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SOME PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF <u>STERCULIA</u> <u>FOETIDA</u> OIL ON ANIMAL SYSTEMS

by

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I hereby recommend that this dissertation prepared under my direction by Donald L. Schneider

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STATEMENT BY AUTHOR

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ABSTRACT

SOME PHYSIOLOGICAL AND BIOCHEMICAL EFFECTS OF STERCULIA FOETIDA OIL ON ANIMAL SYSTEMS

by

Donald L. Schneider - Doctor of Philosophy

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Dissertation Director - Professor Mitchell G. Vavich

Sterculia foetida oil is a rich, natural source of the Halphen reactive cyclopropene fatty acid, sterculic. Feeding 200 mg of this oil per day by gelatin capsules for different periods up to 19 weeks to sexually developing S. C. White Leghorn pullets on a low fat practical type diet, resulted in retardation of comb, ovary and oviduct development; inhibition of egg production, enlargement of gall bladder and liver; increased *in vivo* synthesis of saturated fatty acids and increased deposition of saturated fatty acids in the omental and egg fat. Feeding of 3000 mg of oleic acid in addition to 200 mg of *S. foetida* oil per day permitted normal egg production, but did not prevent the increased deposition of saturated fatty acids.

Feeding 200 mg of this oil per day to mature hens stopped egg production within 3 weeks. Egg production returned to normal within 3 weeks after feeding was stopped.

Results of these experiments give strong evidence that during normal egg production egg fat is derived primarily from newly synthesized fatty acids, but some is continuously derived from body depot fat. When egg production is stopped newly synthesized fatty acids are deposited in body depots.

Feeding this oil to male weanling rats in an 18% casein-10% corn oil purified diet resulted in retardation of growth when 1, 2.5 and 5% of the corn oil was replaced with *S. foetida* oil. At 5% *S. foetida* oil level and above, death occurred between the second and third week. This was prevented at the 5% *S. foetida* oil level by feeding 10 to 15 times the optimal level of combinations of thiamine, riboflavin, pyridoxine, calcium pantothenate and biotin or pyridoxine, calcium pantothenate and biotin; growth was 63% of the corn oil controls. High levels of pyridoxine, calcium pantothenate and biotin alone prevented death, but growth was only 45% of the controls.

Feeding 1 or 2.5% *S. foetida* oil increased the saturated fatty acids of the epididymal fat.

INTRODUCTION

Occasionally, a material is used in the dietary of animals for many years before specific physiological and biochemical effects lead to the discovery of a new class of compounds. One of these is the unusual class of fatty acids found in certain plant lipids which contain the cyclopropene ring.

Two such fatty acids, which are characterized by the general structure,

$$\begin{array}{c} CH_2 \\ / \quad \backslash \\ R_1 - C = C - R_2 - CO_2H \end{array}$$

have been isolated and identified. Sterculic, a C_{19} acid found predominantly in *Sterculia foetida* (*S. foetida*) oil, has the ring in the 9, 10 position (Nunn, '52) and malvalic, a C_{18} acid found predominantly in cottonseed oil, has the ring in the 8, 9 position (MacFarlane, *et al.*, '57). Other cyclopropene fatty acids have been postulated and Dijkstra and Duin ('55) partially isolated and identified a C_{17} acid, bombacic, from kapokseed oil.

This class of fatty acids has been further characterized by the Halphen color reaction originally developed by Halphen ('97) to measure qualitatively the contamination of olive oil with cottonseed oil. According to Faure ('56), this test is specific for the cyclopropene ring. It has aided considerably in studies of these fatty acids.

The lipids of approximately three dozen plant species have been shown to be Halphen reactive and to contain cyclopropene fatty acids (Phelps, '62). Most of these do not appear in the normal dietary of animals and, therefore, have not been of much interest. However, small amounts of the cyclopropene fatty acids have been found in several varieties of cottonseed. Inclusion of these cottonseed meals, containing some residual oil, in the dietary of chickens caused the development of the "pink white" discoloration phenomenon in stored eggs. Confirming proof that the cyclopropene fatty acids were responsible for this phenomenon was shown by Masson, *et al*. ('57) by feeding either *S. foetida* oil or sterculic acid.

Few other biological effects have been attributed to these acids. Some investigators attributed certain biological effects to cottonseed oil, but it was not recognized that these might be due to the cyclopropene fatty acids, probably because of the low content of these acids in cottonseed oil. Most interesting of these is the "hardening effect of cottonseed oil" on depot fat described by Deuel ('55). Ellis and coworkers ('26, '31) working with the "soft pork" problem observed that 4% cottonseed oil in the diet of swine resulted in a lard with a lower iodine number than when 4% corn oil was fed, even though these oils had similar iodine values. Spadola and Ellis ('36) continued this work on rats and observed a depot fat iodine number of 60 when feeding a cottonseed oil having an iodine number of 108. Echles and Palmer ('16) and Keith ('32) observed similar effects in butterfat, obtained from cows fed cottonseed meal, and believed it to be due to the cottonseed oil in the meals. That this hardening was caused by cyclopropene fatty acids in the cottonseed oil was implied in the work of Shenstone and Vickery ('57, '59), who found that feeding Halphen-reactive fractions of cottonseed oil to hens resulted in putty-like yolks. Doberenz, et al. ('62) corroborated this by observing this condition when S. foetida oil was fed. Further evidence that cyclopropene fatty acids caused this "hardening effect" was reported by Schneider, et al. ('61a, '62). They found that feeding S. foetida oil to immature pullets decreased the iodine number of the omental fat from 71 to 42. Furthermore, Evans, et al. ('61) observed that feeding crude cottonseed oil or S. foetida seed to hens increased the stearic acid and decreased the oleic acid content of the egg fat. This change in the saturated and mono-unsaturated fatty acids was also observed in tissue and plasma lipids (Evans, et al., '62).

Some indication that cottonseed oil might affect ovary and oviduct development in pullets is found in the work of Almquist, et al.('34). These authors reported that small ovaries were found in 2 of 9 pullets (at age 33 and 36 weeks, respectively) after they had been fed a cottonseed oil mash for 8 weeks, starting at 21 weeks. That *S. foetida* oil could affect ovary and oviduct development was reported by Schneider, *et al.* ('61a, '62).

A hatchability depression factor in the saponifiable fraction of crude cottonseed oil was observed by Ringrose, Morgan and Lease ('41) and by Naber and Morgan ('57). By calculation, the content of cyclopropene fatty acids of crude cottonseed oil were present at about the same level that Schneider, *et al.* ('61b) found effective for *S. foetida* oil.

Many investigations have been carried out on rats fed cottonseed oil without an indication that cyclopropene fatty acids might be involved. One exception was the observation of Spadola and Ellis ('36) on deposition of more saturated fatty acids. Certain other Halphen-reactive oils have been fed. Thomasson ('55) fed kapokseed oil to rats at a level of 40% of the calories of the diet and found that this oil caused death of the rats between the thirteenth and eighteenth day of the experiment. At lower levels he observed retarded growth. Schneider ('60) fed *S. foetida* oil at the level of 10% of the diet and also observed death of the rats between the second and third weeks. When 5% *S. foetida* oil was fed, growth was retarded, but no effect was observed when 1% *S. foetida* oil was fed.

The work reported here is concerned with additional effects of the cyclopropene fatty acids in animal systems. S. foetida oil was used because of its high content of sterculic acid. These investigations have been developed in two parts as follows:

Part I. The effect of S. foetida oil on the delay of sexual maturity and egg production in the immature and mature female chicken.

Part II. The effect of S. foetida oil on growth of the male weanling rat.

PART I. The Effect of *Sterculia foetida* Oil on the Delay of Sexual Maturity and Egg Production in the Immature and Mature Female Chicken

MATERIALS AND METHODS

Sterculia foetida oil was prepared by extracting ground *Sterculia foetida* nuts two or three times with equal parts of Skellysolve F and B, or with Skellysolve F (1 liter/kg). The solvent was removed from the oil under reduced pressure at 35° C in a glass flash evaporator and a rotating evaporator. Previously, Schneider ('60) showed that the fatty acid components of the hulls and meats were identical, except that one fatty acid component found in the meats was missing from the hulls.

The basal diet used throughout all the experiments is shown in table 1. Feed and water were given *ad libitum,* and the oil supplements were given daily by gelatin capsule.

The pullets were Single-Comb White Leghorns (Kimber 137). All birds were maintained in individual wire laying cages in an open cage-house. Records were kept of egg production and in some experiments, food consumption and body weights.

Table 1. Composition of basal diet.

	%
Ground yellow corn	10.00
Ground milo	55.00
Alfalfa meal (17% protein)	5.00
Soybean oil meal (44% protein)	18.00
Fish meal (63% protein)	1.00
Meat and bone scraps (50% protein)	1.00
Fat[1]	1.00
Salt[2]	0.50
Ground limestone	5.00
Phosphorus supplement[3]	1.50
Techmangam[4]	0.025
Vitamin premix[5]	2.00

[1] Hydrolyzed animal-vegetable fat.

[2] Morton (TM), Morton Salt Co., Chicago, Ill.

[3] Dynafos (P 18.5%, Ca 22.5%), International Minerals Co., Skokie, Ill.

[4] 77% $MnSO_4 \cdot 5H_2O$, Tennessee Eastman Co., Kingsport, Tenn.

[5] Calculated to supply per kg of diet:

Stabilized vitamin A	4400 I.U.
Vitamin D_3	1540 I.C.U.
Riboflavin	4.8 mg.
Niacin	11.0 mg.
d-Calcium pantothenate	5.5 mg.
Choline chloride	499.4 mg.

At the termination of the experiment, the birds were sacrificed by jugular vein exsanguination and the livers, ovaries, oviducts, adrenals and kidneys were removed and weighed.

Experiment 1:Seventy 16 week old pullets were randomly selected into two groups of 30 birds each and one group of 10 birds. The latter group was sacrificed immediately to establish normal baseline organ weights. The other two groups were fed the basal diet and supplemented daily. Group 1 received 200 mg of corn oil[1] and Group 2 received 200 mg of S. foetida oil. Birds from each group were selected at random and sacrificed for organ weight analyses after 5, 10 and 19 weeks of supplementation which corresponded to 21, 26 and 35 weeks of age, respectively.

Experiment 2: Fifty-four 15 week old pullets were randomly selected into six groups of 9 birds each and placed on the basal diet. The 1% hydrolyzed animal-vegetable fat in the basal diet was replaced with glucose monohydrate.[2] Group 1 was supplemented daily with an empty capsule, Group 2 with 200 mg of oleic acid[3], Group 3 with 200

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[1] Mazola, Corn Products Co., New York.

[2] Cerelose 2001, Corn Products Co., New York.

[3] Nutritional Biochemicals Corp., Cleveland, Ohio. This oleic acid was used throughout these investigations. Tentative analysis by gas-liquid chromatography shows it to contain approximately 71-75% oleic acid. Other fatty acids possibly present are myristic, myristoleic, palmitic, palmitoleic, stearic, linoleic and linolenic. Courtesy of Harold E. Nordby, this laboratory.

mg of S. foetida oil, Group 4 with 200 mg of S. foetida oil plus 200 mg of oleic acid, Group 5 with 200 mg of S. foetida oil plus 1200 mg of oleic acid, and Group 6 with 200 mg of S. foetida oil plus 2000 mg of oleic acid. Supplementation was continued for 24 weeks, then discontinued to allow recovery of egg production. After 12 weeks without supplementation, the 16 birds remaining in Groups 1 and 2 were randomly reselected into two groups of 8 birds each. Those in the new Group 1 were supplemented with an empty capsule and Group 2 with 200 mg of S. foetida oil. The remaining 24 birds in Groups 3, 4, 5 and 6 were also randomly reselected into four groups of 6 birds each with the new Group 3 supplemented with an empty capsule, Group 4 with 2000 mg of oleic acid, Group 5 with 200 mg of S. foetida oil, and Group 6 with 200 mg of S. foetida oil plus 2000 mg of oleic acid. Therefore, this experiment was divided into three consecutive periods as follows: (1) a supplemented period, (2) a non-supplemented period, and (3) a regrouping and resumed supplemented period. After 10 weeks of additional supplementation, the birds were sacrificed for organ weight and omental fat iodine number analyses.

Egg fats for iodine number determinations were extracted from samples of yolks, pooled by group, collected over a 2 to 3 day period where possible during the nonsupplemented and resumed supplemented period.

