Effects of Feeding *Cuphea* Oil to Three Generations of CBA/2 and C57B1/6 Mice

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Three generations of CBA/2 and C57B1/6 mice were reared on semipurified diets containing either 17.2% beef tallow and 3.5% corn oil or 8.6% beef tallow, 8.6% crude *Cuphea* oil and 3.5% corn oil. The *Cuphea* oil contained 76% decanoic acid; therefore, health effects of long-term feeding of moderate amounts of medium-chain triacylglycerols were evaluated. The reproductive performance of both strains of mice varied little with diet but, compared with the F1 generation, survival of F2 and F3 pups was diminished. At several time points during 13 wk, *Cuphea* feeding suppressed body weights and food intakes of males of three generations of both strains. But during long-term feeding of males (5–12 mon), *Cuphea* did not suppress body weight or food intake. Mice of both strains developed fatty livers. Mice of the CBA/2 strain had hepatic nodular hyperplasia. *Cuphea* oil feeding caused no specific pathological changes. Although medium-chain triacylglycerols have been reported to be hypocholesterolemic, the substitution of *Cuphea* for half of the dietary beef tallow did not suppress serum cholesterol concentrations in males aged 4–13 mon. The effects of long-term substitution of medium-chain triacylglycerols for beef tallow do not differ from feeding the beef tallow diet. Long-term and multigenerational feeding of crude *Cuphea* oil does not cause any specific toxic effect in mice.

KEY WORDS: Cholesterol, *Cuphea*, medium-chain triacylglycerols, mice, toxicity.

Cuphea viscosissima is a weed native to temperate climates. If hybridization studies prove successful, *Cuphea* could supply significant quantities of edible oil. *Cuphea* varieties have promise as oilseed crops because *Cuphea* triacylglycerols are composed largely of decanoic acid (1). Therefore, *Cuphea* species may be important oilseed alternatives to coconut oil for use in the human food supply and for the production of medium-chain triacylglycerols (MCTs) as nutritional therapeutics (2). Medium-chain triacylglycerols suppress body fat deposition and weight gain (3,4) and reduce plasma cholesterol (5,6). The long-term, multigenerational effects of feeding diets containing relatively large amounts of MCTs have not been studied. This study was designed to assess the effects on three generations of mice of substituting crude *Cuphea* oil for half of the beef tallow in their diet. C57B1/6 and CBA/2 mice that are relatively hyper- and hypocholesterolemic, respectively (7), were chosen to assess *Cuphea's* effect on cholesterol status. CBA/2 mice are among the inbred strains of mice that have a great incidence of hepatomas (8). The use of this strain permitted analysis of the effects of dietary fat composition on spontaneous tumorigenesis. Long-term health and histopathology were evaluated to determine the toxicity of *Cuphea* oil feeding.

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EXPERIMENTAL PROCEDURES

Crude *Cuphea* oil was obtained from the National Center for Agricultural Utilization Research (Peoria, IL). The crude oil was pressed by French Oil Mill Machine Co. (Piqua, OH). *Cuphea* oil fatty acid composition was determined by K.D. Carlson, (USDA, Peoria, IL), according to methods described by Morrison and Smith (9) for the preparation of fatty acid methyl esters. The gas chromatography (GC) system was a Hewlett-Packard 5890 gas chromatograph (Avondale, PA) equipped with a 15 m × 0.24 mm (i.d.) DB1 column. During fatty acid chromatography, column temperature increased 5/min from 150 to 250°C. Helium was the carrier gas. *Cuphea* oil contained predominately decanoic acid. *Cuphea* oil fatty acid composition was 4.8% octanoate, 75.9% decanoate, 2.5% dodecanoate, 2.2% myristate, 3.4% palmitate, 0.7% stearate, 3.3% oleate and 5.5% linoleate.

