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Conjugated linoleic acid and its effects on animal products and health in single-stomached animals

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Conjugated linoleic acids (CLA) have been shown to have anti-carcinogenic, anti-obesity, anti-atherogenic and immunomodulatory functions. The basis for these effects has not been fully explained, but probably involves effects of CLA on eicosanoid metabolism, cytokine production and/or gene expression. The predominant isomer (85–90 %) in the natural sources of CLA has the *cis*-9, *trans*-11 configuration. As interest in CLA grew and synthetic forms became available, the number of studies examining the effects of dietary CLA in rodents, human subjects and livestock has increased greatly. In the late 1990s the observation that CLA had anti-obesity effects was reported. Subsequently, it was determined that this effect in mice could be attributed to the *trans*-10, *cis*-12 isomer that, along with the *cis*-9, *trans*-11 isomer, predominates in the synthetic forms of CLA. The anti-obesity response varies in magnitude depending on species, and has not been consistent in non-rodents. In general, the response is greatest in mice and less or absent in other species. The basis for this lack of consistency is not clear and is unlikely to be accounted for by differences in the source of CLA. In the pig variation in body fat of animals may account for differences in responsiveness. There is no direct evidence of an anti-carcinogenic effect of CLA in human subjects or livestock. Indirect evidence from *in vitro* studies with cell lines, as well as epidemiological studies, suggest that CLA may be relevant as a natural anti-carcinogen. The immunomodulatory effects of CLA may have application in livestock production as an alternative to the use of feed antibiotics, or as a means of improving the response to vaccination and conferring disease resistance. The recent literature on the effects of CLA, with emphasis on its anti-obesity effects, is reviewed.

Conjugated linoleic acid: Animal products: Animal health: Single-stomached animals

Conjugated linoleic acids (CLA) are a mixture of positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12-octadecadienoic acid). The double bonds in linoleic acid, as with most other polyunsaturated fatty acids, are in a methylene-interrupted arrangement, where a C with single bonds separates the two double bonds. The term 'conjugated' in CLA refers to the conjugated diene structure in which the double bonds are separated by C linked by a single bond. CLA were first identified in rumen fluid as an intermediate of the biohydrogenation process (Bartlett & Chapman, 1961). Later, the primary organism responsible (*Butyrivibrio fibrisolvens*) for the formation of CLA was identified (Kepler *et al*. 1966).

Stearic acid is the end product of the biohydrogenation process in the rumen. It is estimated that about 20 % of the dietary linoleic acid escapes the rumen intact and 74 % is hydrogenated to stearic acid. *Trans*-vaccenic acid (*trans*-11-octadecenoic acid) accounts for most of the remaining (6 %) fatty acid chain. CLA in duodenal fluid accounts for only about 0·2 % of the original linoleic acid (Scollan *et al*. 2001). The majority (80–90 %) of the CLA found in milk and tissues is present as the *cis*-9, *trans*-11 isomer (Chin

Abbreviation: CLA, conjugated linoleic acids.

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et al. 1992; Ip *et al*. 1999). Most of the CLA found in tissues of ruminant animals is probably derived from the desaturation of *trans*-vaccenic acid. Several research groups have demonstrated that *trans*-vaccenic acid is absorbed from the digestive tract, is a substrate for stearoyl desaturase and, thus, can be converted to the *cis*-9, *trans*-11 isomer of CLA (Santora *et al*. 2000; Corl *et al*. 2001). Feeding pigs a hydrogenated fat source that contained *trans*-fatty acids (but no CLA) was also shown to result in increased tissue levels of CLA (Glaser *et al*. 2000).

The levels of CLA in various food products were first reported in the early 1990s (Ha *et al*. 1989; Chin *et al*. 1992; Lin *et al*. 1995). In general, the levels are much lower in tissues of non-ruminants than in those of ruminants. In milk samples CLA content was lowest in mare's and sow's milk, intermediate in human milk and highest in goat's, cow's and ewe's milk (Jahreis *et al*. 1999). It is likely that the higher levels in human milk compared with those in milk from the sow or mare can be attributed to dietary CLA, particularly from dairy products. The CLA found in sow's milk was primarily the *cis*-9, *trans*-11 isomer and is most probably of gut origin. Initial evidence of CLA originating from hindgut fermentation in non-ruminants was provided by Chin *et al*. (1994*b*), who showed that feeding linoleic acid to conventional, but not germ-free, rats resulted in increasing tissue concentrations. However, the observation was disputed by other work (Kamlage *et al*. 1999) that failed to show tissue CLA accumulation despite evidence of intestinal microorganism production.