Experiment 3: Twenty 40 week old adult pullets were randomly selected into four groups of 5 birds each and placed on the basal diet without the added fat. After three weeks, daily supplementation was started, Group 1 received an empty capsule, Group 2 received 3000 mg of oleic acid, Group 3 received 200 mg of S. foetida oil and Group 4 received 200 mg of S. foetida oil plus 3000 mg of oleic acid. Supplementation was continued for 6 weeks, discontinued for 6 weeks, resumed for 7 weeks and discontinued for another 6 weeks. Egg production and weekly iodine numbers of the egg fat were followed throughout the experiment. Iodine values were determined on egg fats obtained by pooling the yolks of groups of eggs laid on the same day $\pm$ 3 of each week.

Chemical Methods: Omental fat was extracted by homogenizing a 5 g sample with 50 ml of 98% ethanol-diethyl ether-Skellysolve F (1:3:3). The extract was dried over Na_2SO_4, filtered and heated to 70°C to remove the solvent. Egg fats were extracted with chloroform and treated with Na_2SO_4, according to the method of Murtz, et al. ('60). Iodine numbers were determined by the Hanus Method (A.O.A.C., 7th Ed.). Liver solids and fats were determined according to the method of Sarett and Jandorf ('57).

RESULTS AND DISCUSSION

Experiment 1: No significant difference in food consumption between the two groups was noted. The corn oil controls consumed an average of 106 g and the S. foetida oil group 100 g of feed per bird per day.

Figure 1 reflects the weight change of the birds. S. foetida oil supplementation significantly affected weight gain during the early part of the experiment so that by the fifth week (21 weeks of age) the corn oil group had gained an average of 306 ± 83 g[4], while the S. foetida oil group had only gained 212 ± 74 g ($P < .001$[5]). The difference in weight gain between the two groups expanded until the corn oil controls began to level off at the ninth and tenth week; so that by the tenth week of supplementation (26 weeks of age) this difference was no longer significant (figure 1). By the nineteenth week of supplementation (35 weeks of age) the S. foetida oil group had actually surpassed the corn oil group, although the difference was not statistically significant. For the entire 19 weeks the corn oil group gained an average of 678 ± 102 g and the S. foetida oil group 750 ± 109 g.

These differences may be partially explained by differences in ovary and oviduct development in the two groups. In the early part of the experiment the ovaries and oviducts of the corn oil group developed normally, but they were retarded in the S. foetida oil group. These organs develop over a short period of time (Nalbandov and Card, '46; Nalbandov and James, '49), and this may account for some of the difference in weight gain seen at ages 20 to 26 weeks between the two groups.

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[4] Standard deviation; Snedecor ('46).

[5] Probability; Snedecor ('46).

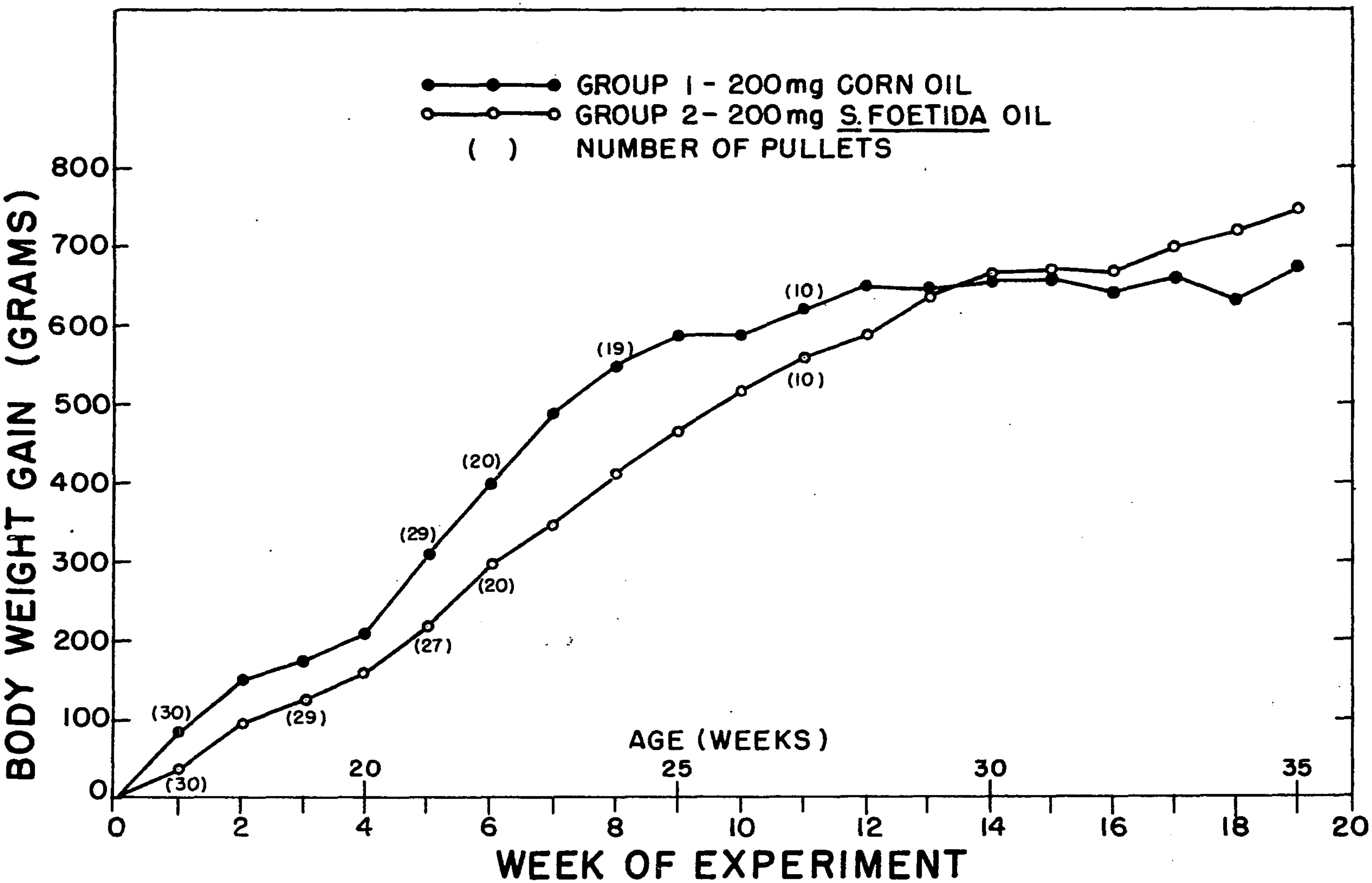

FIGURE 1:- COMPARISON OF WEIGHT GAIN OF PULLETS FED 200 mg DAILY OF CORN OIL OR S. FOETIDA OIL

This developmental difference was further reflected in egg production (figure 2). About two weeks after the change in body weight was noted, the corn oil fed controls started to lay. After 12 weeks of supplementation, all of the corn oil controls were in production. The average age at the onset of egg production was 24 weeks. Yet, none of the S. foetida oil group laid until the 14th week of supplementation (age 30 weeks), and then only two of the remaining 10 birds were sexually mature. One bird laid six eggs over a ten day period while the other laid only one egg.

This extreme difference in egg production may also explain why the essentially nonlaying S. foetida oil group surpassed the corn oil group in weight gain in the last weeks of the experiment. According to Sturkie ('54), there is a direct relationship between metabolic rate and egg production with higher producing hens having a higher metabolic rate.

After 3-4 weeks of supplementation, considerable differences were noted in comb development. Figure 3 shows the lack of comb development in the S. foetida oil group as compared with the corn oil controls. Not all the combs of the treated birds were retarded to this extent but an obvious difference between the groups persisted throughout the experiment.

No significant difference in liver weights was noted between the two groups after 5 or 10 weeks of supplementation (table 2). However, after 19 weeks the livers of the corn oil group averaged 20.23 g per kg of body weight; whereas the S. foetida oil group was significantly heavier,

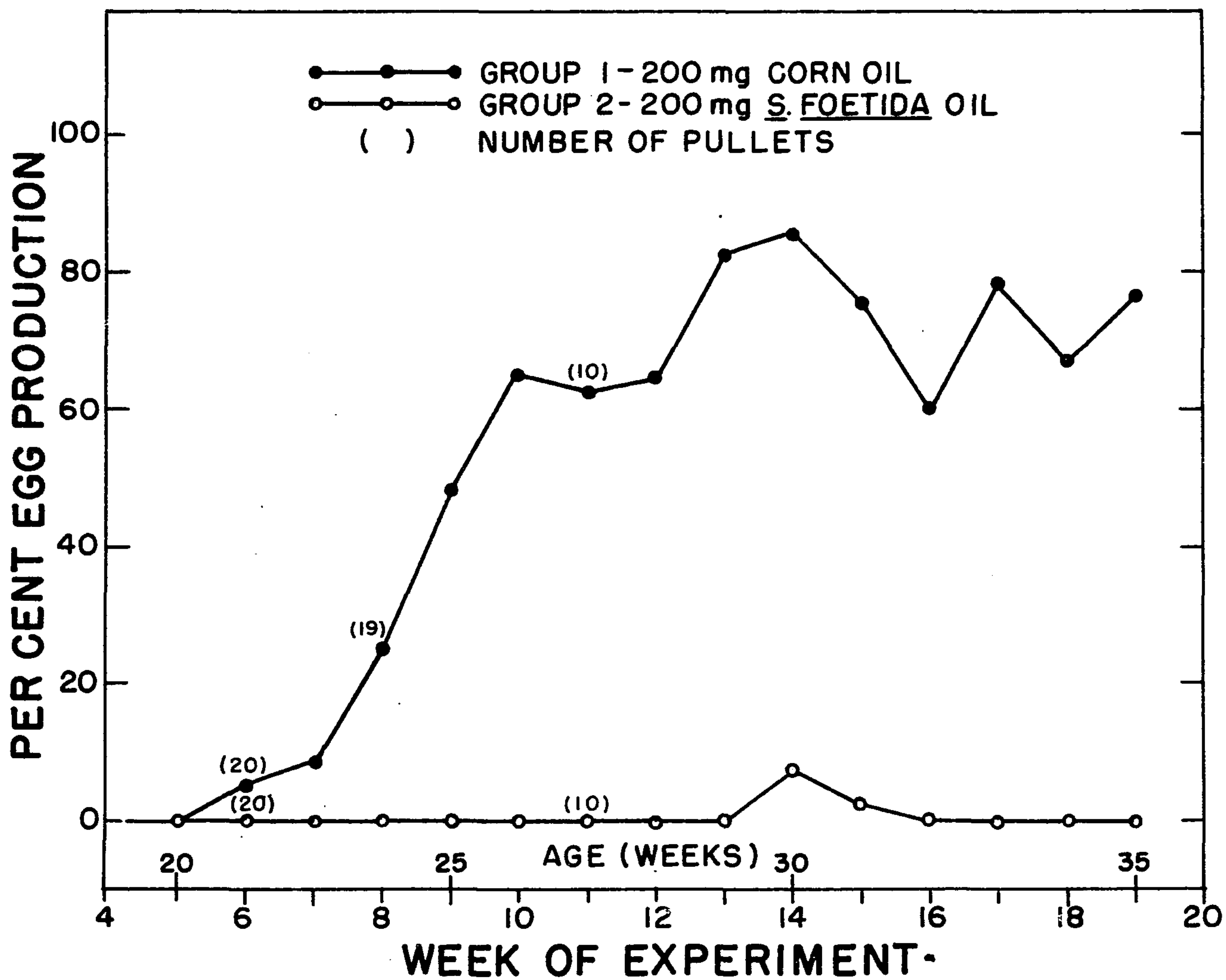

FIGURE 2:- COMPARISON OF PER CENT EGG PRODUCTION OF PULLETS FED 200 mg DAILY OF CORN OIL OR S. FOETIDA OIL.

Figure 3. Comparison of comb development of pullets fed 200 mg daily of corn oil S. foetida oil after 5 weeks of supplementation.

averaging 27.16 g ($P < .001$). Analysis of the per cent solids and fats of the livers (table 3) did not reveal any significant differences between the groups, thus the reason for this difference in weight is not readily apparent unless it is related to egg production.

A significant difference in gall bladder weights (i.e., organ plus fluid content) was found between the two groups at all three periods of supplementation (table 2). At 5, 10 and 19 weeks of supplementation gall bladders from pullets fed corn oil averaged 0.47, 0.71 and 0.79 g; those of the S. foetida oil group averaged 1.80, 2.37 and 1.62 g per kg body weight, respectively. The P values were < .001, < .01 and < .05. Since these weights were determined by difference, no data is given for the actual tissue weight; however, all the enlarged gall bladders were engorged with bile; thus the increased weight was largely due to increased bile collection. Gross examination of the bile and cystic ducts did not reveal any blockage. A sloughing off of the inner lining of the bladder was noted, but this could be due to prolonged standing of the bile within the bladder. Whether the increased collection of bile within the bladder was due to increased bile production by the liver, or to a lack of concentrating or emptying by the bladder, is unknown.

