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Review

Australian Journal of Experimental Agriculture, 2005, **45**, 1189–1203

Rumen protected protein and fat produced from oilseeds and/or meals by formaldehyde treatment; their role in ruminant production and product quality: a review

S. K. Gulati[A,C], *M. R. Garg*[B] *and T. W. Scott*[A]

[A]Faculty of Veterinary Science (B19), Sydney University, NSW 2006, Australia.
[B]National Dairy Development Board, Anand, 388001, Gujarat, India.
[C]Corresponding author. Email: sureshg@vetsci.usyd.edu.au

Abstract. The nutritional characteristics of rumen-protected protein and fat supplements produced by formaldehyde treatment of oilseeds and meals are reviewed. The proportion of rumen undegraded protein (RUP) in different protein sources can be controlled by this process, bio-available lysine is 82–84% and the proportions of acid detergent and neutral detergent insoluble nitrogen are unchanged by formaldehyde treatment; this is in contrast to heat treatment of proteins where significant increases in these nitrogen components can occur if the RUP content exceeds 60% of the crude protein (CP). A RUP content of 75–80% of CP is optimal when using protein supplements for milk production, and for body growth in steers a lower RUP content is desirable (i.e. 50–55% of CP). Both the fat and protein constituents in rumen-protected fat supplements derived from the emulsification and formaldehyde treatment of oilseeds are highly protected from ruminal metabolism (75–90%) and are readily digested in the small intestine (90% for C_{18} unsaturated fatty acids, 82% for the essential amino acids). Protected fat/protein supplements are designed and fed to lactating and non-lactating ruminants to increase efficiency of production, enhance product quality, augment n-3, n-6 and n-9 fatty acid content of meat and milk, and to improve reproductive performance. The challenges and potential role for these protected fat/protein supplements in improving productivity and quality of ruminant derived foods are discussed.

Additional keywords: formaldehyde-treated, rumen-protected nutrients, protein, milk, meat.

Introduction

This review will focus on the properties and use of rumen-protected protein and fat supplements produced by formaldehyde treatment of either oilseed meals or whole oilseeds in the diet of ruminants. The reasons why it is potentially beneficial to protect dietary protein and fat from ruminal metabolism have been detailed elsewhere (Faichney 1970; Ferguson 1975; McDonald and Scott 1977; Ashes *et al.* 1995; NRC 2001; Schroeder *et al.* 2004). Briefly they include: (i) increased supply of rumen undegradable protein (RUP) and essential rate limiting amino acids (e.g. lysine and methionine) for milk, meat and fibre production; and (ii) increased supply of rumen undegradable fat (RUF) with the capacity to enhance the energy density of the diet and provide sources of essential/bioactive fatty acids (n-6 fatty acids, e.g. linoleic $C_{18:2}$; n-3 fatty acids, e.g. linolenic $C_{18:3}$) and conjugated linoleic acids (CLAs) to improve production efficiency and quality of meat and milk products.

Before detailing the nutritional characteristics of formaldehyde-treated protein and fat supplements and their role in ruminant production, a comment on the occurrence, metabolism and safety issues of formaldehyde is needed. Formaldehyde is widely used in industry and occurs naturally as a constituent of many foods including dairy and meat products, coffee, fruits, smoked fish e.g. 0.2 μg/g in meat; 0.1 μL/L in milk; 10 μg/g in cheese; 180 μg/g in fish (Owens *et al.* 1990). Formaldehyde is a normal product of intermediary metabolism in mammals and is involved in the biosynthesis of amino acids. Endogenous levels of formaldehyde in human tissue range from about 3–12 ng of formaldehyde per gram of tissue. Formaldehyde is converted to formic acid by the action of the formaldehyde dehydrogenase enzyme, formic acid is metabolised to carbon dioxide and water, or incorporated into the one carbon pool or excreted in the urine as a sodium salt (Owens *et al.* 1990). Hence, mammalian systems have the biological pathways to effectively metabolise ingested formaldehyde and there is no evidence to suggest that formaldehyde is a carcinogen when consumed orally (FDA 1998). However, formaldehyde vapour can cause sensory irritation of the eyes, nose and throat and is a potential carcinogen (Owens *et al.* 1990; WHO 2004). Therefore, when it is used to treat feedstuffs, closed systems are required and occupational health and safety guidelines for formaldehyde use in industry must be

10.1071/EA04131 0816-1089/05/101189

followed. A sealed silo system also enhances the cross-linking reaction that occurs between the formaldehyde and protein (Ashes *et al.* 1984). The majority of formaldehyde is bound to the protein. However, before opening the silos they should be vented to allow any traces of free aldehyde to disappear. The formaldehyde present in treated feedstuffs is metabolised by ruminants and does not significantly change the naturally occurring levels of formaldehyde in meat and milk (Mills *et al.* 1972; Bitman *et al.* 1975; Atwal and Mahadevan 1994). Formaldehyde is approved for use as a feed additive to protect proteins from ruminal degradation, to preserve silages, to maintain animal feeds or feed ingredients free of salmonella, to control fungi and to improve the handling characteristics of oilseeds and meals, and animal fat pre-mixes (FDA 2004).

Nutritional properties and use of rumen undegraded protein (RUP) produced by formaldehyde treatment of oilseed meals

Nutritional properties of formaldehyde-treated oilseed protein meals

The amount of formaldehyde required to optimally treat different protein sources to ensure maximal ruminal protection without decreasing the digestibility of protein and essential amino acids is very important (Ashes *et al.* 1984; Spears *et al.* 1985; Hamilton *et al.* 1992; Ashes *et al.* 1995). For example, treating sunflower seed meal with 0.5% formaldehyde by weight of crude protein (CP) gave an RUP of 75%, while a level of 0.9% formaldehyde gave an RUP of greater then 90%. If excess formaldehyde is used to treat proteins then the complexes formed between formaldehyde and the reactive group of the protein (e.g. ε-amino group of lysine) are acid resistant (Ashes *et al.* 1984) and this will reduce protein digestibility in the small intestine and bio-availability of essential amino acids (e.g. lysine). In effect the protein will be 'over protected' from ruminal degradation and metabolism (see Fig. 1 for a presentation of these concepts with reference to milk production). Friesian cows, 30 days into lactation and producing on average 18 L milk/day, grazed pasture alone, or were supplemented with cracked barley, sunflower meal or sunflower meal supplemented with 0.5 or 0.7% formaldehyde on a CP basis. Cows fed the sunflower meal with a lower level of protection (i.e. 35%) had a significantly higher rumen ammonia nitrogen level due to protein degradation (Fig. 1); as the level of protein protection increased there was less protein available for ruminal degradation, resulting in lower rumen ammonia (Ashes *et al.* 1995). However, when assessing the degree of protection as a result of formaldehyde treatment as well as other procedures e.g. heat, Stern *et al.* (1994) stressed the difficulties associated with the different techniques used and concluded that it was more realistic to obtain relative measurements of ruminal degradation. The *in vitro* procedure, used to measure the degree of protection, involved anaerobic incubations of replicates with treated and untreated meals together with pure proteins such as casein, and blank tubes with strained rumen fluid from fasted donor sheep and/or cattle. Net rumen ammonia release was measured by steam distillation. The protection values obtained using the *in vitro* procedure were similar to those obtained *in situ* [Table 1; White *et al.* (2004)] where nitrogen degradability was determined on a similar batch of treated canola oilseed meal. Furthermore, there is a positive correlation between soybean meal equivalent values obtained by *in vitro* ammonia release and *in situ* protein degradation in bone, meat and poultry by-products ($r = 0.92$; $P<0.01$) (Herold *et al.* 1996).

