EFFECTS OF INGESTION OF *STERCULIA FOETIDA* OIL ON THE SEXUAL DEVELOPMENT OF THE FEMALE RAT

by

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Edward T. Sheehan. EFFECTS OF INGESTION OF STERCULIA FOETIDA OIL ON THE SEXUAL DEVELOPMENT OF THE FEMALE RAT. M. S. Thesis, Committee on Agricultural Biochemistry and Nutrition. The University of Arizona, 1964.

Previous workers have shown that Sterculia foetida oil is a rich source of the Halphen-active cyclopropenoid fatty acid, sterculic. This fatty acid produces several abnormal biological effects in the avian species, one of which is delay of the sexual development of pullets. In this thesis the effect of this oil on the sexual development of the female rat is reported.

Groups of 10 weanling female Sprague-Dawley albino rats were fed purified diets with 4% corn oil or 4% safflower oil control diets, or control diets substituted with 2% or 3% Sterculia foetida oil to determine the influence of Sterculia foetida oil on sexual maturity as indicated by the age of the rat at the time the vagina opened and the length of the estrous cycles. In addition, the effects of Sterculia foetida oil on growth and weights of certain organs were noted.

Feeding of 2% safflower oil-2% Sterculia foetida oil diet delayed sexual maturity as shown by delayed opening of the vagina

compared with rats fed either control diet. Feeding of 1% safflower oil-3% Sterculia foetida oil diet caused a greater delay in the opening of the vagina and increased the average length of the estrous cycle to eight days as compared with an average of five days for rats fed control diets. After sixteen weeks of dietary feeding, the rats were sacrificed and certain organs were excised and weighed. The liver and ovaries-oviducts-uterus weights for the 1% safflower oil-3% Sterculia foetida oil groups were significantly different from the control oil groups, but brain, kidney and heart weights were not.

INTRODUCTION

Sterculia foetida oil contains from 35 to 70% of the cyclopropenoid fatty acid, sterculic, as glycerides in the form of mono-, di- and tristerculin (Varma, *et al.*, 1957). The structure of this acid was named and identified by Nunn (1952) as a 19-carbon acid with a cyclopropene ring in the 9, 10 position as shown in Figure 1.

A second cyclopropenoid fatty acid, malvalic, has been identified in cottonseed oil. According to MacFarlane (1957), this acid contains 18-carbons with the cyclopropene ring in the 8, 9 position as shown in Figure 1. The content of cyclopropenoid fatty acids in cottonseed oils varies but seldom exceeds a concentration of 1 per cent. Phelps (1962) reported that at least three dozen plants of the order Malvales contain cyclopropenoid fatty acids.

The earliest reports of biological effects of the cyclopropenoid fatty acids were attributed to cottonseed meal or cottonseed oil. Sherwood (1931) noted pink-white discoloration of stored eggs obtained from hens fed cottonseed oil. This was confirmed by Lorenz and Almquist (1934) and Kemmerer, *et al.* (1962). Confirmation that the cyclopropenoid fatty acids were responsible for this phenomenon in stored eggs was provided by Masson, *et al.* (1957) and by Shenstone and Vickery (1956, 1959). Subsequent work showed that

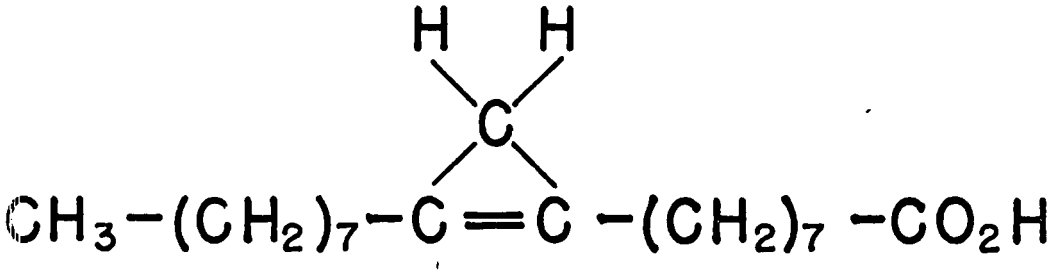
the pink-white phenomenon produced changes in the egg as follows: increased pH of the yolk; changes in the fatty acid composition of the yolk oil, particularly an increase in stearic acid and a decrease in oleic acid content; passage of water and soluble proteins across the vitelline membrane.