The wide variation in sexual development of the pullets did not allow for comparison of the groups statistically for significant differences in ovary and oviduct weights. The group averages plus the range of variations of the organ weights are presented in table 4 and figure 4.

Table 2. Comparison of liver and gall bladder weights from pullets fed 200 mg daily of corn oil or *S. foetida* oil for 5, 10 and 19 weeks.

Oil supplement	Number of pullets	Age (weeks)	Average body weight (g)	Liver		Gall bladder[2]	
				Weight[1] (g)	g per kg body weight	Weight (g)	g per kg body weight
			Baseline				
	10	16	1220 ± 21[3]	22.32	18.30 ± 1.65		
			5 Weeks				
Corn	9	21	1534 ± 72	31.27	20.34 ± 1.91	0.72(9)[4]	0.47 ± 0.11
S. foetida	7		1418 ± 69	29.65	20.92 ± 1.83	2.56(5)	1.80 ± 0.84
			10 Weeks				
Corn	9	26	1787 ± 99	34.21	19.81 ± 3.10	1.11(7)	0.71 ± 0.20
S. foetida	10		1731 ± 149	38.42	21.88 ± 5.12	4.11(8)	2.37 ± 1.21
			19 Weeks				
Corn	10	35	1902 ± 95	38.52	20.23 ± 1.31	1.35(7)	0.79 ± 0.45
S. foetida	10		1975 ± 115	53.56	27.16 ± 3.83	3.19(7)	1.62 ± 0.78

[1] Group average.

[2] Determined by difference; i.e., Liver and gall bladder weight - liver weight = gall bladder weight.

[3] Standard deviation.

[4] Number of organs weighed.

Table 3. Comparison of per cent liver solids and fats from pullets fed 200 mg daily of corn oil or S. foetida oil for 10 and 19 weeks.

Oil supplement	Number of animals	% Solids	% Fats
		10 Weeks	
Corn	9	32.34 ± 2.64[1]	9.13 ± 4.00
S. foetida	9	30.70 ± 1.59	7.35 ± 2.56
		19 Weeks	
Corn	10	31.50 ± 2.33	9.76 ± 3.12
S. foetida	9	31.24 ± 1.55	8.23 ± 1.40

[1] Standard deviation.

After 5 weeks of supplementation (21 weeks of age), the ovaries of the corn oil fed birds averaged 4.96 g, range 0.42-24.90 g; while the S. foetida oil group averaged 0.44 g, range 0.34-0.51 g per kg of body weight. At this age many of the control birds had begun to develop sexually and showed wide individual variation in organ weights. The S. foetida oil group's development was delayed and changed very little from that observed in the baseline values. Also, there was little variation within the group. This same pattern continued at the ten week period (26 weeks of age). The corn oil group showed increased development and wider individual variation while the S. foetida oil group showed only limited development. By the nineteenth week the ovaries of the corn oil group averaged 25.60 g, range 21.10-33.01 g and the S. foetida oil group averaged only 7.76 g, range 0.78-22.60 g per kg body weight. Although these birds were 35 weeks old, many of the S. foetida oil group did not develop mature sex organs (figure 4).

Ovaries from the S. foetida oil group varied from pinpoint size ova to some with almost completely developed ova. However, there did not appear to be as many large ova per ovary in the more normally developing S. foetida oil birds as in the corn oil controls. Also, many of the developing ova were partially or totally degenerated into dark red or blackish masses.

Table 4. Comparison of ovary and oviduct weights from pullets fed 200 mg daily of corn oil and *S. foetida* oil for 5, 10 and 19 weeks.

Oil supplement	Ovary			Oviduct		
	Weight (g)[1]	g per kg body weight		Weight (g)[1]	g per kg body weight	
		average	range[2]		average	range
			Baseline			
	0.40	0.33	0.22- 0.46	0.24	0.20	0.14- 0.25
			5 Weeks			
Corn	8.01	4.96	0.42-24.90	14.09	8.78	0.33-28.06
S. foetida	0.61	0.44	0.34- 0.51	1.17	0.82	0.37- 1.49
			10 Weeks			
Corn	31.18	17.66	0.82-27.30	37.57	20.63	1.91-32.32
S. foetida	3.57	1.97	0.39- 6.57	13.19	7.25	0.37-15.99
			19 Weeks			
Corn	48.69	25.60	21.10-33.01	55.21	29.04	24.88-33.18
S. foetida	14.76	7.76	0.78-22.06	28.64	14.67	6.80-23.36

1 Group average.

2 Range within the group on a g per kg body weight basis.

Figure 4. Comparison of organ development of pullets fed 200 mg daily of corn oil or S. foetida oil after 19 weeks of supplementation. From left to right: ovary, oviduct, and liver and gall bladder. Corn oil control on the bottom; S. foetida oil treated at the top.

Oviduct development at various periods of the experiment followed a pattern similar to that noted in ovarian development (table 4 and figure 4).

Concurrently, with the physiological changes noted during S. foetida oil supplementation, there was a lowering of the amount of unsaturated fatty acids in the omental fat as shown by the iodine numbers in table 5. By the fifth week of supplementation, this change was not sufficient to be noted at the time the birds were sacrificed. However, by the tenth week slight cooling of the omental fat gave it the appearance of "beef fat." After 10 weeks of supplementation the omental fat of the corn oil group had an average iodine number of 70 and the S. foetida oil group averaged 43, a value significantly lower ($P < .001$). At 19 weeks these values were 72 and 41, respectively, and significantly different ($P < .001$). A similar observation was noted by Evans, et al. ('61), who found that feeding S. foetida seeds or crude cottonseed oil to laying hens resulted in eggs with an increased proportion of saturated fatty acids and a decreased proportion of monoenoic acids.

Experiment 2: In this study it was shown that the supplementary feeding of the monoenoic acid, oleic, partially overcame the deleterious effect of S. foetida oil on pullets. Figure 5 contains the egg production data which was used as an indicator of ovary and oviduct development.

Table 5. Comparison of iodine number of omental fat from pullets fed 200 mg daily of corn oil or S. foetida oil for 10 and 19 weeks.

Oil supplement	Iodine absorption number[1]
	10 Weeks
Corn	70 ± 3[2]
S. foetida	43 ± 5
	19 Weeks
Corn	72 ± 3
S. foetida	41 ± 3

[1] Hanus method.

[2] Standard deviation.

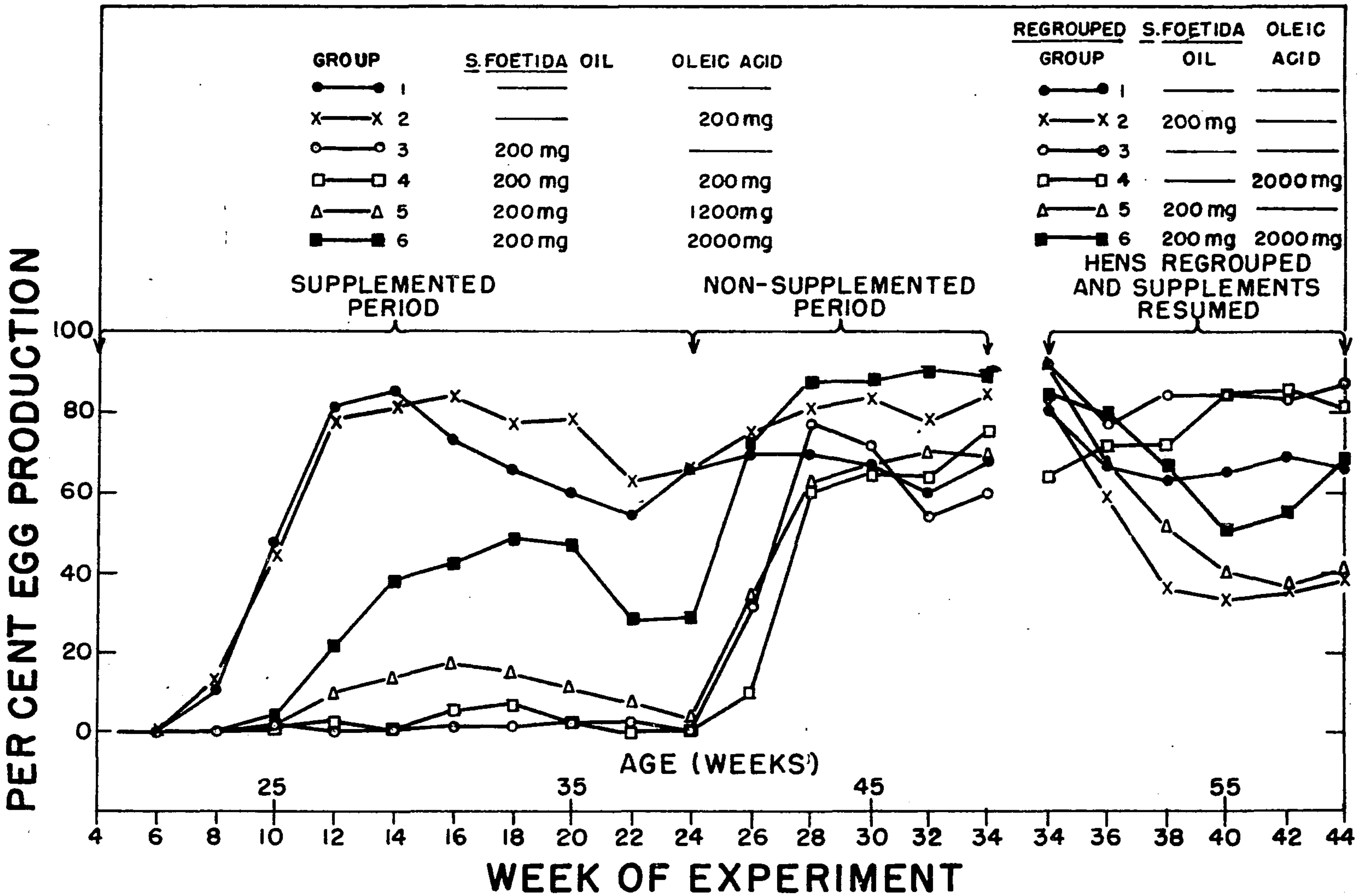

FIGURE 5:- COMPARISON OF PER CENT EGG PRODUCTION OF PULLETS FED 200 mg DAILY OF S. FOETIDA OIL AND VARIOUS LEVELS OF OLEIC ACID

As noted in experiment 1, the controls (Groups 1 and 2) started laying about the seventh and eighth week (age 23 and 24 weeks) of the experiment, and by the twelfth week all of the birds in these groups were laying normally. However, of the birds in Group 3 receiving 200 mg of S. foetida oil per day, only one had laid three eggs and another had laid two eggs during the 24 weeks of supplementation (table 6).

Supplementation with a mixture of S. foetida oil and various levels of oleic acid partially overcame this lack of egg production, although the onset of egg production was delayed 4 weeks compared to the controls. Supplementing 200 mg of S. foetida oil with 200 mg of oleic acid (Group 4), or with 1200 mg of oleic acid (Group 5), had very little effect on egg production. Group 4 reached top production at 18 weeks (7.1%) and Group 5 at 16 weeks (17.5%). However, Group 5 did have a more substantial earlier onset of production (figure 5). Group 6, supplemented with 200 mg of S. foetida oil plus 2000 mg of oleic acid, showed significantly that oleic acid can partially overcome the deleterious effect of S. foetida oil. By 18 weeks of the experiment, Group 6 reached 48.2% production, or 72% of the nonsupplemental controls.[6]

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[6] Some caution should be noted in assuming that the oleic acid is the actual alleviating agent since other fatty acids are known to be present in this preparation (see footnote 3). However, crude calculations based on the work of Wheeler, et al. ('59) of the amount of fatty acids required per day to fulfill the requirements for normal egg production show that when 200 mg of S. foetida oil is fed alone with the basal diet, 587.0% of the linoleic acid requirement is met, by only 56% of the oleic acid requirement is present.

Table 6. Comparison of per cent egg production of pullets fed 200 mg daily of S. foetida oil and various levels of oleic acid.

