Functional foods from animal production

F.R. Dunshea[1], E. Ostrowska[1], J.M. Ferrari[1], J.W. Heard[2], P.T. Doyle[2] and G.P. Walker[2]

[1]National Centre of Excellence in Functional Foods, Department of Primary Industries, 600 Sneydes Rd., Werribee Vic 3030, Frank.Dunshea@dpi.vic.gov.au
[2]Department of Primary Industries, Cooma Road, Kyabram Vic 3620

Summary

Contrary to dogma, many compounds found in animal products appear to have health benefits. For example, conjugated linoleic acids (CLA) and *trans*–11 vaccenic acid (TVA), which are found in ruminant fats, particularly those from grazing animals, are protective against some diseases. In the Victorian Northern Irrigation Area, the concentration of CLA in milk fat ranged from 1.1–35.4 mg/g fat (average 9.1 mg/g), with a major peak in spring. However, there was a 3–fold variation in the concentration of CLA between sites. Milk *trans* isomers varied in a similar manner (5.7–164.2 mg/g of fat, average 60.2 mg/g fat). These peak CLA and TVA concentrations were greater than those found to confer protection against cancers in rodent models. Selenium is also protective against some cancers, and protein–bound selenium is more effective than inorganic selenium. Milk selenium concentrations can be increased through supplemental feeding of yeast–bound selenium and this may be a means of delivering selenium to humans. Dairy proteins may contain factors that reduce appetite and aid in weight control. Data obtained from obese minipigs suggests that high whey–protein diets may reduce feed intake and fat deposition and improve insulin resistance. In conclusion, there appear to be opportunities to make use of inherent components of animal products to develop foods that may confer health benefits on humans.

Keywords: functional foods, conjugated linoleic acid, *trans*–vaccenic acid, selenium, whey protein isolate

Introduction

For many years, the cholesterol and fatty acid profiles of meat, eggs and dairy products have been considered deleterious for human health. However, there is now mounting evidence that many of these messages were over–simplified and that some of the fatty acids found in animal products, particularly those of ruminant origin, may have health benefits. In particular, conjugated linoleic acids (CLA) and vaccenic acid, which are found in ruminant fats such as butter, have been found to be protective against some cancers in both animal and *in vitro* models, atherosclerosis and possibly other diseases. Simple nutritional or management manipulation can increase these fatty acids to levels that have been shown to be protective against some cancers in animal models. While CLA is not found in appreciable levels in pork, chicken meat or eggs, the levels of CLA can be greatly augmented by feeding CLA, obtained by heat–alkali treatment of vegetable oils high in linoleic acid, to livestock. Other successful manipulations of the fatty acid content of animal products include increasing the omega–3 fatty acid levels by feeding fish or flax oils to ruminants, pigs or chickens. This concept of health–through–nutrition or functional foods was introduced to this audience by Dave Farrell in 1991 with the omega–3 enriched egg (Farrell and Gibson 1991). Some of these designer foods, particularly eggs high in omega–3 fatty acids, have been successfully launched commercially and will not be discussed here.

Selenium is another compound with functional properties that is found in animal products. There is a growing body of evidence that suggests that selenium is protective against some cancers and that protein–bound organic selenium is more effective than inorganic selenium (Clark *et al*. 1996). Feeding supplemental selenium–yeast complexes has been shown to increase the organic selenium content of pork and eggs (Mahan and Kim 1996; Kim and Mahan 2001b), and branded products are now sold in Korea and possibly elsewhere. It is highly likely that on–farm innovation will result in the development of more animal products that can be differentiated from commodities based on their enhanced levels of health–active compounds. This is particularly true of eggs and dairy products because they are frequently harvested from the animal. In addition, there are inherent components of milk, meat and eggs that also offer health benefits, protein and lipid–bound (or soluble) molecules offering the greatest promise. In this paper, we focus on some of our recent research on farm manipulation of milk quality to produce milk or

dairy products that confer health benefits. We also discuss some of our recent research with a whey protein isolate that was derived from what was previously a waste stream.

Conjugated linoleic acid and trans–vaccenic acid

Ruminant fat contains conjugated linoleic acid (CLA), which has been shown to be anticarcinogenic *in vitro*, in animal–model *in vivo* studies and in human epidemiological studies. Conjugated linoleic acid is a mixture of positional and geometric isomers of linoleic acid with conjugated double bonds located at positions 7,9; 8,10; 9,11; 10,12 or 11,13 on the carbon chain. There is extensive literature that suggests that the *cis/trans*–9,11 has anti–cancer and other positive health properties (Whigham *et al*. 2000; Pariza *et al*. 2001; Parodi 2002; Bauman *et al*. 2004); the *trans/cis*–10,12 isomer is thought to cause a reduction in lipid deposition in growing animals (de Deckere *et al*. 1999). In milk fat, the predominant CLA isomer is the *cis/trans*–9,11 isomer, comprising approximately 85% of CLA isomers. Another important fatty acid with health benefits is *trans*–11 vaccenic acid (TVA) (Bauman *et al*. 2004), accounting for approximately 54% of the *trans* 18:1 fatty acids in milk fat. Dietary TVA is an important source of CLA because humans and other species have the capacity to produce CLA from TVA. There are several reviews on factors that affect milk CLA concentrations. It is of particular interest to the Australian dairy industry that both CLA and TVA concentrations appear to be higher in milk fat from cows grazing pasture (Walker *et al*. 2004).

Manipulation of milk fat

There is scope to vary milk fat composition of grazing dairy cows by implementing changes to the feeding and management of cows (Table 1). Changes in milk fat composition have usually been achieved in one of two ways. The first method is based on minimising the action of microbial isomerases and hydrogenases on unsaturated lipid in the rumen, i.e., the use of protected lipid supplements. Various protection techniques have been successful. They result in higher amounts of unmodified dietary fatty acids available for milk fat production. The advantages of these techniques are that they result in changes to milk fat composition that can be predicted from the composition of the supplement and have minimal effects on the capacity of the rumen to digest fibre. However, the processing required to produce the protected lipid supplement can increase the cost of the supplement to uncommercial levels. The second method relies on increasing the amount of 18 C fatty acid substrate available to Δ–9–desaturase in the mammary gland and other tissues. This method results in milk fat with significantly increased concentrations of mono–unsaturated fatty acids in milk fat (Fearon 2001); changes in concentrations of other fatty acids depend on feeding conditions.