Almquist (1934) reported finding several birds with small ovaries among pullets fed cottonseed oil rations. Later, Schneider (1961, 1962) showed that feeding of Sterculia foetida oil to pullets greatly retarded growth of ovaries and oviducts of pullets. He also reported complete cessation of egg production in laying hens fed Sterculia foetida oil.

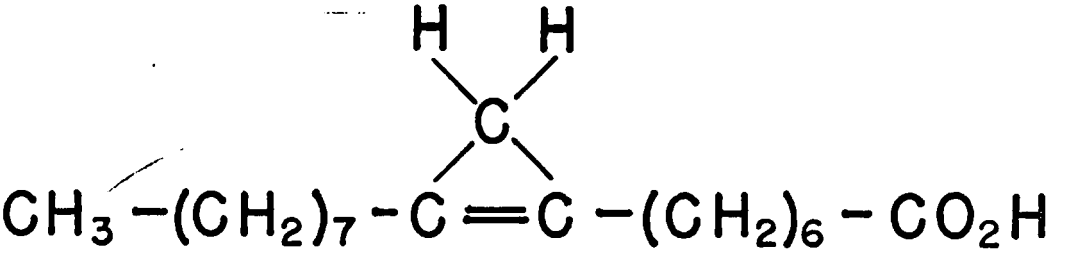
The experiments in this thesis are concerned with the effect of Sterculia foetida oil on the maturation of the female rat and are reported in two sections as follows: 1) the growth of female rats on Sterculia foetida oil diets as indicated by weight gains and feed utilization and 2) sexual maturity as determined by age when the vagina opens and the length and variation of the estrous cycles.

EXPERIMENTAL PROCEDURES

Preparation of Sterculia foetida oil. The Sterculia foetida oil used for these experiments was extracted from Sterculia foetida seeds obtained from the Philippine Islands. The whole seeds were ground and extracted twice with 2 liters of Skellysolve F per kilogram of seed. The solvent was removed from the extracted oil in a



STERCULIC ACID



MALVALIC ACID

FIG. I. – CYCLOPROPENOID FATTY ACIDS.

continuous flow rotary vacuum evaporator at 35°C with reduced pressure (water-pump aspirator). Last traces of solvent were removed from the oil with a rotary vacuum evaporator at 35°C and reduced pressure (high vacuum oil pump). A sample of oil was taken and assayed for Halphen activity using the method of Schneider and Sheehan (unpublished). The various batches of oils ranged from 33% to 35% Halphen-active acids.

The two other oils used in these experiments for control diets were commercially available corn oil[1] and safflower oil[2]. The oils were used at the levels and combinations indicated in Table 1. The oil levels were calculated to supply a minimum of 95 mg of linoleic acid per 10 g of the 1% corn oil-3% Sterculia foetida oil diet and 115 mg linoleic acid per 10 g of the 1% safflower-3% Sterculia foetida oil diet. Although there is not complete agreement on the minimum requirement of the rat for this essential fatty acid, the level of 80 mg per day for the growing rat is the accepted daily requirement as reported by Hansen, et al. (1962).

Composition of diets. The composition of the basal diet is given in Table 2 and is similar to the diet of Sarrett and Snipper (1954) as modified by Schneider (1962). Four per cent oil in the diet was supplied at the expense of the carbohydrate. The diets

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[1] Mazola, Corn Products Refining Company, New York, N. Y.