The CLA content of milk and meat of single-stomached animals can be increased by feeding CLA. As an example, the effect of feeding CLA on adipose tissue and milk fatty acid profile in the pig is shown in Table 1. This effect has been observed in numerous studies in which synthetically-produced CLA has been fed to fish, rodents, poultry, pigs and human subjects. The synthetic forms of CLA differ from natural sources in that the isomer content is markedly different. While the CLA found in ruminants is largely the *cis*-9, *trans*-11 isomer, synthetic CLA usually contains about equal amounts of this isomer and the *trans*-10, *cis*-12 isomer. It was when the synthetic CLA became available that the observations of anti-obesity effects were first reported.

Chin *et al*. (1994*a*) fed a mixed CLA isomer to rats during gestation and lactation and observed increased growth and improved feed efficiency in the offspring. They did not, however, determine effects on carcass lipid content. Park *et al*. (1997) reported that mice fed mixed CLA had approximately 60 % less carcass fat than control animals. This reduction was achieved with little change in intake and was confirmed by other research groups (West *et al*. 1998; DeLany *et al*. 1999). In these early studies CLA was fed at approximately 5 g/kg diet and was in the form of a free fatty acid. In some studies linoleic acid was used as a control, but the response was not different from when CLA was simply substituted for a portion of the oil in the diet. Dietary CLA has also been reported to reduce fat-pad weights in growing rats and this effect was accounted for by a reduction in adipocyte size rather than number (Azain *et al*. 2000). The response to CLA in the rat is of lower magnitude than that seen in the mouse. Feeding CLA to the genetically-obese Zucker rat resulted in an unexpected increase in fat-pad weight (Sisk *et al*. 2001).

Pure forms of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers are now available, but their cost makes it prohibitive to feed these forms to non-rodent species. In mice Park *et al*.

Table 1. Effect of dietary conjugated linoleic acid (CLA) on adipose tissue and milk fatty acid profile

Dietary group	Control	CLA-fed	SEM	*P* statistical significance of difference between groups:
Adipose Tissue*				
16 : 0	21·8	21·7	0·4	NS
16 : 1	2·9	2·5	0·1	NS
18 : 0	10·9	12·5	0·4	0·05
18 : 1	39·0	35·5	0·6	0·01
18 : 2	22·4	23·6	0·5	NS
18 : 3	1·32	1·35	0·03	NS
CLA:				
Cis-9,*trans*-11	0·16	0·67	0·06	0·01
Trans-10,*cis*-12	0·04	0·34	0·06	0·01
Milk†				
16 : 0	27·6	29·3	1·0	NS
16 : 1	9·11	6·3	0·4	0·01
18 : 0	4·2	6·3	0·2	0·01
18 : 1	36·5	34·1	1·4	NS
18 : 2	17·4	17·1	0·5	NS
18 : 3	0·81	0·64	0·03	0·01
CLA:				
Cis-9,*trans*-11	0·27	0·79	0·04	0·01
Trans-10,*cis*-12	0·00	0·70	0·04	0·01

*Adipose tissue collected from growing gilts fed diets containing 5 g CLA/kg for 6 weeks. Values are expressed as mg/100 mg total fatty acids. There was no difference in the lipid content of the adipose tissue between treatments.

†Milk samples were collected on day 21 of lactation from sows fed 0 or 5 g CLA/kg, beginning during mid gestation and continuing through lactation. Results are expressed as mg/100 mg total fatty acids. CLA feeding resulted in a 15% ($P<0·05$) decrease in milk lipid content.

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(1999*a,b*) reported that the *trans*-10, *cis*-12 isomer was responsible for the reductions seen in carcass fat in mice. Similar observations have been made in the rat (Table 2; Azain & Chi, 2001). Rats fed mixed CLA isomers showed reduced fat-pad weights and content of monounsaturated fatty acids. These effects were accounted for by the *trans*-10, *cis*-12 isomer of CLA.

Applications in livestock

As the anti-carcinogenic and anti-obesity effects of CLA were demonstrated in rodents and *in vitro* (Ip *et al*. 1994), interest in the applications of CLA in both animal and human nutrition has grown. Studies with CLA in species other than rodents and human subjects generally fit into three categories: (1) those aimed at increasing CLA content in tissues destined for human consumption as a means to increase CLA intake in human subjects; (2) those aimed at using the repartitioning effect of CLA to reduce body fat in the animal; (3) those related to a direct health benefit of CLA in the animal based on its immunomodulatory effects.