An increase in intake of 18 C fatty acid is usually achieved by incorporating a vegetable oil, as the free oil or whole or processed seed, in the diet or by increasing the intake of highly digestible pasture by cows. The fatty acid composition of vegetable oils varies with species, variety and growing conditions; the fatty acid composition of temperate pasture species tends to be rich in cis–9–cis–12–cis–15 18:3 and cis–6–cis–9–cis–12 18:3 (linolenic acid) (Table 2) (Harwood and Geyer 1964; Harfoot 1981). Because of

Table 1 The range in milk fatty acid concentrations achieved under experimental conditions by researchers at the Hannah Research Institute. Adapted from Banks (1987).

Fatty acid	Fatty acid composition (g/kg)	Fatty acid composition (mmol/mol)
Saturated	350–750	400–800
Mono–unsaturated	200–550	170–500
Poly–unsaturated	<10–300	<10–250

Table 2 Typical fatty acid composition for canola, sunflower and tuna oils and temperate pasture (Harwood and Geyer 1964; [†]Harfoot 1981, [‡]B Kerrison, Nu–Mega Ingredients Pty. Ltd., Nathan, Qld., unpublished data). The predominant fatty acid in each is in bold.

	Fatty acid concentration (g/kg total fatty acids)							
	14:0	16:0	18:0	16:1	18:1	18:2	18:3	20:5/22:6 n–3
Canola Oil	1	43	17	3	**621**	233	82	0
Sunflower Oil	1	62	40	0	200	**695**	2	0
[†]Temperate Pasture	3	111	9	18	29	152	**678**	0
[‡]Tuna Oil	30	220	80	50	160	10	10	**440**

bio–hydrogenation, the fatty acids leaving the rumen will usually consist of 18:0 (stearic acid) and various isomers of 18:1, particularly trans–11 18:1 (trans–vaccenic acid) (Noble 1981). However, as biohydrogenation is usually incomplete, smaller and more variable amounts of cis–9–18:1 (oleic acid), various isomers of CLA, cis–9–cis–12 18:2 (linoleic acid) and linolenic acid also leave the rumen. Their concentrations are largely dependant on diet composition and level of intake of the cow (Kemp *et al*. 1975; Kemp and Lander 1984; Kemp *et al*. 1984a, b; Ekeren *et al*. 1992; Fotouhi and Jenkins 1992a, b; Beam *et al*. 2000). Putative pathways for the biohydrogenation of oleic, linoleic and linolenic acids are shown in Figure 1.

The action of Δ–9–desaturase in the mammary gland converts a high proportion of stearic and trans–vaccenic acids to oleic acid and cis–9–trans–11 CLA, respectively (Griinari *et al*. 2000) (Figure 2). Consequently, milk fat in cows supplemented with vegetable oils rich in oleic or linoleic acids tends to have higher concentrations of stearic, oleic, trans– vaccenic and linoleic acids, and cis–9–trans–11 CLA. Importantly, oleic acid does not contribute to the synthesis of CLA in the rumen or the mammary gland, and ruminal cis–9–trans–11 CLA is derived from linoleic, but probably not linolenic acid. However, both linoleic and linolenic acids contribute to CLA via the desaturation of trans–vaccenic acid in the mammary gland and other tissues. For this reason, there is a positive linear relationship between the concentrations of CLA and TVA in milk fat (Figure 3).

Effect of pasture consumption on CLA and TVA

Considerable research effort has gone into the identification and formulation of diets with the objective of maximising the concentration of unsaturated fatty acids in milk fat. More recently, this focus has shifted to include cis–9–trans–11 CLA. Pasture–based production systems produce milk fat with higher concentrations of CLA and unsaturated fatty acids than feeding systems based upon conserved forages and cereal–grain concentrates. Kelly *et al*. (1998) reported concentrations of CLA and 18:1 in milk fat of cows fed a total mixed ration containing 5 g/kg and 284 g/kg of these compounds, respectively. Concentrations of these fatty acids increased to 11 and 347 g/kg milk fat when cows were offered temperate pasture (Kelly *et al*. 1998). However, the change in diet from a total mixed ration to pasture reduced dry matter intake and yields of milk, milk fat and milk protein (Kolver and Muller 1998). Lock and Garnsworthy (2000) reported a CLA concentration of 9.1 g/kg in milk fat of cows fed a total mixed ration consisting of grass silage, maize silage and concentrates. The concentration of CLA increased to 28.1 g/kg milk fat when cows grazed pasture. Dry matter intakes, milk production and concentrations of other fatty acids were not reported.

Level of intake and composition of fresh pasture affect the concentration CLA in milk fat. In one study, there was a positive linear relationship between unsupplemented pasture intake (2–4 kg DM/100 kg liveweight) and concentration of CLA in milk fat (12–16 g/kg) (Stockdale *et al*. 2001). MacGibbon *et al*. (2001) reported that the average concentration of CLA in samples of milk fat from commercial dairy herds in pasture–based production systems in New Zealand varied from 7–11 g/kg milk fat depending on time of year and amount of supplement fed. Others (Collomb *et al*. 2001) reported that the concentration of TVA and CLA in milk fat of cows grazing pasture in Switzerland increased with altitude, which was associated with changes in pasture composition (less grass and more dicotyledonous herbaceous species).
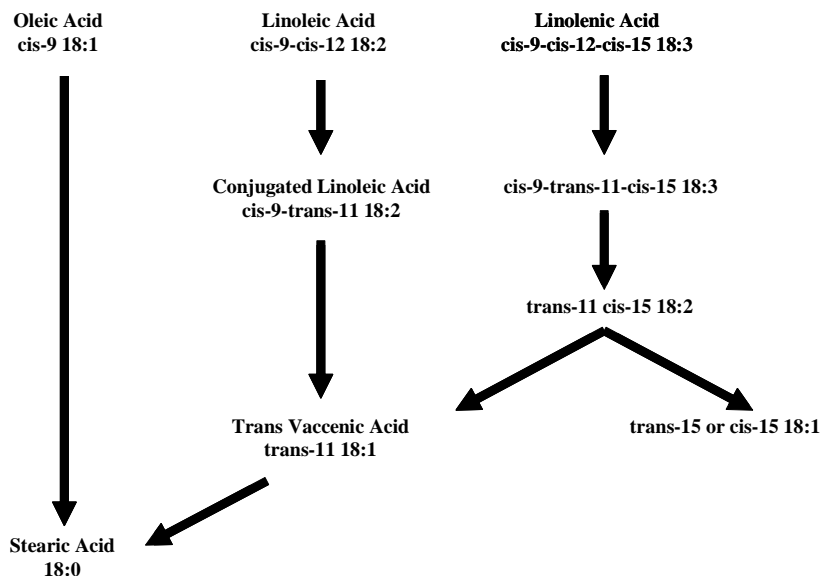