[2] Saffola, Pacific Vegetable Oil Corporation, San Francisco, California.

TABLE 1

Levels of oil fed in diets

Diet No.	% of oil in the diet
1	4% corn oil
2	1% corn oil + 3% Sterculia foetida oil
3	4% safflower oil
4	2% safflower oil + 2% Sterculia foetida oil
5	1% safflower oil + 3% Sterculia foetida oil
6	1% safflower oil + 3% Sterculia foetida oil + vitamin supplement

were mixed in a Hobart dough-type mixer and stored in plastic bags at 4°C. In all experiments the diet was weighed into individual feed cups and replaced daily to prevent running into a problem with rancidity. Two of the experiments were ad libitum feeding and the third was a pair-feeding experiment. In all experiments water was supplied ad libitum. In diet 6 (Table 1) the vitamin supplement was made up of the following vitamins: thiamine, riboflavin, pyridoxine, calcium pantothenate and biotin. These vitamins, in addition to being present in the vitamin mix of the diet, were fed at a level of 10 times the amount of each present in the basal diet.

Animals. The animals used were 21-day-old weanling female albino rats of the Sprague-Dawley strain[1]. Upon being received the rats were put on a standard laboratory diet for 24 hrs to equilibrate their weights. They were distributed into comparable groups of 10 according to weight, and housed in individual hanging screen-bottom cages in a constant-temperature room. The cages were mounted on portable racks holding a total of sixty cages with thirty cages on each side. The racks were placed to provide even light on each side.

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[1] Holtzman & Company, Madison, Wisconsin.

TABLE 2

Composition of basal diet

	%
Casein[1]	18.0
Fat	4.0
Glucose monohydrate[2]	69.62
Non-nutritive fiber[3]	4.0
Salt mixture[4]	4.0
Vitamin mixture[5]	0.38
Total	100.0

[1] Vitamin free; Nutritional Biochemicals Corp., Cleveland, Ohio.

[2] Cerelose 2001; Corn Products Company, New York.

[3] Solka-Floc; Brown Company, Boston, Massachusetts.

[4] Jones and Foster (1942) with NaF added to give 10 ppm in salt mixture.

[5] Vitamin mixture, supplies per 100 g of diet:

Thiamine hydrochloride	0.4 mg	Folic acid	0.2 mg
Riboflavin	0.5 mg	Biotin	0.02 mg
Niacinamide	5.0 mg	Vitamin B_{12} (0.1% trit. in mannitol)	10.0 mg
Pyridoxine hydrochloride	0.25 mg		
Calcium pantothenate	2.0 mg		
Choline bitartrate	200.0 mg	Menadione	0.2 mg
Inositol	100.0 mg	α-tocopherol	5.0 mg
p-Aminobenzoic acid	10.0 mg	Vitamin A*	1000 USP units
		Vitamin D_3**	120 USP units

* PGB-10; Distillation Products Industries, Rochester, New York.

** Super Nopdex-30; NOPCO Chemical Company, Richmond, California.

Sexual maturity methods. The sexual maturity of the rats was determined using two biological markers: the opening of the vagina as signified by the degeneration of the membrane covering the vagina, and the vaginal smear technique of Long and Evans (1922) which characterizes the estrous cycle by the appearance of cornified cells in the vaginal smear. The animals were examined daily and after the vagina opened a vaginal smear was made daily for each rat.

After 16 weeks of experimental feeding the animals were fasted for 24 hrs and sacrificed by the intraperitoneal injection of Surital[1]. The brain, liver, heart, kidney, ovaries-oviducts-uterus were excised and weighed individually. The livers and brains were frozen and placed in cold storage for fatty acid analysis at a later date.

During these experiments feces were collected and stored so that a fat balance study may be reported at a later date.

