above. The challenge is now to understand the gene expression and metabolic switches that might influence adipogenesis and lipogenesis. The THRSP locus has shown some promise as a gene that might regulate fat developmental pathways. It has been extensively studied in liver and adipose tissue of the rat and mouse and while all its functions are not fully understood, it is associated with lipogenesis. Expression of THRSP is regulated by several nutritional and hormonal factors. Studies on THRSP have indicated the gene isolated from both the human and rodent has a similar structure and response to both thyroid hormone and glucose (Campbell et al. 2003). However, the THRSP response to thyroid hormone and glucose differ in the magnitude. The human THRSP promoter responds robustly to thyroid hormone whereas rat THRSP robustly response to glucose (Campbell et al. 2003). Given that we have established a clear bias toward glucose metabolism in intramuscular fat in the bovine more detailed nutritional and hormonal intervention work in young (pre-weaning) cattle would seemed justified. Moreover double muscle cattle, which show low levels of intramuscular fat development, are also characterized by lower levels of triiodothyronine, insulin and glucose plasma concentrations (Hocquette et al., 1999).

Nutritional modulation of intramuscular fat

Manipulating protein and energy

Nutritional manipulation of intramuscular fat levels in pork via altering the dietary protein: energy ratio has been investigated in a number of studies. Of course the basic premise is that by restricting muscle development through a subtle protein deficiency, total carcase fatness will be increased sufficiently to elevate intramuscular fat. The data in pigs shows very clearly that this approach can lead to increases in intramuscular fat (Essen-Gustavsson et al (1994), Cisneros et al. (1996), Eggert et al. (1998), D'Souza et al. (2003))

The results of manipulating the protein:energy ratio in beef cattle diets is less conclusive. The conclusions from 2 studies (Oddy et al. 2000; Pethick et al. 2000) were that diets which contain more or less protein than recommended amounts for feedlot animals do not lead to significant differences in marbling or intramuscular fat. However, there was a trend for high protein diets to produce less and low protein diets more marbling than control diets in both experiments. In the case of Oddy et al. (2000) the low protein diets in combination with added dietary fat (to decrease the protein:energy ratio) significantly decreased feed conversion ratio and cost of gain relative to control and high protein diets. However in the case of Pethick et al. (2000) the low protein diets did not change feed conversion ratio perhaps because in this case they did not include dietary fat. Certainly the data of Pethick et al. (2000) suggest that a simple diet based on barley and hay (with no additional protein source in the form of grain legumes or urea) fed to Angus steers at a starting live weight of 540 kg (P8 back fat = 12 mm) produced equal performance to more traditionally formulated rations containing additional protein sources at an extra cost.

The literature reporting the effects of supplemental fat on marbling scores in beef cattle are mixed and are well discussed by Andrae et al. (2001). These authors argue that marbling responses to dietary fat have been more consistent when supplemental fat is added to diets based on grains that contain less fat than corn (i.e. wheat, barley) and this is supported by their study where high oil maize (7% fat in DM) was fed in comparison to traditional maize (4.7% fat in DM) to finishing cattle (final HCW = approx. 330 kg). Simple measures of carcass fatness (fat thickness) and intramuscular fat (visual marbling score) where similar when cattle were fed isoenergetic diets. However when the high and low oil maize diets were formulated at the same inclusion level of maize the marbling score was higher for the high oil maize based ration. This is predictable since at equal inclusion in the ration along with equal dry matter intake, the high oil maize grain would supply more net energy for fat synthesis.