Conjugated linoleic acid studies in swine

The first reports of the effects of dietary CLA in the pig appeared in the late 1990s. The objective of these studies was to extend observations from studies in the rodent and determine whether CLA had potential as a feed additive to reduce fat in the carcass of pigs. A positive outcome would have benefit for both the producer (decreased fat is associated with increased efficiency) and the consumer. These studies utilized low levels (< 10 g/kg diet) of feed-grade CLA products containing approximately equal amounts of the *cis*-9, *trans*-11 and *trans*-10, *cis*-12 isomers. The total content of these isomers was 50–80 %. The first of these studies to appear in referred journals reported 7·7 % (Dugan *et al*. 1997) and 20 % (Ostrowska *et al*. 1999) decreases in carcass fat in pigs fed 10–20 g CLA/kg for several weeks before slaughter. A summary of these and subsequent published reports on the effects of CLA on growth performance and carcass fat in pigs is shown in Table 3. It is obvious that the response to CLA is not consistent. In addition, many other studies have been conducted but have not been published as full journal articles. Examples of these studies can be found in University reports and meeting abstracts. Reasons for the inconsistency are listed in Table 4. To generalize, it appears that CLA reduces carcass fat in pigs with > 23 mm subcutaneous fat thickness at 100 kg body weight (Ostrowska *et al*. 1999; O'Quinn *et al*. 2000*a,b*; Thiel-Cooper *et al*. 2001; Waylan *et al*. 2002; Wiegand *et al*. 2002), but not in studies where fat thickness was < 20 mm (Bee, 2001; Averette-Gatlin *et al*. 2002; M Azain, unpublished results). Related to this observation, the response to CLA was greater in barrows (26 mm fat in control group) than in gilts (20 mm fat in control group; Tischendorf *et al*. 2002). In addition, the response was greater with low-energy diets than with diets with added fat (Dugan *et al*. 2001). This latter response is in contrast to the results reported in mice where it was shown that similar reductions in carcass fat were seen in diets with 15 or 45 % energy from fat (West *et al*. 1998).

In rats a growth-promoting effect of CLA was observed in the progeny of dams fed CLA during pregnancy and lactation (Chin *et al*. 1994*b*). This same response was also observed in the pig (Bee, 2000), where it was reported that progeny of dams fed diets containing 20 g CLA/kg had 14 %

Table 2. Effect of conjugated linoleic acid (CLA) products and pure isomers on growth, intake, and selected fat-pad weights in growing female Sprague–Dawley rats* (From Azain & Chi, 2000)

Values are least-significant means for seven to eight rats per treatment								
Treatment group … Level in diet (g/kg) …	Control	CLA-60† 12	CLA-80† 10	*c*-9, *t*-11‡ 3·2	*t*-10, *c*-12‡ 3·2	Nu-Chek§ 8	SEM	Statistical significance of this effect of diet: $P <$
Body wt (g):								
Day 0	86	86	85	85	86	84	2	NS
Day 49	207	208	217	220	221	221	5	NS
Body wt gain (g/d)	2·5	2·5	2·7	2·8	2·8	2·8	0·1	NS
Intake (g/d)	15·3^{ab}	13·0^{c}	13·8^{c}	15·7^{ab}	15·8^{a}	14.2^{bc}	0·5	0·005
Feed intake/wt gain	6·2^{a}	5·2^{b}	5·1^{b}	5·7^{ab}	5·7^{ab}	5·1^{b}	0·2	0·001
Liver wt (g)	7·01	7·63	7·65	7·45	8·30	7·97	0·34	0·20
Relative wt (g/kg body wt)	3·41	3·75	3·56	3·36	3·74	3·69	0·12	0·15
Retroperitoneal pad: wt (g)	1·53^{a}	1·15^{ab}	0·81^{b}	1·43^{ab}	1·08^{b}	0·94^{b}	0·14	0·01
Monounsaturates (mg/100 mg total fatty acids):	40·5^{a}	33·7^{b}	34·0^{b}	40·2^{a}	34·2^{b}	32·2^{b}	1·3	0·01
Parmetrial pad: wt (g)	2·51^{ab}	1·43^{c}	1·60^{c}	2·71^{a}	1·99^{bc}	1·76^{c}	0·25	0·005
Monounsaturates (mg/100 mg total fatty acids)	40·6^{a}	33·8^{b}	35·4^{b}	39·9^{a}	32·3^{b}	31·1^{b}	1·0	0·01