RESULTS AND DISCUSSION

Growth and Development

Weight gain and feed utilization. The weight gained and feed intake are listed in Table 3 for the ad libitum and pair-feeding experiments. The 4% corn oil and 4% safflower oil control groups in the ad libitum feeding experiment showed no significant differences in weight gains or feed intake. Similarly, results for the 4% corn

[1] Parke, Davis & Company, Detroit, Michigan.

TABLE 3.

Comparison of efficiency of feed utilization by female rats fed various oil-containing diets *ad libitum* or by pair-feeding.

% Oil in diet	No. of Rats	Avg. Initial Wt.	Avg. Wt. Gain	Avg. Feed Intake	Feed Utilization ①
Ad libitum		g	g	g	
4% Corn	10	51	190	1314	14.5
4% Safflower	9	51	191	1305	14.6
2% Safflower + 2% *S. foetida*	9	51	157	1219	12.9
1% Safflower + 3% *S. foetida*	10	51	127	995	12.8
1% Safflower + 3% *S. foetida* + Vitamin supp.	10	51	143	1113	12.9
Paired Feeding					
4% Corn	9	50	193	1558	12.4
1% Corn + 3% *S. foetida*	8	50	169	1532	11.0
4% Safflower	10	50	203	1575	12.9
2% Safflower + 2% *S. foetida*	10	50	187	1613	11.6
1% Safflower + 3% *S. foetida*	9	50	165	1498	11.1
1% Safflower + 3% *S. foetida* + Vitamin supp.	10	50	164	1510	10.9

① g gain/g Feed consumed (x 100)

oil and 4% safflower oil control groups on the pair-feeding experiment showed no significant differences in the body weights or feed intake. The Student "t" test, Snedecor (1955) and a probability of 5% was taken as the minimal level of statistical significance.

When half of the safflower oil in the 4% control diet was replaced with Sterculia foetida oil so that the dietary content was 2% safflower oil-2% Sterculia foetida oil, the weight gains and feed utilization were diminished (Table 3). In the ad libitum feeding experiment the Sterculia foetida oil group had a weight gain to feed intake ratio of 157 g to 1219 g, and its control group had a ratio of 191 g to 1305 g. In the pair-feeding experiment the Sterculia foetida oil group had a weight gain of 187 g while its control group gained 203 g and both groups had approximately the same feed intake. The difference in weight gain between the control group and 2% safflower oil-2% Sterculia foetida oil group on either experiment was significant ($P < 0.05$), but there was no significant difference in feed intake.

Increasing the proportion of Sterculia foetida oil so that the dietary oil content was 1% safflower oil-3% Sterculia foetida oil further decreased the weight gain and feed utilization. The weight gain to feed intake ratio for this group on the ad libitum experiment was 127 g to 995 g. On the pair-feeding experiment the weight gain was 165 g, and the feed intake was similar to the control group.

In the ad libitum experiment the feed intake of the 1% safflower oil-3% Sterculia foetida oil group was only 77% of the safflower control group and was highly significant ($P < 0.01$). The weight gain of the 1% safflower oil-3% Sterculia foetida oil group was 67% of the safflower oil group and was highly significant ($P < 0.001$). There was also a significant difference in feed utilization between the 4% safflower oil group and the 1% safflower oil-3% Sterculia foetida oil group (Table 3).

In the pair-feeding experiment (Table 3) the total feed intake for the 4% safflower oil group and the 1% safflower oil-3% Sterculia foetida oil group were similar since both groups were fed equal amounts of feed, but the weight gains were significantly different ($P < 0.001$). The feed utilization between these groups on this experiment were significantly different; the safflower oil group gained 12.9 g per 100 g of feed intake, and the 1% safflower oil-3% Sterculia foetida oil group gained only 11.1 g per 100 g feed intake.

These differences in feed utilization are apparent when the overall growth curves are examined. Growth curves are presented in the discussion of sexual maturity (Figure 2).