Vitamin A

Vitamin A deficiency is associated with an elevated intramuscular fat content (Harper and Pethick, 2004). Indeed recent gene marker studies in beef cattle (Barendse, 2004) have shown an association between intronic and exonic alleles within the retinoid related orphan receptor C (gamma) gene and marbling scores. It has been reported that a low intake of ß-carotene or vitamin A in young Wagyu steers increases marbling (Oka et al., 1998). This was confirmed by Adachi et al., (1999) who showed that vitamin A levels in cattle blood were negatively associated with marbling scores. More recent work by the Beef CRC (Kruk et al. 2005) showed a 35% increase in intramuscular fat of the LT muscle (but not m. semitendinosus) in Australian Angus cattle fed a diet deficient in Vitamin A for 300 days. This was also associated with increased seam fat thickness suggesting that total carcase fatness had been influenced. D'Souza et al. (2003) also reported that feeding pigs a grower and finisher diet deficient in vitamin A significantly improved the intramuscular fat levels in the m. longissimus thoracis muscle from 1.3 to 2%. It has been proposed that the effect of Vitamin A on intramuscular fat deposition is mediated by retinoic acid, a derivative of Vitamin A, which regulates the adipogenic differentiation of fibroblasts, inhibiting the terminal differentiation of intramuscular adipose tissue in cattle (Kuri-Harcuch, 1982). It has also been proposed that retinoic acid regulated growth hormone gene expression (Bedo et al. 1989), which in turn decreases fat deposition and intramuscular fat in steers (Dalke et al. 1992). Deficiencies in retinoic acid, therefore, may result in lower growth hormone concentrations and increased fat deposition including intramuscular fat. However in the case of the pig, D'Sousa et al. (2003) found no change in carcase composition, measured using dual X-ray absorptiometric analysis, suggesting that at least in the pig the effects of Vitamin A deficiency might be more localized to intramuscular fat. However further work to assess whole body fatness in the live animal would be needed to validate this suggestion.

Fermentation pattern in ruminants

Pethick et al. (1997, 2004) postulated that diets which promote both: (i) maximal fermentation in the rumen to produce gluconeogenic precursors (propionate), and (ii) which maximise starch digestion in the small intestine might increase intramuscular fat deposition. Such diets are usually associated with high levels of processing which increase the accessibility of the dietary starch granule to both microbial and animal amylases and so maximise the availability of glucose to the fattening animal (Rowe et al. 1999). The logic behind this hypothesis was that (i) such diets would promote increased levels of anabolic hormones (insulin) which are known to stimulate lipogenesis; (ii) the logic parallels the observation in humans that diets with a high glycaemic index (i.e. diets that allow rapid glucose absorption and concomitant high insulin levels) promote obesity (Ludwig 2000); (iii) Such diets will also deliver increased levels of net energy for lipogenesis (the reason why grain feeding promotes more intramuscular fat development compared with grass finishing, Pethick et al. 2004) and (iv) there is evidence that marbling adipocytes show a preference for glucose/lactate carbon while subcutaneous adipose tissue uses mainly acetate as a source of acetyl units for lipogenesis (see above). The potential for this strategy to specifically stimulate intramuscular adipocyte development is by no means clear, but as discussed above experiments targeted at early life interventions at this axis would seemed warranted in cattle where the marbling genetics is well defined.

Conclusions

Intramuscular fat is clearly an important but not exclusive determinant of the consumers response to beef. A major determinant of intramuscular fat content is the potential for muscle growth. Thus animals which display high muscle growth either have reduced expression or show no development of intramuscular during the so called fattening phase. In this scenario genetic approaches for increasing intramuscular fat will need to focus on changing fat distribution (toward intramuscular fat) if the potential for muscle growth is to be maintained. Our knowledge of fat development at the intramuscular site is now being underpinned by new metabolic and genomic characterization of pathways for adipogenesis and lipogenesis and the hope is that this might allow for some nutritional management of marbling. Nutritional manipulation of intramuscular fat independently from total carcase or whole body fat depots has proved difficult to achieve. However there is now a growing body of evidence that intramuscular adipocytes are metabolically different to at other depots (subcutaneous fat) and further research is needed to see if this offers the possibility for targeted stimulation of fat development within muscle.

References

Adachi, K., H. Kawano, K. Tsuno, Y. Nomura, N. Yamamoto, A. Arikawa, A. Tsuji, M. Adachi, T. Onimaru, and K. Ohwada. 1999. Relationship between serum biochemical values and marbling scores in Japanese Black Steers. Journal of Veterinary Medicine and Science 61(18): 961-964.