CBA/2 and C57B1/6 mice of breeding age (8–10-wk-old) were obtained from SASCO (Omaha, NE). Two females and one male were housed together and fed the experimental diets. *Cuphea* oil was substituted for half of the beef tallow in the basal diet (Table 1). Beef tallow was composed of 4.7% myristate, 32.4% palmitate, 7.9% palmitoleate, 12.9% stearate, 39.2% oleate and 1.1% linoleate, as determined by GC (9). Diet ingredients were supplied by Teklad Test Diets (Madison, WI). Diets were prepared monthly and stored at 4°C. After observation of the presence of vaginal plugs or rapidly increasing body weights, pregnant females were housed individually. Offspring were weaned at four weeks of age, and then housed together until sexual maturation, when they were housed in groups of two females and one male. Within each diet group, females were mated with males from different

TABLE 1

Experimental Diets[a]

Ingredient	Basal diet (g/kg)	Cuphea oil diet (g/kg)
Beef tallow	172	86
Corn oil	35	35
Cuphea oil	—	86
Casein	192	192
dl-Methionine	3	3
Cornstarch	214	214
Sucrose	236	236
Dextrin	45	45
Cellulose	37	37
Vitamins (AIN76)	10	10
Minerals (AIN76)	53	53
Choline	2	2
Cholesterol	1	1

[a]Ascorbate was added at 0.1 g/kg as an antioxidant. All ingredients, with the exceptions of *Cuphea* oil and corn oil (ISU Food Stores, Ames, IA) were obtained from Teklad Test Diets (Madison, WI).

litters. Three generations of mice of each strain were fed each diet. Males of each generation were housed individually and fed for 13 wk. Food intakes and body weights were measured weekly. These mice were housed in a reverse light/dark cycle. Some males of each generation were fed for 5–12 mon, and body weights and food intakes were determined. Because *Cuphea* oil was in short supply, the F1 generation of the C57B1/6 strain was fed for 10 mon, the F2 generation was fed for 8 mon and the F3 generation was fed for 5 mon; whereas, in the CBA/2 strain, the F1 generation was fed for 11–12 mon, the F2 generation was fed for 9–11 mon and the F3 generation was fed for 6–8 mon.

Total serum cholesterol was determined in 10 μL of serum by using a cholesterol assay kit and cholesterol calibrators (Sigma Chemical Co., St. Louis, MO). Serum cholesterol was determined colorimetrically at an absorbance of 500 nm on a Gilford UV/visible spectrophotometer (Oberlin, OH) (10).

Body weights, food intakes, liver weights (13-wk feeding study only) and total serum cholesterol were analyzed by analyses of variance on the Statistical Analysis System, ISU Computation Center. The number of pups born and surviving to weaning was analyzed by Chi-square tests ($P < 0.05$). Groups of *Cuphea*- and basal-fed mice were compared within each strain and generation. The overall effect of strain and generation was also assessed. For the purpose of calculating the Chi-square statistic, the expected numbers of pups born and surviving to weaning in F1 and F2 mice were proportionate to the pups born and surviving to weaning in the basal-fed FO generation of each strain.

Mice were necropsied by a standard procedure (11), and gross lesions were noted. After fixation in buffered neutral formalin, specimens for microscopy were routinely processed, sectioned at 5 μm and stained with hematoxylin and eosin. The following tissues were evaluated microscopically: all gross lesions, two sections of liver (one each of the median and left lobes), cross section of right kidney, and a mid-sagittal section of left kidney, spleen, lung, heart and one testis.

RESULTS

Cuphea feeding did not affect reproduction, with the exception that CBA/2 F1 mice produced significantly fewer offspring than the expected 6.5 pups/litter produced by females fed the basal diet (Chi-square analysis, $P < 0.05$) (Table 2). The fewer numbers of breeding females in the F2 generations reflect the limited availability of *Cuphea* oil. C57B1/6 mice produced significantly more offspring per litter than CBA/2 mice (Chi-square, $P < 0.01$). Nurturing of young was significantly impaired in the second and third generations (Chi-square, $P < 0.01$), especially in CBA/2 mice (Table 2).

No consistent variations were observed in body weights, liver weights or food intakes, although where significant differences were seen between the diets, *Cuphea*-fed mice had lighter body weights and reduced food intakes.

The following significant differences were noted. In mice fed for 13 wk, *Cuphea*-fed F1 C57B1/6 mice had 10% lower body weights after 8 and 13 wk (Table 3), and food intakes were 18% less in *Cuphea*-fed mice after 8 wk (Table 4). In F2 C57B1/6 mice, body weights were 20% less in *Cuphea*-fed mice at four weeks of age. In F3 C57B1/6 mice, body weights were 16% less in *Cuphea*-fed mice at 13 wk of age, and liver weights were diminished by 15% (data not shown).