Organ weights. In Table 4 the organ weights of the control groups were compared to the organ weights of the test group in two ways. The average organ weight of the control group was compared with the average organ weight of the Sterculia foetida oil group and

TABLE 4

Comparison of organ weights and organ weights as percent of body weight of female rats pair-fed various oil-containing diets.

Oil in the diet	4% Corn	1% Corn 3% *S. foetida*	4% Safflower	2% Safflower 2% *S. foetida*	1% Safflower 3% *S. foetida*	1% Safflower 3% *S. foetida* + Vitamins
No. of rats	10	8	10	10	9	10
Body wt. g	243.9 ± 12.9[1]	219.1 ± 17.7	254.1 ± 16.6	238.1 ± 14.9	215.7 ± 16.5	215.1 ± 17.4
Liver g	5.71 ± .94	10.94 ± .94	6.22 ± 1.41	11.78 ± 1.59	11.03 ± 1.76	10.22 ± 1.18
% of body wt.	2.33 ± .29	5.03 ± .69	2.47 ± .62	4.98 ± .55	5.13 ± .86	4.80 ± .77
Ovary-oviduct						
Uterus g	.71 ± .26	.42 ± .07	.50 ± .06	.46 ± .09	.36 ± .12	.48 ± .20
% of body wt.	.29 ± .09	.19 ± .04	.20 ± .02	.20 ± .03	.17 ± .05	.22 ± .07
Heart g	.84 ± .10	.80 ± .06	.80 ± .05	.83 ± .10	.82 ± .09	.79 ± .08
% of body wt.	.34 ± .03	.37 ± .04	.33 ± .03	.35 ± .04	.38 ± .03	.37 ± .04
Kidney g	1.44 ± .13	1.50 ± .09	1.46 ± .09	1.63 ± .10	1.49 ± .25	1.49 ± .23
% of body wt.	.59 ± .03	.68 ± .05	.58 ± .04	.69 ± .05	.69 ± .07	.70 ± .10
Brain g	1.79 ± .09	1.79 ± .08	1.76 ± .12	1.80 ± .27	1.87 ± .29	1.73 ± .09
% of body wt.	.74 ± .03	.82 ± .07	.70 ± .05	.76 ± .05	.87 ± .15	.81 ± .07

[1] Standard deviation

also the organ weight as per cent of body weight. Only the data for the pair-feeding experiment was presented in this table since the data on the ad libitum feeding experiments were similar in all respects. The liver and body weights of the Sterculia foetida oil group were significantly lower ($P < 0.001$) than those of the safflower oil control group. This is in agreement with data published by Schneider (1962) for the chicken. It is interesting to note that the ovaries-oviduct-uterus weights were significantly different. In Table 5 are presented the results of the statistical analyses for the body and organ weights presented in Table 4. There were statistically significant differences in body, liver and ovaries-oviduct-uterus weights between control and Sterculia foetida oil groups; no significant differences in brain, kidney or heart weights were noted.

Sexual Maturity

Since onset of sexual maturity and ovulation in the rat may be subject to variations such as environmental conditions, certain environmental factors were controlled as uniformly as possible. The room was kept at constant temperature; the rats were all handled only once a day; the light was approximately even on both sides of the rack and the rats from each group were distributed so that two from each group were on each level of the rack's five levels.

TABLE 5.

Comparison of liver, ovaries-oviducts-uterus, and body weights of female rats fed various oil-containing diets.

Group	Liver(g)	Ovaries-oviduct-uterus	Body wt.(g)
	Mean ± S.E.[1]	Mean ± S.E.	Mean ± S.E.
4% Corn oil	5.71 ± 0.31	0.71 ± 0.087	243.9 ± 4.31
1% Corn oil + 3% *S.foetida* oil	10.94 ± 0.34 P < 0.001[2]	0.42 ± 0.024 P < 0.003	219.1 ± 6.25 P < 0.001
4% Safflower oil	6.22 ± 0.45	0.50 ± 0.019	254.1 ± 5.24
2% Safflower oil + 2% *S. foetida* oil	11.78 ± 0.50 P < 0.001	0.46 ± 0.028 P < 0.23	238.1 ± 4.72 P < 0.05
1% Safflower oil + 3% *S.foetida* oil	11.03 ± 0.59 P < 0.001	0.36 ± 0.040 P < 0.003	215.7 ± 5.49 P < 0.001
1% Safflower oil + 3% *S.foetida* oil + Vitamin supplement	10.22 ± 0.37 P < 0.001	0.48 ± 0.064 P < 0.32	215.1 ± 5.51 P < 0.001

