

Growth Parameters and Plasma-tissue Fatty Acid Profiles of Rats Fed Rubber Seed Oil

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ABSTRACT

Rats fed rubber seed oil (RSO) at the 10% level grew significantly less than those fed RSO at the 5% level or corn oil (CO) at the 5% or 10% levels, these rats showing disproportionately lower liver weights. There was no incidence of fatty liver due to oil source or dietary level. Similarly, no adverse effects were observed in levels of haematocrit, serum glucose and serum α -amino nitrogen in all rats fed RSO or CO. Fatty acid profiles of plasma and liver of RSO- or CO-fed rats were normal, although RSO-fed rats had significantly elevated levels of linolenic acid.

INTRODUCTION

Tropical countries of South East Asia, Africa and Latin America which provide almost all the natural rubber of the world, have a potential to

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produce considerable amounts of rubber seed oil. Malaysia, Indonesia and Thailand have about 5, 3 and 1.5 million hectares, respectively, of rubber plantations (FAO, 1982). We have calculated that these countries have a capacity to produce 137 500, 82 500 and 41 250 metric tonnes, respectively, of rubber seed oil. Rubber seed oil is rich in polyunsaturated fatty acids especially linoleic and linolenic acids and is a semi-drying oil like rapeseed oil but, unlike rapeseed oil, it has no content of erucic acid (Nwokolo *et al.*, 1987). It seems therefore to have a potential as a dietary oil when it refined and its nutritional quality has been exhaustively investigated.

Nutritional and growth studies are essential components of evaluation of new dietary oils. In the 1960s and 1970s, numerous scientists extensively experimented with high erucic acid rapeseed (HEAR) oil and low erucic acid rapeseed (LEAR) oil in diets of a wide range of experimental animals. Results of these studies showed that erucic acid interfered with growth processes and that whereas HEAR oil was associated with growth depression (Thomasson, 1955; Thomasson & Boldingh, 1955) and myocardial lipidosis (Abdellatif & Vles, 1973), LEAR oil was not associated with these problems. These investigations led to the world wide acceptance of canola oil (low erucic acid rapeseed oil) as a dietary oil.

Very little is known about the nutritional quality of rubber seed oil. Rubber seed oil and corn oil have been fed at 8% and 20% levels to broiler chicks (Nwokolo & Sim, 1987). Results indicated no significant differences between the oils for growth, feed consumption, feed conversion ratio and lipid digestibility. Liver lipid content (but not heart lipid content) at the 20% dietary level was significantly higher in chicks fed rubber seed oil than those fed corn oil. These workers reported evidence of myocardial lipidosis in chicks fed either rubber seed oil or corn oil at the 20% level.

The objective of this study was to provide additional information on the nutritional quality of rubber seed oil (RSO), using commercially available corn oil as a control. Growth rate, organ weights, blood chemistry and tissue fatty acid profiles of weanling rats fed these oils were measured after a 21-day feeding period.

MATERIALS AND METHODS

Test diets consisting of casein, starch, sucrose, alfalfa (cellulose), vitamins and minerals were supplemented with rubber seed oil or corn oil, at both the 5% and 10% levels (Table 1). The fatty acid composition of the corn oil and rubber seed oil used in these experiments is shown in Table 2.

Weanling Wistar rats used in the feeding trials were raised on a standard rat chow for two weeks prior to the onset of the experiments. At the

TABLE 1
Composition of Diets Containing RSO or CO at 5% and 10% Dietary Levels

Ingredients (w/w %)	RSO diets		CO diets	
	5% RSO	10% RSO	5% CO	10% CO
Casein	20.0	20.0	20.0	20.00
Corn starch	15.00	15.00	15.00	15.00
Sucrose	49.85	39.85	49.85	39.85
Alfacel (Fibre)	5.00	10.00	5.00	10.00
Vegetable oil	5.00	10.00	5.00	10.00
Vitamin mix ^a	1.00	1.00	1.00	1.00
Mineral mix ^b	3.50	3.50	3.50	3.50
Choline chloride	0.20	0.20	0.20	0.20
DL-Methionine	0.30	0.30	0.30	0.30
L-Lysine	0.15	0.15	0.15	0.15
Calculated Compositions				
Crude protein (%)	17.00	17.00	17.00	17.00
Metab. energy (kcal/kg)	3671	3614	3675	3624
DL-Methionine (%)	0.86	0.86	0.86	0.86
L-Lysine (%)	1.75	1.75	1.75	1.75

^a Vitamin mixture ICN 76, from Nutritional Biochemicals Corporation, Cleveland, OH, USA.

