Effects of dietary *trans*-fatty acids on reproductive performance of Wistar rats

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1. Wistar rats were fed for three successive generations on a semi-purified diet, in which the fat was provided by butter, sunflower oil, rapeseed oil or hydrogenated vegetable fat, differing in the content of *cis,cis*-18:2 and *trans*-18:1 fatty acids. Effects of these fats on the composition of adipose tissue and reproductive performance were studied. Fatty acids were analysed using high-performance liquid chromatography.

2. The fatty acid pattern of adipose tissue was closely related to dietary fat composition and, established in the first generation, did not change significantly in successive generations of rats.

3. Hydrogenated fat adversely affected litter size, sperm morphology and regularity of oestrous cycle, and prolonged the period of gestation in experimental animals. Differences observed between the generations were not significant.

4. Hydrogenated fat decreased the level of serum testosterone in males, but the differences observed in levels of serum progesterone in females were not apparently related to the dietary *trans*-fatty acids.

Fats are important constituents of our diet and, beside their functions as sources of energy and carriers for fat-soluble vitamins, they supply essential fatty acids that are vital components of cell membranes (Hansen & Jensen 1985), and precursors for potent hormone-like compounds such as prostaglandins and leukotrienes (Gorman, 1979; Lewis & Austen, 1981; Walker, 1983; Mead, 1984).

Widespread use of partially hydrogenated vegetable oils containing large amounts of *trans*-fatty acid has raised questions concerning the biological effects of consumption of significant amounts of these isomers (Alfin-Slater *et al.* 1965). Although biochemical and physiological research on isomeric fatty acids in hydrogenated oils have substantially increased the understanding of their metabolism, there is still no absolute consensus on the role of isomeric fats in nutrition and health (Emken, 1983). One aspect of *trans*-fatty acids that has received great attention is their relation to essential fatty acid metabolism. Numbers of investigations in vivo have provided evidence that hydrogenated fats and specific fatty acid isomers can influence the activity of the desaturases, elongases, acyltransferases, oxygenases and prostaglandin synthetases (Emken, 1984). Particularly important is the ability of *trans,trans*-18:2 fatty acids to inhibit the elongation–desaturation reactions of linoleic acid (Kurata & Privett, 1980). Monoenoic *trans*-fatty acids may also undergo desaturation to dienoic acids in rat tissue, particularly if the animals are essential-fatty-acid deficient. Therefore, it is obvious that giving hydrogenated fat containing a high concentration of *trans*-18:1 acid may accentuate essential-fatty-acid deficiency in rats (Holman, 1964; Nugteren, 1970; Hill *et al.* 1979; Walker, 1983; Zevenbergen *et al.* 1988).

Since Burr & Burr (1930) demonstrated the essentiality of linoleic acid in reproduction and growth of animals, a wide range of physiological functions has been shown to be affected by essential-fatty-acid deficiency (Holman, 1968, 1973; Alling *et al.* 1972; Cornwell & Morisaki, 1984; Nugteren *et al.* 1985; Guesnet *et al.* 1986; Hansen, 1986). Impairment of reproductive functions is one of the earlier symptoms of the lack of essential fatty acids (Menon *et al.* 1981; Ravel *et al.* 1985).

It is well established that essential-fatty-acid deficiency in the rat leads to degeneration

of the testis, with concomitant infertility, in males (Ahluwalia *et al.* 1967; Marzouki & Coniglio, 1982). In females, though they may conceive, fetuses are often reabsorbed, animals abort or young ones are stillborn (Mohrhauer & Holman, 1967; Menon *et al.* 1981); the frequency of irregular oestrous cycles, with an increased incidence of prolonged dioestrus, prolonged period of gestation, reduced litter size, and increased pup mortality, are usually significantly higher (Satomi & Matsuda, 1973; Parlanti & Orellana, 1985). It seems possible that high concentrations of *trans*-fatty acid isomers, as found in hydrogenated dietary oils, could also adversely affect the reproduction of laboratory rats, though that effect would be more probable at lower levels of essential fatty acids (Holman & Aaes-Jorgensen, 1956; Alfin-Slater *et al.* 1957; Emken, 1984).