[a,b,c]Mean values with unlike superscript letters were significantly different (P<0·05).
c, *cis*; *t*, *trans*.
*Rats were fed experimental diets for 7 weeks. The CLA content of diets with the mixed CLA products (CLA-60), CLA-80 and Nu-Chek) contained approximately 4 g/kg of both the *c*-9, *t*-11 and *t*-10, *c*-12 isomers, which corresponds to the level of each isomer in the pure products.
†Natural Lipid, Vernon Hills, IL, USA.
‡Matreya Inc., State College, PA, USA.
§Nu-chek Prep, Elysian, MN, USA.

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Table 3. Summary of published reports on the effect of dietary conjugated linoleic acid (CLA) on carcass composition in the pig

Reference	Gender	Wt range	CLA source	Diet		Response
				Control	Treatments	
Dugan *et al.* (1997)	Barrows, gilts *n* 108	62–106 kg	CLA-50 (Natural Lipid*)	Wheat–barley–SBM 13·8 MJ DE/kg No added fat	0 *v.* 20 g CLA/kg	↓SC fat (g/kg carcass), 7 % ↑Lean, 2 % No change in IM fat
Ostrowska *et al.* (1999)	Gilts *n* 60	57–107 kg	CLA-55 (Natural Lipid*)	Wheat–SBM–peas–blood meal 14·3 MJ DE/kg 200 g Soyabean oil/kg	Six diets (0–10 g CLA/kg)	↓SC fat (linear), 20 % ↑Lean Control 23·5 mm P2 fat
O'Quinn *et al.* (2000*a*) Expt 1	Barrows *n* 36	38–106 kg	MTO *v.* CLA-60	Maize–SBM No added fat	0 *v.* 5 g/kg	↓SC fat, 5 % Control 23·4 mm 10th rib
Expt 2	Barrows *n* 80	33–119 kg	MTO	Maize–SBM No added fat	Four diets 0–1 g MTO/kg	Quadratic response Average fat ↓, 5 % Control 24·4 mm 10th rib
O'Quinn *et al.* (2000*b*)	Barrows *n* 80	45–118 kg	MTO	Maize–SBM No added fat	0 *v.* 5 g MTO/kg	↓SC fat, 6–8 % Control 24.5 mm fat
Bee (2001)	Barrows, gilts *n* 24	70–98 kg	Selin-CLA (600 g CLA/kg)	Wheat–barley–oat No added fat 13·1MJ DE/kg	20 g lard/kg *v.* 20 g CLA/kg	No change in carcass fat or lean ↓SC fat, 18 % over loin, but not at rump Control 18 mm fat CLA incorporated into tissues
Dugan *et al.* (2001)	Barrows *n* 216	36–115 kg	CLA-65 ME	Wheat–Barley–SBM 14·2 or 14·5 MJ DE/kg 20 or 50 g added fat/kg	0 *v.* 2·5 and 5 g CLA/kg	↓SC fat, 11 % at 20 g added fat/kg, 3 % at 50 g added fat/kg Lean ↑, 5 % at 20 g added fat/kg fat, no change at 50 g added fat/kg
Eggert *et al.* (2001)	Gilts *n* 30	75–120 kg	CLA-60	Maize–SBM	10 g SFO/kg *v.* 10 g CLA-60/kg	CLA decreased intake ↓SC fat, 8 % (NS) Control 19·1 mm fat Increased belly firmness
Ramsay *et al.* (2001)	Barrows, gilts *n* 48	20–55 kg	Bioriginal CLA (670 g/kg)	Maize–SBM–DSM No added fat	Five diets (0–20 g CLA/kg)	No effect of CLA on composition (grower pigs)
Thiel-Cooper (2001)	Barrows *n* 40	26–114 kg	CLA-60	Maize–SBM No added fat	Five diets (0–10 g CLA/kg for SBO)	CLA, linear ↑gain ↓SC fat, quadratic, 10 % Control 28·6 mm fat Loin area ?
Tischendorf *et al.* (2002)	Barrows, gilts *n* 80	24–120 kg	CLA (550 g/kg)	Barley–SBM 20 g added fat/kg	20 g rapeseed oil/kg *v.* 20 g CLA/kg	Barrows, ↓SC fat, 11 % Control, 26 mm fat Gilts, no change Control, 20 mm fat
Waylan *et al.* (2002)	Barrows *n* 72	45–115 kg	MTO	Maize–SBM No added fat	0 *v.* 5 g MTO/kg	↓SC fat, 6 % Control, 26 mm fat
Wiegand *et al.* (2002)	Barrows *n* 92	28–115 kg	CLA-60	Maize–SBM No added fat Four treatments 0, 29, 56, 87 d on CLA	0 *v.* 7·5 g CLA/kg	↓SC fat, 14 % at 29 d 14 % at 56 d 20 % at 87 d Control, 26 mm fat
Averette-Gatlin *et al.* (2002)	Gilts *n* 144	49–113 kg	CLA-60	Maize–SBM 0 *v.* 40 g added fat/kg 14·6–15·5 MJ DE/kg	0 *v.* 10 g CLA/kg for maize oil	No effect of CLA on SC fat CLA ↑ marbling Control, 15 mm fat
Azain *et al.* (unpublished results)	Gilts *n* 33	60–105 kg	CLA-60	Maize–SBM 49 g added fat/kg 14·7 MJ DE/kg added fat	0, 5, 20 g CLA/kg	CLA, ↓SC fat, 6 % (NS) Control, 18 mm fat

