Effect of *Sterculia foetida* Oil on Weanling Rat Growth and Survival

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Sterculia foetida oil is a rich source of the cyclopropene fatty acid, sterculic. Feeding this oil to male weanling rats resulted in poor growth at moderate levels of oil in the diet and death at levels of 5% and above. The symptoms leading to death were characteristic of B-vitamin deficiency. Although high dietary levels of pyridoxine, pantothenate, and biotin prevented death, growth improved only slightly.

Several seed oils are known to contain significant amounts of the cyclopropenoid fatty acids which have been implicated in various detrimental biological effects (Phelps *et al.*, 1965). However, little work has been reported on the effect of these oils on typical weanling rat growth. An early study by Thomasson (1955) showed that rats fed kapok seed oil, containing the C_{17} cyclopropenoid fatty acid bombacic (Dijkstra and Duin, 1955), died within 18 days when the oil was fed at 40% or more of the calories, and that lower levels resulted in slower growth. The present paper reports the results of feeding *Sterculia foetida* oil, containing the C_{19} cyclopropenoid fatty acid sterculic (Nunn, 1952), to male weanling rats, and the effect of supplementing the diet with specific B-vitamins on growth and survival.

EXPERIMENTAL

Two types of experiments were conducted. In Experiment 1, the effect of graded levels of *S. foetida* oil (0 to 10% of the diet) on growth, organ weights, liver composition, and iodine values of epididymal fat was studied. In Experiment 2, several trials were conducted with groups of rats fed 5% *S. foetida* oil–5% corn oil diets, and the capacity of high dietary levels of B-vitamins alone or in combination to prevent death and promote growth was studied.

The basal diet (18% casein) was that reported by Sheehan and Vavich (1965) but contained 10% corn oil. The vitamin mixture in this diet supplied per 100 grams of diet (in milligrams): thiamine·HCl, 0.4; riboflavin, 0.5; niacinamide, 5.0; pyridoxine·HCl, 0.25; Ca pantothenate, 2.0; choline bitartrate, 200.0; inositol, 100.0; *p*-aminobenzoic acid, 10.0; folic acid, 0.2; biotin, 0.02; vitamin B_{12} (0.1% trit. in mannitol), 10.0; menadione, 0.2; α-tocopherol, 5.0; and 1000 U.S.P. units of vitamin A and 120 U.S.P. units of vitamin D_3.

S. foetida oil was prepared by extracting ground *S. foetida* seeds three times with three volumes of equal parts of Skellysolve F and B. The solvent was removed from the oil under reduced pressure at 35° C. in a glass circulating evaporator and a rotating evaporator. The extent of solvent removal was tested by treating corn oil with the same process. Rats fed this treated corn oil exhibited the same growth, food efficiency, and organ weights as the corn oil controls (data not shown). The *S. foetida* oil contained approximately 35% cyclopropenoid fatty acid (modified Halphen test, Sheehan, 1967), and was added to the basal diet in lieu of corn oil. All diets contained 10% added fat. Sufficient diets were prepared to last three weeks and were kept refrigerated. Fresh diet was fed daily since diets containing *S. foetida* oil become rancid within a few days at room temperature. [TBA values as measured by the method of Sidwell *et al.* (1954) of the corn oil control diet and 5% *S. foetida* oil–5% corn oil diet were 0.095 and 0.124 absorption unit when stored at room temperature for 24 hours and 0.297 and 0.315 absorption unit when stored for 21 days in the refrigerator, respectively.]

Sprague-Dawley male weanling rats were randomly selected into groups of 10 each of comparable weight. The animals were housed in individual screen-bottomed cages in a constant temperature (26° C.) room; food and water were fed *ad libitum*. The rats were sacrificed at the end of six weeks by intraperitoneal injection of sodium thiamylal (Surital, Parke, Davis and Co.) after an overnight fast.

Liver solids and fats were determined according to the method of Sarett and Jandorf (1947). The epididymal fat pads were extracted, and iodine values of the extracted fat were determined (Schneider *et al.*, 1962).

RESULTS AND DISCUSSION

Experiment 1. The data on the growth experiment are summarized in Table I. Six-week weight gain of the rats fed 1% *S. foetida* oil–9% corn oil diet was 81% of the corn oil controls, significantly less. Feeding 2.5% *S. foetida* oil–7.5% corn oil and 5% *S. foetida* oil–5% corn oil diets resulted in still poorer growth of the rats and only four of the 10 rats fed the latter diet survived. On the average, death occurred at 15 days. The 7.5% *S. foetida* oil–2.5% corn oil and 10% *S. foetida* oil diets caused death of all animals with average time of death occurring at 14 and 13 days, respectively.