Some nutritional properties of protein meals optimally treated with formaldehyde for use in the diet of lactating ruminants are given in Table 1.

Several criteria were used to define these nutritional features including *in vitro* and *in vivo* protein degradation, acid detergent insoluble nitrogen (ADIN), neutral detergent insoluble nitrogen (NDIN) content and bio-available lysine. The degree of protein protection is around 70–75% and this produces a ratio of about 3:1 RUP/RDP. The bio-available lysine content is about 82–85% (Gulati *et al.* 2002*a*) and the proportions of ADIN/NDIN, indicators of non-usable nitrogen (NRC 2001; Schroeder *et al.* 1996) in formaldehyde-treated proteins, remains low (Table 1). In

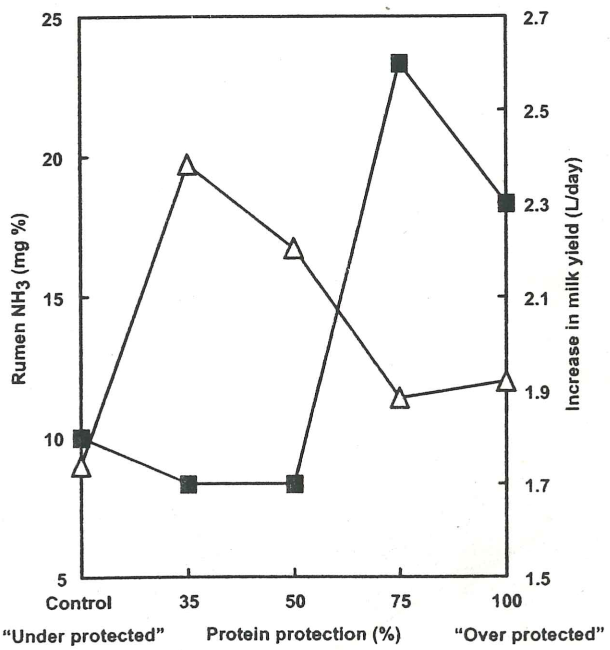

Figure 1. Effect of increasing the degree of protein protection (%) on rumen ammonia (mg %) and changes in milk production (L/day). Friesian cows grazed pasture alone, or were supplemented with cracked barley, untreated sunflower meal or sunflower meal treated with formaldehyde. (■) Increase in milk yield; (Δ) Rumen ammonia. (Hamilton *et al.* 1992).

contrast, if heat treatment of proteins is used to achieve the same degree of rumen protection (70–75%), there is a significant increase in the proportions of ADIN/NDIN and the bio-available lysine is reduced (Faldet *et al.* 1991; Schroeder *et al.* 1996). The temperature and duration of heat treatment needs to be carefully controlled to avoid 'over protection' of the protein (Satter *et al.* 1994; Schroeder *et al.* 1996).

Effects on milk production

The data presented in Table 2 are examples of the production responses in lactating ruminants fed untreated or formaldehyde-treated oilseed meal supplements. In higher yielding dairy cows, feeding these protein supplements increased milk yield in early lactation where energy and protein were limiting (Verite and Journet 1977; Broderick and Lane 1978; Kaufmann and Lupping 1979; Madsen 1982; Kaim *et al.* 1987; Hamilton *et al.* 1992; Gulati *et al.* 2002*a*). In low yielding dairy cows in India, for example, the feeding of 1 kg of formaldehyde-treated oilseed protein meal supplement containing 248 g RUP significantly increased milk yield by 10% compared with untreated protein meal supplements, in cows and buffaloes producing 8–12 L and 12–14 L of milk respectively (Garg *et al.* 2002, 2003). Although it is generally accepted that RUP supplements have a more beneficial role in high producing cows, where microbial protein synthesis is not capable of supplying adequate protein and essential amino acids to meet requirements (NRC 2001), there is clearly a use for these supplements in lactating ruminants fed low quality forage and/or straw-based diets. The beneficial effects of RUP supplements in these latter situations may be due to an increase in dry matter intake, which has been reported previously (Egan 1977; Lee *et al.* 1985) as well as the increased supply of metabolisable protein and amino acids. Crop residues form the bulk of the basal diet of ruminant animals in India, resulting in a deficiency of nutrients for optimal microbial output and metabolisable protein and/or amino acids. As indicated above, cows supplemented with 1 kg of protected sunflower meal will provide an additional 248 g of RUP. Assuming an 80% digestibility of the protein and an efficiency of use of metabolisable protein for lactation of 0.67 (NRC 2001) then about 133 g of additional protein would be available from the supplement; this would be sufficient to meet the extra protein requirement for the 10% increase in milk yield in low yielding animals. Furthermore, the recent results of White *et al.* (2004) suggest that this type of RUP supplement may be beneficial in increasing the protein yield of cows' milk (control 636 g/day *v.* RUP 672 g/day; $P = 0.03$) in Mediterranean environments during the summer months to overcome a deficiency of metabolisable amino acids.

In a comprehensive review of the effects of RUP on dairy cow performance, Santos *et al.* (1998) concluded that although responses were variable, chemically treated soybean meal and fish meal were the most effective in increasing milk yield. Other sources of rumen protected vegetable proteins such as heat-treated rapeseed meal or soybean meal produced variable responses in milk yield and protein content (Santos *et al.* 1998) Their reasons for this include variation in the degree of protein protection and digestibility of the constituent amino acids in the small intestine. This variation demonstrates the need to ensure that RUP supplements should be of consistent quality with respect to rumen protection, bio-availability and digestibility of the essential amino acids in the small intestine. Such characteristics are essential with respect to improving milk yield, protein content and also in investigating the benefit of RUP on the pattern of nitrogen excretion. The challenge here is to reduce nitrogen losses to the environment by

Table 1. Nutritional properties of formaldehyde-treated oilseed protein meals for milk production

RUP, rumen undegraded protein; RDP, rumen degradable protein; n.d., not determined

	Crude protein (%)	Protein protection[A] (%)	ADIN[B]	NDIN[B]	RUP (g/kg)	RDP (g/kg)	Bioavailable lysine (%)[C]
Sunflower	33 ± 0.9	73 ± 1.1	4.1 ± 0.11	5.3 ± 0.12	241	89	82
Soybean	51 ± 1.3	77 ± 0.5	5.5 ± 0.25	1.5 ± 0.09	393	117	84
Canola	40 ± 0.9	75 ± 0.9	4.2 ± 0.13	2.8 ± 0.16	300	100	85
Canola[D]	32 ± 0.4	78 ± 0.4	n.d.	n.d.	250	70	n.d.