Dietary *trans*-fatty acids have been shown to be incorporated into many tissues (Johnston *et al.* 1958; Egwim & Kummerow, 1972; Wood, 1979; Moore *et al.* 1980; Emken, 1984). Adipose tissue, known to accumulate high amounts of *trans*-fatty acids (Moore *et al.* 1980), is interesting in that chronic feeding of hydrogenated fat leads to elevation of lipogenic enzyme activity, although these enzymes are not apparently affected in other tissues, for example, liver (Wilck, 1982; Walker, 1983). Of interest also are the recent findings that conversion of androgens to oestrogens takes place in adipose tissue, which suggests that adipose tissue may be a significant extra-gonadal source of oestrogens (Frisch, 1984). Fatness and adipose tissue fatty acid pattern also influence the direction of oestrogen metabolism to the most potent or least potent forms (Fishman *et al.* 1975).

In the present study we have tried to evaluate possible effects of several dietary fats differing in their content of essential as well as *trans*-fatty acids on fatty acid profiles of adipose tissue and the reproductive performance of the Wistar rat in a multi-generation study.

EXPERIMENTAL

Materials

In the present experiment the following dietary fats were used: butterfat (BF), sunflower oil (SO), rapeseed oil (low in erucic acid content; RO), hydrogenated vegetable oil (HO). These fats added were included at 50 g/kg in an experimental semi-purified diet (SED) derived from the AIN 76 diet (American Institute of Nutrition, 1977) based on sucrose. As a control, diet DOS 2b (Velaz, Prague), based on natural ingredients, was used. The composition of the diets and their fatty acid profiles are given in Tables 1 and 2. Diet HO contained the highest concentration of *trans*-18:1 and *trans,trans*-18:2 fatty acids, approximately 19 and 3% of total fatty acids respectively. Diet BF contained the lowest concentration of *cis,cis*-18:2 fatty acid, approximately 2·9% of total fatty acids, while diet HO contained approximately 4%, diet DOS 14%, diet SO 64% and diet RO 22% of this fatty acid. These concentrations represented 0·38 and 0·54% of total energy in diets BF and HO, 1·85% in diet DOS, 2·89% in diet RO and 8·35% in diet SO. The highest levels of *cis*-18:1 fatty acid were found in diet HO, 45% and diet RO 56%. The diets were prepared every two weeks and stored at −18°.

Animal experiments

Weanling Wistar rats (100; Ipcv:WIST), weighing 40–60 g, were randomly allocated to five groups of ten males and ten females each. Rats were housed in groups of five animals of the same sex in polypropylene cages (Velaz, Prague).

All animals were fed *ad lib.* and had free access to drinking water. Food intakes were measured weekly on a cage (five rats) basis. Body-weights were measured twice weekly.

After 85 d on their respective diets, six animals randomly selected from each group were fasted for 12 h and anaesthetized with Thiopental (Spofa, Prague; 50 mg/kg animal weight

Table 1. *Composition of the diets (g/kg)*

Diet... DOS 2b		SED	
Ground wheat	600	Sucrose	500
Dried milk*	130	Casein†	200
Casein†	130	Starch‡	150
Wheat bran	90	Cellulose	50
DOS-1-komplex-stabil§	50	Fat	50
		Vitamin-mineral mix‖	50

* Fat content 280 g/kg.

† Extracted (fat- and vitamin-free).

‡ Potato starch.

§ Vitamin content (mg/kg): thiamin 240, riboflavin 160, pyridoxine 80, cyanocobalamin 0·6, vitamin E 2 g, vitamin A 30, vitamin D 2·5, folic acid 120, calcium pantothenate 160, inositol 2 g. Mineral content (g/kg): calcium carbonate 84, calcium phosphate 221, sodium chloride 150, ferrous sulphate 32, cupric sulphate 392 mg, zinc sulphate 440 mg, manganous carbonate 2, potassium iodide 26 mg (Velaz, Prague, Czechoslovakia).

‖ Vitamin content (mg/kg): thiamin 370, riboflavin 282, pyridoxine 200, cyanocobalamin 0·8, vitamin E 2·5 g, vitamin A 36, vitamin D 2·5, vitamin K 1·5, folic acid 131, calcium pantothenate 1·0 g, inositol 4·3 g, biotin 5, nicotinic acid 1·3 g, choline chloride 23 g, L-methionine 72 g. Mineral content (g/kg): calcium carbonate 92, calcium phosphate (dibasic) 340, sodium chloride 164, ferrous sulphate 35, cuprous sulphate 427 mg, zinc sulphate 479 mg, manganous carbonate 207 mg, potassium iodide 29 mg, magnesium sulphate (heptahydrate) 196, potassium chloride 166, sodium selenite 8 mg, sodium fluoride 22 mg, potassium chromate 109 mg.