① Mean ± standard error of the mean

② "t test" compared with appropriate control group.

Opening of the vagina. In Figure 2 the weight and age of the rat at the time the vagina opened is indicated under the two different experimental conditions of ad libitum and pair-feeding. In the ad libitum feeding experiment the average age at the time the vagina opened was 37 days with a range of 31 to 46 days for the 4% corn oil or 4% safflower oil control groups. This agrees with the report of Long and Evans (1922) that in albino rats the vagina opens between 32 and 109 days of age. The 3% Sterculia foetida oil group had an average age of 68 days with a range of 53 to 81 days at the time the vagina opened. The average body weights when the vagina opened were 101 g and 126 g, respectively for the control and 3% Sterculia foetida oil group.

In the pair-feeding experiment the growth of the control group was slower than in the ad libitum feeding experiment, since the control rats were allowed only as much diet as the 1% safflower oil-3% Sterculia foetida oil rats had ingested on the previous day. The average age at the time the vagina opened in the pair-fed control group was 57 days with a range of 43 to 86 days; whereas the 1% safflower oil-3% Sterculia foetida oil group averaged 75 days with a range of 53 to 101 days (Table 6). At the time the vagina opened the average body weight was 129 g for the control group and 139 g for the 1% safflower oil-3% Sterculia foetida oil group. In both experiments the age at the time the vagina opened was significantly

greater for the 1% safflower oil-3% Sterculia foetida oil group than for the control groups; whereas the body weights at this time were not significantly different in any case.

The purpose of the pair-feeding experiment was to test whether the greater feed intake and faster growth of the control group in the ad libitum feeding was a factor in the earlier opening of the vagina. In either experiment the average weight of the Sterculia foetida oil group at the time the vagina opened was always slightly greater than the average weight of the control group. Therefore, the data is conclusive in showing that the difference in age at the time the vagina opened was dependent on the Sterculia foetida oil and not simply on growth.

Length of estrous cycle. For the sake of simplicity Table 7 and Table 8 were prepared for ad libitum and pair-feeding experiments separately, and the consecutive estrous cycles of one typical animal from each group chosen at random are shown. The length of the estrous cycle was determined by counting the number of days between "peak" estrous days. The "peak" day was the day associated with the greatest number of cornified cells noted on the slide of the daily vaginal smears. The control corn oil and safflower oil groups both showed regular consecutive estrous cycles with an average cycle length of 5 days with 90% of the cycles falling in the range of 4 to 6 days. This agrees with the report of Long and Evans (1922) who noted an