[A]Protection of protein from rumen degradation was measured by *in vitro* incubation with rumen fluid and measuring ammonia release (Ashes *et al.* 1979). See section on nutritional properties of treated oilseed meals for details of procedures. *In vitro* rumen protection of unprotected protein meals were: sunflower meal 29%; soybean meal 31%; canola meal 51% and are similar to *in situ* values of Stern *et al.* (1994); NRC (2001).

[B]Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were determined by the methods of Schroeder *et al.* (1996).

[C]Bioavailable lysine was determined by the method of Carpenter (1966).

[D]*In situ* nylon bag protein protection on this batch of canola protein meal was 71% (White *et al.* 2004). The correlation between soybean meal equivalent value obtained by *in vitro* ammonia release and *in situ* protein degradation for meat, bone and poultry by-product meals is $r = 0.92$, $P<0.01$ (Herold *et al.* 1996).

strategically using RUP supplements to decrease the amount of crude protein required in the diet to sustain milk production and quality (Castillo *et al.* 2001). Moreover, the use of blends of formaldehyde-treated oilseed meals provides the opportunity to improve the amino acid balance of the RUP supplements and also ensure that other rate limiting amino acids beyond methionine and lysine are readily available; this is the advantage of using RUP supplements rather than protected individual amino acids.

Effects on wool and body growth

The original concept to use formaldehyde to protect proteins from ruminal degradation was pioneered by Ferguson *et al.* (1967) and Ferguson (1975); the aim was to deliver more RUP and in particular, sulfur-amino acids to the small intestine to increase wool growth. Research has concentrated on the use of protected proteins to increase nitrogen and amino acid flow to the small intestine, wool growth and body growth of lambs, calves and steers (Faichney 1970; Faichney and White 1977; Faichney and Lloyd Davies 1973; Spears *et al.* 1985; Ashes *et al.* 1995; White *et al.* 2000). Two important criteria have been defined: (i) the degree of protein protection required to improve bio-available lysine, nitrogen utilisation and growth rates of steers is around 55–60% (Fig. 2); (Spears *et al.* 1985; Ashes *et al.* 1995); and (ii) more positive responses to the inclusion of RUP supplements is likely to occur in ruminants exhibiting compensatory growth or in the physiological stage of maximum growth where protein and essential amino acids requirements are highest (Owens *et al.* 1993; NRC 1996). As with high producing dairy cows, the role of RUP supplements in reducing urea and ammonia excretion in beef feedlots, without compromising performance merits further investigation. Moreover, the strategic use of RUP supplements to offset the negative effects of intestinal parasites on protein and amino acid metabolism and to improve immuno-competence and resilience to infection, requires more experimentation (Walkden-Brown and Kahn 2002; Steel 2003). There is also a need to further identify the mechanisms and physiological significance of RUP supplements on hormones controlling intermediary metabolism including plasma insulin, which regulates protein and fat synthesis and is elevated by increased supply of protein to the small intestine.

Table 2. Effect of formaldehyde-treated oilseed meals on milk parameters

Untreated protein meals were compared with formaldehyde treated (F-Treated) protein meals in each trial
$*P<0.05$; $**P<0.001$

Reference	Oilseed protein meal (kg/day)	Lactating ruminants	Stage of lactation (days)	Diet	Comparison	Milk parameters Yield (kg/day)	Protein (%)	Fat (%)
Verite and Journet (1977)	Soybean/Rapeseed (Trial 1) 3:1 1.5	Cows (*n* = 24) Holstein	91	Maize silage, grain/lucerne hay (fed ~*ad libitum*)	Untreated	24.6	3.1	2.5
					F-Treated	25.5	3.0	2.6
	(Trial 2) 1:1 1.5	Cows (*n* = 24) Holstein	91	Maize silage, grain/lucerne hay (diet ~restricted)	Untreated	27.0	3.2	3.9
					F-Treated	28.9*	3.2	3.8
Madsen (1982)	Soybean 1.7	Cows (*n* = 24) Red Danish	70	Fodder beets/concentrate Barley straw	Untreated	22.8	3.3	4.0
					F-Treated	25.8**	3.1	3.9
Lundquist *et al.* (1986)	Soybean 1.0	Cows (*n* = 48) Holstein	112	Corn silage/alfalfa hay/concentrate	Untreated	28.7	3.2	3.4
					F-Treated	29.5*	3.0	3.2
Kaim *et al.* (1987)	Soybean 1.4	Cows (*n* = 93) Holstein	21	Maize silage/vetch hay/concentrate	Untreated	34.6	3.3	3.6
					F-Treated	36.3	3.3	3.5
Hamilton *et al.* (1992)	Sunflower 1.1	Cows (*n* = 45) Fresian	30	Pasture Kikuyu	Untreated	17.8	2.9	3.4
					F-Treated	18.9*	3.0	3.4
Sampath *et al.* (1997)	Groundnut 1.0	Cows (*n* = 14) Crossbred	15	Straw-based	Untreated	7.8	not available	
				Straw-based	F-Treated	9.4*	not available	
Gulati *et al.* (2002*a*)	Sunflower 1.0	Cows (*n* = 20) Fresian	90	Pasture Kikuyu	Untreated	35.3	3.0	3.5
					F-Treated	36.7*	2.9	3.8
Garg *et al.* (2002)	Sunflower 1.0	Cows (*n* = 20) Hf × Jersey Crossbred	100	Straw-based	Untreated	8.4	3.3	4.4
					F-Treated	9.5*	3.5	4.6
Garg *et al.* (2003)	Sunflower 1.0	Buffaloes (*n* = 16) Mehsani	40	Straw-based	Untreated	8.5	3.5	6.7
					F-Treated	9.3*	3.7	7.1
White *et al.* (2004)	Canola 2.2	Cows (*n* = 60) Fresian	83	Grass silage + concentrate	Control	21.7	2.9	4.0
					F-Treated	22.7	3.0[A]	3.8

[A]In White *et al.* (2004), the control group was untreated lupin meal *v.* a formaldehyde-treated canola meal. An increase in milk protein was observed (control 636 g/day *v.* RUP 672 g/day; $P = 0.03$).