^b Mineral mixture ICN 76, from Nutritional Biochemicals Corporation, Cleveland, OH, USA.

TABLE 2
Composition of Vegetable Oils used in Experimental Diets

Fatty acids (%)	Corn oil	Rubber seed oil
C14:0	0.01	0.08 ^a
C14:1	0.00	0.00
C16:0	13.10 ^a	9.27
C16:1	0.27	0.14
C18:0	2.09	10.58 ^a
C18:1	26.55	26.64
C18:1	56.17 ^a	34.92
C20:0	0.00	0.57 ^a
C18:3	0.75	17.27 ^a
C20:1	0.46 ^a	0.18
C22:0	0.00	0.15 ^a

^a Significantly different by 't' test $P < 0.05$.

commencement of the feeding trials, average rat weight was 162.1 ± 6.3 g. Rats were housed individually in a stainless steel metabolism cage in a temperature (22°C) and fluorescent light (12 h, 0700–1900 h) controlled room. There were four dietary treatments; 5% RSO, 5% CO, 10% RSO, 10% CO and six individual rat replications. Feed and water were provided daily to appetite and daily feed consumption recorded. Rats were weighed every seven days, preceded by 16-h withdrawal of feed and water.

At the end of the 21-day experimental period, all rats in each treatment group were weighed at 0900–1000 h. Animals were anaesthetized with ether and 10 ml of blood was taken from each rat from the vena cava. Haematocrit and haemoglobin measurements were performed immediately. Serum was obtained by centrifugation ($3000 \times g$, 20 min) and stored at -20°C until analyses of serum glucose (glucose oxidase) and α -amino nitrogen (Goodwin, 1968) were performed. The carcasses were immediately dissected after blood sampling with liver, heart, kidney, adipose tissue and spleen being removed, weighed and frozen. Frozen blood plasma and tissue homogenates of adipose tissue and liver were extracted with Folch's reagent (Folch *et al.*, 1957), methylated with BF_3 (Metcalf *et al.*, 1961) and analyzed for component fatty acids using a Varian Model 3700 gas chromatograph. Total lipid content of rat heart and liver were determined by AOAC methods (AOAC, 1984). All data were analyzed statistically by analysis of variance procedure (Steel & Torrie, 1984).

RESULTS AND DISCUSSION

Rats fed rubber seed oil at the 10% dietary level exhibited significantly ($P < 0.05$) slower growth rate compared with animals fed the 5% rubber seed oil or the 5% and 10% corn oil diets (Table 3). These results suggest that at high dietary levels (up to 23.3% of total dietary energy) crude rubber seed oil may depress animal growth. Inclusion of most oils at the 10% dietary level may be considered high but certainly not excessive and is not expected to depress growth. Why growth depression occurred in rats fed 10% rubber seed oil diets but not 10% corn oil diets is therefore not clear. The test diets were isocaloric and isonitrogenous, which would indicate that the growth depression could not be attributed to any disparity in the energy or protein content of these test diets.

The physico-chemical properties of rubber seed oil were compatible with an oil that was unstable in storage and prone to rancidity and autoxidation. The oil had high iodine value (138), saponification number (192), free fatty acid content (4.51%) and peroxide value (7.5 mEq/kg) in comparison with the corn oil control which also had high iodine value (124) and

TABLE 3
Growth Rate, Feed Consumption, Feed Conversion Ratio and Blood Chemistry of Rats fed Corn Oil or Rubber Seed Oil

