INFLUENCE OF CERTAIN HORMONES ON DELAY OF SEXUAL MATURITY OF THE FEMALE CHICKEN BY *STERCULIA FOETIDA* OIL

by

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ABSTRACT

The deleterious effects resulting from the ingestion of S. foetida oil by sexually immature pullets have been cited many times. These generally include reduced growth, inhibition of egg production, and a lack of comb, ovary, and oviduct development.

In this study each of six groups of 18 White Leghorn pullets was supplemented with S. foetida oil, and a seventh group of 18 pullets was supplemented with corn oil. Feeding of the oils by gelatin capsules was started at 15 weeks of age. At 23 weeks of age five of the six S. foetida oil fed groups were injected intramuscularly with one of the following hormones: 17-beta estradiol, progesterone, follicle stimulating hormone, luteinizing hormone, and chorionic gonadotropin. The sixth group of S. foetida oil fed pullets received no injections and the corn oil fed group was injected with propylene glycol. Hormone injections were given every other day for 18 days. Two days later six birds from each group were sacrificed and their ovary-oviduct weights were compared.

Delayed sexual maturity caused by the feeding of S. foetida oil was not reversed or significantly altered by any of the hormones. In agreement with published

reports values for percent egg production, growth, and combined ovary-oviduct weights of the six groups fed S. foetida oil were all significantly lower than those for the corn oil fed group ($P < .05$). This indicates that delayed sexual maturity in the female chicken caused by S. foetida oil may not be due to an insufficiency of any of the hormones tested. However, this does not eliminate the possibility that combinations of the hormones may exert an effect.

INTRODUCTION

To date the literature dealing with the deleterious effects of ingesting cyclopropenoid fatty acids has focused on the "pink white" disorder found in hens' eggs following storage. There has also been some significant work regarding the effects on reproduction in rats and chickens, but no conclusions have been made regarding the mechanism or site of action of these compounds.

It was initially observed by Sherwood (1931) that hens on a diet containing cottonseed products laid eggs which discolored when stored. Kemmerer, Heywang, and Lowe (1967) demonstrated that cottonseed oil lowered egg production if fed at 2% or more in the diet and lowered egg quality if fed at the 1% level or more in the diet. Numerous other physiological effects in the female chicken have been described and attributed to the ingestion of cyclopropenoid fatty acids including retardation of growth, poor comb development, delayed sexual maturity, reduced egg hatchability, enlarged liver, and enlarged gall bladder. Similar effects have been noted by Sheehan and Vavich (1965) in the female rat, including growth retardation, delay of sexual maturity, irregular and prolonged estrus cycle, poor reproduction, high mortality of newborn, and enlarged

liver. Histological changes in the ovaries and uterii of Sterculia foetida oil fed rats were reported by Rascop, Sheehan, and Vavich (1966).

Sterculic acid, 8-(2-octyl-1-cyclopropenyl) octanoic acid is the principal cyclopropenoid fatty acid extracted from S. foetida seeds. Nunn (1952) elucidated its structure. It gives a positive Halphen test which is characteristic of cyclopropenoid compounds. Hydrogenation of the ring double bond causes a negative Halphen test. This same loss of reactivity is brought about by the addition of halogens or opening of the ring.

In 1939 Morgan and Ringrose reported that feeding crude cottonseed oil to hens reduced egg hatchability, but hydrogenated cottonseed oil did not. In 1957 a similar report was made by Naber and Morgan. In 1957 Masson et al. showed that hydrogenation of sterculic and malvalic acids destroyed their biological activity. The work of Shenstone and Vickery (1959) supported Masson's findings. This is evidence that the physiological effects noted are caused by the cyclopropene ring of these fatty acids.