Table 2. *Fatty acid composition of dietary fats (weight % total fatty acids)*

Fatty acid	Diet*... DOS	BF	SO	RO	HO
8:0	1·66	3·45	0·18	0·06	0·12
12:0	2·32	4·76	0·86	0·35	0·76
14:0	6·78	10·97	0·06	0·05	0·37
14:1	0·79	1·49	—	—	0·02
16:0	21·14	30·44	6·11	5·18	8·89
16:1	2·24	3·29	0·14	0·32	0·82
18:0	7·14	10·21	3·72	1·71	8·11
c-18:1	18·29	23·16	21·45	56·23	45·22
t-18:1	1·34	3·05	—	—	19·45
cc-18:2	14·24	2·90	64·23	22·26	4·13
tt-18:2	0·43	0·98	—	—	3·19
18:3	2·55	2·22	2·02	10·54	1·52
20:0	0·05	—	0·34	0·17	0·19
c-20:1	0·45	0·24	0·12	0·06	0·10
20:2	2·00	—			
20:3	0·37	—	0·63	0·37	0·39
22:1	—	—	—	0·33	—

c, *cis*-isomer; *t*, *trans*-isomer; DOS, control diet; BF, butterfat; SO, sunflower oil; RO, rapeseed oil low in erucic acid; HO, hydrogenated vegetable oil.

* For details of DOS (DOS 2b; Velaz, Prague) and diets BF, SO, RO and HO (semi-purified experimental diet + 50 g BF, SO, RO or HO/kg respectively), see Table 1 and p. 520.

intraperitoneally). Blood samples for progesterone and testosterone determinations were taken from the inferior vena cava and stored at −18° until analysis.

Liver, kidney, heart and testis and epididymis or ovary were then removed, washed in cooled saline (9 g sodium chloride/l) and wet weighed. Samples of adipose tissue were taken from the linea alba abdominal subcutaneous region and fatty-acid profiles were analysed.

The remaining animals, four males and four females in each group, were then mated 1:1 in a cage. The number of newborn pups were reduced to eight for each dam with sexes equalized. At the age of 35 d the animals were weaned and their number was again reduced to ten males and ten females in each dietary group by random selection. These animals were kept on their respective diets to the age of 85 d, and then treated in the same manner as their parents. The procedures were repeated so that three successive generations of the rats on the same experimental diets could be investigated. The experiment was repeated during the following year with new animals in the same months to avoid seasonal differences in physiological activities of the animals. In both cases, F1 generation animals were born in February, F2 generation in May, and F3 generation in September. In all, 600 Wistar rats were used in the experiment.

Lipid analysis

Lipids from adipose tissue were extracted by the method of Folch *et al.* (1957), using methanol – chloroform (2:1, v/v) and a solvents: tissue value of 20:1. Fatty acid profiles of the lipid extracts were analysed by high-performance liquid chromatography after derivatization of fatty acids with phenacyl bromide (Hanis *et al.* 1986, 1988).

Reproduction tests

The percentage of pregnant females and the litter size were recorded for each dietary group and nest. The age of vaginal opening was determined by daily observations. The age of the first oestrus and regularity of the oestrous cycle were determined from daily vaginal smears. Rats with three consecutive regular, 4 d, oestrous cycles were considered as regular-oestrous-cycle rats. The length of pregnancy was calculated from the first day of spermatozoa presence in the vaginal fluid smear. The morphological abnormality of spermatozoa was determined by staining with Giemsa-Romanowski reagent (Washington *et al.* 1983); 400 sperm were used for mean value calculations. Plasma concentrations of progesterone and testosterone were determined by radioimmunoassay (Abraham, 1974).

Statistical analysis

The Student–Newman–Keuls multiple-range test (Miller, 1981) was used for evaluation of the significance of the differences between experimental groups.

RESULTS AND DISCUSSION

During the experimental period the animals showed no apparent symptoms of nutritional deficiency. Feed intakes between 32 and 85 d increased from 14 to 23 g/d for males, and from 14 to 21 g/d for females; no significant differences related to the diets were observed.