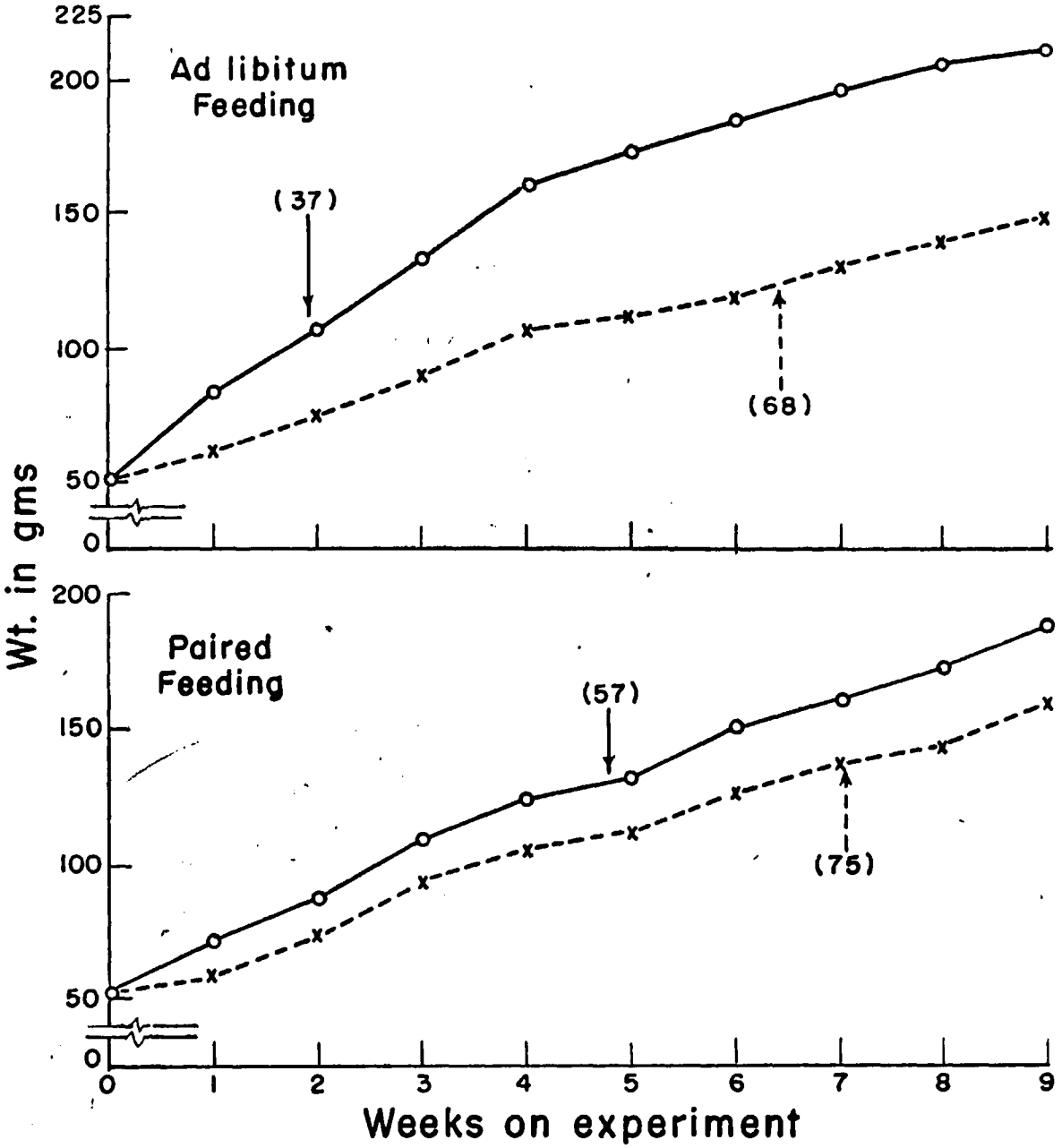

Fig. 2.– Effect of *ad libitum* or paired-feeding on growth curves of female rats with or without *S.foetida* oil in the diets.

Legend

o——o 4% Control oil diet

x- - - -x 3% *S.foetida* oil + 1% Control oil

( ) Days of age at time vagina opens

TABLE 6.