Supplement	Groups 1	2	3	4	5	6
S. foetida oil	--	--	200 mg	200 mg	200 mg	200 mg
Oleic acid	--	200 mg	--	200 mg	1200 mg	2000 mg
No. of birds	9	9	9	9	9	9
Wk. of expt.	% Egg Production					
2	0	0	0	0	0	0
4	0	0	0	0	0	0
6	0	0	0	0	0	0
8	10.3	11.9	0	0	0	0
10	46.8	43.7	0.8	0(8)	0	3.2
12	81.0	78.6	0	0.9	10.3	21.4(8)
14	84.9	81.0	0	0	13.5	37.5
16	73.8	84.1	0.8	5.4	17.5	42.9
18	66.7	77.0	0.8	7.1	15.1	48.2
20	59.5	77.8	1.8(8)[1]	1.8	10.3	46.4
22	54.0	62.7	1.2(6)	0(7)	7.1	27.7
24	65.1	65.1	0(5)	0(6)	0.8	27.7
	9/9[2]	9/9	2/5	2/6	6/9	7/8
	Nonsupplemented Period					
26	69.0	75.4	31.4	9.5	34.1	71.4(7)
28	69.0	81.0	77.1	60.7	61.9	87.8
30	66.7	83.3	72.9	65.5	66.7	87.8
32	60.7(8)	77.8	54.3	64.3	70.6	90.8
34	67.0	84.1	60.0	76.2	69.0	88.8
	7/8[2]	9/9	5/5	5/6	8/9	7/7

[1] Number in parenthesis represents number of birds remaining in the group because of deaths.

[2] Number of birds that laid over number of birds remaining in the group.

There was a drop in egg production among all the groups during the last six weeks of supplementation. This is partially explained by the fact that these birds were maintained in an open cagehouse, and there was a substantial decrease and variation in environmental temperature during this period (Romanoff and Romanoff, '49).

After 24 weeks (39 weeks of age), supplementation was discontinued and the hens were continued on the basal diet in order to determine the period of time required for resumption of egg production of the S. foetida oil treated birds. Within four weeks all of the S. foetida oil treated birds with the exception of one hen each in Groups 4 and 5 were laying, and the per cent egg production was equivalent or better than the controls (see figure 5 and table 6).

The nonsupplemented period was continued for a total of ten weeks after which the animals were regrouped by random selection (as described in Materials and Methods) and placed on supplementation to again follow the regression of egg production by S. foetida oil. This data is shown in figure 5 and table 7.

Even though the birds in Group 2 (age 49 weeks) had never received S. foetida oil, there was an immediate suppression of egg production from 80.4% to 35.7% after 4 weeks of supplementation, and 37.5% after 10 weeks of supplementation. Groups 3, 4, 5 and 6 had previously recieved S. foetida oil with or without various levels of

Table 7. Comparison of per cent egg production of pullets regrouped and supplemented daily with 200 mg of S. foetida oil and 2000 mg of oleic acid.

Supplement	Groups					
	1	2	3	4	5	6
S. foetida oil	--	200 mg	---	--	200 mg	200 mg
Oleic acid	--	--	---	2000 mg	--	2000 mg
No. of birds	8	8	6	6	6	6
Wk. of expt.	% Egg Production					
34	79.5	80.4	91.7	64.3	92.9	85.7
36	66.1	59.8	76.2	72.6	67.8	78.5
38	63.4	35.7	84.5	72.6	51.1	67.8
40	65.2	33.0	84.5	84.5	40.4	50.0
42	69.6	33.9	83.3	85.7	35.7	54.8
44	66.3	37.5	87.2	80.8	41.0	67.9

oleic acid. Group 3 which only received an empty capsule continued its normal egg production. Group 4 which had a rather low initial egg production actually increased egg production by 15% when it was supplemented with 2000 mg of oleic acid daily. Supplementing with 200 mg of S. foetida oil alone (Group 5), again resulted in suppression of egg production. When 2000 mg of oleic acid was fed with 200 mg of S. foetida oil (Group 6) the egg production decreased, but not to the same extent as Group 5. This shows again that oleic acid partially overcame the deleterious effect of S. foetida oil on egg production as noted in the first supplemental period of the experiment.

As noted in table 6, long term feeding of S. foetida oil to pullets occasionally caused death. This occurred after 10 to 25 weeks of supplementation in both experiments 1 and 2. The syndrome had definite characteristics. The feces became quite light colored and fluid. The animal became emaciated and appeared dehydrated with shrinking and pale discoloration of the comb and wattles. Within a couple of weeks after development of these symptoms, the bird died. Autopsy revealed extreme emaciation throughout the body with very little fat present, and that was a highly saturated type. Most striking was the enlarged gall bladder which varied from 50 to 80 mm in length and 20 mm in diameter. However, examination of the ducts which empty the gall bladder did not show any blockage. Occasionally, the liver and kidneys appeared enlarged and a white opaque stringy material appeared in the ureters of the kidneys. This has not been identified. No areas of hemorrage or fat appeared on the liver, but small areas of bile storage were occasionally seen within the liver, especially after storage for some time in formaldehyde. The ultimate cause of death was unknown.

Along with the per cent egg production, the iodine number of the egg fats were followed during the periods of nonsupplementation and resumption of supplementation after regrouping. These data are shown in figures 6 and 7. During the nonsupplemental period, very little difference or change was found in iodine numbers between the two control groups (Groups 1 and 2, figure 6). They averaged 69 and 67, respectively, which is in agreement with the average iodine number, 71, found for omental fat in the control group of experiment 1 (table 5). The egg fats of the treated birds were considerably lower at the end of the first supplemental period than the controls (average of 51 for four groups). However, they were considerably higher than the 42 found for the omental fat of the treated birds in experiment 1. There was a gradual rise in iodine number of the egg fats of the S. foetida oil treated birds during the 63 days of the nonsupplemental period, from 51 at the start to 60 at the end. The fact that some of these birds had received various levels of oleic acid previously did not appear to influence the rise in egg fat iodine number.

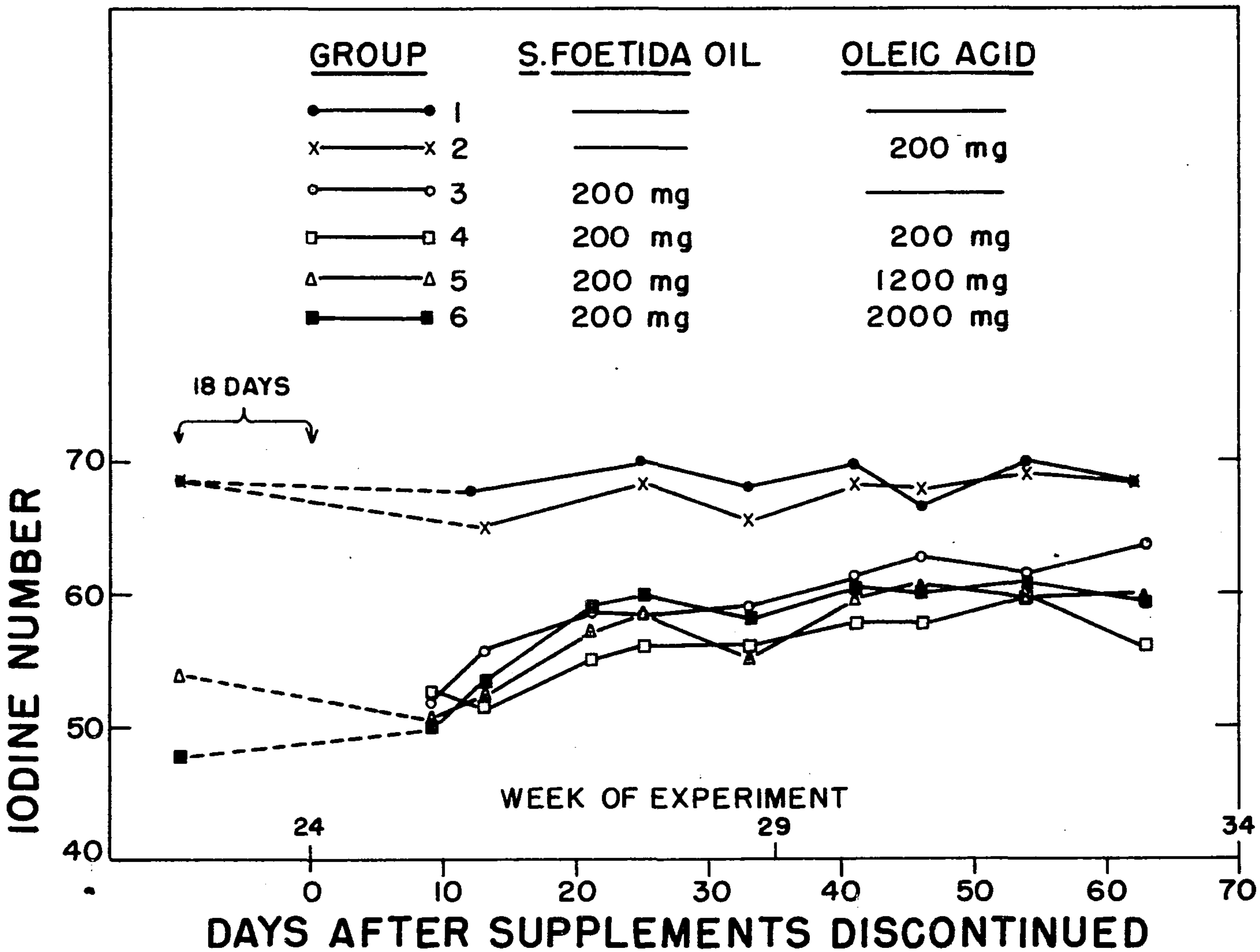

FIGURE 6:–COMPARISON OF IODINE NUMBERS OF FATS FROM EGGS LAID AFTER SUPPLEMENTS WERE DISCONTINUED.

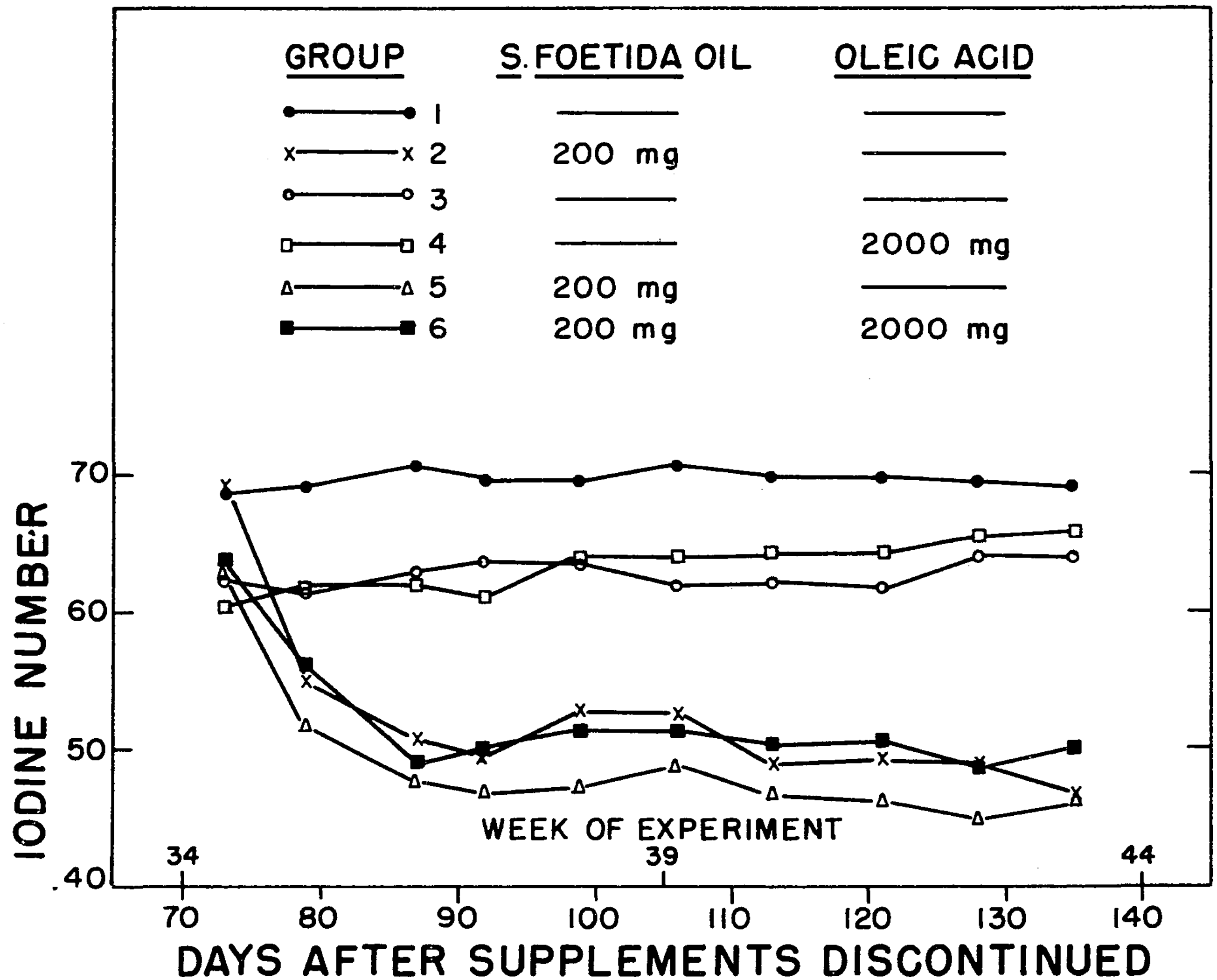

FIGURE 7:- COMPARISON OF IODINE NUMBERS OF FATS FROM EGGS LAID DURING REGROUPING AND RESUPPLEMENTATION.