Body-weight gains are presented in Figs 1 and 2. The lower weights of the rats fed on diets BF or HO, with the proportion of energy derived from *cis,cis*-18:2 fatty acid at the lower margin of rat requirement (as determined by Pudelkewicz *et al.* (1968)) were observed at the end of the experiment. However, these weights were not significantly different from those of rats in the other dietary groups. No significant differences were observed in the weights and gross pathology or morphology of hearts, livers, kidneys, testes, epididimides and ovaries. These results are in agreement with the observations of other workers conducting long-term and multiple-generation studies with rats fed on hydrogenated fats (Alfin-Slater *et al.* 1957; Alfin-Slater & Aftergood, 1979; Duthie & Barlow, 1982; Kritchevsky, 1982; Svaar, 1982; Zevenbergen *et al.* 1988).

Fatty-acid profiles of adipose tissue fat of animals fed on different diets are summarized in Table 3. These profiles were related to the fatty-acid composition of dietary fats (Table 2), demonstrating that adipose tissue fatty acid reflects dietary fat intake (Emken, 1983).

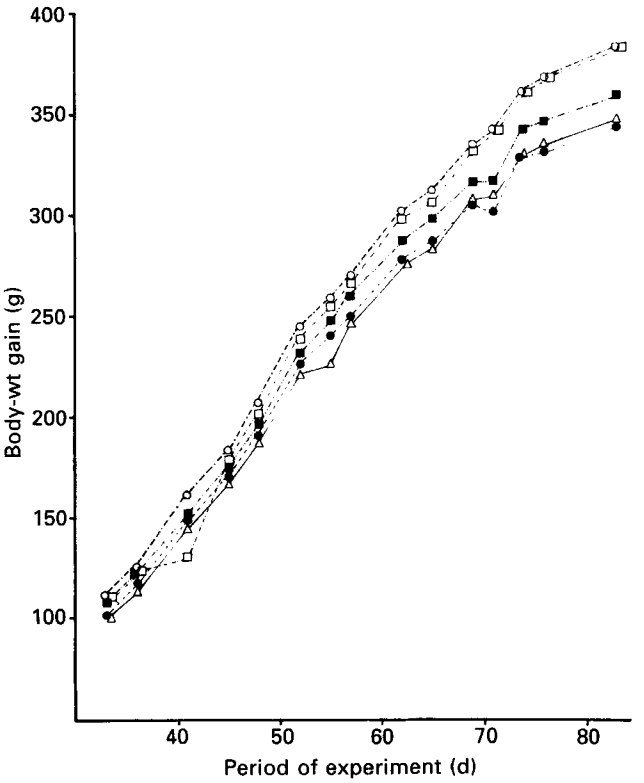

Fig. 1. Body-weight gains of male Wistar rats fed on different dietary fats. (○), Control diet (DOS); (●), butterfat; (□), sunflower oil; (■), rapeseed oil; (△), hydrogenated oil. For details of control diet DOS and diets containing different dietary fats, see p. 520. Standard errors of the means: 0·80 (32–45 d), 1·39 (48–57 d), 2·03 (62–85 d).

That dietary *trans*-fatty acids are incorporated into both depot and structural fats has been well documented (Egwim & Kummerow 1972; Emken, 1984). We found significantly higher concentrations ($P < 0·05$) of *trans*-18:1 and *trans,trans*-18:2 fatty acids in adipose tissue of animals fed on diets HO and BF than those of the animals on the other diets. The highest concentrations of these isomers were observed in diet HO rats: approximately 15% of *trans*-18:1 and 2% of *trans,trans*-18:2. Adipose tissue of the animals on diet BF contained approximately 2% of *trans*-18:1 and 1% of *trans,trans*-18:2. Traces of these *trans*-fatty acids were also detected in adipose tissue of animals fed on diets free of *trans*-fatty acids, which may indicate a possible endogenous source for a small proportion of *trans*-isomers, also observed in human tissues (Ohlrogge *et al.* 1982). Animals fed on diets HO or BF also exhibited significantly lower concentrations of *cis,cis*-18:2 and all-*cis*-20:4 fatty acids, and significantly higher concentrations of 20:1 and 20:3 fatty acids, as compared with the animals in other dietary groups. The lowest content of *cis,cis*-18:2 fatty acid was found in adipose tissue of rats fed on diet BF, which reflects the low content of linoleic acid in diet BF. The lowest content of all-*cis*-20:4 fatty acid was found in the group given diet HO, probably because the highest concentrations of various fatty-acid isomers were found in HO, and through the possible interference of these isomers with the metabolic pathway of linoleic (*cis,cis*-9,12-18:2) and arachidonic (all-*cis*-5,8,11,14-20:4) acids (Nugteren, 1970; Kurata & Privett, 1980; Mahfouz *et al.* 1980; Walker, 1983). Though a