	5% Oil diets		10% Oil diets	
	CO	RSO	CO	RSO
Average daily gain (g)	6.99 ^a ± 0.02	7.30 ^a ± 0.02	7.09 ^a ± 0.05	6.28 ^b ± 0.04
Average daily feed consumed (g)	20.2 ^b ± 1.8	20.8 ^a ± 0.7	20.6 ^a ± 1.1	19.2 ^b ± 1.0
Feed conversion ratio	2.92 ^b ± 0.01	2.85 ^b ± 0.01	2.91 ^b ± 0.01	3.10 ^a ± 0.11
Haematocrit (%)	47.9 ^{ab} ± 0.93	50.2 ^a ± 1.89	46.9 ^b ± 1.11	49.3 ^a ± 0.74
Haemoglobin (g/dl)	13.8 ^b ± 0.48	14.1 ^a ± 0.41	14.0 ^a ± 0.37	14.1 ^a ± 0.81
Serum glucose (mg/dl)	130 ^{ab} ± 4.35	131 ^a ± 3.47	124 ^b ± 6.00	132 ^a ± 3.33
Serum α amino nitrogen (mg/dl)	7.95 ^b ± 0.18	9.38 ^a ± 0.22	7.95 ^a ± 0.10	9.07 ^{ab} ± 0.57

^{a,b} Means with different superscripts within rows are significantly different at $P < 0.05$.

Feed conversion ratio = gram feed consumed/gram weight gain.

Values are mean \pm SEM.

saponification number (188) but very much lower peroxide value (0.14 mEq/kg) and no free fatty acid content.

A peroxide value of 1 mEq/kg is judged borderline in terms of rancidity (Weiss, 1970). The primary products of lipid oxidation are hydroperoxides (or simply peroxides) and the concentration of peroxides in an oil is considered a measure of the extent of oxidation of the oil. The high peroxide value of rubber seed oil in comparison with corn oil indicated that either during production or storage of the oil, considerable oxidation occurred. Hydroperoxides formed during oil oxidation, rapidly break down to form aldehydes which have strong disagreeable flavours and odours. There was a definite objectionable odour to the rubber seed oil and to the test diets containing the oil, in contrast with the corn oil and corn oil diets. Caution must, however, be exercised in suggesting that the growth restriction in rats fed 10% rubber seed oil was purely due to the oil. It could also be due to the unrefined state of rubber seed oil, a situation which could not have been prevented in this study, because refined rubber seed oil was not available. There is every indication that rubber seed oil is neither being produced nor refined at any appreciable level anywhere in the world; hence there is a need for this study. It is noteworthy, however, that in a previous experiment (Nwokolo & Sim, 1987) in which crude rubber seed oil was also fed at 8% and at 20% dietary level to chicks, a serious incidence of feed refusal was observed at the 20% levels (but not at the 8% level). In the present experiment, there was a significant depression in feed consumption by rats

fed the 10% RSO diet, which may indicate some anti-palatability at the higher dietary levels of rubber seed oil.

Growth rates observed in rats fed RSO at the 5% level were similar to values previously reported in animals fed other dietary oils such as olive oil or peanut oil (Craig & Beare, 1968), corn oil (Hung *et al.*, 1977) or soybean oil (Kramer *et al.*, 1973). The severe growth depression which was observed in rats fed rapeseed oil (Kramer *et al.*, 1973) was not observed in the present study at either the 5% or 10% dietary levels of RSO. This may be explained on the basis that our analysis of RSO showed no detectable level of erucic acid (C22:1, *n*9) and only an extremely low content of gandoic acid (C20:1, *n*9).

Table 4 presents the body weights and individual organ weights of rats fed corn oil and rubber seed oil. Absolute liver weights (not shown) as well as relative weights (g/100 g body wt) of this organ in rats fed the 10% RSO diet were found to be disproportionately lower ($P < 0.05$) than in other rats fed the 5% RSO and the corn oil diets. We have mentioned earlier the probability of auto-oxidation products being responsible for toxicity and reduced growth in rats fed 10% RSO. In the absence of any other plausible explanation, it is suggested that oxidation-derived anti-nutritional factors

TABLE 4
Body and Tissue Weights and Tissue Lipid Content of Rats fed Corn Oil or Rubber Seed Oil