The iodine numbers of the egg fats, after the birds were regrouped and the supplements were fed again, shows the tremendous effect of feeding only 200 mg of S. foetida oil daily, on raising the level of the saturated fatty acids and lowering the level of the unsaturated fatty acids in egg fat (figure 7). Birds which had never received S. foetida oil (Groups 1 and 2) showed rapid decline in iodine number from 69 to 55 within 9 days when S. foetida oil was fed (Group 2). This group stabilized at about 47 to 49, while the nonsupplemented controls (Group 1) maintained a number of 69 to 70 throughout the 10 week supplemental period.

A similar precipitous drop in iodine number was noted when S. foetida oil was fed to birds which had received S. foetida oil previously (Groups 5 and 6). However, it appears that the presence of 2000 mg of oleic acid in the S. foetida oil supplement (Group 6) sustained a slightly higher iodine number than when S. foetida oil was fed alone (Group 5). The drop in iodine number of the egg fats from Group 6 also shows that even though egg production was satisfactory (figure 5 and table 7) the increased formation of the saturated fatty acids took place when S. foetida oil was fed.

The gradual increase in iodine number continued when S. foetida oil supplements were discontinued (Group 3), or when supplements of 2000 mg oleic acid were fed (Group 4). Although the iodine number of

Group 4 was slightly higher than that of Group 3, neither reached the level of the nonsupplemented controls (Group 1).

Table 8 contains the iodine numbers of the omental fat obtained at termination of the experiment. Comparison of Groups 1 and 2, which had not received S. foetida oil before, showed that S. foetida oil significantly lowered the iodine number of the omental fat ($P < .001$).

Groups 3, 4, 5 and 6 (all previously supplemented with S. foetida oil) were all significantly lower than the nonsupplemented controls ($P < .001$). Group 3 had not been supplemented with S. foetida oil for 135 days; yet its low iodine number, 61, compared with 76, found for the nonsupplemented Group 1, points to the slow turnover of "storage" fat in the laying pullet.

Significant differences ($P < .02$) were found between Group 3 and Groups 4, 5 and 6. Thus, on the one hand, supplementing with 2000 mg of oleic acid per day (Group 4) raised the omental fat iodine number of the group to 67, as compared with a value of 61 for its nonsupplemented control (Group 3). On the other hand, supplementing Group 5 with 200 mg of S. foetida oil lowered the omental fat iodine number to 52. Group 6 with an omental fat iodine number of 57 showed that supplying 2000 mg of oleic acid with 200 mg of S. foetida oil only partially overcame the iodine number lowering effect of S. foetida oil (Group 5).

Table 8. Comparison of the iodine numbers of the omental fat from pullets fed 200 mg of S. foetida oil and 2000 mg of oleic acid daily.

Group	Supplement		Iodine number of omental fat
	S. foetida	Oleic acid	
1	---	---	76 ± 3 [1]
2	200 mg	---	62 ± 7
3	---	---	61 ± 3
4	---	2000 mg	67 ± 3
5	200 mg	---	52 ± 5
6	200 mg	2000 mg	57 ± 2

[1] Standard deviation.

Comparison of the omental fat iodine number of 76 for the non-supplemented controls (Group 1) in this experiment with the 71 for the corn oil controls in experiment 1 shows a considerable difference. This can be explained on the basis of the differing iodine numbers of the fats ingested by the two groups. In experiment 1, 1% hydrolyzed animal-vegetable fat (iondine number 56) was used in the diet, and the iodine number of the extracted fat was 74. In experiment 2, the animal-vegetable fat was replaced with glucose monohydrate, and all the fat present in the diet was mainly derived from corn or other vegetable sources (approximately 2.2% total fat). This extracted fat had an iodine value of 100. Thus, the addition of the hydrolyzed animal-vegetable fat significantly lowered the iodine number of the dietary fat, and this was reflected in the iodine numbers of the omental fat of the respective control groups. Although the corn oil controls in experiment 1 were supplemented daily with 200 mg of corn oil (iodine number 122), this was insufficient to affect the omental fat significantly.

Comparison of the iodine numbers of the egg fats obtained the last week of the experiment with those of the omental fats (table 9), showed that the egg fats were consistently of the same magnitude or lower in each group except Group 3, which had previously received S. foetida oil and probably had a more saturated omental fat. This is in agreement with Feigenbaum and Fisher ('59) and is especially true in

Table 9. Comparison of the iodine number of the pooled egg fat, individual omental fat and egg production.

Bird no.	No. of eggs[1]	Omental fat I_2 no.	Pooled egg fat I_2 no.[2]	Bird no.	No. of eggs	Omental fat I_2 no.	Pooled egg fat I_2 no.
Group 1 - No supplement				Group 4 - 2000 mg oleic acid			
391	56	73		413	53	68	
392	55	79		414	48	68	
393	4	76		415	58	70	
394	50	78		416	52	63	
395	52	79		417	63	72	
396	42	79		418	54	64	
397	55	74			Average	68	66
398	52	71					
	Average	76	69				
Group 2 - 200 mg S. foetida oil				Group 5 - 200 mg S. foetida oil			
399	10	55		419	40	56	
400	6	53		420	13	48	
401	3	59		421	11	43	
402	42	72		422	48	56	
403	54	67		423	37	54	
404	49	66		424	48	53	
405	45	67			Average	52	46
406	12	55					
	Average	62	47				
Group 3 - No supplement				Group 6 - 200 mg S. foetida oil plus 2000 mg oleic acid			
407	57	64		425	37	59	
408	57	61		426	33	56	
409	59	62		427	39	54	
410	66	57		428	50	56	
411	62	63		429	50	60	
412	43	--		430	47	57	
	Average	61	64		Average	57	50

[1] Laid during a period of 10 weeks.

[2] Pooled value for last week of experiment.

low fat diets. Of special interest are the differences found in Groups 2, 5 and 6 where S. foetida oil was fed alone or with oleic acid. The iodine numbers of the egg fat are low (46-50) as compared with the other groups (64-69), as are also the omental fat values (52-57 versus 61-76) if S. foetida oil had been previously fed (Groups 5 and 6 averaged 52 and 57, respectively; whereas, Group 3 was 63). Apparently, S. foetida oil caused increased formation of saturated fatty acids and a large part of these were stored in the egg. This concept is further strengthened by comparing the iodine numbers of the omental fat in these groups with egg production. For the most part, where egg production was fairly good the iodine number was average, but where egg production was low (bird number 399, 400, 401 and 406 of Group 2 and number 420 and 421, Group 5), the iodine number of the omental fat was also considerably lower than the rest of the group.

It is interesting to compare the above data with the general concepts about the degree of saturation in the depot fats of animals and their products, and the dynamics of lipid mobilization in the fowl. Cruickshank ('34) reviewed the early work and reported on further work which showed that dietary fat can alter body fat composition in the direction of greater saturation or unsaturation; whereas, egg fat was influenced only by the ingestion of unsaturated fats. This concept has been further corroborated by the work of Reiser ('50), Fisher and

Leveille ('57), Feigenbaum and Fisher ('59) and Wheeler, Peterson and Michaels ('59). For the most part, these investigators used 4 to 20% levels of fat in the diet and yet were unable to lower the iodine number of egg fat substantially. Feigenbaum and Fisher, feeding 10% coconut oil which is over 90% saturated fat (predominantly lauric acid), were able to lower body fat to an iodine number of 39 after 5 weeks, but the egg fat iodine number was 62. Yet, by feeding only 200 mg of S. foetida oil (iodine number 76) the body fat iodine number was lowered to about 40, if there was little production of eggs, and the egg fat to about 46. This emphasizes that the effect of the S. foetida oil cannot be explained solely on quantity of intake, and that the cyclopropene fatty acid inhibits the normal in vivo conversion of saturated fatty acids to mono-unsaturated fatty acids.

The inhibitory action of S. foetida oil provides some new insight into fatty acid deposition in the egg. When egg production is normal the egg fat is fairly saturated, but when egg production is low the omental fat becomes more saturated (table 9), showing that during normal egg production most of the circulating fatty acids are going into the yolk; whereas, when this requirement is not necessary then deposition in the "fixed" fat stores takes place. This is substantiated by the work of Budowski, Bottino and Reiser ('61), who found with labeling techniques that these depots were far from being in "isotopic steady state" after

3 weeks of feeding. However, there is some depot fat utilization for egg fat formation as shown by failure of the egg fat to become as unsaturated as the controls even after 135 days (figure 7).

These data also point to the possibility of fatty acid selectivity by the ovarian membrane. When S. foetida oil was fed there was an increase in normally appearing saturated fatty acids, both in the body depot and in the egg fat. But, when coconut oil, which is predominantly lauric acid, was fed (Feigenbaum and Fisher, '59) there was an increase in the saturated fatty acids in the body depot but the egg fat was changed very little.

Comparison of the organ weights at termination of the experiments are shown in tables 10, 11 and 12. No significant differences were observed between the various groups in liver, heart, kidney and adrenal weights.

Significant differences in gall bladder weights (table 10) were noted as in experiment 1. Of the two groups which previously had not received S. foetida oil (Groups 1 and 2), Group 2 receiving 200 mg of S. foetida oil had significantly larger gall bladders than the nonsupplemented Group 1; 1.75 versus 1.01 g per kg of body weight ($P < .001$). Among the other four groups which had been supplemented previously with S. foetida oil, only Group 5 had significantly larger gall bladders than the nonsupplemented control, Group 3; 2.30 versus 1.16 g per kg

Table 10. Comparison of liver and gall bladder weights from pullets fed 200 mg *S. foetida* oil and 2000 mg oleic acid daily.

Group	Oil supplement	No. of birds	Average body weight	Liver: Weight (g)	Liver: g per kg body weight	Gall bladder[1]: Weight (g)	Gall bladder[1]: g per kg body weight
1	none	8	1784 ± 184[2]	40.23	22.62 ± 3.73	1.82	1.01 ± 0.41
2	200 mg *S. foetida* oil	8	1804 ± 224	41.56	22.84 ± 3.76	3.20	1.75 ± 0.14
3	none	6	1752 ± 208	41.03	23.35 ± 1.93	2.03	1.16 ± 0.51
4	2000 mg oleic acid	6	1975 ± 265	49.95	25.54 ± 4.46	3.48	1.83 ± 0.65
5	200 mg *S. foetida* oil	6	1653 ± 241	40.88	24.73 ± 3.02	3.75	2.30 ± 0.81
6	200 mg *S. foetida* oil + 2000 mg oleic acid	6	1783 ± 283	46.99	25.94 ± 2.82	3.26	1.75 ± 0.36

[1] Determined by difference; i.e., Liver and gall bladder weight - liver weight = gall bladder weight.

[2] Standard deviation.

Table 11. Comparison of ovary and oviduct weights from pullets fed 200 mg S. foetida oil and 2000 mg oleic acid daily.

Group	Oil supplement	No. laying[1]	Ovary Weight (g)	Ovary g per kg body weight avg.	Ovary g per kg body weight range[2]	Oviduct Weight (g)	Oviduct g per kg body weight avg.	Oviduct g per kg body weight range[2]
1	none	7/8	41.03	23.20	3.04-33.65	49.99	28.30	5.54-37.50
2	200 mg S. foetida oil	4/8	23.71	12.72	1.64-23.03	31.27	16.66	2.59-31.91
3	none	6/6	38.03	27.28	22.01-35.97	51.07	29.77	25.13-41.18
4	2000 mg oleic acid	6/6	47.20	28.35	18.44-26.64	59.41	30.33	25.90-35.97
5	200 mg S. foetida oil	4/6	31.10	18.82	2.37-29.08	43.68	26.63	9.24-37.42
6	200 mg S. foetida oil + 2000 mg oleic acid	6/6	52.10	28.96	22.40-34.34	54.87	30.59	24.27-39.12

[1] Number of birds laying over number of birds in the group.

[2] Range within the group on a g per kg body weight basis.