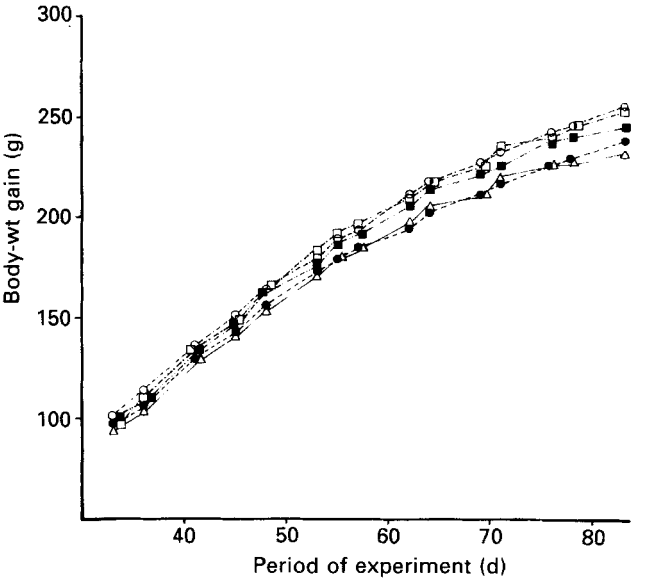

Fig. 2. Body-weight gains of female Wistar rats fed on different dietary fats. (○), Control diet (DOS); (●), butterfat; (□), sunflower oil; (■), rapeseed oil; (△), hydrogenated oil. For details of control diet DOS and diets containing different dietary fats, see p. 520. Standard errors of the means: 0·79 (32–45 d), 1·10 (48–57 d), 1·50 (62–85 d).

decrease in linoleic and arachidonic acids is usually observed in animals fed on essential-fatty-acid-deficient and high-*trans*-fatty-acid diets, our findings suggest that even giving a hydrogenated-fat diet alone could affect the tissue concentration of arachidonic and linoleic acids. Our observations seem to be in line with previous reports suggesting similar changes in the fatty-acid composition of tissues of animals fed on partially hydrogenated fats, even with a supply of about 4% of linoleic acid (Kirstein *et al.* 1983, Thomassen *et al.* 1983). No significant differences were observed in fatty acid profiles between males and females, or between the successive generations of the rats on their respective diets. The fatty-acid composition of adipose tissue established during the 85 d in the first generation of rats was found to be unchanged in the following generations.

Giving diet HO to Wistar rats resulted in significantly increased incidences of irregular oestrous cycles and prolonged gestation periods, compared with other dietary groups. Half the females on diet HO exhibited irregular oestrous cycles compared with 25% for females given diets BF or RO, and those fed on diets DOS or SO showed a regular 4 d oestrous cycle. Dams fed on diet HO had prolonged gestation periods: over 22 d in 50% of the dams while, with diet DOS, only in 9% was the gestation period longer than 22 d, and with diets BF, SO and RO only 19–20%. Each group consisted of sixty females.

There were no statistically significant differences among the dietary groups in the age of vaginal opening and the age of the first oestrus, but the animals fed on diets HO or BF usually showed higher mean values for these variables. First oestrus onset occurred at the age of 37·7 (SE 1·5)–41·2 (SE 2·8) d, which was 4–6 d after the vaginal opening. Also, no significant differences were observed in the fecundity rate of females fed on these diets. The mean number of pregnant females after 48 h mating was about 85%.

The average size of litters in diet HO group was significantly smaller than that for the other diets ($P < 0·05$), as may be seen in Table 4. The differences among the other dietary groups were not statistically significant.