	5% Oil diets		10% Oil diets	
	CO	RSO	CO	RSO
Final body weights (g)	297.0 ^b ± 5.1	311.5 ^a ± 6.0	317.5 ^a ± 6.1	297.0 ^b ± 5.8
Body weight gain (g)	147.0 ^a ± 4.8	154.8 ^a ± 5.1	150.8 ^a ± 4.9	132.0 ^b ± 4.3
Liver weight (g/100 g body wt)	4.65 ^b ± 0.10	4.78 ^{ab} ± 0.11	4.85 ^a ± 0.14	4.17 ^c ± 0.08
Heart weight (g/100 g body wt)	0.34 ± 0.02	0.33 ± 0.01	0.34 ± 0.02	0.33 ± 0.02
Kidney weight (g/100 g body wt)	0.84 ± 0.04	0.83 ± 0.03	0.84 ± 0.03	0.81 ± 0.04
Spleen weight (g/100 g body wt)	0.25 ± 0.01	0.26 ± 0.02	0.26 ± 0.01	0.27 ± 0.02
Liver lipid content (% wet weight)	4.79 ^b ± 0.11	4.80 ^b ± 0.13	5.06 ^a ± 0.18	5.04 ^a ± 0.15
Heart lipid content (% wet weight)	2.95 ^b ± 0.11	2.97 ^b ± 0.17	3.15 ^a ± 0.29	3.17 ^a ± 0.21

^{a,b,c} Means with different superscripts within rows are significantly different at $P < 0.05$. Values are mean ± SEM.

were responsible for the growth restriction observed. The processes of refining which include degumming, acid neutralization, bleaching and deodorization, are usually enough to remove any anti-nutritional factors in oils. Refining can therefore improve the nutritional quality of RSO. In tropical countries, for which rubber seed oil is being proposed as a source of dietary oil, there is little or no refining of vegetable oils for human use. Many tropical oils including palm, palm kernel, coconut, groundnut and melon seed oil have always been utilized in the unrefined state. The rubber seed oil evaluated in this experiment was not refined because primarily, the study was designed to investigate the existence of any toxicity in crude rubber seed oil. It was also necessary to evaluate the unrefined rubber seed oil against commercially available corn oil. Results of this study suggest that indeed rubber seed oil needs to be refined and stabilized with anti-oxidants before further evaluation as a possible dietary oil for human use.

The fat content of liver and heart tissues in rats fed rubber seed oil or corn oil at the two dietary levels is presented in Table 4. Morphological examination of rat livers and staining of liquid-nitrogen frozen samples with Sudan III did not indicate a fatty liver in animals fed rubber seed oil. This was confirmed later with liver lipid determinations.

Similarly, there was no significant difference in heart lipid content among rats fed the two sources of dietary oils. These results demonstrate that rubber seed oil can be fed up to the 10% dietary level or 19.9% of total dietary calories without inducing cardiac lipidosis or fatty liver in rats.

The blood chemistry analysis involving haemoglobin, haematocrit, serum glucose and α -amino nitrogen in rats fed corn oil and RSO has been presented along with growth data in Table 3. There was no evidence of metabolic or physiological disorders attributable to RSO at either the 5 or 10% dietary levels despite the fact that the RSO was not refined. While it has been reported that the kind as well as content of fat in the diet can influence net protein utilization in rats (Kluszczyńska, 1979), there was no sign of abnormal levels of serum α -amino nitrogen in rats fed RSO. Naismith (1962) reported increased nitrogen excretion and marked reduction in nitrogen retention in rats fed diets deficient in essential fatty acids. Our observation that RSO contains an equivalent level of EFA, compared to corn oil (see Table 1) and that all dietary treatments received the same level of protein, probably explains why there were no apparent differences in the serum α -amino nitrogen levels between groups.

Finally, the fatty acid profiles of lipids in plasma, heart, liver and adipose tissue of rats fed RSO and corn oil, respectively, are presented in Tables 5–7. These results demonstrate that both the total fatty acid composition of the dietary oil, as well as the level in which it is fed, are important factors in influencing the relative disposition of fatty acids in tissue lipids. Rats fed