In F1 CBA/2 mice, body weights were less in *Cuphea*-fed mice at 13 wk of age, as were food intakes at 8 and 13 wk. Body weights and food intakes were less at eight weeks in F2 CBA/2 mice.

In CBA/2 mice fed for 5–12 mon, there were no significant diet-related differences in body weights at any time (Table 5). Body weights of C57B1/6 mice were less in *Cuphea* groups only after 13 wk (F1) and 4 wk (F2). A

TABLE 2

Reproductive Performance of Three Generations of Two Strains of Mice

Generation	Diet	Pregnant (%)		Pups born/ female	Pups at weaning/ pups born (%)
Strain: CBA/2					
F0	Basal	19	(95)	5.9	42[a]
	Cuphea	17	(85)	5.3	51
F1	Basal	18	(90)	6.5	18
	Cuphea	23	(88)	5.3[b]	14
F2	Basal	22	(100)	5.4	15
	Cuphea	8	(100)	6.1	12
Strain: C57B1/6					
F0	Basal	14	(88)	7.5[c]	50[a]
	Cuphea	12	(86)	7.8	60
F1	Basal	19	(100)	8.6	28
	Cuphea	17	(100)	8.2	24
F2	Basal	19	(95)	8.0	28
	Cuphea	14	(93)	7.3	28

[a]The survival of pups to weaning was significantly affected by generation, Chi-square, $P < 0.01$.
[b]The number of pups born per mother was significantly affected by diet only in F1 CBA/2 mice, Chi-square, $P < 0.05$.
[c]The number of pups born per mother was significantly affected by strain, Chi-square, $P < 0.01$.

TABLE 3

Mean Body Weights of Three Generations of Two Strains of Mice Fed for Thirteen Weeks[a]

Strain	Generation	Diet	n	Body wt[b] (4 wk)	(8 wk)	(13 wk)
C57B1/6	1	Basal	15	15.8 ± 2.2	26.4 ± 3.3[c]	34.3 ± 4.3[c]
	1	*Cuphea*	16	16.1 ± 2.0	23.5 ± 2.8[d]	30.6 ± 2.9[d]
	2	Basal	17	15.6 ± 2.3[c]	23.8 ± 2.3	28.6 ± 3.7
	2	*Cuphea*	16	12.5 ± 1.3[d]	23.2 ± 1.5	28.6 ± 2.6
	3	Basal	17	11.6 ± 2.5	23.2 ± 2.9	31.6 ± 5.0[c]
	3	*Cuphea*	13	11.8 ± 3.2	21.5 ± 2.4	26.7 ± 3.8[d]
CBA/2	1	Basal	25	14.2 ± 2.7	27.4 ± 2.4	33.1 ± 2.9[c]
	1	*Cuphea*	29	15.1 ± 2.6	26.0 ± 3.2	31.1 ± 3.8[d]
	2	Basal	13	14.3 ± 2.7	25.2 ± 2.0[c]	30.9 ± 3.4
	2	*Cuphea*	11	13.6 ± 2.6	22.7 ± 1.9[d]	28.3 ± 3.1
	3	Basal	11	15.1 ± 2.9	25.8 ± 2.0	32.0 ± 3.2
	3	*Cuphea*	5	12.8 ± 2.3	22.9 ± 3.9	29.2 ± 5.3

[a]All data are expressed as means ± SD. Pairs of basal- and *Cuphea*-fed groups that were significantly different are marked by different superscript letters, $P < 0.05$, Student's *t*-test.
[b]In grams.

few diet-related differences in food intakes were noted, but these differences did not correlate with body weight differences (Table 6).

Serum cholesterol was significantly greater after three months in C57B1/6 mice than in CBA/2 mice (227 ± 40 *vs.* 181 ± 23 mg/dL, respectively), and also after 5–12 mon (329 ± 87 *vs.* 217 ± 37 mg/dL, respectively). But there were no significant differences in cholesterol levels due to diet, generation or length of feeding (data not shown), and there were no significant interactions among these variables (ANOVA, $P < 0.05$).