Table 3. *Main fatty acid profiles of adipose tissue of Wistar rats fed on different dietary fats (weight % total fatty acids)*
(Mean values with their standard errors)

Diet†...	DOS		BF		SO		RO		HO	
Fatty acid	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
12:0	2·03	0·042	1·18	0·013	1·65	0·022	0·40	0·011	0·29	0·008
14:0	6·80	0·131	4·86	0·052	7·76	0·026	1·96	0·022	2·70	0·023
14:1	0·62	0·016	0·77	0·013	0·45	0·020	0·69	0·022	0·30	0·005
16:0	30·01	0·063	29·25	0·105	28·12	0·556	27·88	0·145	25·35	0·129
16:1	13·12	0·016	13·93	0·109	9·69	0·132	9·26	0·087	12·78	0·066
18:0	4·15	0·019	2·55	0·035	5·81	0·120	4·33	0·041	3·27	0·023
c-18:1	33·08	0·067	40·27	0·147	34·08	0·423	44·71	0·134	32·40	0·052
t-18:1	0·96	0·021	2·17*	0·016	0·47	0·031	0·33	0·011	15·60*	0·048
cc-18:2	5·12	0·060	1·68*	0·056	8·70	0·051	6·56	0·060	2·10*	0·017
tt-18:2	0·50	0·005	1·10*	0·013	0·37	0·019	0·44	0·023	2·18*	0·019
18:3	1·35	0·040	0·14*	0·004	0·62	0·005	1·32	0·015	0·29*	0·005
c-20:1	0·20	0·008	0·60*	0·004	0·12	0·004	0·15	0·004	0·79*	0·017
20:3	0·49	0·007	1·06*	0·010	0·43	0·008	0·38	0·010	1·59*	0·012
20:4	0·32	0·008	0·20*	0·005	0·46	0·011	0·44	0·008	0·12*	0·005
22:1	—		—		—		0·25	0·004	—	
22:6	1·25	0·011	0·24	0·011	1·27	0·014	0·90	0·008	0·24	0·010

c, *cis*-isomer; *t*, *trans*-isomer; DOS, control diet; BF, butterfat; SO, sunflower oil; RO, rapeseed oil low in erucic acid; HO, hydrogenated vegetable oil.

* Mean values in the same horizontal rows were significantly different from values for diets DOS, SO, RO ($P < 0·05$).

† For details of DOS (DOS 2b; Velaz, Prague) and diets BF, SO, RO and HO (semi-purified experimental diet +50 g BF, SO, RO or HO/kg respectively), see Table 1 and p. 520.

Table 4. *Litter size of rats fed on different fats (no. of new born pups for a dam)*
(Mean values with their standard errors)

Generation...	F1 (*n* 12)‡		F2 (*n* 12)‡		F3 (*n* 20)‡	
Diet†	Mean	SE	Mean	SE	Mean	SE
DOS	12·0	0·61	10·9	0·66	10·4	0·51
BF	11·0	0·64	10·8	0·72	9·4[a]	0·40
SO	11·0	0·90	10·3	0·87	10·3	0·56
RO	10·4	0·38	10·6	0·52	10·9	0·45
HO	8·5*	0·75	8·0*	0·78	8·8*	0·63

DOS, control diet; BF, butterfat; SO, sunflower oil; RO, rapeseed oil low in erucic acid; HO, hydrogenated vegetable oil.

* Mean values in the same vertical column were significantly different from other values, except the value marked 'a' ($P < 0·05$).

† For details of DOS (DOS 2b; Velaz, Prague) and diets BF, SO, RO and HO (semi-purified experimental diets +50 g BF, SO, RO or HO/kg respectively), see Table 1 and p. 520.

‡ No. of dams.

In males fed on diet HO a significantly higher incidence of abnormal sperm morphology occurred as compared with males on diet DOS ($P < 0·05$). Differences among other dietary groups were not statistically significant (Table 5).

The levels of serum concentrations of progesterone and testosterone of the experimental rats are summarized in Tables 6 and 7. The concentration of testosterone was significantly

Table 5. *Incidence (%) of abnormal morphology of spermatozoa of male rats fed on different dietary fats*
(Mean values with their standard errors)

Generation...	F1 (*n* 20)‡		F2 (*n* 20)‡		F3 (*n* 40)‡	
Diet†	Mean	SE	Mean	SE	Mean	SE
DOS	6·8	0·20	7·1	0·29	6·3	0·33
BF	7·8	0·18	8·2	0·43	8·9	0·59
SO	7·1	0·36	6·2	0·36	9·2	0·52
RO	7·3	0·31	5·9	0·36	7·6	0·38
HO	9·2*	0·31	9·8*	0·53	12·5*	0·84

DOS, control diet; BF, butterfat; SO, sunflower oil; RO, rapeseed oil low in erucic acid; HO, hydrogenated vegetable oil.
* Mean values in the same vertical column were significantly different from other values ($P < 0·05$).
† For details of DOS (DOS 2b; Velaz, Prague) and diets BF, SO, RO and HO (semi-purified experimental diets +50 g BF, SO, RO or HO/kg respectively), see Table 1 and p. 520.
‡ No. of males. Age of animals 85 d.