Figure 1 Putative pathways of ruminal biohydrogenation of oleic, linoleic and linolenic acids. Adapted from Bauman *et al.* (2001).

A study of the relationship between milk composition, time of year and nutrition and management practices typical of irrigated, pasture–based dairy farms in northern Victoria supported these findings and added significantly to knowledge of the concentrations of isomers of TVA and CLA in milk fat (Walker *et al.* 2005). In this study, the concentration of total CLA in milk fat ranged from 1.1–35.4 mg/g milk fat (average 9.1 mg/g milk fat). The average concentration of CLA peaked twice: in April–May (autumn) and in September–October (spring). However, there was up to 3–fold variation in the concentration of CLA between sites within collection periods (Ostrowska *et al.* 2004). These values represent are consistent with the lower and upper concentrations reported in the literature (Bauman *et al.* 2001), although concentrations of CLA in milk from cows fed total mixed rations are generally about 4–5 mg/g of milk fat (Kelly *et al* 1998; Dhiman *et al.* 2000; Chouinard *et al.* 2001). The major isomer, *cis,trans* 9,11, accounted for 84% of total CLA. Given this value, it is not surprising

that the concentration of the *cis,trans* 9,11 CLA isomer also peaked in August/September; the lowest concentrations were recorded between April and June (Figure 4) when the total intake of pasture was at its lowest. The next most abundant group of CLA isomers comprised *cis/trans* 11,13 (4.2%), *cis/trans* 8,10 and 7,9 (collectively, 3.1% of the total; 0.25 ± 0.029 mg/g) and *cis/trans* 10,12 (0.4%).

Similar to CLA, the concentration of *trans*–18:1 fatty acids peaked in August/September, while the lowest concentrations were recorded between April and June. The pattern for the major *trans*–18:1 isomer, TVA, was similar although there was considerable variation, particularly in the higher concentration catagory (data not shown). Total milk fat concentrations of trans isomers also varied with time of year (5.7–164.2 mg/g fat, average 60.2 mg/g fat). The composition of *trans* 18:1 fatty acid isomers was, in decreasing order of concentration: TVA (*trans*–11) (53.4% of total *trans*), *trans*–14 (10.7%), *trans*–10 (9.6%), *trans*–12 (9.1%),
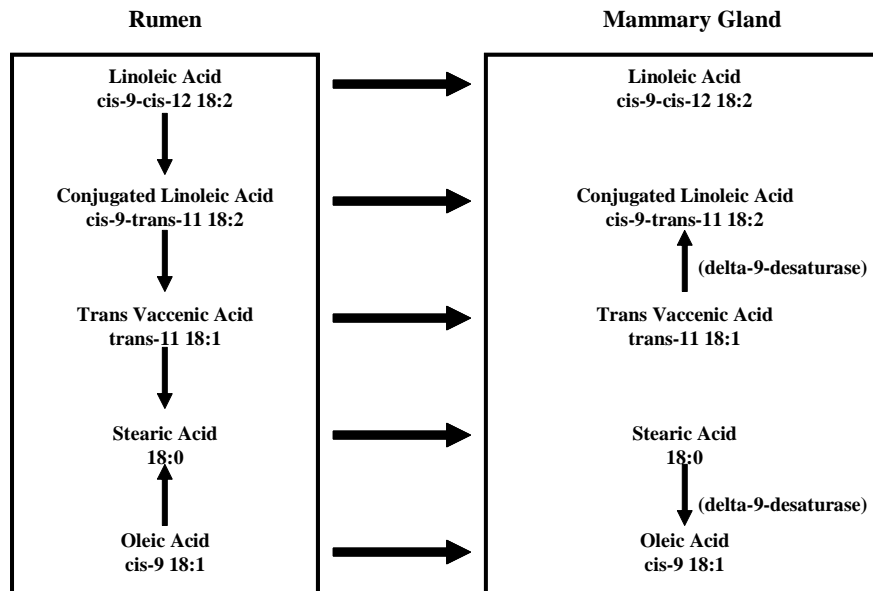
Figure 2 Pathways by which fatty acids derived from dietary oleic and linoleic acids can become available for milk fat production. Adapted from Bauman *et al.* (2001).
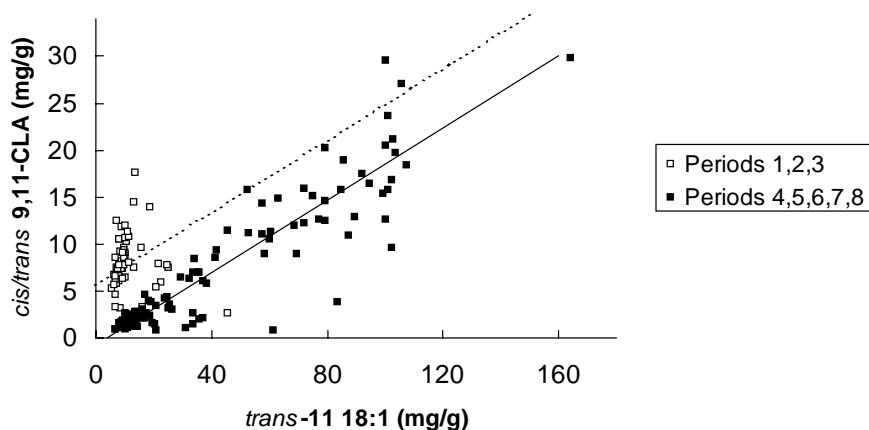
Figure 3 Relationship between *cis,trans*–9,11 CLA and *trans*–11 18:1 in milk fat.
Y = −0.724 (SE 0.504) + 0.193 (SE 0.00899) X + 6.348 Periods 1,2,3;
R^2 = 0.76; *P*<0.001.

trans–13 (7.5%), 4.94 ± 0.53 mg/g; *trans*–15 (6.0%), 1.44 ± 0.31 mg/g; *trans*–9 (3.5%).