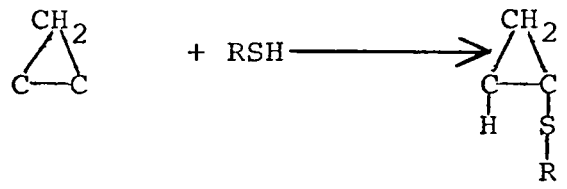
Feeding of cyclopropenoid fatty acids results in a definite alteration of the pH of stored eggs by causing a shift in pH of the yolk from 6.2 to 8.6. This pH change has been attributed to an alteration in permeability of the vitelline membrane. In 1960 Doberenz et al. supported

this theory by demonstrating a definite uptake in water by the yolks of eggs laid by hens fed cyclopropenoid fatty acids.

Schneider et al. (1962) reported an alteration of the ratios of fatty acids in rats fed cyclopropenoid fatty acids. There appeared to be a shift towards a higher concentration of saturated fatty acids at the expense of the monoenoic fatty acids. This was interpreted as an indication that unsaturated fatty acids other than linoleic could be important in functioning of the pituitary-gonadal axis as no deficiency of linoleic acid was found. Less oleic acid appears to be converted from stearic acid.

Raju and Reiser (1966) attempted to demonstrate the mechanism of action of cyclopropenoid fatty acids in the fatty acyl desaturase system. They suggested that the inhibition of this system is caused by irreversible binding of some essential thiol groups of the enzyme. This theory is based on in vitro studies, but there is indirect in vivo evidence that the fatty acyl desaturase system is impaired and the physiological disorders are not due to a simple deficiency of oleic acid. The report of Johnson et al. (1967) tends to support this theory.

Ory and Altschul (1964) demonstrated that one possible mechanism of action is the irreversible reaction of the cyclopropene ring with mercaptans to bind the sulfur in

a thioether linkage: 

This reaction could irreversibly inactivate enzymes requiring free sulfhydryl groups. Kircher (1964) demonstrated reactions of mercaptans with methyl sterculate and sterculene (1, 2-dioctylcyclopropene) and suggested that this type of reaction might take place *in vivo*. Ory and Altschul (1964) demonstrated that lipolysis was reduced 50% by the addition of *S. foetida* oil in *Ricinus communis* and that no inhibition occurred if lipolysis took place in the presence of *S. foetida* oil and cysteine. This countering of inhibition is evidence that the oil preferentially reacts with the sulfhydryl group of cysteine rather than that belonging to the acid lipase. Increasing rates of lipolyses were noted with the addition of larger amounts of cysteine. These results suggest that other sulfhydryl-containing enzymes may be inhibited by cyclopropenoid fatty acids. Kircher (1964) also noted that the high reactivity of the cyclopropene ring *in vitro* indicates that it may readily react with sulfhydryl groups under physiological conditions.

According to Nalbandov (1958 and 1964), the functioning of the pituitary-gonadal axis in the hen is quite similar to that in other vertebrates with a few exceptions.

The ovary of the hen is dependent on pituitary gonadotrophins for normal functioning. It has been shown that clutch length is governed by the amount of gonadotrophin produced by the pituitary gland. However, because of the almost continuous and cyclic maturation and ovulation of eggs in chickens with long clutches, it is thought that the rate of follicle stimulating hormone (FSH) secretion is not controlled by the variation in estrogen concentration produced by the follicles. It is probable that the flow of FSH is continuous and its concentration controls the clutch size; that is, the higher the concentration of FSH the larger the clutch. Also some Luteinizing hormone (LH) is produced at all times, but at certain intervals, greater amounts of LH are released and these "ovulatory peaks" cause ovulation. McLaren (1966) stated that progesterone can also cause ovulation, but it is not known if its action causes an increase in LH or if it works in some other manner. It may be secreted by preovulatory follicles and thereby cause ovulation when progesterone is being released in sufficient amounts.