DE, digestible energy; SBM, soyabean meal; SC, subcutaneous; IM, intramuscular; ↑, increase; ↓, decrease; MTO, modified tall oil, a source of CLA; ME, methyl esters.
*Vernon Hills, IL, USA.

greater body weights and 17 % greater muscle mass at 70 d of age than the corresponding controls. In response to reports that CLA reduces adipocyte proliferation *in vitro* (Brodie *et al.* 1999; Satory & Smith, 1999) CLA was fed during gestation and lactation to both rats (Poulos *et al.* 2001) and pigs (Poulos *et al.* 2000), but no indication of differences in adiposity due to maternal diet was observed.

As mentioned earlier, one of the objectives of feeding CLA has been to create a product with human health benefits. This approach is analogous to the efforts to

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Table 4. Possible explanations for the lack of a consistent anti-obesity response in the pig

Variables	Discussion
Source of CLA	Several sources of CLA have been fed. However, if tissue CLA levels are measured, it is unlikely that this factor accounts for the lack of response
Level of fat in the diet	Most of the CLA studies showing a response had low-energy diets, with little or no added fat other than the CLA and the oil used as the control or reference. A study specifically looking at responses in pigs fed 20 or 50 g added fat/kg concluded that the response was greater in low-fat diets (Dugan *et al.* 2001). Studies in rodents (West *et al.* 1998) showed that mice fed diets with 15 or 40 % energy from fat had similar responses to CLA
Gender	In Sprague–Dawley rats females respond to CLA better than males (Azain & Chi, 2001). In mice both genders seem to respond. In pigs it is reported that barrows show a greater response than gilts (Tischendorf *et al.* 2002)
Genetics and percentage lean	This factor has not been directly addressed in the pig. However, the gender effect suggests that pigs with more subcutaneous fat respond better than those with less fat
Duration of feeding	In rodents changes in fat content are seen in 1–2 weeks. In pigs changes have been noted after as little as 29 d of feeding CLA

CLA, conjugated linoleic acids.

increase *n*-3 fatty acids in animal products that were prevalent in the 1990s (for example, see Taugbol, 1993; Overland *et al*. 1996; Leskanich *et al*. 1997). In a study in which 7·5 g active CLA isomers/kg diet were fed for 87 d, the CLA content in muscle increased from being undetectable in the control group to reaching 0·71 mg/100 mg fatty acids present (Wiegand *et al*. 2002). Since intramuscular fat was approximately 5 % of the muscle weight, consumption of 100 g pork from a CLA-fed animal would contribute < 50 mg CLA to the diet. Tissue accumulation of CLA is dependent on dose in the diet (Ramsay *et al*. 2001) and, in general, accumulation in adipose tissue is greater than that in muscle (Ramsay *et al*. 2001; Wiegand *et al*. 2002). At best, it would be expected that < 10 mg/100 mg fatty acids in a muscle would be CLA, resulting in a level of approximately 5 g/kg serving of pork meat. Current estimates of CLA intake in human subjects range from 300 mg (Riserus *et al*. 2001) to 1 g/d (Ha *et al*. 1989). In order to achieve the 5 g/kg diet level of CLA used in laboratory animals to prevent cancer and reduce body fat, 2·5–5 g CLA/d (5–10 g/kg DM intake) is required in human subjects.