TABLE 5
Plasma Fatty Acid Profile of Rats fed Corn Oil or Rubber Seed Oil

Fatty acids (%)	5% Oil diets		10% Oil diets	
	Corn oil	Rubber seed oil	Corn oil	Rubber seed oil
C14:0	0.37 ^{ab} ± 0.03	0.34 ^b ± 0.04	0.46 ^a ± 0.06	0.43 ^a ± 0.08
C16:0	17.26 ^b ± 1.00	18.23 ^{ab} ± 1.15	18.76 ^a ± 1.31	18.75 ^a ± 1.21
C16:1	2.94 ^a ± 0.06	2.18 ^c ± 0.11	2.44 ^b ± 0.19	2.85 ^a ± 0.21
C18:0	8.36 ^b ± 0.41	8.54 ^{ab} ± 0.34	8.92 ^a ± 0.53	9.28 ^a ± 0.71
C18:1	18.08 ^b ± 1.81	18.86 ^a ± 2.01	18.71 ^a ± 1.95	18.88 ^a ± 2.18
C18:2	23.61 ± 1.30	23.91 ± 1.39	23.63 ± 1.41	23.86 ± 1.33
C18:3	0.0	0.99 ± 0.03	0.0	0.98 ± 0.02
C20:0	0.31 ± 0.02	0.32 ± 0.02	0.33 ± 0.03	0.34 ± 0.03
C20:1	—	—	—	—
C20:4	20.93 ^a ± 1.13	20.05 ^a ± 1.09	18.76 ^b ± 1.27	18.95 ^b ± 1.11
C20:5	0.35 ^b ± 0.02	0.37 ^b ± 0.03	0.46 ^a ± 0.03	0.38 ^b ± 0.03
C22:0	—	—	—	—
C22:5	2.18 ± 0.41	2.08 ± 0.47	2.13 ± 0.61	2.17 ± 0.44
C22:6	1.27 ^b ± 0.04	1.30 ^b ± 0.05	1.37 ^a ± 0.04	1.40 ^a ± 0.05

^{a,b,c} Means with different superscripts within rows are significantly different at $P < 0.05$. Values are mean ± SEM.

TABLE 6
Fatty Acids of Liver Tissue of Rats Fed Corn Oil or Rubber Seed Oil

Fatty acids (%)	5% Oil diets		10% Oil diets	
	Corn oil	Rubber seed oil	Corn oil	Rubber seed oil
C14:0	0.33 ± 0.03	0.32 ± 0.03	0.36 ± 0.04	0.35 ± 0.06
C14:1	—	—	—	—
C16:0	22.33 ± 1.13	22.23 ± 1.26	22.49 ± 1.18	22.43 ± 1.23
C16:1	1.96 ± 0.07	1.92 ± 0.09	2.01 ± 0.08	2.05 ± 0.12
C18:0	13.70 ± 1.16	13.86 ± 1.18	14.09 ± 1.22	14.65 ± 1.09
C18:1	18.40 ^a ± 1.09	18.2 ^{ab} ± 1.11	18.47 ^a ± 0.93	18.02 ^b ± 0.96
C18:2	17.37 ^{bc} ± 1.10	17.105 ^c ± 1.11	18.23 ^a ± 1.23	17.57 ^b ± 1.08
C20:0	0.19 ^c ± 0.01	0.32 ^{ab} ± 0.02	0.29 ^b ± 0.02	0.35 ^a ± 0.02
C18:3	0.00 ^c	1.34 ^b ± 0.05	0.00 ^c	1.56 ^a ± 0.06
C20:1	0.21 ± 0.01	0.20 ± 0.01	0.22 ± 0.01	0.20 ± 0.01
C20:4	15.31 ^c ± 1.00	16.46 ^b ± 0.93	18.07 ^a ± 0.99	18.01 ^a ± 1.01
C22:0	0.35 ^b ± 0.02	0.55 ^a ± 0.05	0.38 ^b ± 0.03	0.53 ^a ± 0.05
C22:5	3.15 ^b ± 0.21	2.89 ^c ± 0.26	3.36 ^a ± 0.36	3.18 ^b ± 0.28
C22:6	1.75 ± 0.08	1.79 ± 0.06	1.78 ± 0.08	1.82 ± 0.05

^{a,b,c} Means with different superscripts within rows are significantly different at $P < 0.05$. Values are mean ± SEM.