As expected, there was a significant positive correlation between the concentrations of TVA and the *cis/trans*–9,11 isomers of CLA ($R^2 = 0.54$, $P<0.001$). However, it appeared that there was little relationship between the two fatty acids from late autumn to mid–winter; separate inclusion in the regression analysis dramatically improved the relationship ($R^2 = 0.76$, $P<0.001$; Figure 3). Cows tend to be underfed on pasture in northern Victoria at these times of year, which may explain the lack of relationship, given the importance of pasture in providing lipid precursors for both TVA and CLA. Significantly, these data show that at times of the year when cows consume much of their diet as highly digestible (lush) pasture, the CLA and TVA concentrations can be as high as those known to be protective against mammary cancers in rats (Ip *et al.* 1999; Lock *et al.* 2004).

Effect of supplements on CLA and TVA

Walker *et al.* (2000) reported that the concentration and yield of CLA in milk fat varied quadratically with the amount of cereal–grain concentrate consumed by cows grazing 25 kg DM/day perennial pasture. The concentration of CLA in milk fat was reduced from 16 g/kg in unsupplemented cows to 10 g/kg when cows were supplemented with cereal–grain concentrates at 6 kg DM/day. The concentration of CLA increased to 14 g/kg milk fat when the level of supplementation increased to 11 kg DM/day (Walker *et al.* 2000). This response was associated with increased yields of milk and milk protein, but the yield of milk fat was depressed (Walker *et al.* 2001b).

With the exception of herbage ME consumed, feeding or management practices had no effect on the concentration of total CLA in milk in the study of Ostrowska *et al.* (2004). For every MJ increase in ME/ kg DM, there was an increase in the concentration of *cis/trans* 9,11 CLA (+1.74 mg/g fat, $P<0.001$; Figure 5), which is consistent with higher intakes of lipids from pasture that has a higher ME content (Walker et al. 2004). A similar response was observed for milk fat TVA in which concentrations increased by 8.92 mg/g for every MJ increase in pasture ME ($P<0.001$), but it was 38.7 mg/g lower from late Autumn to mid–winter ($P<0.001$) than the rest of the year regardless of the pasture ME content (data not shown).
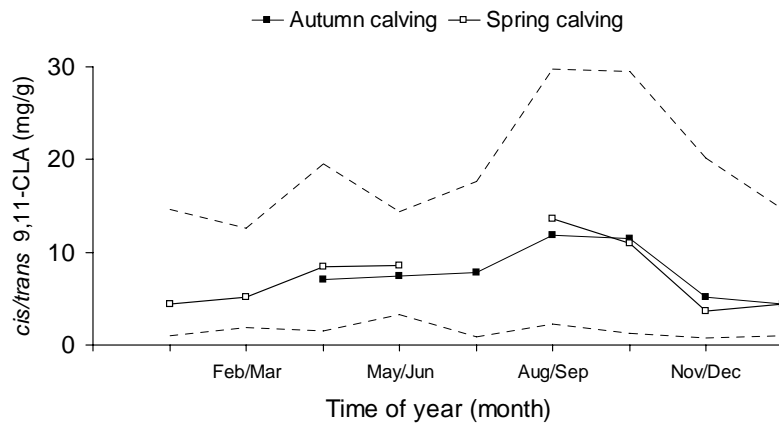

Figure 4 Effect of time of year and season of calving on milk fat cis/trans 9,11–CLA (rumenic acid) concentrations. Data are presented as means and dotted lines indicate the upper and lower values observed at each time of year (pooled across calving season). The SED for calving season × period was 2.28 mg/g.
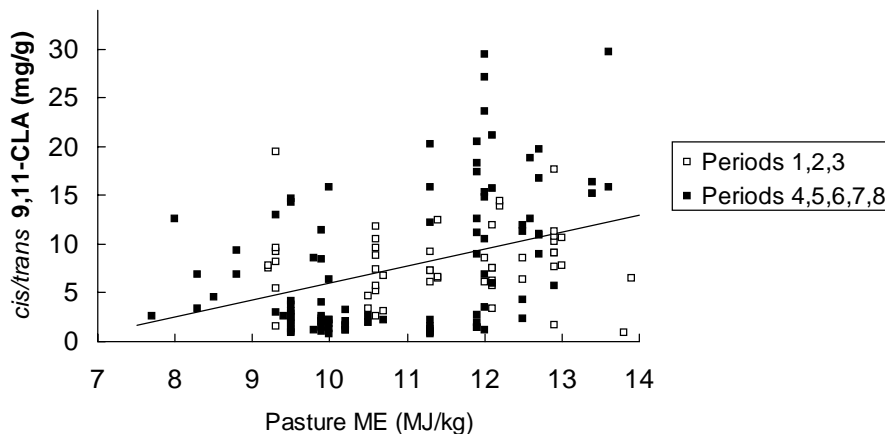

Figure 5 Relationship between *cis,trans*–9,11 CLA in milk fat and pasture ME content. Y= – 11.43 (SE 3.83) + 1.742 (SE 0.344) X; R^2 = 0.14; $P<0.001$.