Water consumption was similar in all groups, and food consumption of the groups fed *S. foetida* oil did not vary greatly from the corn oil controls until the level of *S. foetida* oil was 5% of the diet. However, food efficiencies

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Table I. Effect of *S. foetida* Oil on Weight Gain, Food Efficiency, and Organ Weight and Composition of Male Weanling Rats

	Per Cent Oil in Diets					
S. foetida oil	...	1	2.5	5.0	7.5	10.0
Corn oil	10	9	7.5	5.0	2.5	...
No. of rats, survivors	10	10	10	4	0	0
6-Week data						
Wt. gain, grams	208 ± 19[a]	168 ± 16[b]	126 ± 25[b]	63 ± 11[b]	...	...
Water intake, grams	894	902	903	910	...	...
Food intake, grams	586	650	559	219	...	...
Food efficiency[c]	32.1 ± 3.2	25.9 ± 2.8[b]	22.5 ± 3.8[b]	21.6 ± 5.7[b]	...	...
Organ weights and composition						
Liver wt., grams	6.88	6.93	6.60	6.26	...	...
% body wt.	2.7 ± 0.3	3.3 ± 0.6[b]	4.0 ± 0.6[b]	5.9 ± 1.1[b]	...	...
% solids	29.8 ± 0.5	29.4 ± 1.7	29.4 ± 1.7	28.1 ± 1.4	...	...
% fat	4.6 ± 0.2	4.7 ± 0.9	4.7 ± 0.7	3.8 ± 0.5	...	...
Kidney wt., grams	1.63	1.42	1.26	0.93	...	...
% body wt.	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	...	...
Adrenal wt., mg.	47.0	42.7	36.7	31.6	...	...
Mg. per 100 grams of body wt.	19.2 ± 1.7	20.5 ± 1.7	22.1 ± 3.1	30.1 ± 4.5	...	...
Epididymal fat I_2 value	87 ± 7	69 ± 8[b]	69 ± 3[b]	...	...	...

[a] Mean ± standard deviation.
[b] $P < 0.02$ or less.
[c] Grams gained per 100 grams of food.

of all groups fed *S. foetida* oil were significantly lower than the corn oil controls.

The effect of *S. foetida* oil on rat growth was similar to that found for kapok seed oil (Thomasson, 1955). Poorer growth and food efficiencies were also observed in female weanling rats fed low levels of *S. foetida* oil (Sheehan and Vavich, 1965) and in sexually immature hens fed 200 mg. of *S. foetida* oil daily (Schneider *et al.*, 1962).

Relative liver weights (per cent of body weight) increased as the dietary level of *S. foetida* oil was increased and were significantly higher than the corn oil controls. However, composition analyses of the livers showed no consistent difference in solids or fat, suggesting that the increase in relative liver weights of the *S. foetida* oil–fed rats was probably due to the smaller body weights of these animals.

Relative kidney and adrenal weights of the group fed 1% *S. foetida* oil–9% corn oil diet were not different from the corn oil controls, but these organs were significantly heavier in the animals fed higher levels of *S. foetida* oil. These differences were also probably related to the smaller body weights of the animals fed *S. foetida* oil.

The iodine values of the epididymal fat pads from the groups fed 1% *S. foetida* oil–9% corn oil and 2.5% *S. foetida* oil–7.5% corn oil diets were significantly lower than found in the corn oil–fed controls (Table I). Considering that the iodine values of these oils were 76 for *S. foetida* oil and 122 for corn oil, the iodine values of the epididymal fat pads from animals fed *S. foetida* oil were much lower than could be expected from the amount of oil present in the diets. This decrease in fat iodine values was also noted in the depot fat of chickens fed *S. foetida* oil (Schneider *et al.*, 1962). It typified the increased deposition of saturated fatty acids that occurs when seed oils containing cyclopropenoid fatty acids are fed, and the apparent interference of sterculic acid with the metabolic conversion of stearic acid to oleic (Evans *et al.*, 1962; Schneider, 1962).