Nutritional properties and role of rumen undegraded fat and rumen undegraded protein (RUF/RUP) supplements produced by emulsification and formaldehyde treatment of oilseed

Background

Since Reiser (1951) discovered that the C_{18} unsaturated fatty acids present in the diet of the ruminants were effectively biohydrogenated by rumen microorganisms, the following aspects of lipid metabolism in ruminants have been reported:

(i) The major pathways of biohydrogenation of linolenic, linoleic and oleic to stearic acid have been elucidated (Harfoot 1981);

(ii) C_{18} *trans* fatty acids are the final precursors for the formation of stearic acid in the hydrogenation sequences and their accumulation in the rumen is indicative of metabolic disturbances to the normal pattern of fat metabolism (Harfoot 1981);

(iii) Conjugated linoleic acid, for example 9 *cis* 11 *trans* octadecadienoic has been identified as intermediary in the sequence of biohydrogenation or can also be synthesised *de novo* from *trans* 11 octadecadienoic acid (Griinari *et al.* 2000);

(iv) C_{20} and C_{22} polyenoic fatty acids present in fish oil are hydrogenated at low concentrations in the rumen (e.g. <1 mg/mL of rumen fluid) and as their concentration increases the degree of hydrogenation is reduced and abnormal amounts of C_{18} *trans* and hydroxy fatty acids accumulate in the rumen (Gulati *et al.* 1999*a*; Chilliard *et al.* 2001; Kitessa *et al.* 2002) and;

(v) Inclusion of fat supplements in the diets of high producing ruminants is potentially beneficial because of their increased energy density, the direct transfer of long-chain fatty acids into milk and body tissues and the bioactive role of specific n-3/n-6 fatty acids in improving the efficiency of nutrient partitioning and reproductive function (Bauman *et al.* 2001; Wilkins *et al.* 1996). To achieve these benefits, ideally the fat supplements should be highly protected from ruminal metabolism (e.g. >75%) and the constituent fatty acids be designed in terms of composition and proportions to produce the desired production and quality goal, e.g. reduced milk fat as a result of protecting CLAs, soft healthy milk fat or improved reproductive performance by feeding rumen protected n-6 and n-3 fatty acids.

However, inclusion of unprotected or poorly protected fat supplements containing, in particular, C_{18} and C_{20} unsaturated fatty acids in the diet of ruminants, reduces dry matter intake, fibre digestion and often decreases the protein content of milk; these effects have been comprehensively reviewed (Schroeder *et al.* 2004).

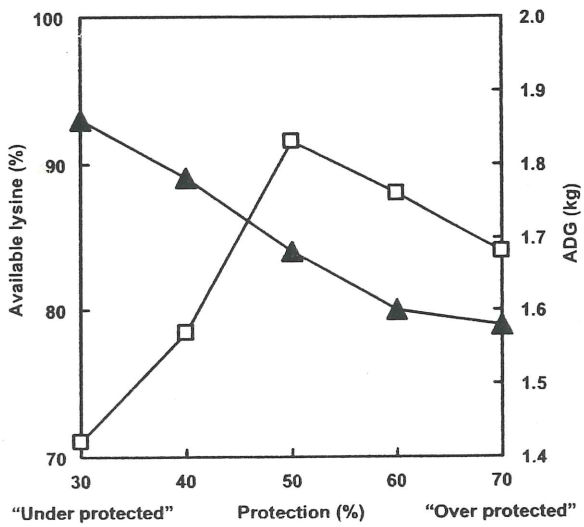

Figure 2. Effect of increasing the protection of sunflower protein meal on the bioavailable lysine (▲) and average daily weight gain in steers (□) (Ashes *et al.* 1995).

Nutritional properties and design characteristics of RUF and RUP supplements derived from oilseeds

The original concept to protect dietary fat supplements from ruminal metabolism was developed in the 1970s by Scott and Cook (1971); much of the early research concentrated on the use of vegetable oil and casein formulations in which the protein was solubilised in water under alkaline conditions (pH 10–11) and emulsified with oil before treatment with formaldehyde. The formaldehyde cross-linked with the protein primarily via the ɛ́-NH2 group of lysine and formed an envelope or matrix of rumen inert protein, which in turn protected the oil from ruminal lypolysis and biohydrogenation (Fig. 3) (Scott and Cook 1971).

During this early phase the major thrust of the research effort focussed on using this type of RUF and RUP supplement to significantly increase the proportions of C_{18} polyunsaturated fatty acids, particularly linoleic acid in meat and milk — at that time a target of >20% $C_{18:2}$ was considered nutritionally desirable (McDonald and Scott 1977). In recent years, the methods of manufacturing rumen protected fat and protein from oilseeds have been substantially modified using computer controlled process engineering; this has improved the quality and enabled the manufacture of fat supplements with specific fatty acids designed for different production and quality end-points (Scott and Ashes 1993; Ashes *et al.* 1995; Gulati *et al.* 1995).

A comparison of the degree of rumen protection for a range of fat supplements is shown in Figure 4; this demonstrates that the emulsification and formaldehyde treatment of oilseeds is the most effective process for protecting fats from ruminal metabolism. Recent results of Petit (2003) show that the direct treatment of oilseeds with formaldehyde without prior emulsification procedures is ineffective in protecting polyunsaturated fats against ruminal