Andrae JG, Duckett SK, Hunt CW, Pritchard GT, Owens FN. 2001. Effects of feeding high-oil corn to beef steers on carcass characteristics and meat quality. Journal of Animal Science 79, 582–588

Aoki, Y., N. Nakanishi, T. Yamada, N. Harashima & T. Yamazaki. 2001. Subsequent performance and carcass characteristics of Japanese Black x Holstein crossbred steers reared on pasture from weaning at three months of age. Bulletin National Grasslands Research Institute 60: 40–55

Bejerholm, C., Barton-Gade, P.A. (1986) Effect of intramuscular fat level on eating quality of pig meat. In 'Proceedinmgs of the 32nd European Meeting of Meat Research Workers, Ghent, Belgium'. pp. 389-391.

Barendse, W. (2004) DNA markers for marbling. Patent WO 2004/070055

Bedo, G., Santisteban, P. and Aranada, A. (1989). Retinoic acid regulates growth hormone gene expression. Nature 339, 231-234.

Bonnet, M., Y. Faulconnier, J.F. Hocquette, C. Leroux, P. Boulesteix, Y. Chilliard & D.W. Pethick, 2003. Lipogenesis in subcutaneaous adipose tissue and in oxidative or glycolytic muscles from Angus, Black Japanese x Angus and Limousin steers. In: Progess in Research on Energy and Protein Metabolism, W.B.Souffrant, C.C. Metges (editors), 2003. EAAP Publication N° 109, Wageningen Pers., Wageningen, The Netherlands, 469-472

Campbell MC, Anderson GW, Mariash CN (2003) Human spot 14 glucose and thyroid hormone response: characterization and thyroid hormone response element identification. Endocrinology 144, 5242-8.

Channon, H.A., Reynolds, J., and Baud, S. (2001). Identifying pathways to ensure acceptable eating quality of pork. Final Report, Australian Pork Limited, Canberra.

Cisneros, F., Ellis, M., Barker, D.H., Easter, R.A. and McKeith, F.K. (1996). The influence of time of feeding of amino-acid deficient diets on the intramuscular fat content of pork. Animal Science 63, 517-522.

Dalke, B.S., Roeder, R.A., Kasser, T.R., Neenhuizen, J.J., Hunt, C.W., Hinman, D.D. and Schelling, G.T. (1992) Dose response effects of recombinant bovine somatotropin implants of feedlot performance in steers. Journal of Animal Science 70, 2130-2137.

Dikeman, M.E., 1987. Fat reduction in animals and the effects on palatability and consumer acceptance of meat products. Recent Meat Conference 40: 93-103

D'Sousa, D.N., Pethick, D.W., Dunshea, F.R., Pluske, J.P. and Mullan, B.P. (2003) Nutritional manipulation increases intramuscular fat levels in the Longissimus muscle of female finishers pigs. Australian Journal Agricultural Research 54, 745-749.

Duckett, S.K., D.G. Wagner, L.D. Yates, H.G. Dolezal & S.G. May SG, 1993. Effects of time on feed on beef nutrient composition. Journal of Animal Science 71: 2079–88

Dunshea, F.R. and D'Sousa, D.N. (2003) Fat deposition in the pig. In Manipulating pig production (Edited by Paterson J. A.), Vol. IX, p. 127-150. Australasian Pig Science Association, Werribee.

Eggert, J.M., Farrand, E.J., Mills, S.E., Schinkel, A.P., Forrest, J.C., Grant, A.L. and Watkins, B.A. (1998). Effects of feeding poultry fat and finishing with supplemental beef tallow on pork quality and carcase composition. http://www.ansc.purdue.edu/swine/swineday/sday98/psd06-98.htm.

Essen-Gustavsson B, Karlsson A, Lundstrom K, Enfalt AC (1994) Intramuscular fat and muscle fibre contents in halothane gene free pigs fed high or low protein diets and its relation to meat quality. Meat Science 38, 269–277.