A reduction in the concentration of CLA in milk fat in response to feeding up to 6 kg DM cereal–grain supplement/day to cows grazing perennial pasture has been consistently observed in a number of experiments (Walker *et al*. 2001a). However, supplementing cows grazing pasture with vegetable oils rich in linoleic and or linolenic acids appears to increase the concentrations of CLA and unsaturated fatty acids in milk fat. Lawless *et al*. (1998) reported concentrations of CLA and total 18:1 of 16.6 and 249 g/kg milk fat, respectively, when cows were offered temperate pasture at *ad libitum* intake and supplemented with 3 kg DM/day unmolassed beet pulp. Partially replacing the beet pulp with either 550 g full fat soybean meal or the same amount of full fat rapeseed meal increased the concentrations of CLA in milk fat to 22.3 and 24.9 g/kg and the concentrations of 18:1 to 313 and 315 g/kg, respectively (Lawless *et al*. 1998). Interestingly, the rapeseed meal treatment resulted in a higher concentration of CLA than the soybean meal treatment. This occurred despite the rapeseed meal having a lower concentration of 18:2 (195 vs. 545 g 18:2/kg total fatty acids for rapeseed and soybean meal, respectively). Milk, milk fat and milk protein yields were not affected by treatments. Kay *et al*. (2004) reported concentrations of CLA and cis–9 18:1 of 22.3 and 181 g/kg milk fat, respectively, when cows grazed temperate pasture at an allowance of 35 kg DM/day. Drenching cows twice daily with sunflower oil at a dose equivalent to 40 g/kg pasture DM intake increased concentrations of CLA and total 18:1 to 28.5 and 253 g/kg milk fat, respectively (Kay *et al*. 2004). Milk and milk protein yields were not affected by the treatment, but milk fat yield decreased by 25%.

Not all diets that increase intakes of 18C fatty acids are effective in increasing the concentration of CLA in milk fat. For example, feeding silage made from corn high in oil was only marginally effective at increasing the concentration of CLA in milk fat compared with standard corn silage (Chouinard *et al*. 2001). This occurred despite the increase in lipid content of the diet (32–54 g/kg DM), and may have resulted from changes in the fatty acid composition of pasture during the ensiling process. Noftsger *et al*. (2000) supplemented cows fed a total mixed ration with expanded expelled cottonseed (140, 210 or 280 g/kg DM) or whole cottonseed (140 g/kg DM). These treatments supplied about 10, 15, 20 and 55 g cottonseed oil/kg DM, respectively, but had no significant effect on the concentration of CLA in milk fat. As the cottonseed oil contained 540 g 18:2/kg, a significant response of CLA to increasing intake of cottonseed oil was expected. It was suggested that the absence of a response might have been the result of heating of the expanded expelled cottonseed, which may have protected the oil from biohydrogenation in the rumen (Noftsger *et al*. 2000). However, cottonseed can also contain malvalic acid (Badami *et al*. 1982). This fatty acid, along with sterculic acid, is an inhibitor of Δ–9–desaturase in mammary and other tissues (Corl *et al*. 2001). Inhibition of desaturase

in the mammary gland may also have been responsible for the lack of response observed in this study.

Supplementing cows with either fish oil or marine algae increases the concentration of trans–vaccenic acid and, consequently, CLA in milk fat (Bauman *et al*. 2000). Fish oil and marine algae are sources of very long chain polyunsaturated fatty acids. They can contain high levels of 20:5 n–3 (EPA) and/or 22:6 n–3 (DHA), but the fatty acid composition of fish oils varies considerably with species, season and the geographical location from which they are harvested (Moffat and McGill 1993). The increase in CLA achieved by fish oil supplementation is the result of inhibition of the biohydrogenation of trans octadecanoic fatty acids in the rumen and is better correlated with the intake of EPA and DHA than the intake of total marine lipids per se (Chilliard *et al*. 2001).

Fish oils and other marine lipids contain negligible levels of linoleic and linolenic acids (Harwood and Geyer 1964; Moffat and McGill 1993; Franklin *et al*. 1999). The response of trans–vaccenic acid and CLA to marine lipids is often much greater than would be expected from the provision of precursors for their synthesis. Therefore, it may be possible to enhance the already high endogenous levels of CLA and TVA in milk from cows grazing temperate pasture by supplementation with cereal–grain supplements containing vegetable– and tuna oils, although this remains to be tested. It is also possible that similar dietary regimens will be used to manipulate the CLA and TVA content of ruminant meat.

Manipulation of CLA and TVA content of meat

Conjugated linoleic acid and TVA concentrations are greater in the meat from animals with high intakes of pasture than that from animals fed concentrate diets (Aurousseau *et al*. 2004; Realini *et al*. 2004). For example, concentrations of *cis/trans*–9,11 CLA and total trans–18:1 fatty acids were 16 and 62 mg/g intramuscular fat in lambs fed pasture, and 6 and 36 mg/g muscle fat in lambs fed concentrate diets (Aurousseau *et al*. 2004). CLA content of meat can be further enhanced by feeding rumen–protected CLA (Gillis *et al*. 2004). The opportunities that exist for ruminant meats are to position intramuscular fat as being an excellent source of CLA and polyunsaturated fatty acids and to develop markets for products that are further enriched through dietary manipulation. Use of technologies such as supplementation with cereal–grain supplements containing vegetable and tuna oils may further increase the concentrations of these fatty acids, although on–farm delivery for grazing beef cattle is more problematic than for dairy cattle.