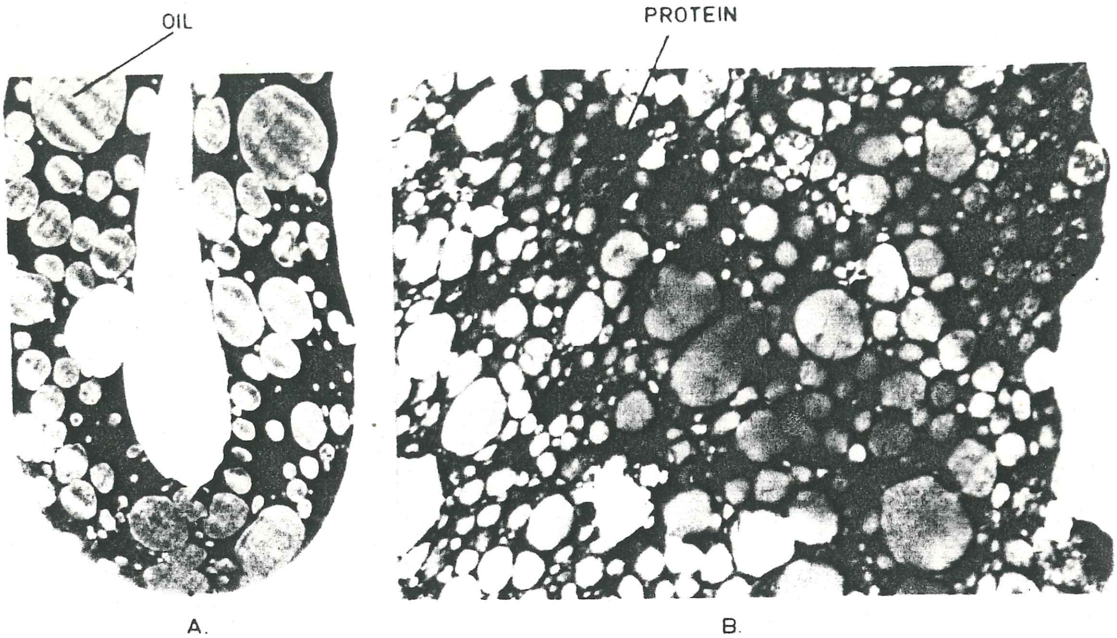

Figure 3. Electron micrograph of a protected oilseed supplement. Oil droplets are embedded in a matrix of inert protein: (A) spray dried; (B) flash dried.

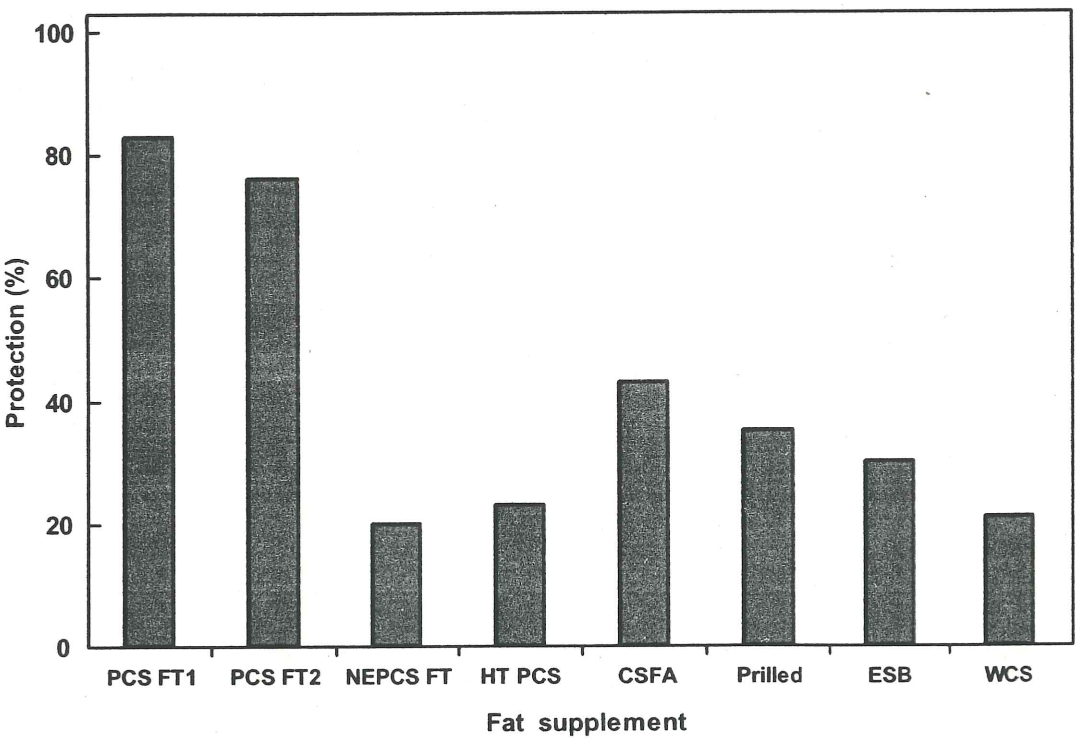

Figure 4. Comparison of the degree of rumen protection of a range of fat supplements. Protection was determined by measuring the bio-hydrogenation of $C_{18:2}$ fatty acids occurring after a 24 h *in vitro* incubation of fat supplements with rumen fluid (for further details see Gulati *et al.* 1997). PCS FT1, canola/soybean protected with formaldehyde (Gulati *et al.* 1997); PCS FT2, canola/soybean protected with formaldehyde (Tymchuk *et al.* 1998; NEPCS FT, non emulsified canola/soybean protected with formaldehyde (Gulati, unpublished data); HT PCS, heat treated protected Canola/soybean (Tymchuk *et al.* 1998); CSFA, calcium salts of fatty acids; prilled fat; ESB, extruded soybean seed; WCS, whole cotton seed (Adapted from Gulati *et al.* 1997).

biohydrogenation. A more detailed summary of the nutritional components of a formaldehyde treated canola–soybean oilseed blend (70/30; w/w) is given in Table 3. This supplement contains about 26% protein, 35% fat and the dry matter content is around 90% (Zinn *et al.* 2000).

The supplement contained 75–80% of rumen undegradable fat and the digestibility of the individual C_{18} fatty acids in feed-lot cattle ranged from 89 to 92% (Table 3; Zinn *et al.* 2000). Moreover, there was a synergistic effect on the absorption of saturated fatty acids, e.g. stearic acid; Zinn *et al.* (2000) calculated that for every 1% increase in oleic acid reaching the small intestine, there was a 1% increase in digestibility of stearic acid. Such synergism improves the nutritional value of fat supplementation and also signals the need for more experimentation on optimising the combination of specific fats, e.g. medium-chain triglycerides (C_{10}–C_{12} fatty acids) blended with C_{16} and C_{18} unsaturated triglycerides for milk production and intramuscular fat deposition. This synergy is also likely to occur if calcium salts of predominantly long chain saturated fatty acids are blended with formaldehyde-treated rumen protected oilseed supplements — this strategy has the potential to significantly improve the net energy value of dietary fat supplements and to reduce the proportion of saturated fats in milk and meat (Scollan *et al.* 2003).

In terms of the protein component of the blended oilseed supplement, about 80–90% is rumen undegradable and about 80% of the individual essential amino acids are digested in the small intestine (Table 3). Hence, these forms of oilseed supplements significantly increase protein and essential amino acid supply as well as enhancing the nutritional properties of the fat component for human consumption.

Production and quality effects from feeding RUF and RUP supplements derived from oilseeds

The primary reason for feeding formaldehyde-treated oilseed supplements to ruminants is that the fat composition can be designed to achieve very specific goals with respect to production parameters and/or quality of derived animal products.