Livers of most mice had excessive lipid accumulation (fatty change) (Table 7). The fatty vacuoles varied somewhat in size and number between strains, but varied more in distribution within the hepatic lobule. CBA/2 mice tended to accumulate fat as large vacuoles in periportal hepatocytes, with smaller vacuoles in centrilobular hepatocytes. C57B1/6 mice tended to have a more diffuse fatty change, with large vacuoles in centrilobular areas. There were no visible differences within mice of the same strain and generation in the amount of lipid in livers of mice fed the basal diet *vs.* those fed the *Cuphea* oil diet. CBA/2 mice often had hepatic nodules (termed hepatomas by some classification schemes), but these occurred with similar frequency in mice fed either diet. They were more common in older mice than in younger mice.

In kidneys, proximal cortical tubules of all mice contained fat vacuoles (Table 7). There were no apparent differences in the amount of fat. Numerous mice had lymphocyte aggregates and/or active purulent pyelitis or pyelonephritis. There were no apparent differences in lesions between groups of CBA/2 mice on the different diets. In C57B1/6 mice, those on the *Cuphea* diets tended toward slightly higher scores.

Preputial gland lesions, including abscesses and cysts, occurred occasionally in mice on both diets, but in CBA/2

TABLE 4

Food Intakes of Three Generations of Two Strains of Mice Fed for Three Months.[a]

Strain	Generation	Diet	n	Food intake[b] (8 wk)	(13 wk)
C57B1/6	1	Basal	15	94 ± 15[c]	112 ± 7
	1	*Cuphea*	16	77 ± 12[d]	104 ± 12
	2	Basal	17	74 ± 10	108 ± 12
	2	*Cuphea*	16	79 ± 6	113 ± 8
	3	Basal	17	71 ± 7	106 ± 12
	3	*Cuphea*	13	74 ± 7	98 ± 12
CBA/2	1	Basal	25	100 ± 16[c]	128 ± 27[c]
	1	*Cuphea*	29	83 ± 9[d]	112 ± 12[d]
	2	Basal	13	102 ± 16[c]	128 ± 12
	2	*Cuphea*	11	84 ± 11[d]	118 ± 9
	3	Basal	11	80 ± 16	119 ± 11
	3	*Cuphea*	5	79 ± 7	110 ± 10

[a]All data are expressed as means ± SD. Pairs of basal- and *Cuphea*-fed groups that were significantly different ($P < 0.05$, Student's *t*-test) are marked by different superscript letters.
[b]In total grams.

TABLE 5

Body Weights of Three Generations of Two Strains of Mice Fed for 5–12 Mon[a]

Strain	Generation	Diet	n	Body wt (4 wk)	(13 wk)	(26 wk)	(39 wk)	(45 wk)
C57B1/6	1	Basal	7	16 ± 3	35 ± 4[b]	45 ± 4	51 ± 4	
	1	*Cuphea*	5	16 ± 2	29 ± 2[b]	41 ± 5	46 ± 6	
	2	Basal	5	16 ± 2[b]	28 ± 4	42 ± 4	42 ± 4[c]	
	2	*Cuphea*	6	13 ± 2[b]	26 ± 2	39 ± 6	44 ± 5[c]	
	3	Basal	4	13 ± 1	32 ± 5	40 ± 2[d]		
	3	*Cuphea*	4	12 ± 4	29 ± 5	37 ± 8[d]		
CBA/2	1	Basal	5	14 ± 2	34 ± 1	43 ± 2	47 ± 2	48 ± 3
	1	*Cuphea*	5	15 ± 3	33 ± 5	38 ± 7	44 ± 6	43 ± 4
	2	Basal	5	15 ± 2	31 ± 2	38 ± 3	45 ± 3	
	2	*Cuphea*	5	15 ± 4	29 ± 4	38 ± 5	46 ± 2	
	3	Basal	4	14 ± 4	31 ± 2	42 ± 3	44 ± 2[c]	
	3	*Cuphea*	2	13 ± 2	27 ± 6	39 ± 10	41 ± 10[c]	

[a]All data are expressed as means ± SD.
[b]These pairs of basal- and *Cuphea*-fed groups are significantly different at $P < 0.05$, Student's *t*-test.
[c]29 wk.
[d]18 wk.

mice more than C57B1/6 mice (Table 7). Lungs of some mice had increased numbers of lymphoid aggregates or follicles, typically associated with airways or around vessels. These occurred more in F2 CBA/2 mice and in F1 C57B1/6 mice. Spleens of some mice were enlarged. These usually were found in mice with active inflammation, usually due to urinary tract infections.