It is interesting that only the reproductive system of the female of the species appears to be affected by the ingestion of cyclopropenoid fatty acids. Histological studies have shown that the testes and epididymides of male rats fed S. foetida oil are normal. Also sperm production in the seminiferous tubules and storage of sperm in the

epididymides are normal. It is probable that there is no upset in the testosterone balance, a precursor to estrogen. It might be considered in the syntheses of estrogen from cholesterol that testosterone is formed first and then follows one of two pathways: (a) Testosterone ⟶ 19 Hydroxytestosterone ⟶ Estradiol-17B or (b) Testosterone ⟶ Androstene-3, 17-dione ⟶ 19-hydroxyandrostene-3, 17-dione ⟶ estrone ⟶ estriol. Both pathways include a desaturation step as ring A of the phenanthrene nucleus contains one unsaturation in testosterone and three unsaturations in the estrogen molecule.

This study was undertaken to determine if the delay of sexual maturity in the female chicken, caused by feeding S. foetida oil, results from a lowered level of one of the sex hormones.

METHODS AND MATERIALS

Oil Supplements

The Sterculia foetida oil used in these experiments was prepared by extraction of Sterculia foetida seeds according to Schneider et al. (1961). The seeds were ground and then extracted three times with two liters of Skellysolve F per kilogram of seed. The solvent was removed from the oil in a continuous flow rotary vacuum evaporator at 35°C with reduced pressure provided by a water-pump aspirator. A high vacuum oil pump was used to remove the last traces of the solvent at a temperature of 35°C.

Corn oil[1] was used in these experiments as the control oil.

Experimental Animals and Diets

The experimental animals were Single-comb White Leghorn female chickens. At 15 weeks of age they were housed two in a cage in a room with controlled lighting and temperature (14 hours of light and 10 hours of darkness; 26°C). They received a starter diet for the first 20 weeks. At 21 weeks of age they were changed to a laying

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

diet and fed this diet until the termination of the study. The compositions of the diets fed are shown in Tables 1 and 2. Feed and water were given ad libitum for the entire period, and the oil supplements were given daily in gelatin capsules beginning at 15 weeks of age.

Hormones Injected

The hormones and dosages used in this experiment are listed in Table 3. Propylene glycol was used as the carrier for estrogen (17-beta estradiol) and progesterone. Chorionic gonadotropin, Luteinizing hormone, and follicle stimulating hormone were dissolved in distilled water. The powder was weighed and dissolved in a volumetric flask at room temperature. Luteinizing hormone, follicle stimulating hormone, and chorionic gonadotropin were dissolved in distilled water in their original vials. All hormone solutions were kept in refrigerated storage and warmed to room temperature before injection. Solvents, containers, and measuring devices were autoclaved before being used. Antiseptic techniques were employed in making hormone solutions and in dispensing them.

Experimental Procedure

One hundred and fifty pullets were divided into two groups at 16 weeks of age. The first group consisted of 120 birds which were supplemented with 200 mg of Sterculia

Table 1. Composition of starter diet.

	%
Ground yellow corn	27.000
Ground milo	29.230
Soybean meal (solvent)	20.400
Fish meal, sardine	6.000
Meat and bone scraps	3.000
Whey, dried whole cheese	0.500
Corn dist. dried solubles	0.700
Dehydrated alfalfa meal (17% protein)	3.000
Animal fat	5.000
MHA, meth, hydroxy analog	0.130
Pr-9 vitamin mix[c]	2.500
Calcium carbonate	1.000
Dicalcium phosphate[a]	1.000
Salt (tm)[b]	0.300
Trace mineral mix	0.200
Sulfa-Quinoxaline	0.040

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

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

[c]Calculated to supply per kg of diet:

Stabilized vitamin A Palmitate	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

Table 2. Composition of laying diet.

	%
Ground milo	64.100
Soybean meal (solvent)	8.500
Fish meal, sardine	5.000
Meat and bone scraps	2.500
Whey, dried whole cheese	2.500
Dehydrated alfalfa meal (17% protein)	5.000
Animal fat	1.000
Pr-9 mix[c]	2.500
Calcium carbonate	6.700
Dicalcium phosphate[a]	1.500
Salt (tm)[b]	0.500
Trace mineral mix	0.200

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

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

[c]Calculated to supply per kg of diet:

Stabilized vitamin A Palmitate	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

Table 3. Hormones and doses injected.