Experiment 2. The symptoms accompanying death of rats fed *S. foetida* oil were characteristic of a vitamin-B complex deficiency. Prior to death, the animals developed a general malaise followed by porphyrin deposits about the nose, mouth, whiskers, and on the feet; autopsy revealed a flaccid intestinal tract distended with gas and a general lack of muscle tone. Thus, several trials were conducted to determine if B-vitamin supplementation would overcome the effect of *S. foetida* oil. Throughout these trials, survival and growth of rats fed the 5% *S. foetida* oil–5% corn oil diet containing higher than normal levels of B-vitamins were compared with corn oil controls (Table II).

High levels of riboflavin alone and a combination of riboflavin, thiamine, pyridoxine, pantothenate, and biotin were studied in Trial I. Riboflavin alone (Group 2) had no effect, but the combination of the 5 vitamins (Group 3) provided 100% survival and growth was 72% of the corn oil controls.

Thiamine levels of 5, 15, and 30 times normal dietary level (Trial II, Groups 5, 6, and 7, respectively) had no effect. Poor growth and deaths occurred in all of these groups, and the study was terminated after 2 weeks.

In Trial III, increased dietary levels of the single vitamins pyridoxine, pantothenate, and biotin; combination of these three vitamins; and the five-vitamin combination listed above were studied. Pyridoxine or pantothenate

Table II. Effect of High Levels of B-Vitamins on Survival and Growth of Male Weanling Rats Fed 5% *S. foetida*–5% Corn Oil Diets for Six Weeks

	Vitamins Added								
Group No.	Ribo.	Thia.	Pyri.	Pant.	Biotin	Niacin	Multiples of Normal Level[a]	% of Survivors	Weight Gain, % of Corn Oil Controls[b]
Trial I (riboflavin)									
1	−	−	−	−	−	−	−	33	40
2	+	−	−	−	−	−	10	33	35
3	+	+	+	+	+	−	10	100	72
Trial II (thiamine)[c]									
4	−	−	−	−	−	−	−	80	20
5	−	+	−	−	−	−	5	90	8
6	−	+	−	−	−	−	15	70	9
7	−	+	−	−	−	−	30	70	2
8	+	+	+	+	+	−	15	100	46
Trial III (pyridoxine, pantothenate, biotin)									
9	−	−	−	−	−	−	−	70	37
10	−	−	+	−	−	−	15	100	47
11	−	−	−	+	−	−	15	100	43
12	−	−	−	−	+	−	15	80	46
13	−	−	+	+	+	−	15	100	58
14	+	+	+	+	+	−	15	100	56
Trial IV (niacin)									
15	−	−	−	−	−	−	−	70	33
16	−	−	−	−	−	+	10	60	23
17	−	−	+	+	+	+	10	100	42
18	−	−	+	+	+	−	10	100	39
19	+	+	+	+	+	+	10	90	54

[a] Normal levels of vitamins (mg. per 100 grams of diet): thiamine hydrochloride, 0.4; riboflavin, 0.5; pyridoxine hydrochloride, 0.25; calcium pantothenate, 2.0; biotin, 0.02; and niacinamide, 5.0.
[b] Corn oil controls included in each trial, but data not shown. Growth of these groups was normal; average 204 grams of weight gain in 6 weeks.
[c] Two-week data.

alone (Groups 10 and 11) provided 100% survival and improved growth slightly. Increasing the level of biotin (Group 12) improved growth but survival was 80%. With the combination of pyridoxine, pantothenate, and biotin (Group 13) survival and growth were equal to those obtained when riboflavin and thiamine were included with these three vitamins (Group 14).

Increasing the level of niacin to 10 times the normal level (Trial IV, Group 16) did not affect survival nor improve growth (Group 15). These parameters were only slightly improved when it was included with the pyridoxine-pantothenate-biotin combination (Group 17 *vs.* Group 18) or with all six vitamins (Group 19).

Of the six vitamins studied, increased levels of pyridoxine or pantothenate alone or in combination with biotin were most effective in sustaining life and improving growth. However, in no case did growth reach that of the corn oil controls.

The sequelae of events which lead to poor growth and death of rats fed *S. foetida* oil remain unknown. *S. foetida* oil has been shown to inhibit dehydrogenation of saturated fatty acids to the corresponding monoenes (Reiser and Raju, 1964), but that complete blockage of this enzyme system would cause death has not been clarified. Similarly, the B-vitamins found effective in the above studies are known to be involved in lipid metabolism, but further work is needed to determine their exact role.

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