Dietary CLA supplementation also results in modified fatty acid composition in pig tissue (O'Quinn *et al*. 2000; Eggert *et al*. 2001; Ramsay *et al*. 2001; Wiegand *et al*. 2001; Joo *et al*. 2002; D'Souza and Mullan

2002; Wiegand *et al.* 2002; Ostrowska *et al.* 2005). Dietary CLA is incorporated into adipose tissue and to a lesser extent into intramuscular fat in a dose–dependant manner in pigs (Eggert *et al.* 2001; Ostrowska *et al.* 2003), which offers the opportunity to improve the quality of fat. Although the concentration of individual CLA isomers increases with the level of dietary CLA, there is obviously some selectivity in the uptake or incorporation of certain isomers, especially the *cis/trans* isomers (Ostrowska *et al.* 2003). These authors calculated transfer efficiencies for the individual isomers and showed that the *cis/trans*–9,11 isomer was incorporated most efficiently (46.4%) into subcutaneous adipose tissue, whereas the *cis/trans*–11,13 isomer was incorporated most efficiently (0.74%) into in intramuscular fat (Ostrowska *et al.* 2003). The preferential enrichment of *cis/trans*–9,11 is of particular value with respect to potential health benefits as there is extensive evidence showing that it has anti–cancer properties (Whigham *et al.* 2000; Pariza *et al.* 2001). The *trans/cis*–10,12 isomer, which is thought to be responsible for the reduction in lipid deposition, was incorporated less efficiently in both subcutaneous and intramuscular fat (13.31% and 0.31%, respectively), than the remaining isomers in the *trans/cis* group (Ostrowska *et al.* 2003). The implication of these findings is that levels of CLA in pig adipose tissue and intramuscular fat can be increased to levels similar to those of dairy products and red meat (typically 2–24 mg/g of fat), currently the major sources of dietary CLA (Ritzenthaler *et al.* 2001). Thus, there is potential to increase the level of CLA substantially by supplementing animal diets with CLA. This is particularly true of the *cis/trans*–9,11 isomer, which is readily incorporated into animal fat.

The effects of dietary CLA on the contents of other fatty acids in muscle and fat depends on the dietary fat that the CLA replaces; some differences in the fatty acid profiles of tissue lipids could be due to differences in the fatty acid profile of the diets. Therefore, care should be taken in interpreting effects of CLA supplementation on changes in the profiles of other fatty acids. Despite this caveat, it does appear that some of the changes in the fatty acid composition of porcine adipose tissue can be attributed to dietary CLA. For example, (Ostrowska *et al.* 2003) found that the concentrations of palmitoleic (16:1) and palmitic (16:0) acids in both intramuscular and subcutaneous fat increased in a linear fashion with increasing dietary CLA. This was due to dietary CLA because the level of dietary palmitic acid decreased as CLA replaced soy oil (Ostrowska *et al.* 2003). As palmitoleic acid was not detected in the diets, it must have been derived from desaturation of the respective saturated fatty acid, palmitic acid, which is in turn regulated by the fatty acyl–CoA Δ^9–desaturase complex. The Δ^9–desaturase enzyme complex is also responsible for desaturation of stearic acid to oleic acid. In the study of Ostrowska *et al.* (2003), the concentration of stearic acid was not affected by dietary CLA despite a decrease in the dietary level of stearic acid as the dietary CLA content

increased, but a significant reduction in oleic acid was detected despite similar levels in all the six diets. Others have also reported a distinct shift toward lower oleic acid concentrations in pigs fed diets supplemented with CLA (Bee 2000; Ramsay *et al.* 2001). This could be due to the depressed synthesis of oleic acid in adipose tissue lipids, possibly due to decreasing stearoyl–CoA desaturase activity. The resulting increase in the ratio of saturated to unsaturated fatty acids is unfavourable because increased consumption of saturated fatty acids is associated with increased risk of coronary heart disease. The calculated index of atherogenicity (Ulbricht and Southgate 1991), which takes into consideration the protective properties of fatty acids, is also negatively affected by CLA supplementation (Ostrowska *et al.* 2003). Therefore, applying nutritional techniques to increase the poly– and mono–unsaturated fatty acid content of pork to ameliorate the effects of dietary CLA should be considered. It should also be borne in mind that the index of atherogenicity was derived before the potential anti–atherogenic effects of CLA were known (Parodi 2002).

Milk protein–bound selenium

Because of the relatively low level of selenium in Australian and New Zealand soils, many primary products from these countries have relatively low Se levels. While it is generally accepted that Se intakes of Australian and New Zealand consumers are sufficient to ensure that there are no overt signs of deficiency, there is a growing feeling that the relatively low intakes may contribute to elevated levels of risk for some cancers (e.g., bowel cancer). There is a growing body of evidence that suggests that selenium is protective against some cancers and that protein–bound organic selenium is more effective than inorganic selenium (Clark *et al.* 1996). However, Se supplementation is problematic because high Se intakes can be toxic, particularly if the source is inorganic. Protein–bound Se is more bioactive and less toxic than organic forms of Se and there is increasing interest in delivering Se in organic forms in the food we eat. These organic forms may include yeast, meat or milk proteins. Feeding supplemental selenium–yeast complexes has been shown to increase the organic selenium contained in pork, meat and eggs (Mahan and Kim 1996; Kim and Mahan 2001a); branded products are now sold in Korea.

In the dairy survey discussed previously, we also had the opportunity to investigate the effects of on–farm management factors and season on milk Se concentrations. The mean (± SE) and range in milk selenium concentrations were 12.7 ± 0.56 and 3–37.1 µg/kg milk, respectively. Milk collected in spring had a lower concentration of selenium than milk collected in autumn (9.8 vs. 16.2 µg/kg milk, *P*<0.05). The average concentration of selenium in milk from cows fed mineral supplements was greater than that of milk

from unsupplemented cows (16.3 vs. 8.7 mg/kg milk, $P<0.01$), and increased by 0.7 ± 0.15 mg/kg milk for each kg of cereal–grain concentrate fed. There was no effect of time of calving on milk Se concentrations. However, season and mineral supplements were important in determining the concentration of Se in milk, suggesting that targeted feeding strategies may be effective in enhancing the Se concentration of milk. In this context, milk selenium concentration responses to supplementation with organic selenium are greater than responses to inorganic selenium (Ortman and Pehrson 1999), and concentrations in milk peak within one week of feeding organic selenium supplements (McIntosh *et al.* 2004; Heard *et al.* 2005) (Figure 6). More recently, Heard *et al.* (unpublished data) found that the selenium concentration in milk of grazing cows is affected by the selenium content of grain supplements. From the information available from our survey, it was not possible to determine how much of the variation in milk selenium concentration was due to supplements of organic or inorganic selenium or to determine the concentrations of selenium in concentrates and herbage. However, there is considerable variation in the content of Se in pasture and concentrates used in the dairy industry. For example, pasture Se concentrations vary from 10–50 µg/kg DM, the upper limit of which is considered adequate for grazing livestock (Caple *et al.* 1980). Milk Se concentrations of 3–5 µg/kg and 6–11 µg/kg have been detected in milk from cattle in 'low' and 'high' Se areas in the Netherlands, respectively (Binnerts 1979). While variations in Se concentration in pastures may lead to variation in milk concentrations, the effects are likely to be relatively small compared to those obtained when cows are fed organic Se in yeast supplements unless significant amounts of Se are added to the fertiliser used (Aro *et al.* 1998). The Se concentrations in concentrate supplements such as wheat (mean 23, range 1–117 µg/kg) and lupins (mean 89, range 10–488 µg/kg) vary considerably (White *et al.*

1981) and are likely to affect concentrations in milk, as was observed in our survey.