While it is possible to create designer heart-healthy meat and eggs enriched with *n*-3 fatty acids from fish oil or flaxseed, the benefits are offset by the negative effects of these polyunsaturated fatty acids on product shelf-life and firmness. In contrast, CLA has been reported to have beneficial effects on processing of pork. CLA has been shown to increase intramuscular fat content (Wiegand *et al*. 2002), increase product firmness (Eggert *et al*. 2001; Thiel-Cooper *et al*. 2001; Waylan *et al*. 2002) and, because of its effects on fatty acid profile, might be expected to increase product shelf-life. However, as with the carcass fat effect, the repeatability of the marbling and firmness responses has been problematic. O'Quinn *et al*. (2000*a*,*b*) reported reduced carcass fat but no change in intramuscular fat content or firmness. Eggert *et al*. (2001) saw no change in carcass or intramuscular fat, but did observe an increase in firmness with CLA feeding.

With the likelihood that use of antibiotics as feed additives will be reduced, there has been interest in the application of the immunomodulatory effects of CLA in the pig. Weber *et al*. (2001) reported that CLA-fed pigs had greater antibody titres to *Mycoplasma hyopneumoniae*, but no difference in titres to porcine reproductive and respiratory syndrome virus. It has also been shown that killer cell counts, a measure of the cytotoxic potential of mononuclear cells, were enhanced in CLA-fed pigs. Bassaganya-Riera *et al*. (2001) concluded that CLA might enhance response to viral infections in pigs. In this work CLA feeding induced increases in the percentages of CD8+ lymphocytes, which may have practical application as a means to enhance responsiveness to certain vaccines and to control diseases that cause mucosal inflammation.

Poultry

There are limited published reports on body fat in poultry. Du & Ahn (2002) reported no effect on abdominal-fat-pad weight in broilers fed up to 10 g CLA/kg from weeks 3–6 of age. They reported a 15 % reduction in body fat content of birds fed 20 or 30 g CLA/kg for 5 weeks. There were negative effects of these higher levels of CLA on meat quality (meat was harder, drier and darker). Relative abdominal-fat-pad size was reduced in broilers fed 15 g CLA/kg from 8–42 d of age (Szymczyk *et al*. 2001). However, interpretation of this response is confounded by a reduced intake (6 %), gain (11 %), and efficiency (6 %) in the CLA-fed group. CLA accumulated in breast- and leg-muscle lipid and accounted for approximately 10 g/100 g fatty acids present in birds fed 15 g CLA/kg diet.

Feeding CLA to laying hens results in incorporation into the egg (Chamruspollert & Sell, 1999); however, it also results in changes in the physical properties (colour and hardness) of the eggs that would make them unacceptable for consumption and cause a severe reduction in hatchability (Aydin *et al*. 2001). Feeding diets containing 5 or 50 g CLA/kg to hens for 1 month resulted in eggs with a CLA content of 0·8 or 11 g/100 g total fatty acid respectively. The net effect was that the CLA content of the egg went from being undetectable in control birds to reaching approximately 350 mg per egg in birds fed the 50 g CLA/kg diet. There was no effect on egg weight or production rate (Chamruspollert & Sell, 1999). The negative effects on

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physical properties and hatchability were seen in as little as 3–4 d of feeding diets containing 5 g CLA/kg. It should be noted that these changes were seen when CLA was fed as the only supplementary fat source in the diet and could be prevented by the addition of a small amount of another oil to the diet. Combining CLA with another oil reduced the amount of CLA incorporated into egg lipids.

Birds fed CLA have attenuated responses to the negative effects of endotoxins on body weight (Cook *et al*. 1993; Takahashi *et al*. 2002). Similar effects have been observed in mice (Miller *et al*. 1994). It is suggested that these effects result from a reduced cytokine response to endotoxin, which may be a direct effect or may be mediated through changes in prostaglandin production (Takahashi *et al*. 2002). It is not clear whether a reduced response to endotoxin is always a desirable effect.