Comparison of body weights and ages at time vagina opens of female rats pair-fed various combinations of oil-containing diets

Group	Diet	Body wt. in g		Age in days	
		Mean ± S.D.	Range	Mean ± S.D.	Range
I	4% Corn oil	124.8 ± 12.89	95-143	55.4 ± 5.89	43-65
II	1% Corn oil 3% *S. foetida* oil	136.4 ± 23.87 P < 0.19 ①	115-195	73.5 ± 15.3 P < 0.003	58-101
III	4% Safflower oil	133.1 ± 17.52	107-140	59.8 ± 10.7	46-86
IV	2% Safflower oil 2% *S. foetida* oil	141.3 ± 11.08 P < 0.23	126-159	66.5 ± 8.34 P < 0.10	56-79
V	1% Safflower oil 3% *S. foetida* oil	142.1 ± 23.14 P < 0.32	111-184	76.3 ± 16.3 P < 0.01	57-98
VI	1% Safflower oil 3% *S. foetida* oil + 10 x B-Vitamins	143.0 ± 17.04 P < 0.23	112-173	79.1 ± 22.7 P < 0.02	52-128

① "t test" Compared with appropriate control oil group.

average estrous cycle of 5 days for normal albino rats. When 2% safflower oil-2% Sterculia foetida oil was fed the cycles were fewer but not very different in length from the control group. But, when the 1% safflower oil-3% Sterculia foetida oil was fed the consecutive cycles were fewer in number and more irregular with an average cycle length of 8 days. The patterns for comparable groups in the three experiments were in agreement with each other.

When 5% corn oil-5% Sterculia foetida oil was fed there was a high mortality rate which confirmed the report by Schneider (1960, 1962). After 3 weeks on experiment with a survival rate of 50%, the rats which survived showed a delayed opening of the vagina and variations in length and number of estrous cycles similar to that found in the experiments where the total oil level was 4% of the diet.

In the experiments where the vitamin supplements were added to the regular vitamin mix in the diets of the 1% safflower oil-3% Sterculia foetida oil groups, the growth was slightly improved but the maturation characteristics were essentially the same as the 1% safflower oil-3% Sterculia foetida oil groups with the normal level of vitamin mix in the diets.

TABLE 7.

Comparison of length of consecutive estrous cycles of female rats fed various oil-containing diets by *ad libitum* feeding.

Diet	Length of estrous cycles in days	Total No. of Cycles	Total No. of Days
4% Corn oil	7-5-5-5-5-6-6-5-5-5-6-5-5-9-4-5-14	17	102
4% Safflower oil	6-4-6-5-5-5-5-5-5-6-6-4-7-5-4-6-5-3	18	92
2% Safflower oil 2% *S. foetida* oil	8-5-6-6-5-6-3-6-4-5-5-5-5-5	14	74
1% Safflower oil 3% *S. foetida* oil	8-6-8-10-9-8-3-7-8-12	10	79
1% Safflower oil 3% *S. foetida* oil + Vitamin supplement	11-6-6-17-4-6-6	7	56

TABLE 8.

Comparison of length of consecutive estrous cycles of female rats pair-fed various oil-containing diets.

Diet	Length of estrous cycles in days	Total No. of Cycles	Total No. of Days
4% Corn oil	5-5-5-5-5-5-5-5-5-5-5-4-9-4	15	77
1% Corn oil 3% *S. foetida* oil	14-7-11-16-5-4-6	7	63
4% Safflower oil	6-5-6-5-5-5-5-5-5-5-5-4-5-5-5-5	17	86
2% Safflower oil 2% *S. foetida* oil	12-5-5-5-5-5-14-5-4	9	60
1% Safflower oil 3% *S. foetida* oil	13-8-8-4	4	33
1% Safflower oil 3% *S. foetida* oil + Vitamin supplement	15-8-6	3	29

CONCLUSIONS

In a comparison with female rats fed 4% corn oil or 4% safflower oil, the feeding of 3% Sterculia foetida oil with either 1% corn oil or 1% safflower oil delayed sexual maturity as measured by two biological markers. The age of the rat at the time the vagina opened was significantly greater and the length of the estrous cycle was longer and more variable.

Sterculia foetida oil also caused an inhibition of growth of the rats, an increase in liver weight and a decrease in ovary-oviduct-uterus weight.

At this time the exact mechanism that produces these effects is not known.

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