The growing interest in the selenium content of dairy products has to a large extent been fuelled by McIntosh and colleagues, who worked with casein processed from milk obtained from cows that were supplemented with yeast–bound selenium (Sel–Plex®) (McIntosh *et al.* 2004). Using an azoxymethene–induced colon cancer model, these workers found significant reductions in colonic tumour incidence (number of rats with tumours) and tumour burden (number of tumours per rat) in rats consuming selenised casein (1 ppm Se) relative to control and yeast Se treatments. There was an effective reduction in the number of benign and malignant tumours in the selenised casein group relative to that of the control and yeast selenium treatments (McIntosh *et al.* 2004). Therefore, it is possible that delivery of selenium via enriched milk and dairy products will be a means of providing health benefits to people at risk of colon or other cancers. This may be due to an additive effect of selenium and other components in dairy protein that have been found to protect against cancer in rodent models (McIntosh *et al.* 1995).

Whey protein isolates

Milk contains a mixture of proteins, each having unique attributes for nutritional, biological and human food ingredient applications (Smithers *et al.* 1996). The major proteins present in milk include β–lactalbumin, α–lactoglobulin, immunoglobulin, bovine serum albumin, κ–casein, β–casein and the α–caseins (Etzel 2004). Lactoferrin and lactoperoxidase are minor but commercially important proteins. In addition, rennet whey contains glycomacropeptide (GMP), which is cleaved from κ–casein by chymosin to initiate precipitation of the caseins, forming curd. Protein
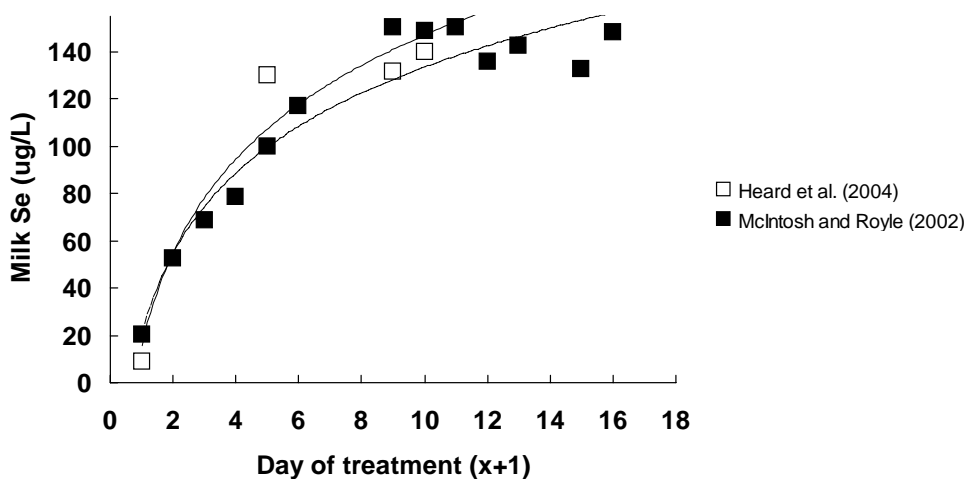

Figure 6 Relationship between milk selenium concentrations and time after commencing supplementation with SelPlex.

from bovine whole milk consists of approximately 20% whey protein. When casein is removed from whole milk, liquid whey remains; whey has a protein concentration of about 65%. A number of different proprietary processes exist to treat or purify whey protein, resulting in a various whey protein isolates, some of which may be rich in bioactive peptides such as GMP.

There has also been increased interest in the role of protein in weight control. In a recent review, four lines of evidence were presented to support a role for dietary proteins in the regulation of food intake and weight maintenance (Anderson and Moore 2004). Firstly, proteins suppress food intake more than fats or carbohydrates do, and the extent of this reduction is greater than can be accounted for by their energy content alone. Secondly, proteins make a stronger contribution to satiety than fat and carbohydrates, and delay the return of hunger for longer. Third, high–protein diets support the maintenance of lean body mass under circumstances of energy restriction, thereby promoting weight loss primarily as adipose tissue. Finally, protein digestion leads to the stimulation of many physiological and metabolic responses involved in the regulation of feed intake. In addition, some protein sources contain peptides that may have a direct effect upon satiety. For example, GMP is thought to have a satiating effect. Certain whey protein isolates (WPI) are relatively rich in GMP.

In order to test whether a WPI rich in GMP has an effect on feed intake, body weight and other indices of obesity, we conducted a study using obese minipigs as models for obese humans. The minipig is an excellent model for obesity because it contains 50% body fat and displays insulin resistance with respect to both glucose and amino acid uptake (Dunshea *et al.* 2005a; Dunshea *et al.* 2005b). Sixteen obese female minipigs (133 kg, 50% body fat) were randomly allocated to a 2×2 factorial design in which the factors were source of protein (WPI; NaturaPro MG2460, MG