Table 12. Comparison of heart, kidneys, adrenals and thyroid weights from pullets fed 200 mg S. foetida oil and 2000 mg oleic acid daily.

Group	Oil supplement	Heart		Kidneys		Adrenals		Thyroid	
		Wt. (g)	g per kg body weight	Wt. (g)	g per kg body weight	Wt. (mg)	mg per kg body weight	Wt. (mg)	mg per kg body weight
1	none	6.48	3.63 ± 0.30[1]	12.62	7.09 ± 1.08	120.1	66.7 ± 8.6	200.3	111.8 ± 27.2
2	200 mg S. foetida oil	6.41	3.47 ± 0.52	11.90	6.60 ± 1.09	106.3	58.8 ± 9.0	250.6	137.7 ± 13.3
3	none	6.32	3.59 ± 0.46	11.10	6.34 ± 0.96	122.5	69.1 ± 11.9	160.9	93.7 ± 22.4
4	2000 mg oleic acid	6.63	3.35 ± 0.34	13.53	6.94 ± 1.21	111.0	54.8 ± 12.1	279.5	140.8 ± 26.9
5	200 mg S. foetida oil	6.13	3.72 ± 0.41	11.99	7.26 ± 0.51	114.0	70.5 ± 10.5	226.6	135.5 ± 20.7
6	200 mg S. foetida oil + 2000 mg oleic acid	6.83	3.79 ± 0.52	13.55	7.48 ± 1.34	104.1	66.5 ± 15.4	192.8	104.8 ± 19.2

[1] Standard deviation.

of body weight ($P < .02$). It should also be noted that Group 4 (2000 mg S. foetida oil; 2.30 g per kg of body weight) and Group 6 (200 mg S. foetida oil plus 2000 mg oleic acid; 1.75 g per kg of body weight), which had previously received S. foetida oil, had significantly larger gall bladders than Group 1 (not supplemented; 1.01 g per kg of body weight). However, Group 3 (not supplemented but received S. foetida oil previously; 1.16 g per kg of body weight) was not significantly different from Group 1. From this it would appear that the oleic acid caused the enlargement of the gall bladder as well as S. foetida oil. However, this was not the case of the corn oil fed controls in experiment 1 (table 2).

As in experiment 1, variation in ovary and oviduct weights did not allow statistical comparison. The data is presented in table 11, along with the number of birds laying per group. Generally, those groups which received only S. foetida oil had smaller ovaries and oviducts than their respective nonsupplemented controls, or those which received oleic acid alone, or S. foetida oil and oleic acid together. However, the range of organ weights in table 11 (Groups 1, 2 and 5) and figure 8 shows the atrophy of the ovary that takes place when a chicken ceases laying for some time. Degeneration of the ova is particularly notable.

No significant difference was noted in thyroid weights between the two groups not previously supplemented, Groups 1 and 2 (table 12). Group 4 (2000 mg oleic acid) and Group 5 (200 mg S. foetida oil) were

Figure 8. Comparison of ovaries and oviducts from hens in full production then placed on supplements. That on the right received 200 mg of S. foetida oil for 10 weeks; that on the left received an empty capsule.

significantly larger than Group 3 (nonsupplemented control); 140.8 and 135.5 versus 93.7 mg per kg, respectively ($P < .02$). Group 6 was not significantly different; the reason for this is not apparent. The differences do not appear to follow a logical pattern.

Experiment 3: The effect of feeding *S. foetida* oil on the egg production of adult hens (42 weeks of age) is shown in figure 9. Very little difference was noted during the experiment between the groups receiving no supplement (Group 1), 3000 mg of oleic acid (Group 2) or 200 mg of *S. foetida* oil and 3000 mg of oleic acid (Group 4). However, when 200 mg of *S. foetida* oil was fed alone, it suppressed egg production completely within 6 weeks. Withdrawal of the *S. foetida* oil allowed normal recovery of production to occur within 3 weeks. Supplementing with S. foetida oil again suppressed egg production, but only to 34% of normal even though supplementation was continued for an additional week. Apparently, this level of *S. foetida* oil was not as effective in decreasing egg production during the second supplementation period (see also experiment 2, figure 5).

It is noteworthy that throughout this experiment (experiment 3), supplementation of 200 mg of *S. foetida* oil with 3000 mg of oleic acid allowed normal egg production.

Figure 10 contains the iodine numbers of the egg fats obtained in this experiment. Unfortunately, not all the eggs were collected

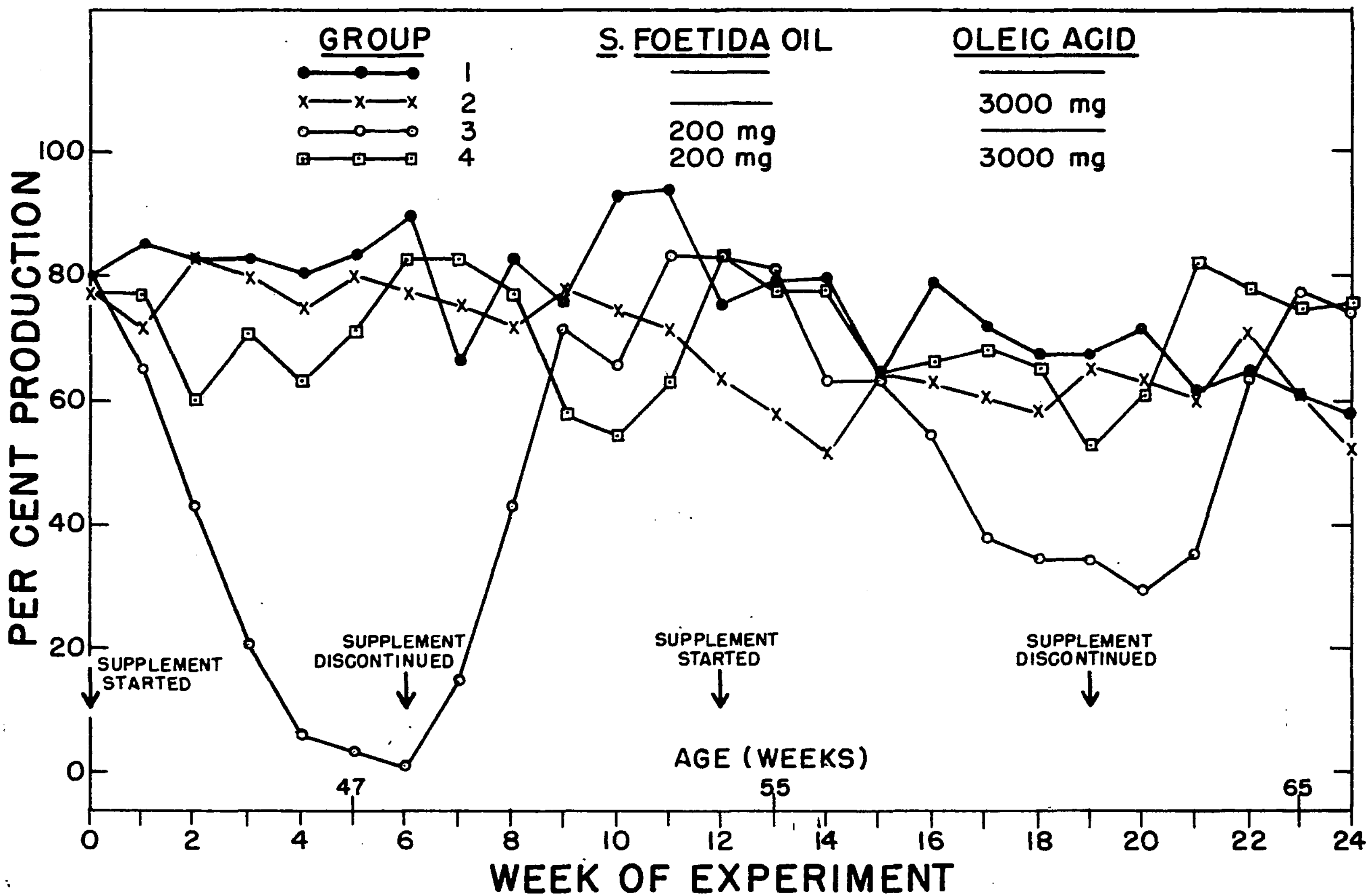

FIGURE 9: COMPARISON OF PER CENT EGG PRODUCTION OF MATURE HENS FED S. FOETIDA OIL OR OLEIC ACID.

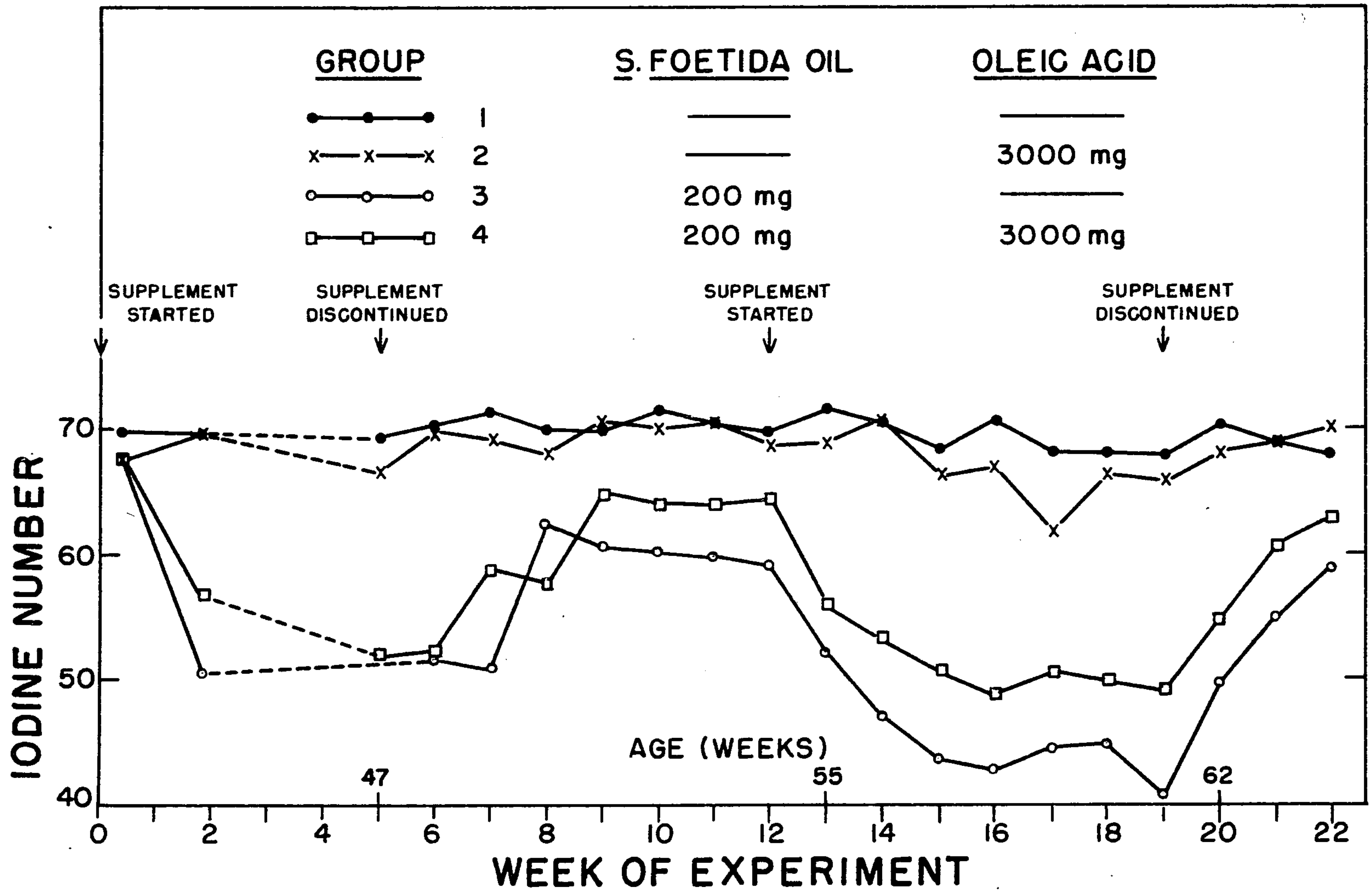

FIGURE 10:- COMPARISON OF IODINE NUMBERS OF FATS FROM EGGS LAID BY MATURE HENS FED S. FOETIDA OIL OR OLEIC ACID.

during the early part of the experiment. However, the data show that whenever S. foetida oil was given there was an immediate response culminating in an increase of the saturated fatty acids; i.e., lowering of iodine number. The iodine numbers of the egg fats from Groups 3 and 4 immediately dropped. There was also a rather rapid recovery. One week after supplementation had been discontinued, the iodine numbers of the S. foetida oil groups started to increase again. An indication that whatever the effect of the S. foetida oil was on the formation of saturated fatty acids, it disappeared rather quickly allowing partial recovery of the normal pathway.