Gardner, G. E., Pethick, D. W., Greenwood, P. L. and Hegarty, R. S. (2005). The effect of genotype and plane of nutrition on rate of pH decline post mortem and the expression of enzymatic markers of metabolism in lamb carcases. Australian Journal of Agricultural Research, submitted.

Gondret, F., J. Mourot & M. Bonneau, 1997. Developmental changes in lipogenic enzymes in muscle compared to liver and extramuscular adipose tissues in the rabbit (Oryctolagus cuniculus). Comparative Biochemistry and Physiology 117B: 259-265

Gondret, F., J. Mourot & M. Bonneau, 1998. Comparison of intramuscular adipose tissue cellularity in muscles differing in their lipid content and fibre type composition during rabbit growth. Livestock Production Science 54: 1-10

Gondret F., J.F. Hocquette & P. Herpin P., 2004. Age-related relationships between muscle fat content and metabolic traits in growing rabbits. Reproduction Nutrition and Development 44, 1-16.

Harper, G.S. and Pethick, D.W (2004) How might marbling begin ? Australian Journal of Experimental Agriculture 44, 653-662.

Hegarty, R.S., Hopkins, D.L., Farrell, T.C., Banks, R. and Harden, S. (2005). Effect s of available nutrition and the growth and muscling potential of sires on the development of crossbred lambs. 2: Composition and commercial yield. Australian Journal of Agricultural Research. In press.

Hocquette, J.F. & D. Bauchart, 1999. Intestinal absorption, blood transport and hepatic and muscle metabolism of fatty acids in preruminant and ruminant animals. Reproduction Nutrition and Development 39: 27-48

Hocquette, J.F., C. Jurie, Y. Ueda, P. Boulesteix, D. Bauchart & D.W. Pethick, 2003. The relationship between muscle metabolic pathways and marbling of beef. In: Progress in Research on Energy and Protein Metabolism, W.B.Souffrant, C.C. Metges (editors), 2003. EAAP Publication N° 109, Wageningen Pers., Wageningen, The Netherlands, 513-516.

Hocquette, J.F., Jurie, C., Bonnet, Pethick, D.W. (2005) Bovine intramuscular adipose tissue has a higher potential for fatty acid synthesis from glucose than subcutaneous adipose tissue. In: Proceedings of the 56th European Association of Animal Production p248 (www.eaap.org).

Hopkins, D.L., Hegarty, R.S. and Farrell, T.C. (2005) Relationship between sire EBV'S and the meat and eating quality of meat from their progeny grown on two planes of nutrition. Australian Journal of Experimental Agriculture 45, 525-533.

Johnson ER, Butterfield RM, Pryor WJ (1972) Studies of fat distribution in the bovine carcass. I. The partition of fatty tissues between depots. Australian Journal of Agricultural Research 23, 381.

Kruk, Z.A., Bottema, C.D.K., Davis, J., Siebert, B.D., Harper, G.S., Di, J. and Pitchford, W.S. (2005) Effects of vitamin A on growth performance and carcass quality in steers. Beef CRC, unpublished results

Kuri-Harcuch, W. (1982) Differentiation of 3T3-F442A cells into adipocytes is inhibited by retinoic acid. Differentiation 23, 164-169.

Lehnert, S.A., Wang, Y.H., Tan, S.H. and Reverter, A. (2006) Gene expression-based approaches to beef quality research. Australian Journal of Experimental Agriculture. In press

Ludwig DS (2000) Dietary glycemic index and body weight reduction. Proceedings of the Nutrition Society of Australia 34, 286–293.

McPherron, A.C., Lawler, A.M. and Lee, S.-J. (1997). Regulation of skeletal muscle mass in mice by a new TGF-beta superfamily member. Nature 387, 83-90.

Oka, A., Y. Maruo, T. Miki, T. Yamasaki, and T. Saito. 1998. Influence of vitamin A on the quality of beef from Tajima strain of Japanese Black cattle. Meat Science 48: 159-167.

Oddy VH, Smith C, Dobos R, Harper GS, Allingham PG (2000) Effect of dietary protein content on marbling and performance of beef cattle. Final report of project FLOT 210, Meat and Livestock Australia, Nth. Sydney.