From the examples given in Table 4 and the fatty acid profiles of milk and meat in Tables 5 and 6, the following points can be made:

(i) A blend of rumen protected canola–soybean oilseeds increases the fat content of milk but does not decrease the protein content or yield significantly, enhances the proportion of C_{18} unsaturated fatty acids and reduces the saturated fatty acid content of milk. This modification of milk fat composition is achieved either with cows fed total mixed rations (Ashes *et al.* 1997) or grazing pasture and fed fat supplements during milking (Gulati *et al.* 2002*b*). This type of milk fat is much softer and the butter produced can be spread directly from the refrigerator (Gulati *et al.* 1999*b*, 2000*b*). The inclusion of additional Vitamin E (600 IU) in the diet of the lactating cows fed these fat supplements ensures

Table 3. Nutritional properties of rumen undegradable fat and protein supplements produced from formaldehyde-treated canola/soybean oilseed

Fat (%)	Protection (%)	RUF[A] (g/kg DM)	RDF[B] (g/kg DM)	Fatty acid (%; w/w)		Intestinal digestibility[C]	Reference
26.3	80	210.4	52.6	C18:1	37.5	92.4	Zinn *et al.* (2000)
				C18:2	38.2	89.4	
				C18:3	8.1	92.5	
Protein (%)	**Protection (%)**	**RUP[D] (g/kg DM)**	**RDP[E] (g/kg DM)**	**Essential amino acids (%; w/w)**			
34.9	81	282.7	66.3	Threonine	1.5	79.0	Zinn *et al.* (2000)
				Methionine	0.3	76.2	
				Isoleucine	1.7	78.0	
				Leucine	2.7	83.3	
				Phenylalanine	1.9	79.3	
				Lysine	1.9	81.4	
				Histidine	1.0	84.1	
				Arginine	2.9	86.2	

[A]RUF, Rumen undegraded fat; measured by *in vitro* rumen incubation procedures and estimating the hydrogenation of fatty acids (Gulati *et al.* 1997).
[B]RDF, Rumen degraded fat.
[C]Intestinal digestibility was measured by techniques described by Zinn *et al.* (2000).
[D]RUP, Rumen undegraded protein; measured by *in vitro* rumen incubation procedures described under nutritional properties of treated oilseed protein meals (Ashes *et al.* 1979).
[E]RDP, Rumen degraded protein.

Table 4. Design characteristics and applications of rumen-protected fat/protein supplements

Oil/seed	Fat (%)	Protein (%)	Essential/bioactive fatty acids	Applications: Production	Applications: Quality	Reference
Canola/soybean (70/30; w/w)	27–33	30–34	C18:1, C18:2, C18:3	*Milk* Trend to increased milk yield Increased fat yield Protein yield unchanged	Softer healthier fat Lowers LDL cholesterol in humans	Ashes *et al.* (1992) Noakes *et al.* (1996) Ashes *et al.* (1997) Gulati *et al.* (1999*b*) Poppitt *et al.* (2002)
				Meat Improved NE (m) & feed efficiency Increased fat content & dressing %	Softer fat Reduced saturated fats Improved P:S ratios	Ashes *et al.* (1993) Gulati *et al.* (1995) Zinn *et al.* (2000)
Cotton/soybean (80/20; w/w)	34–38	33–37	C18:2	Improved reproductive performance, higher conception rates, lower embryonic mortality		Wilkins *et al.* (1996)
Soybean/fish oil (70:30; w/w)	32–36	33–37	C20:5; C22:6	*Milk* No negative effect on performance	Higher n-3 fatty acid content & softer fat Increased CLA and trans fatty content	Ashes *et al.* (2000) Gulati *et al.* (2002*b*) Kitessa *et al.* (2004)
				Meat No negative effect on performance	Higher n-3 fatty acid content Leaner carcass?	Ashes *et al.* (2000) Kitessa *et al.* (2001)
CLA/casein (50/50; w/w)	33–36	30–34	9cis, 11trans 10trans, 12 cis	*Milk* 30–40% reduction in milk fat Energy efficiency Nutrient partitioning	Higher CLA content	de Veth *et al.* (2003) Gulati *et al.* (2004)
Soybean/linseed/sunflower (70:22:8; w/w/w)	31–35	36–38	C18:1, C18:2, C18:3	*Meat* Similar dry matter intake, liveweight gain and carcass weight	Reduced saturated fats Improved P:S ratios	Scollan *et al.* (2003)
Soybean/high oleyl sunflower (70:30; w/w)	44–47	35–38	C18:1	*Milk* No change in production performance	Softer butter fat High in C18:1 cis	Gulati *et al.* (2000*a*)
Soybean/linseed (70/30; w/w)	32–35	36–38	C18:3	*Milk* No change in production performance	Increased n-3 content Softer butter fat	Gulati *et al.* (2002*b*)

Table 5. Fatty acid profiles of milk, subcutaneous adipose tissue and muscle from ruminants fed designer oilseed based RUF/RUP supplements

Product	Supplement	Quantity fed (kg)	Fat intake (g/day)	Fatty acid intake (g/day)		Major fatty acid composition (%; w/w) C 12:0	C 14:0	C 16:0	C 18:0	C 18:1 t	C 18:1 c	C 18:2	C 18:3	CLA	C 20:5	C 22:6	Reference
Cow milk	Canola/soybean	0.00				3.1	9.9	26.5	10.8	5.0	25.7	2.6	0.8				Gulati *et al.* (2002*b*)
	(70/30; w/w)	2.00	600	C18:1	282.0												
				C18:2	138.0	3.0	8.9	19.8	12.5	4.8	32.6	7.6	2.2				
				C18:3	42.0												
Cow milk	Soybean/fish oil	0.00				2.4	9.0	25.6	14.7	4.5	23.7	2.7	0.9				
	(70/30; w/w)	2.00	736	C20:5	23.9												
				C22:6	85.4	2.9	8.8	23.0	11.4	5.8	19.7	6.5	1.3		0.6	1.1	Kitessa *et al.* (2004)
Sheep milk	Soybean/fish oil	0.00				2.4	9.0	25.6	14.7	4.5	23.7	2.7	0.9	1.4	0.0	0.0	Kitessa *et al.* (2003)
	(70/30; w/w)	0.12	44	C20:5	1.3												
				C22:6	6.0	2.9	8.8	23.0	11.4	5.8	19.7	6.5	1.3	1.5	0.6	1.1	
Cow milk	Soybean/linseed	0.00				2.8	10.5	30.2	10.4	2.9	22.3	2.5	0.6				Gulati *et al.* (2002*b*)
	(70/30; w/w)	1.50	570	C18:3	223.0	2.8	9.7	26.3	11.0	2.6	21.5	5.5	5.1				
Beef	Canola/soybean	0.00					2.8	26.1	13.8		45.6	1.5	0.3	0.8			Ashes *et al.* (1993)
Subcutaneous	(70/30; w/w)	0.90	270	C18:1	165.0												
adipose tissue				C18:2	53.0		2.7	22.0	12.7		48.0	4.7	1.5	1.2			
Beef	Cottonseed	0.00					2.8	26.4	17.8		44.5	2.2					Gulati *et al.* (1996)
Subcutaneous		1.14	433	C18:2	243.0		3.1	27.3	21.9		32.9	10.4					
adipose tissue																	
Muscle	Megalac control	0.40	360	C18:2	106.7		4.1	28.9	11.9	2.7	36.1	1.1	0.4				Scollan *et al.* (2003)
Subcutaneous	Soybean/linseed/	0.92	337	C18:3	44.6		4.2	26.0	11.2	2.1	34.9	4.9	2.0				
adipose tissue	sunflower																
	(70:22:8; w/w/w)																
Lamb	Soybean/fish oil	0.00					2.4	23.2	16.4	3.3	32.5	4.6	0.8		0.6	0.4	Kitessa *et al.* (2001)
Muscle	(70:30; w/w)	0.11	42	C20:5	1.5												
(L. dorsi)				C22:6	5.6		2.8	23.2	15.1	4.6	25.9	8.3	1.1		1.8	1.5	