These effects of CLA are similar to those reported for *n*-3 fatty acids. More detailed examination of the events associated with the CLA response has been conducted in laboratory animals. Turek *et al*. (1998) showed that CLA-fed rats had reduced basal levels of tumour necrosis factor, but no change in lipopolysaccharide-induced levels. This same research group saw no change in interleukin 1, but did see reduced basal and lipopolysaccharide-stimulated levels of interleukin 6 in CLA-fed animals. In guinea-pigs CLA reduced histamine and prostaglandin E2 release, implying that it would reduce the hypersensitivity reactions seen in, for example, older animals (Whigham *et al*. 2000).

Fish

In hybrid striped bass (*Monroe saxatilis* × *M. chrysops*; Twibell *et al*. 2000) feeding CLA decreased growth rate and feed intake, but improved feed efficiency. Liver lipids were decreased, which is in contrast to some reports in rodents showing that liver lipids are elevated in mice fed CLA (Park *et al*. 1999*a*; DeLany *et al*. 1999) Intraperitoneal fat, expressed relative to body weight, was also reduced in the fish fed CLA. It is difficult to conclude whether the decrease in fat is a direct effect of CLA or a consequence of the reduction in intake. This same research group reported the effects of feeding CLA to perch (*Perca falvescens)*, a species with a lower body lipid content (Twibell *et al*. 2001). In perch CLA did not affect gain, intake, efficiency or relative fat-pad weight, but did reduce liver lipid content. In both species dose-dependent incorporation of CLA was observed. Muscle CLA content was increased from non-detectable levels to 1–3 g/100 g total fatty acids present. A 100 g serving of muscle from fish fed diets with 5 or 10 g mixed CLA isomers/kg for 8 weeks would contain approximately 54 or 115 mg *cis*-9, *trans*-11 isomer and 58 or 150 mg *trans*-10, *cis*-12 isomer respectively.

Applications in man

Since much of the effort to increase CLA content in animal products is based on a benefit to human subjects consuming these products, it is important to examine the evidence for effects in human subjects. The potential health benefits of CLA in man would include reductions in body fat and prevention of cancer. There are at least four reports of studies in human subjects demonstrating that dietary CLA reduces body fat (Blankson *et al*. 2000; Mougios *et al*. 2001; Riserus *et al*. 2001; Thom *et al*. 2001). Patients were supplemented with 1·4–6·8 g CLA/d for 4–12 weeks and body composition was assessed by dual-energy X-ray absorptiometry (Blankson *et al*. 2000), skinfold thickness (Mougios *et al*. 2001), waist:hip ratio (Riserus *et al*. 2001) or i.r. light (Thom *et al*. 2001). As an example of the level of response, Blankson *et al*. (2000) reported that patients supplemented with 6·8 g CLA/d lost 1·5 kg body fat and gained 1·0 kg lean mass over the 12-week trial. The group given the placebo gained 1·5 kg body fat during this period. It should be noted that, as in the pig, other studies have failed to detect changes in body composition (Zambell *et al*. 2000). Human studies with CLA are limited by the number and variation in subjects and by the limitations in detecting changes in fat mass.

Clearly, the body fat reductions in response to CLA feeding in human subjects are of a lower magnitude than those reported in mice. It has been suggested that differences in energy expenditure can account for the relatively minor changes in fat mass in response to CLA in human subjects as compared with those seen in mice (Terpstra, 2001). Central to this argument is the claim that the effects of CLA on fat mass are accounted for by changes in the efficiency of energy utilization. While this effect may be demonstrated in mice in some studies (Terpstra *et al*. 2002) it is not found in others (West *et al*. 1998) and was not observed in rats (Azain *et al*. 2000) or pigs (Muller *et al*. 1998).

As has been demonstrated in dairy cows (Baumgard *et al*. 2001) and lactating sows (Poulos *et al*. 2000) feeding CLA, particularly synthetic forms containing the *trans*-10, *cis*-12 isomer, results in a decrease in milk fat content in human breast milk (Masters *et al*. 2002). In the pig this depression in milk fat had no detectable effect on progeny growth (Poulos *et al*. 2000). Nevertheless, use of CLA supplements containing the *trans*-10, *cis*-12 isomer during lactation in women is discouraged. Research support from the beef and dairy industries in the USA has targeted studies that investigate ways of increasing the CLA content of meat and milk from ruminants, with specific emphasis on the potential for anti-carcinogenic effects in man (National Cattlemans' Beef Association, 2002; National Dairy Council, 2002).