As noted earlier, once S. foetida oil had been fed the iodine numbers of the egg fats did not return completely to that of the normals. Also, feeding a sufficiently high enough level of oleic acid, with S. foetida oil, gave egg fats with iodine numbers slightly higher than when S. foetida oil was fed alone.

PART II. The Effect of Sterculia foetida Oil on Growth of the Male Weanling Rat

MATERIALS AND METHODS

Sterculia foetida oil was prepared as reported earlier (Part I). The basal casein diet (table 13) was similar to that of Sarett and Snipper ('54), except that glucose monohydrate was used as the carbohydrate, Solka-Floc as the source of non-nutritive fiber, and the source of oil and amounts of vitamins were varied. Sufficient diet was prepared at one time to last throughout the experiment and stored at 45°F. Fresh diet was fed daily.

Holtzman Albino[1] strain of male weanling rats were used. They were maintained on a stock diet for 1 to 2 days after being received to allow equilibration of weight and were then selected, according to weight, into groups of 10 rats. They were housed in individual screen-bottom cages in an air-conditioned room, maintained at 80°F. Food and water were fed ad libitum. Records were kept of food and water consumption and weight gain during a 6 week period.

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[1] Holtzman Co.; Dallas, Texas and Madison, Wisconsin.

Table 13. Composition of basal diet.

		%
Casein[1]		18.0
Fat		10.0
Glucose monohydrate[2]		63.62
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 Co., New York.

[3] Solka-Floc; Brown Co., Boston, Mass.

[4] Jones and Foster ('42) 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 "	Biotin	0.02 "
Niacinamide	5.0 "	Vitamin B_{12} (0.1% trit. in mannitol)	10.0 "
Pyridoxine hydrochloride	0.25 "		
Calcium pantothenate	2.0 "		
Choline bitartrate	200.0 "	Menadione	0.2 "
Inositol	100.0 "	α - tocopherol	5.0 "
p-Aminobenzoic acid	10.0 "	Vitamin A*	1000 USP units
		Vitamin D_3**	120 " "

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

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

At termination of some experiments, the animals were fasted for 24 hours and then sacrificed by intraperitoneal injection of Surital[2] solution. The livers, kidneys and adrenals of each animal were weighed and recorded, and the livers were analyzed for per cent total solids and lipids, according to the method of Sarett and Jandorf ('47).

TBA values (a measure of fat oxidation) of the dietary fats were determined according to the thiobarbituric acid method of Sidwell, et al. ('54). The epididymal depot fat was extracted and iodine number determined as in Part I.

Experiment 1. Seventy male weanling rats were equally distributed, according to weight, into 7 groups of 10 animals each. Each group was assigned to one of the 7 experimental diets shown in table 14. The composition of these diets is listed in table 13, except for changes in the type of fat. The experiment was terminated at the end of six weeks.

Experiment 2. Three similar experiments were combined to study the effect of various vitamins on growth of rats fed 5% S. foetida-5% corn oil. The diets are listed in table 19.

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[2] Parke-Davis and Co.; Detroit, Michigan.

Table 14. Level of fat in the diet of experiment 1.

Diet	Fat
1	10% corn oil[1]
2	10% corn oil, Skellysolve F treated
3	1% S. foetida oil-9% corn oil
4	2.5% S. foetida oil-7.5% corn oil
5	5% S. foetida oil-5% corn oil
6	7.5% S. foetida oil-2.5% corn oil
7	10% S. foetida oil

[1] Mazola; Corn Products Company, New York.

RESULTS AND DISCUSSION

Experiment 1. The effect of feeding various concentrations of S. foetida oil on growth of male weanling rats is shown in table 15. Contrary to the report of Schneider ('60), replacing 1% of the 10% corn oil of the diet with S. foetida oil (Group 3) significantly retarded rat growth at 6 weeks as compared to the corn oil controls (Group 1); 168 g gained versus 208 g gained, respectively ($P < .001$). Increasing the level of S. foetida oil in the diet at 2.5% further retarded rat growth as shown by Group 4; 6 weeks gain, 126 g ($P < .001$). In the 5% S. foetida oil group (Group 5), over 50% of the rats died by the third week of the experiment, with an average survival time of 15 days. The remaining 4 rats gained an average of only 63 g for six weeks. All the rats fed 7.5% S. foetida oil (Group 6) and 10% S. foetida oil (Group 7) were dead by the third week of the experiment. Average survival times were 14 and 13 days, respectively. This parallels the observation of Thomasson ('55) for kapokseed oil.

The symptoms accompanying death of the rats were characteristic of a vitamin B-complex deficiency. Prior to death there was a general malaise followed by porphyrin deposits about the nose, mouth and whiskers and on the feet. Autopsy revealed a flaccid intestinal tract distended with gas. There was a general lack of muscular tone.

Table 15. Comparison of cumulative weekly weight gains of male rats receiving various levels of S. foetida oil and corn oil in the diet.

Group	Oil in the diet	No. of rats	Initial weight	Average weekly gain					
				1	2	3	4	5	6
			g	g	g	g	g	g	g
1	10% corn oil	10	51	29	62	104	147	184	208 ± 19 [1]
2	10% corn oil, Skellysolve F treated	10	51	29	56	92	141	175	196 ± 14
3	1% S. foetida oil-9% corn oil	10	51	23	54	86	126	148	168 ± 16
4	2.5% S. foetida oil-7.5% corn oil	10	51	12	29	44	88	117	126 ± 25
5	5% S. foetida oil-5% corn oil	10	51	4	6(8) [2]	22(4)	47(4)	49(4)	63(4)
6	7.5% S. foetida oil-2.5% corn oil	10	51	-6	-8(5)	all animals died (average survival time 14 days)			
7	10% S. foetida oil	10	51	-11(9)	-12(4)	all animals died (average survival time 13 days)			

[1] Standard deviation.

[2] Number of animals remaining.

No difference was noted when the weight gain of the corn oil treated with Skellysolve F (Group 2) was compared with that of the corn oil controls (Group 1), showing that the method of extraction or solvents used in the extraction of the S. foetida oil was not the cause of the deleterious effect of S. foetida oil.

Table 16 contains the comparison of the food and water consumption during the six week growth period. No difference was noted in food or water consumption between the corn oil controls (Group 1) and the corn oil treated with Skellysolve F (Group 2). Significant differences in food efficiency (i.e., g gained/100 g of food consumed) were found between the S. foetida oil Groups 3 and 4 and the controls, Group 1. The food efficiency of Groups 3 and 4 were 25.9 and 22.5, respectively; whereas, the corn oil controls (Group 1) were 32.1 ($P < .001$ in both cases) showing poorer utilization of the S. foetida oil diets.

No differences in water consumption per 100 gms of food were found between Groups 1, 2 and 3; but the difference between Group 4 (161.6 ml per 100 g of food) and Group 1 (136.9 ml per 100 g of food) approached significance ($P < .05$).

Organ weights and liver composition are shown in table 17. Significant differences were found in the liver weights (g/100 g body weight). Group 3, 1% S. foetida oil had an average liver weight of 3.33 ($P < .02$) and Group 4, 2.5% S. foetida oil 3.98 ($P < .001$) compared to

Table 16. Comparison of food and water consumption for 6 weeks of rats receiving various levels of S. foetida oil and corn oil in the diet.

Group	Oil in the diet	Food		Water	
		Total intake g	Food efficiency[1]	Total intake ml	ml/ 100 g of food
1	10% corn oil	586	32.1 + 3.2[2]	894	136.9 ± 17.9
2	10% corn oil, Skellysolve F treated	649	30.2 ± 2.5	820	127.1 ± 16.5
3	1% S. foetida oil-9% corn oil	650	25.9 ± 2.8	902	138.8 ± 10.3
4	2.5% S. foetida oil-7.5% corn oil	559	22.5 ± 3.8	903	161.6 ± 26.4

[1] Gms gained per 100 gms of food.

[2] Standard deviation.

Table 17. Comparison of organ weights and per cent liver solids and fats from rats receiving various levels of *S. foetida* oil and corn oil in the diet.

	Groups				
	1	2	3	4	5
Oil in the diet	10% corn oil	10% corn oil-Skellysolve F treated	1% *S. foetida* oil-9% corn oil	2.5% *S. foetida* oil-7.5% corn oil	5% *S. foetida* oil-5% corn oil
No. of rats	10	10	10	10	4
Fasted wt., % wt. loss/24 hr. fast	5.26 ± 0.89[1]	4.80 ± 0.80	4.58 ± 0.88	5.34 ± 0.95	6.84 ± 1.62
Liver					
Weight, g	6.88	7.04	6.93	6.60	6.26
% body wt.	2.74 ± 0.27	3.01 ± 0.48	3.33 ± 0.60	3.98 ± 0.56	5.90 ± 1.10
% solids	29.84 ± 0.52	28.70 ± 2.81	29.36 ± 1.71	29.44 ± 1.74	28.14 ± 1.37
% lipids	4.62 ± 0.24	4.34 ± 0.78	4.71 ± 0.86	4.69 ± 0.72	3.76 ± 0.51
Kidney					
Weight, g	1.63	1.56	1.42	1.26	0.93
% body wt.	0.66 ± 0.04	0.66 ± 0.05	0.67 ± 0.10	0.75 ± 0.06	0.88 ± 0.04
Adrenals					
Weight, g	47.0	43.3	42.7	36.7	31.6
mg/100 g	19.2 ± 1.7	18.5 ± 1.9	20.5 ± 1.7	22.1 ± 3.1	30.1 ± 4.5

[1] Standard deviation.

the corn oil controls which had 2.74. This corroborates the earlier finding of Schneider ('60) when 5% S. foetida oil was fed. However, no difference in the percentage of liver fat was found in any group as had been reported for the 5% S. foetida oil diet. Kidney and adrenal weights of the rats fed the 2.5% S. foetida oil diet (Group 4) were significantly different from the controls (Group 1). Group 4 kidneys averaged 0.75 g/100 g body weight compared to 0.66 for Group 1 ($P < .001$). Group 4 adrenals averaged 22.1 mg/100 gm body weight compared to 19.2 for Group 1 ($P < .02$). No other significant differences were found.

Table 18 contains the iodine numbers of the epididymal fat pads. Groups 3 and 4 which received S. foetida oil had a significantly lower iodine number (69) than did corn oil (87), ($P < .001$). As noted in the chicken experiments, feeding S. foetida oil resulted in increased formation of saturated fatty acids (lower iodine number) of depot fats. In this experiment the level of dietary fat was considerably higher than in the chicken experiments, and there was a considerable drop in the iodine number of the epididymal fat. It would not seem that this drop was attributable to dietary intake since the calculated iodine number for the fats of diets 1, 3 and 4 were 122, 117 and 111, respectively (based on corn oil, 122; S. foetida oil, 76).

Since Schneider ('60) had reported that rancidity developed when S. foetida oil and corn oil were mixed together and allowed to

Table 18. Comparison of the iodine number of the epididymal fat pad from rats receiving various levels of S. foetida oil and corn oil in the diet.

Group	Oil in the diet	Iodine number
1	10% corn oil	87 ± 7 [1]
2	10% corn oil-Skellysolve F treated	88 ± 3
3	1% S. foetida oil-9% corn oil	69 ± 8
4	2.5% S. foetida oil-7.5% corn oil	69 ± 3

[1] Standard deviation.

stand for a few days in the rat cage, peroxidation of the fats in the diets was measured according to the thiobarbituric acid method of Sidwell, et al. ('54). No peroxide formation or rancidity was noted in any of the diets which were stored under refrigeration and fed daily. However, samples of the diets stored in the open at room temperature became rancid. The 2.5% S. foetida oil-7.5% corn oil diet had a 20-fold increase in TBA value. The 5% S. foetida oil-5% corn oil had a 15-fold increase in TBA value, and both the 1% S. foetida oil-9% corn oil and the 7.5% S. foetida oil-2.5% corn oil TBA values increased about 10-fold.

Experiment 2. Increased levels of thiamine, riboflavin, pyridoxine, calcium pantothenate and biotin enhanced growth and increased survival time of the rats fed the 5% S. foetida oil-5% corn oil diet as shown in table 19.

As noted above, when 5% S. foetida oil was fed (Group 2) 11 of the 20 rats on the diet died with a mean survival time of 14 days. However, when a mixture of vitamins containing thiamine, riboflavin, pyridoxine, calcium pantothenate and biotin were added to the diet to give 10 or 15 times the optimal level (Groups 3 and 3a), no deaths occurred. Averaged together, these groups gained 63% as much weight as the corn oil controls (Group 1) in 6 weeks.