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, p151-158 (University of New England).

Pethick DW, McIntyre BL, Tudor GE (2000). The role of dietary protein as a regulator of the expression of marbling in feedlot cattle (WA). Final report of project FLOT 209, Meat and Livestock Australia, Nth. Sydney.

Pethick, D.W., Harper, G.S. and Oddy, V.H. (2004) Growth, development and nutritional manipulation of marbling in cattle: a review. Australian Journal of Experimental Agriculture 44, 705-715.

Pethick, D.W., Harper, G.S. and Dunshea, F.R. (2005a) Fat Metabolism and Turnover. In 'Quantitative Aspects of Ruminant Digestion and Metabolism 2nd Edition' edited J. Dijkstra, J.M. Forbes and J. France. CABI Publishing, Oxford, UK. pp345-371.

Pethick, D.W., D'Souza, D.N., Dunshea, F.R. and Harper, G.S. (2005b). Fat metabolism and regional distribution in ruminants and pigs – influences of genetics and nutrition. Recent Advances in Animal Nutrition in Australia 2005, P.B. Cronje and N. Richards (editors), University of New England. Pp39-45.

Pethick, D.W., Fergusson, D.M., Gardner, G.E., Hocquette, J.F., Thompson, J.M. and Warner, R. (2005c) Muscle metabolism in relation to genotypic and environmental influences on consumer defined quality of red meat. In: Indicators of milk and beef quality, J.F. Hocquette & S. Gigli (editors), Wageningen Academic Publishers, Wageningen, The Netherlands. Pp 95-110.

Pugh, A.K., B.L. McIntyre, G. Tudor & D.W. Pethick, 2005. Understanding the effect of gender and age on the pattern of fat deposition in cattle. In: Indicators of milk and beef quality, J.F. Hocquette & S. Gigli (editors), Wageningen Academic Publishers, Wageningen, The Netherlands. Pp 405-408.

Rowe JB, Choct M, Pethick DW (1999) Processing cereal grain for animal feeding. Australian Journal of Agricultural Research 50, 721–736.

Savell, J.W. & H.R. Cross, 1986. The role of fat in the palatability of beef, pork and lamb. Meat Res. Update 1 (4): 1-10. Publ. Dept. of Animal Science, The Texas A&M University System, Texas, USA.

Smith, S.B. and Crouse, J.D. (1984) Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. Journal of Nutrition 114, 792-800.

Smith SB, Prior RL, Koong LJ, Mersmann HJ (1992) Nitrogen and lipid metabolism in heifers fed at increasing levels of intake. Journal of Nutrition 70, 152–160.

Thompson, J.M. and Butterfield, R.M. (1987) Food intake, growth and body composition in Australian Merino sheep selected for high and low weaning weight. Animal Production 45, 49-60.

Thompson, J.M. (2004) The effects of marbling on flavour and juiciness scores of cooked beef, after adjusting to a constant tenderness. Australian Journal of Experimental Agriculture 44, 645-652.

Vernon, R.G., 1981. Lipid metabolism in the adipose tissue of ruminants. In: Lipid metabolism in Ruminant Animals, W.W. Christie (editor), Pergamon Press, Oxford, 279–362.

Wang YH, Byrne KA, Reverter A, Harper GS, Taniguchi M, McWilliam SM, Mannen H, Oyama K, Lehnert SA (2005) Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. Mammalian Genome 16, 201-10.

Wegner, J., Albrecht, E. and Ender, K. (1998) Morphological aspects of subcutaneous and intramuscular adipocyte growth in cattle. Arch. Tierz. Dummerstorf. 41, 313-320.

Whitehurst GB, Beitz DC, Cianzio D, Topel DG (1981) Fatty acid synthesis from lactate in growing cattle. Journal of Nutrition 111, 1454–1461.

Wood, J.D. (1984) Fat deposition and the quality of fat tissue in meat animals. In: Wiseman J, ed. Fats in animal nutrition.pp407-35. London: Butterworths.