Table 6. *Serum concentration of progesterone (ng/ml) in female Wistar rats fed on different dietary fats*
(Mean values with their standard errors)

Generation...	F1 (*n* 12)†		F2 (*n* 12)†		F3 (*n* 20)†	
Diet*	Mean	SE	Mean	SE	Mean	SE
DOS	13·85	2·48	18·00	2·41	13·24	2·09
BF	5·83	0·96	16·26	1·81	6·15	0·88
SO	16·23	2·64	19·40	2·71	10·67	1·15
RO	7·47	0·99	17·19	2·41	7·96	0·78
HO	7·84	1·73	16·13	9·32	6·36	4·97

DOS, control diet; BF, butterfat; SO, sunflower oil; RO, rapeseed oil low in erucic acid; HO, hydrogenated vegetable oil.
* For details of DOS (DOS 2b; Velaz, Prague) and diets BF, SO, RO and HO (semi-purified experimental diets +50 g BF, SO, RO or HO/kg respectively), see Table 1 and p. 520.
† No. of females. Age of animals 85 d.

($P < 0·05$) lower in males on diets HO or BF than in other dietary groups. The concentrations of progesterone in females showed a wide dispersion of values, which makes statistical evaluation impossible. Contrary to all other observations the concentration also tended to be higher in the second generation than in the first or the third generations of the females, for which we can find no reasonable explanation. There seems to be no direct relation with the dietary content of linoleic acid or *trans*-fatty acids.

It is well documented that lack of essential fatty acids impairs the reproductive functions of mammals (Menon *et al.* 1981). So far the only studies with hydrogenated fat that reported differences in reproduction were those in which hydrogenated fats were given to essential-fatty-acid-deficient rats (Holman & Aaes-Jorgensen, 1956; Alfin-Slater *et al.* 1957; Emken, 1984), though *trans*-octadecenoic acid interference with the linoleic acid metabolic pathway was observed in vitro, even in animals fed on essential-fatty-acid-sufficient diets (Kirstein *et al.* 1983). The symptoms observed in our experiment, such as prolonged gestation period, reduced litter size and irregular oestrous cycle in the diet HO-

Table 7. *Serum concentration of testosterone (ng/ml) in male Wistar rats fed on different dietary fats*
(Mean values with their standard errors)

Generation ...	F1 (*n* 20)‡		F2 (*n* 20)‡		F3 (*n* 40)‡	
Diet†	Mean	SE	Mean	SE	Mean	SE
DOS	8·38	0·25	8·68	0·22	9·24	0·16
BF	4·20*	0·13	5·60*	0·19	6·20*	0·17
SO	7·67	0·22	7·34	0·39	8·68	0·29
RO	6·15	0·25	7·25	0·36	8·95	0·18
HO	2·80*	0·15	3·20*	0·21	3·54*	0·14

DOS, control diet; BF, butterfat; SO, sunflower oil; RO, rapeseed oil low in erucic acid; HO, hydrogenated vegetable oil.
* Mean values in the same vertical column were significantly different from other values ($P < 0·05$).
† For details of DOS (DOS 2b; Velaz, Prague) and diets BF, SO, RO and HO (semi-purified experimental diets +50 g BF, SO, RO or HO/kg respectively), see Table 1 and p. 520.
‡ No. of males. Age of animals 85 d.

fed group are usual essential-fatty-acid-deficiency symptoms (Waltman *et al.* 1978; Parlanti & Orellana, 1985; Guesnet *et al.* 1986), though diet HO was not essential-fatty-acid deficient.

In conclusion, it would be of great importance to explore intakes of dietary essential fatty acids that would prevent adverse effects of various partially hydrogenated dietary fats on the reproductive performance of animals, because it seems probable that essential fatty-acid intakes considered sufficient at low dietary intakes of hydrogenated oils may substantially differ from essential fatty-acid requirements of animals consuming high amounts of hydrogenated oils.

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