Feeding 10 times the optimal level of riboflavin (Group 4) or 5, 15 and 30 times the optimal level of thiamine (Groups 5, 6 and 7) did

Table 19. Comparison of cumulative weekly weight gain of rats receiving 5% S. foetida oil-5% corn oil diet and increased levels of thiamine, riboflavin, pyridoxine, calcium pantothenate and biotin in the diet.

Group	Excess vitamins added	Times[2] vitamins increased	No. of rats	Initial wt. g	Average gain weeks 1 g	3 g	6 g
1[1]			30	49	32	100[4]	207
2			29	49	5	24(10)[3,4]	80(9)
3	B_1; B_2; B_6; CaPan; biotin	10	10	46	18	68	145
3a	B_1; B_2; B_6; CaPan; biotin	15	20	51	16	54(10)[4]	117
4	B_2	10	9	47	2	27(3)	72(3)
5	B_1	5	10	53	4)		
6	B_1	15	10	53	7)	terminated at two weeks	
7	B_1	30	10	53	5)		
8	B_6; CaPan; biotin	15	10	48	11	50	122
9	B_6	15	10	48	8	38	99
10	CaPan	15	10	48	7	43	91
11	biotin	15	10	48	9	40(9)	97(8)

1 Group 1, controls received 10% corn oil; Groups 2 through 11 received 5% S. foetida oil-5% corn oil.
2 Number of times the normal vitamin level (table 13) was increased.
3 Number of animals remaining.
4 Group of 10 rats terminated.

not protect the rats from the effect of S. foetida oil. Of those receiving riboflavin, 7 of the 10 rats died by the second week with a mean survival time of 11 days. Among those receiving thiamine, deaths also occurred. The thiamine groups were terminated at the end of 2 weeks.

No deaths occurred when 15 times the optimal level of pyridoxine, calcium pantothenate and biotin were fed (Group 8). However, the growth was 84% of that obtained when all five vitamines were fed (Group 3a) and 59% of the controls. Feeding pyridoxine or calcium pantothenate alone (Groups 9 and 10) at the same level did not give quite as good growth as when all three were fed, but each prevented death. When biotin was fed alone (Group 11) growth was equivalent to that obtained with pyridoxine or calcium pantothenate alone. However, two rats died during the experiment, and the general appearance of the rats was not good.

No definite conclusion or hypothesis can be drawn from the above data as to the mechanism by which S. foetida oil affects growth. All of these vitamins are involved in lipid metabolism and, in combination, support life and growth. But in combination or individually, growth was never satisfactory, and only pyridoxine and calcium pantothenate alone prevented death.

SUMMARY

The effect of S. foetida oil, a rich, natural source of the cyclopropene fatty acid, sterculic, on the immature and mature female chicken and the male weanling rat has been investigated. The following observations were made:

Female Chicken:

1. Feeding S. foetida oil to 15 to 16 weeks old immature female chickens at the rate of 200 mg per day by gelatin capsule for 9 weeks resulted in retardation of comb, ovary and oviduct development and inhibited egg production. It also caused enlargement of the gall bladder and, after long term feeding, enlargement of the liver.

2. S. foetida oil interfered with normal lipid metabolism causing an increased formation of saturated fatty acids, as evidenced by a lowering of iodine number of the omental and egg fat.

3. Feeding of 2000 mg of oleic acid with 200 mg of S. foetida oil permitted egg production to increase to 64.2% of that of the controls, although onset of egg production was delayed about four weeks.

4. After long term S. foetida oil feeding was discontinued, egg production returned to normal within 3 to 4 weeks.

5. There was a gradual increase in the iodine number of the egg fat after feeding was discontinued, but the values did not reach those of the controls within 135 days.

6. Feeding 200 mg of S. *foetida* oil to mature female chickens inhibited egg production, but feeding 3000 mg of oleic acid with 200 mg of S. *foetida* oil permitted normal egg production.

7. Although the feeding of oleic acid along with S. *foetida* oil prevented inhibition of egg production, it did not prevent the increased formation of saturated fatty acids which accompanies S. *foetida* oil feeding.

8. The data showed that newly synthesized fatty acids are deposited primarily in the yolk during normal egg production and, secondarily, in the body depot stores. It also showed that a small amount of depot fat is continuously utilized for egg fat.

Male Weanling Rats:

1. Replacing 1% or more of a 10% corn oil-18% casein diet with S. *foetida* oil resulted in growth retardation and decreased food efficiency. At 2.5% level, increased water consumption was noted.

2. Livers of rats fed 1% S. *foetida* oil and livers, kidneys and adrenals of rats fed 2.5% S. *foetida* oil were significantly heavier than the corn oil controls.

3. Feeding 5% S. foetida oil caused some deaths, but 7.5% or more in the diet caused deaths of all rats by the end of the third week.

4. Feeding S. foetida oil at the 1 or 2.5% level lowered the iodine number of the epididymal fat pads to 69, compared to 87 for the corn oil controls.

5. Increasing the amounts of a combination of thiamine, riboflavin, pyridoxine, calcium pantothenate and biotin 10 to 15 times the optimal levels in the diet prevented death in the rats receiving 5% S. foetida oil; growth was 63% of the corn oil controls. Increased levels of thiamine or riboflavin alone did not prevent death.

6. Increased levels of pyridoxine, calcium pantothenate or a combination of these two vitamins with biotin prevented death, but individually these vitamins did not stimulate growth significantly. Increased level of biotin alone resulted in a few deaths.

LITERATURE CITED

Almquist, H. J., F. W. Lorenz and B. R. Burmester 1934 Relation of depot fat to egg yolk fat in laying hens. J. Biol. Chem., 106:365.

Budowski, P., N. R. Bottino and R. Reiser 1961 Lipid transport in the laying hen and the incubating egg. Arch. Biochem. Biophys., 93:483.

Cruickshank, E. M. 1934 Studies in fat metabolism in the fowl. I. The composition of the egg fat and depot fat of the fowl as affected by the ingestion of large amounts of different fats. Biochem. J., 28:965.

Deuel, H. J., Jr. 1955 The Lipids. Vol. II. Biochemistry. Interscience Publishers, Inc., New York, p. 536.

Dijkstra, G., and H. J. Duin 1955 Structure of sterculic acid and other analogous fatty acids. Nature, 176:71.

Doberenz, A. R., D. L. Schneider, A. A. Kurnick, M. G. Vavich and A. R. Kemmerer 1962 Physical changes in eggs produced by hens receiving Sterculia foetida oil supplements. Poultry Sci., 41:700.

Eckles, C. H., and L. S. Palmer 1916 Effects of feeding cottonseed products on the composition and properties of butter. Univ. of Mo. College of Agr., Agr. Expt. Sta. Res. Bul., 27: 44 pp. Abstract: Mo. Agr. Expt. Sta. Bul., 151:18.

Ellis, N. R., and H. S. Isbell 1926 Soft pork studies. II. The influence of the character of the ration upon the composition of the body fat of hogs. J. Biol. Chem., 69:219.

Ellis, N. R., Carmen S. Rothwell and W. O. Pool 1931 The effect of ingested cottonseed oil on the composition of body fat. J. Biol. Chem., 92:385.

Evans, R. J., J. A. Davidson and Selma L. Bandemer 1961 Fatty acid and lipide distribution in egg yolk from hens fed cottonseed oil or Sterculia foetida seeds. J. Nutrition, 73:282.

Evans, R. J., Selma L. Bandemer, Marjorie Anderson and J. A. Davidson 1962 Fatty acid distribution in tissues from hens fed cottonseed oil or Sterculia foetida seeds. J. Nutrition 76:314.

Faure, P. K. 1956 Sterculia acid and its Halphen reaction. Nature, 178:372.

Feigenbaum, A. S., and H. Fisher 1959 The influence of dietary fat on the incorporation of fatty acids into body and egg fat of the hen. Arch. Biochem. Biophys., 79:302.

Halphen, G. 1897 Réaction caractéristique de l'huile de coton. J. de Pharmacie et de Chimie, 6:390.

Keith, J. I., A. H. Kuhlman, Earl Weaver and W. D. Gallup 1932-34 Effect of cottonseed meal upon the dairy products. Report, Okla. A and M College, Agr. Expt. Sta., p. 164.

MacFarlane, J. J., F. S. Shenstone and J. R. Vickery 1957 Malvalic acid and its structure. Nature, 179:830.

Masson, J. C., M. G. Vavich, B. W. Heywang and A. R. Kemmerer 1957 Pink discoloration in eggs caused by sterculic acid. Science, 126:3277.

Murtz, N. L., Mary C. Williams and R. Reiser 1960 Nonsynthesis of linoleic acid from acetate-1-C^{14} by the laying hen. J. Nutrition, 72:451.

Naber, E. C., and C. L. Morgan 1957 Studies on a hatchability depressing factor in cottonseed oil. Poultry Sci., 36:429.

Nalbandov, A. L., and L. E. Card 1946 Effect of FSH and LH upon the ovaries of immature chicks and low producing hens. Endocrinology, 38:71.

Nalbandov, A. L., and M. F. James 1949 The blood vascular system of the chicken ovary. Am. J. Anat., 85:347.

Nunn, J. R. 1952 The structure of sterculic acid. J. Chem. Soc., (66), 313.

Official Methods of Analysis. ed. 7 1950 Association of Official Agricultural Chemists, Washington, D. C.

Phelps, R. A. 1962 A review of cyclopropenyl ("Halphen", Malvalic and Sterculic acids) fatty acid research. Unpublished. Research and Educational Division, National Cottonseed Products Association, Dallas, Texas.

Reiser, R. 1950 Fatty acid changes in egg yolk of hens on a fat-free and a cottonseed oil ration. J. Nutrition, 40:429.

Ringrose, R. C., C. L. Morgan and E. J. Lease 1941 The effect of cottonseed oil on the hatchability of eggs. Poultry Sci. 20:57.

Romanoff, A. L., and A. J. Romanoff 1949 The Avian Egg. John Wiley and Sons, Inc., New York, pp. 48, 248.

Sarett, H. P., and B. J. Jandorf 1947 Effects of chronic DDT intoxication in rats on lipids and other constituents of liver. J. Pharm. Expt. Therap., 91:340.

Sarett, H. P., and L. P. Snipper 1954 Comparison of fructose and glucose in the diet of alloxan-diabetic rats. J. Nutrition, 52: 525.

Schneider, D. L. 1960 Effect of Sterculia foetida oil on rat growth and egg hatchability. M. S. Thesis, Library-Univ. of Arizona, Tucson.

Schneider, D. L., A. A. Kurnick, M. G. Vavich and A. R. Kemmerer 1961a Delay in sexual maturity of pullets by Sterculia foetida oil. Given at informal Poultry Nutrition Conference, Atlantic City, New Jersey; as reported in Feedstuffs, 33:10.

Schneider, D. L., M. G. Vavich, A. A. Kurnick and A. R. Kemmerer 1961b Effect of Sterculia foetida oil on mortality of the chick embryo. Poultry Sci., 40:1644.

Schneider, D. L., A. A. Kurnick, M. G. Vavich and A. R. Kemmerer 1962 Delay of sexual maturity in chickens by Sterculia foetida oil. J. Nutrition, 77:403.

Shenstone, F. S., and J. R. Vickery 1956 A biologically active fatty acid in Malvaceae. Nature, 177:94.

Shenstone, F. S., and J. R. Vickery 1959 Substances in plants of the order Malvales causing pink whites in stored eggs. Poultry Sci., 38:1055.

Sidwell, C. G., H. Salwin, M. Benca and J. H. Mitchell 1954 The use of thiobarbituric acid as a measure of fat oxidation. J. Am. Oil Chem. Soc., 61:603.

Snedecor, G. W. 1946 Statistical Methods. The Iowa State College Press, Inc., Ames, Iowa.

Spadola, J. M., and N. R. Ellis 1936 The effect of the ingestion of cottonseed oil before and after hydrogenation on the composition of the body fat of the rat. J. Biol. Chem., 113:205.

Sturkie, P. O. 1954 Avian Physiology. Comstock Publishing Associates, a division of Cornell University Press, Ithaca, New York, p. 146.

Thomasson, H. J. 1955 The biological value of oils and fats. I. Growth and food intake on feeding with natural oils and fats. J. Nutrition, 56:455.

Wheeler, P., D. W. Peterson and G. D. Michaels 1959 Fatty acid distribution in egg yolk as influenced by type and level of dietary fat. J. Nutrition, 69:253.