At the present time there is a lack of studies demonstrating a direct effect of CLA on immune function or as an anti-carcinogen in human subjects. In one of the few direct tests published it was reported that there was no effect of CLA (3·9 g/d for 63 d) on any measure of immune status in healthy women (Kelly *et al*. 2000). Indirect evidence from epidemiological work suggests that increased dairy product consumption is associated with reduced cancer (Jarvinen *et al*. 1997). However, other studies suggest this effect cannot be accounted for by milk lipid (Hjartaker *et al*. 2001). Numerous *in vitro* studies with human cell lines demonstrate the effects of CLA as an anti-carcinogen (for reviews, see Kritchevsky, 2000; Whigham *et al*. 2000; Pariza *et al*. 2001). Since the anti-carcinogenic effects of CLA were initially observed using natural sources of CLA and before the observation of the anti-obesity effects of the *trans*-10, *cis*-12 isomer, it would have been assumed that

the *cis*–9, *trans*–11 isomer was responsible for the anti-carcinogenic effects of CLA. However, recent studies suggest that the *trans*-10, *cis*-12 isomer may also have this effect (Ip *et al*. 2002; Palombo *et al*. 2002).

Mechanism of action

The basis for the multiple effects of CLA has not been fully explained, but probably involves effects of CLA on eicosanoid metabolism, cytokine production and/or gene expression. A summary of evidence for these various mechanisms can be found in recent reviews (Pariza *et al*. 2001; Roche *et al*. 2001). Changes in eicosanoid metabolism relate to the effect of CLA on the ability to inhibit or act as substrates for enzymes in these pathways. Recent evidence has suggested that the anti-obesity effects of CLA are mimicked by lipoxygenase inhibitors (Park & Pariza, 2001). Other studies point to the ability of CLA to act as ligands for peroxisome proliferator-activated receptors (Pariza *et al*. 2001), which may account for many of the adipose tissue effects. Numerous studies suggest, at least in mice, that part of the effect of CLA on adipose mass is due to apoptosis and lipodystrophy (Tsuboyama-Kasaoka *et al*. 2000). Involvement of apoptosis has also been suggested as playing a role in the anti-carcinogenic effects of CLA (for example, see Park *et al*. 2001). Immunomodulatory effects of CLA are most probably related to cytokine production (Sugano *et al*. 1998; Turek *et al*. 1998; Hayek *et al*. 1999), but it is possible that eicosanoids may also be involved indirectly (Bulgarella *et al*. 2001; Pariza *et al*. 2001; Whigham *et al*. 2002).

Several suggestions have been advanced to account for species differences in the response to CLA. It is unlikely that differences in the source of CLA can account for these differences. As evidence for this observation, it has been shown that the feed-grade forms of CLA (CLA-55, CLA-60 and CLA-80) result in reductions in fat-pad weights in female Sprague–Dawley rats and give responses similar to purer mixed isomers (Azain *et al*. 2000) as well as to the individual isomers (Table 2; Azain & Chi, 2001). It is more likely that biological differences between species account for differences in sensitivity to CLA. It has been suggested that at least part of the difference in response to CLA could be related to species differences in the peroxisome proliferator-activated receptor system (Moya-Camarena & Belury, 1999). Terpstra (2001) has suggested, at least in the case of differences between mice and man, that the lower response in human subjects is due to differences in metabolic rate on a unit body mass basis. As suggested earlier with reference to the differences in response among studies in the pig, differences between species in relative amounts of body fat may also be of importance.

In summary, data from *in vitro* studies and from laboratory animals, particularly mice, clearly show that dietary CLA has anti-carcinogenic, anti-obesity and immunomodulatory effects. These effects are less-well documented in man and livestock species. There are examples of studies in livestock and human subjects showing the anti-obesity effect, but results are inconsistent. In the pig the inconsistency seems to be accounted for by the level of body fat in the animals; those animals with greater amounts of subcutaneous fat seem to respond. There is evidence for immunomodulatory effects of CLA in pigs and chickens, but the practical application for these responses is not clear. With the exception of reduced hatchability of eggs from hens fed CLA, no toxic effects of CLA have been observed. It is feasible by feeding CLA to create foods with altered fatty acid profiles as a means of increasing CLA levels in the human diet. However, the impact or practical importance is questionable. There is no direct evidence of an anti-carcinogenic effect of CLA in human subjects or livestock. Indirect evidence from *in vitro* studies with cell lines, as well as epidemiological studies, suggest that CLA may be relevant as a natural anti-carcinogen.

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