DISCUSSION

Cuphea oil contains a great proportion of MCTs. This is the first three-generation feeding trial performed with such a fat source. Although MCTs have been reported to reduce plasma cholesterol (5,6) and body weight gain of rats (3,4), these effects were not generally observed in CBA/2 or C57B1/6 mice.

Many inbred strains of mice are difficult to breed. The failure of mothers to successfully nurture pups to weanling age (four weeks) was the only reproductive problem noted (Table 2). The F0 mice were exposed to a different diet and environment than were the F1 and F2 mice before breeding, but it is not clear how these differences might have led to the impairment of survival of pups in the latter generations. However, the proportion of pups born and surviving to weaning was similar when either diet was fed.

Body weight and food intakes were suppressed in *Cuphea*-fed mice at some time points during a 13-wk feeding trial (Tables 3 and 4), but *Cuphea* feeding had little effect on body weight or food intake in mice fed for 5–12 mon (Tables 5 and 6). The occasional suppression of food intake or body weight by *Cuphea* oil feeding is probably due to random variation, and not due to unknown antinutritional factors or MCTs. Feeding MCTs has been shown to either reduce (3,4) or to have no effect on body weight gain (12). Overall, crude *Cuphea* oil did not suppress

TABLE 6

Food Intakes of Three Generations of Two Strains of Mice Fed for 5–12 Months[a]

Strain	Generation	Diet	n	Food intake (13 wk)	(26 wk)	(39 wk)	(45 wk)
C57B1/6	1	Basal	7	201 ± 15	291 ± 15[b]	311 ± 17	
	1	*Cuphea*	5	186 ± 18	315 ± 17[b]	322 ± 12	
	2	Basal	5	192 ± 15	303 ± 25	94 ± 12[c]	
	2	*Cuphea*	6	190 ± 6	303 ± 12	96 ± 2[c]	
	3	Basal	4	183 ± 21	123 ± 5[d]		
	3	*Cuphea*	4	186 ± 7	129 ± 5[d]		
CBA/2	1	Basal	5	218 ± 27	339 ± 15[b]	284 ± 20	137 ± 20
	1	*Cuphea*	5	205 ± 18	299 ± 30[b]	303 ± 25	134 ± 10
	2	Basal	5	242 ± 31[b]	312 (n = 1)	322 ± 41	
	2	*Cuphea*	5	200 ± 15[b]	312 ± 16	313 ± 18	
	3	Basal	4	208 ± 10	331 ± 33	72 ± 4[c]	
	3	*Cuphea*	2	191 ± 7	318 ± 9	74 ± 10[c]	

[a]All data are expressed as means ± SD.
[b]These pairs of basal- and *Cuphea*-fed groups are significantly different at $P < 0.05$, Student's *t*-test.
[c]29 wk.
[d]18 wk.

TABLE 7

Histopathology of Mice Fed *Cuphea* Oil for 5–12 Mon[a]

Strain	Group	n	Liver	Kidney	Lung	Other
C57B1/6	F1-Basal	7	4.0	1.7	lf (4)	fb (1)
	F1-*Cuphea*	4	3.8	2.5	lf (1)	fb (2)
						pgn (1)
	F2-Basal	5	3.8	1.2	lf (1)	
	F2-*Cuphea*	6	3.7	1.8		pga (1)
	F3-Basal	4	3.8	1.0		
	Fe-*Cuphea*	4	2.8	1.2		
CBA/2	F1-Basal	6	1.7	1.5	lf (1)	pga (1)
			n (4),e (1)	i (1)	h (1)	pgn (1)
			f (2),			
	F1-*Cuphea*	5	1.8	1.0		
			n (4),			
			ef (1)			
	F2-Basal	5	1.4	2.0	lf (1)	pgn (1)
			nf (4)		h (2)	fb (1)
						cv (1)
	F2-*Cuphea*	5	1.8	2.0	lf (4)	pga (1)
			nf (2)	m (1)	h (1)	fb (1)
			e (1)	i (1)		spgr,pgd (1)
	F3-Basal	4	2.0	2.0	h (1)	pgc (1)
						pga (1)
	F3-*Cuphea*	2	1.5	2.5	lf (1)	pga (1)
			nf (1)		f (1)	