oxidative stability of milk and dairy products with a modified fatty acid composition (Ashes *et al.* 1997). Consumption of milk and dairy products (Noakes *et al.* 1996) or butter alone (Poppitt *et al.* 2002) containing the modified fatty acid profile significantly reduced the plasma low density lipoproteins (LDL) in humans and these have desirable nutritional properties for the dietary management of cardiovascular disease.

(ii) Likewise with meat the feeding of canola–soybean oilseed supplements increases the proportion of total fat and C_{18} unsaturated fatty acids and reduces the content of saturated fatty acids (Tables 5 and 6) (Ashes *et al.* 1995; Gulati *et al.* 1995; Scollan *et al.* 2003).

(iii) If the aim of fat supplementation is to specifically increase the proportion of the C_{20} and C_{22} n-3 fatty acids in meat and milk then a fish oil and soybean supplement is fed.

(iv) Similarly if the goal is to increase the C_{18} n-3 content of ruminant tissues and/or products and/or alter the balance between n-3 and n-6 fatty acids, then a supplement is designed by blending different proportions of linseed (flax), canola and sunflower oilseeds (Tables 4 and 5).

(v) A high oleyl oilseed based supplement can be used to specifically increase the proportions of C_{18} monounsaturated fatty acids in milk fat (Table 4; Gulati *et al.* 2000*a*).

(vi) For improving reproductive performance, a supplement containing cotton and soybean oilseeds has been used; it contained a high proportion (50–60%) of linoleic acid which inhibits cyclo-oxygenase activity and biosynthesis of prostaglandin F2-α in endometrial tissue. This in turn reduces early embryonic mortality and improves pregnancy rates. Hereford cows ($n = 143$) at pasture were fed 1 kg/h.day cottonseed meal (37% crude protein; <3% fat) for 2 weeks thereafter, a group was allocated at random to be fed an equivalent amount of formaldehyde treated cotton oilseed (35% CP and 35% crude fat). The supplements were fed for a total of 8 weeks. The conception rates were higher in cows fed formaldehyde-treated oilseed for both the first (61% *v.* 46%) and second (71% *v.* 56%) cycles resulting in a significantly higher pregnancy rate for the 2 cycles (77% *v.* 61%) ($P<0.05$) (Wilkins *et al.* 1996). In the future there is scope to design fat supplements containing increased proportions of n-3 fatty acids as they also effect prostaglandin synthesis and improve fertility. Moreover, spermatozoa derived from ruminant species contain very high proportions of docosahexaenoic acid ($C_{22:6}$) and there may be scope to improve male fertility by feeding fat supplements containing specific proportions of n-3 fatty acids.

(vii) Where a fat supplement is required to influence nutrient partitioning, reduce milk fat secretion and improve energetic efficiency, then a soybean or casein and CLA product is used (Shingfield *et al.* 2004). Preferably the proportion of the *trans* 10 *cis* 12 CLA should be as high as possible because this is the bioactive fatty acid with respect to inhibiting mammary gland lipogenesis and reducing milk fat yield by 30–40% (de Veth *et al.* 2003; Gulati *et al.* 2004) More research is required on this type of fat supplement because it has the potential to significantly improve the efficiency of milk and meat production and to manipulate fat content.

Efficiency of transfer of rumen protected fatty acids into milk fat

There are 2 principal reasons to examine the apparent transfer efficiency of protected dietary fatty acids into milk fat: (i) it assists in predicting how much fat supplement should be fed to produce the desired change in the proportions of individual fatty acids, and (ii) to establish the most cost effective strategy for fat supplementation.

The apparent transfer efficiency of C_{18}, C_{20} and C_{22} fatty acids into milk fat are summarised in Table 7. There is variation in transfer efficiencies but in general terms, the relative efficiency of incorporation of $C_{18:2}$ and $C_{18:3}$ (i.e. 30–50%) is higher then the C_{20} and C_{22} polyenoic fatty acids (ie 10–25%). The percent transfer is influenced by the amount of fat and proportion of constituent fatty acid; e.g. with respect to $C_{18:2}$ transfer, a maximum is reached around 600 g fat/day of which about 140 g is $C_{18:2}$ (Table 6). With respect to C_{20} and C_{22} unsaturated fatty acids, the transfer efficiency of the protected form is higher than that obtained by Chilliard *et al.* (2001) for the unprotected fatty

Table 6. Comparison of subcutaneous fatty acid composition from lot-fed cattle

Genotype & ration	Saturated (%; w/w) Σ14 + 16 + 18	Monounsaturated (%; w/w) Σ16:1 + 18:1	Polyunsaturated (%; w/w) Σ18:2 + 18:3	Ratio of unsaturated to saturated
Angus, grain fed	42	49	2.5	1.22
Angus, fed grain and protected lipid[A]	32	55	7.5	1.96
Wagyu[B]	31	59	2	1.97

[A]Protected lipid was a combination of canola/soybean (70/30; w/w) oilseed (Gulati *et al.* 1995).
[B]Data from May *et al.* (1993).