Nutritionals, Brunswick, Victoria) vs. soy protein isolate (SPI; Profarm 974, ADM, Palm Beach, Queensland) or level of dietary protein (11% CP (LP) vs. 22% CP (HP)). The WPI contained 46%, 30% and 8% β–lactalbumin, GMP and α–lactoglobulin, respectively. After consuming their respective diets for 10 weeks, the surgically prepared pigs were infused iv. with insulin at a rate of 0.6 and 6.0 mU/kg/min and blood glucose and amino acid concentrations were clamped at pre–infusion levels by simultaneous infusions of dextrose (50% w/v) and a parenteral amino acid mix (10% w/v), respectively. The protocol for the hyperinsulinemic/euglycemic/eulysinemic clamp is outlined in Figure 7. Composition of the ham region was determined by Dual energy X–ray absorptiometry at 0, 4 and 8 weeks. Feed intake was lower in pigs fed the HP diet (2070 vs. 2352 g/d, $P<0.001$), particularly in pigs fed WPC (1951 vs. 2408 g/d) as indicated by an interaction ($P = 0.027$) between source and level (Figure 8). Pigs consuming the HP diet deposited less weight (231 vs. 382 g/d, $P = 0.045$) and had a lower ratio of fat:lean in the ham (0.70 vs. 0.76, $P = 0.026$) at 8 weeks than those fed the LP diet. Protein source had no effect on the amount of dextrose infused to maintain euglycemia (108 vs. 115 mL/h, $P = 0.59$) but the amount infused was lower in the minipigs fed the LP diet (101 vs. 125 mL/h, $P = 0.048$) (Figure 9). The amount of dextrose required to maintain glycemia was greater at the higher dose of insulin (114 vs. 226 mL/h, $P<0.001$). Protein source had no effect on the amino acid infusion rate required to maintain plasma lysine concentrations (50 vs. 50 mL/h, $P = 0.98$) but the amount infused was lower in pigs fed the LP diet (45 vs. 55 mL/h, $P = 0.030$). The amino acid infusion rate was greater at the higher dose of insulin (47 vs. 103 mL/h, $P<0.001$). A HP diet reduced feed intake, weight gain and fat deposition and reduced insulin resistance in obese minipigs. The HP diet containing WPI enriched with GMP had the greatest effect upon feed intake and weight gain.
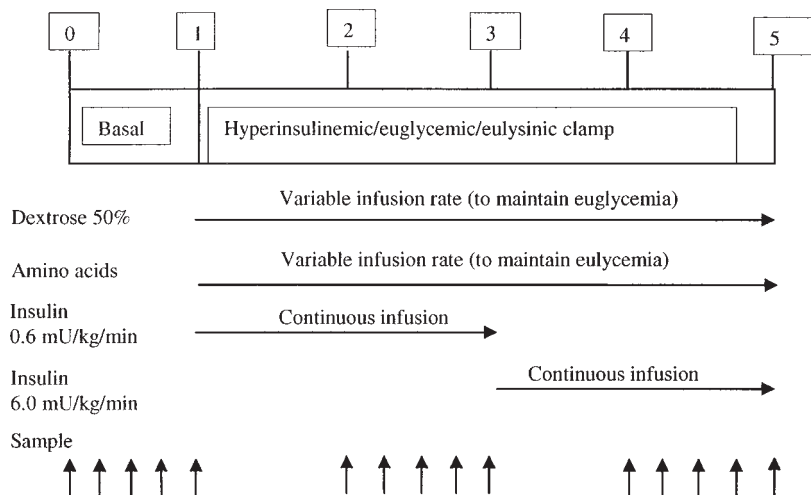

Figure 7 Experimental protocol for the hyperinsulinemic/euglycenic/eulysinic clamp.
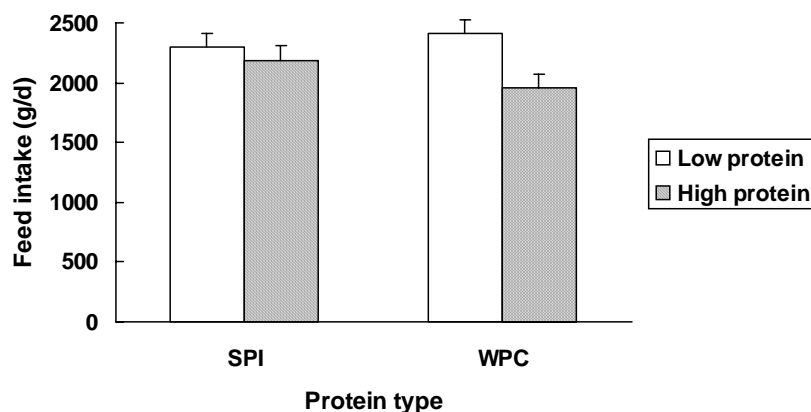
Figure 8 Effect of dietary protein source and level on feed intake of obese minipigs.
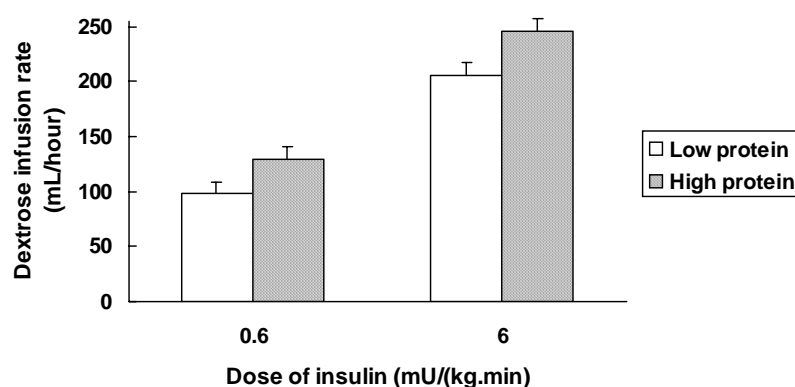

Figure 9 Effect of dose of insulin and dietary protein level on the dextrose (50% w/v) infusion rate to maintain glycemia in obese minipigs.

Conclusion

In conclusion, these case studies demonstrate that it is possible to manipulate animal products either on–farm or in the processing plant to produce food products that have health benefits for humans. It is highly likely that on–farm innovation will result in the development of more animal products that can be differentiated from commodities because of enhanced levels of health–active compounds. This is particularly likely for dairy products because they are harvested from the animal frequently. Australia's pasture–based grazing systems allow for incorporation of compounds either directly from the pasture or as the products of ruminal fermentation of pasture components. Some of these inherent components of milk and meat also offer health benefits with protein and lipid–bound (or soluble) molecules offering the greatest promise.

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