[a]Key to lesions: Liver: 0, No significant lesions; 1, periportal fatty change, large vacuoles; 2, periportal fatty change, large vacuoles with diffuse small vacuoles; 3, diffuse fatty change, small vacuoles, or centrilobular fatty change only; 4, diffuse fatty change, large and small vacuoles, no pattern; n, nodule (typically nodular hyperplasia); f, fatty, e, eosinophilic. Kidney: 1, Mild proximal tubule fat, stout glomeruli; 2, changes in 1 plus one or few lymphocyte aggregates; 3, changes in 1 plus marked chronic or chronic active pyelitis; i, infarcts; m, mineral. Lung: lf, Lymphoid follicles; h, hemosiderin. Other: pg, Preputial gland; a, abscess; d, dilation, c, cyst; n, nodular hyperplasia; spgr, sperm granuloma of epididymus; fb, full bladder; cv, cystitis, vesiculitis. For liver and kidney, data are expressed as mean scores. Numbers in parentheses indicate the number of mice with each type of lesion.

growth and food intake of two strains of mice fed over three generations and for up to 12 mon.

The strain-dependent variation in serum cholesterol was expected because CBA/2 mice are relatively hypocholesterolemic (7). Feeding MCTs can reduce plasma cholesterol (5,6). Replacing half of the calories contributed by beef tallow with *Cuphea* oil, so that *Cuphea* oil contributed 16% of total calories (out of a total of 39% fat calories), was not a sufficient dietary modification to reduce serum cholesterol in mice.

Liver weight, a sign of hepatotoxicity (13), did not differ generally between diets in mice fed for three months (data not shown). Accumulation of hepatic lipid was probably caused by the fat and cholesterol contents of the diets (14), a diet similar in total lipid content to the usual American diet. There were no differences between the diets or the generations in fatty liver development. The two strains showed different patterns of fat accumulation, perhaps because of differences in hepatic fat metabolism. The hepatic nodules were strain-dependent and not diet-dependent. These nodules occur frequently in CBA/2 mice, usually in mice older than those used in this study (8). The development of other neoplasms, specifically N-methylnitrosourea-initiated rat mammary tumors, may be influenced by substituting MCTs for corn oil (14). MCTs (17.6% by weight of the diet fed with 5.9% corn oil) and a diet containing 5% corn oil by weight of diet suppressed tumor promotion to the same extent, compared with 23.5% corn oil. The total tumor incidence was reduced in MCT-fed rats, compared with corn oil-fed rats, but the presence of adenocarcinomas was not suppressed by MCT feeding. Spontaneously occurring hepatic nodules in mice were not affected by *Cuphea* oil; and, therefore, MCT feeding at about 8% by weight of the diet in substitution for beef tallow did not suppress spontaneous hepatoma formation. It is likely that MCTs and other saturated fat sources, such as beef tallow, when substituted for linoleate, might suppress mammary tumorigenesis because, when total fat calories are held constant, mammary tumorigenesis increases as dietary linoleate content increases from 0.5 to 5% by weight of the diet (15). Tumorigenesis also is suppressed by caloric restriction (16), but *Cuphea* oil had no such effect during the long-term feeding of mice.

Renal vacuolar changes were likely caused by the great total lipid content of the feed (Table 7). The inflammatory renal lesions were interesting and may have been more severe with the *Cuphea* diets, but not statistically so. The lesions seen are often caused by partial urinary tract obstruction and ascending bacterial infections following the obstruction. Whether this was related to lipid content in the diets or related to other factors, such as husbandry, is open to speculation, but it is not likely to be directly related to the diets treated.

Lesions in the preputial glands also were strain-dependent and not diet-dependent (Table 7). Lesions in lungs likely resulted from environmental factors and had no strain- or diet-dependence.

In conclusion, feeding 8% *Cuphea* oil as a substitute for half of the dietary beef tallow was no more toxic to mice than was the beef tallow diet. The diet containing *Cuphea* oil did not impair reproductivity or cause any specific disease in the mouse tissues examined. *Cuphea* oil moderately suppressed body weights and food intakes of mice in some groups between 4 and 13 wk of age, but *Cuphea* oil had no long-term effects on body weight, food intake or cholesterol status. *Cuphea* oil seems to be nontoxic, but of no particular health benefit to mice when fed as a partial substitute for beef tallow.

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