Table 7. Apparent transfer efficiency of formaldehyde-treated rumen protected n-3 and n-6 fatty acids into milk fat

Species	Supplement	Supplement intake (kg)	Fat intake (g/day)	Fatty acids	Fatty acid intake (g/day)	Transfer efficiency (%)	Reference
Cow	Canola/soybean (70/30: w/w)	1.70	507	C18:1	313	38	Tymchuk *et al.* (1998)
				C18:2	92	44	
				C18:3	44	38	
Cow	Cottonseed	2.00	640	C18:2	358.4	43	Simos *et al.* (2000)
Cow	Canola/soybean (70/30: w/w)	1.00	300	C18:1	141	34	Gulati *et al.* (2002*b*)
		2.00	600	C18:1	282	49	
		3.00	900	C18:1	423	28	
		1.00	300	C18:2	69	32	
		2.00	600	C18:2	138	41	
		3.00	900	C18:2	207	25	
		1.00	300	C18:3	21	32	
		2.00	600	C18:3	42	41	
		3.00	900	C18:3	63	28	
Cow	Soybean/linseed (70/30: w/w)	1.50	570	C18:3	223	24	Gulati *et al.* (2002*b*)
		3.00	1140	C18:3	446	19	
Cow	Soybean/fish oil (70/30: w/w)	0.60	225	C20:5	8	9	Gulati *et al.* (2002*b*)
		1.50	563	C20:5	19	24	
		3.00	1125	C20:5	39	21	
		0.60	225	C22:6	29	10	
		1.50	563	C22:6	73	14	
		3.00	1125	C22:6	145	10	
Sheep	Soybean/fish oil (70/30: w/w)	0.12	44	C20:5	1.3	21	Kitessa *et al.* (2003)
				C22:6	6	18	
Buffalo	Canola/soybean (70/30: w/w)	0.50	150	C18:2	42	24	Gulati *et al.* (2003)

acids, i.e. $C_{20:5}$ are 2.6% and $C_{22:6}$ are 4.1%. The calculations in Table 8 summarise how this information can be used to design a supplemental fat feeding strategy to produce milk containing a soft, healthier fat that lowers LDL cholesterol in humans (Noakes *et al.* 1996).

Conclusion and future challenges

It is clear that an effective technology exists to optimally protect a range of oilseed -derived nutrients, i.e. protein and fat from ruminal metabolism. These can be used to increase the supply of RUP, RUF, essential amino acids and essential/bioactive fatty acids for improving efficiency of production and quality of animal products. They provide a practical feeding strategy to improve reproductive performance, regulate nutrient and energy partitioning, manipulation of the fat content and fatty acid composition of milk and meat. Fat/protein supplements can now be designed, manufactured and fed to produce a fat with an array of specific fatty acids/triglycerides to meet the diverse market requirements of food manufacturers and consumers. Likewise, with respect to improving efficiency of nutrient use or reproductive performance fat/protein supplements can

Table 8. An example of the calculations used to estimate the amount of protected dietary fat supplement required to produce fat modified milk with 8% (w/w) linoleic acid ($C_{18:2}$)

If milk production per day is ~30 L
Fat content ~3.5%
Fat yield ~1050 g per day
The percentage of C_{18} unsaturated fatty acids required is ~40–45% of which $C_{18:2}$ is ~8%, $C_{18:1}$ 32%, $C_{18:3}$ is 2% (see Table 5) i.e. the cow needs to secrete an additional 60 g of $C_{18:2}$/day in milk fat.
How much canola/soybean supplement (S) is required?
Fat content of supplement is ~35%
Linoleic acid content is 28% of total fat
Degree of rumen protection is 75%
% Transfer of linoleic acid into milk is ~40%
Supplement required = S
$S \times 0.35 \times 0.28 \times 0.75 \times 0.4 = 60$
$0.0294S = 60$
Therefore fat supplement required per day (S) = 2.04 kg

be designed to contain the desired proportions of essential bioactive fatty acids required.

The challenge is to optimise the use of these designed fat/protein supplements in different ruminant production systems that exist globally. For example, in the developed countries more emphasis is placed on efficiency of production and quality of milk/meat from animals of high genetic potential. Therefore, it would seem desirable to design fat/protein supplements that target the rate limiting steps in production and quality, these include:

(i) Reproductive efficiency. Longer inter-calving intervals and low pregnancy rates in the first 100 days post-calving are major problems in high producing dairy cows. Therefore future research could focus on a more precise definition of the type of fatty acids required in a fat supplement for the first 100 days of lactation. For example, should rumen protected CLAs be fed to reduce milk fat secretion and thus decrease the amount of body fat mobilisation? This could have important implications in overcoming the negative energy balance that is so prevalent in early lactation. Alternatively, should CLAs be fed alone or in combination with a fat supplement containing the desired properties of n-3 and n-6 fatty acids, which influence ovarian function, prostaglandin synthesis, pregnancy and embryonic survival?

(ii) Fatty acid utilisation and product quality. For many years the scientific emphasis has focussed on the need to improve the fatty acid composition of ruminant milk and meat products because they contain high proportions of saturated fatty acids in particular myristic (in milk) and palmitic (in meat and milk), which elevates plasma LDL cholesterol, a known risk factor in heart disease. As mentioned above rumen protected fat/protein supplements can be designed and fed to ruminants to significantly lower saturated fats. The challenge is to develop vertically integrated production/processing systems that would encourage the adoption of the technology and ensure the availability of healthy fat-modified meat and milk products.

A further challenge with respect to fat utilisation is to design fat/protein supplements that enhance energetic efficiency and increase intramuscular fat deposition (marbling), which is an important trait in meat quality. With respect to marbling there may be scope to design a fat supplement that does not inhibit endogenous lipogenesis but provides additional fatty acids for either energy or direct incorporation into muscle adipocytes. Ideally this type of fat supplement should contain low proportions of polyenoic fatty acids (e.g. $C_{18:2}$, $C_{18:3}$, $C_{20:5}$, $C_{22:6}$) because they inhibit lipogenesis (Clarke 2000), sufficient medium chain length fatty acids (C_{10-12}) for oxidation/energy and a predominant amount of C_{16}/C_{18} long chain fatty acids for incorporation into muscle triglycerides. Furthermore, should a fat/protein supplement designed to increase marbling contain rumen-protected lecithin to provide a source of choline, a lipotropic factor that influences fat utilisation and meat quality (Bindel *et al.* 2000)?

Further research is required into the potential benefits of rumen protected fats/proteins in alleviating production losses induced by environmental stress. In particular, improved utilisation of long-chain fatty acids in heat-stressed dairy cows to offset reduced dry matter intake and concomitant losses in milk production merits further investigation.

Finally, the availability of protected fat/protein supplements containing specific fatty acid sub-types will facilitate more research in ruminants on the role of essential/bioactive lipids in gene expression, tissue differentiation, cellular signalling pathways, immune response and disease resistance. As the knowledge base expands on the nutritional significance and metabolic role of fatty acids in humans and animals, there will be opportunities to redesign the composition of protected fat supplements to improve ruminant productivity and quality of meat and milk products.

Acknowledgments

Collaborative research project supported by the Australian Centre for International Agricultural Research (ACIAR).

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Received 5 July 2004, accepted 2 May 2005