TABLE 7
Fatty Acids of Adipose Tissue of Rats Fed Corn Oil or Rubber Seed Oil

Fatty acids (%)	5% Oil diets		10% Oil diets	
	Corn oil	Rubber seed oil	Corn oil	Rubber seed oil
C14:0	2.09 ± 0.35	1.87 ± 0.28	2.15 ± 0.30	1.96 ± 0.40
C14:1	0.37 ± 0.04 ^a	0.23 ± 0.06 ^b	0.33 ± 0.06 ^b	0.23 ± 0.04 ^b
C16:0	24.10 ± 1.60 ^b	27.21 ± 1.81 ^a	25.33 ± 1.73 ^{ab}	23.91 ± 1.98 ^b
C16:1	7.24 ± 0.72 ^a	7.89 ± 0.75 ^a	5.33 ± 0.48 ^b	5.22 ± 0.52 ^b
C18:0	4.07 ± 0.58 ^b	4.48 ± 0.60 ^{ab}	4.03 ± 0.38 ^b	5.18 ± 0.78 ^a
C18:1	33.94 ± 3.17 ^a	33.84 ± 2.95 ^a	29.35 ± 2.61 ^b	32.28 ± 3.01 ^{ab}
C18:2	24.12 ± 2.75 ^b	16.46 ± 1.89 ^d	28.26 ± 2.72 ^a	21.36 ± 2.01 ^c
C20:0	0.14 ± 0.02 ^a	0.10 ± 0.01 ^b	0.15 ± 0.06 ^a	0.11 ± 0.07 ^b
C18:3	0.52 ± 0.17 ^c	4.61 ± 0.75 ^b	0.48 ± 0.21 ^c	5.96 ± 0.90 ^a
C20:1	0.20 ± 0.01 ^b	0.38 ± 0.04 ^a	0.14 ± 0.03 ^c	0.34 ± 0.04 ^a
C22:0	0.09 ± 0.00 ^b	0.02 ± 0.00 ^c	0.19 ± 0.00 ^a	0.01 ± 0.00 ^c
C22:5	0.23 ± 0.09 ^b	0.05 ± 0.02 ^c	0.36 ± 0.08 ^a	0.07 ± 0.00 ^c
C22:6	0.19 ± 0.03 ^a	0.02 ± 0.00 ^b	0.20 ± 0.04 ^a	0.02 ± 0.00 ^b

^{a,b,c,d} Means with different superscripts within rows are significantly different at $P < 0.05$. Values are mean ± SEM.

RSO at the 10% dietary level, exhibited a relatively higher proportion of stearic acid in plasma, heart, liver and adipose tissue compared with rats fed 5% RSO, or corn oil at both the 5% and 10% levels. On the other hand, feeding rats RSO at both the 5% and 10% dietary levels resulted in a significantly ($P < 0.05$) higher uptake of linolenic acid (C18:3) than rats fed corn oil. In contrast, the disposition of linoleic acid (C18:2) was significantly less in rats fed RSO, compared to those animals fed corn oil. These results are a reflection of the fatty acid composition of RSO and possibly the preferential uptake and retention of certain polyunsaturated fatty acids (Beare, 1961). The fact that higher tissue levels of linolenic acid (C18:3) occur in rats fed RSO is a reflection of the composition of this particular oil and may be particularly noteworthy in regard to the recent findings that *n*-3 fatty acids are essential for normal development and function of the brain and retina (Neuringer *et al.*, 1986). Moreover, other studies have shown that the deficiency of *n*-3 polyunsaturated fatty acids can impair cognitive performance (Lamprey & Walker, 1976). Thus, in certain areas of the tropics where chronic malnutrition exists, it is feasible to suggest that this particular oil seed could have an advantageous role in supplying the necessary fatty acids required for optimal development.

In summary, there is no evidence produced from this study which would indicate an obvious toxic effect of RSO in growing rats fed at the 5% dietary

level. At this particular level, biochemical indicators of health and body function were not adversely affected by the inclusion of this oil in the diet. Some concern of a relatively mild toxic effect may exist with RSO, when fed at the 10% level, although, we emphasize that this needs to be reevaluated using a refined source of this oil. The preferential uptake and retention of linolenic acid in rats fed RSO may be viewed as a considerable asset, when taking into consideration its importance in both development and function of various organ systems.

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