Group No.	Oil Fed	Bird No.	Hormones and Solvents	Doses Injected
1 (a)	S. foetida	1-9	Follicle Stimulating Hormone (FSH) Lyophilized, Porcine, in water.	125 µg/0.05 ml
1 (b)	S. foetida	10-18		250 µg/0.10 ml
2 (a)	S. foetida	1-9	Luteinizing Hormone (LH) Lyophilized, Equine, in water.	125 µg/0.05 ml
2 (b)	S. foetida	10-18		250 µg/0.10 ml
3 (a)	S. foetida	1-9	Progesterone in propylene glycol.	50 µg/0.20 ml
3 (b)	S. foetida	10-18		100 µg/0.20 ml
4 (a)	S. foetida	1-9	17-beta Estradiol in propylene glycol.	80 µg/0.20 ml
4 (b)	S. foetida	10-18		160 µg/0.20 ml
5 (a)	S. foetida	1-9	Chorionic Gonadotropin, Lyophilized, in water.	300 µg/0.05 ml
5 (b)	S. foetida	10-18		600 µg/0.10 ml

Table 3. (Continued).

Group No.	Oil Fed	Bird No.	Hormones and Solvents	Doses Injected
6	S. foetida	1-18	None	
7	Corn oil	1-18	Propylene Glycol.	0.20 ml

foetida oil daily, and the second group consisted of 30 birds which were supplemented with 200 mg of corn oil daily. At 17 weeks of age the pullets fed S. foetida oil were divided into six groups of 18 birds each and a group of 18 birds was selected from the 30 controls. The birds were grouped according to weight so that the mean weights of all seven groups were practically the same. Oil supplements were continued as before and egg production was recorded. At 22 weeks of age 6.5% of those hens fed S. foetida oil were laying. This was a higher percentage than reported by Schneider (1962). Since Halphen tests showed that the various batches of oil contained between 19 and 21% cyclopropenoid fatty acids, about one-half the concentration reported by Sheehan (1964), the S. foetida oil supplement was increased to 400 mg daily and the corn oil supplement was increased to 400 mg daily. No effect on egg production was noted as a result of the increased supplements of S. foetida oil as egg production continued to rise until it leveled off at 23 weeks of age to 12%. Hormone injections were started at the beginning of the 23rd week and continued every other day for 18 days. The hormones[1] were obtained as a powder and dissolved as needed. The doses given were based on recommended dosage per kilogram of body weight.

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[1]Mann Research Laboratories, New York, New York.

Two days following the final hormone injections, six birds from each of the seven groups were sacrificed and the ovaries and oviducts were removed and weighed.

RESULTS AND DISCUSSION

Weight Gains Before Hormone Injections

At 19 weeks of age a divergence in growth rates was noted between the S. foetida and corn oil fed groups ($P < .05$[1]). This divergence continued more or less for the next seven weeks when the experiment was terminated (Figs. 1 and 2).

Comb Development Before Hormone Injections

Those pullets fed S. foetida oil displayed a general lack of comb development. Several birds of this group did not develop combs throughout the experiment, while all control birds progressed normally in this respect.

Egg Production Before Hormone Injections

In the control group the first egg was layed at 19 weeks of age, and egg production increased to 77.7% by the 23rd week. The S. foetida oil fed groups began laying at 20 weeks of age, but by the 23rd week egg production was only 12% for the six groups. Egg production performance for each of the seven groups for the last 26 days of the experiment are shown in Table 4 and Figs. 3 and 4.

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[1]Probability: Simpson, Roe, and Lewontin (1960).

Effect of Hormones on Weight Gain

Generally growth rates of the S. foetida oil fed groups were slower ($P < .05$) than those of the corn oil fed controls throughout the experiment (Figs. 1 and 2). However, throughout the 18-day period of hormone injections, the six S. foetida oil fed groups experienced both gains and losses, and therefore not all of the S. foetida fed groups were in the range of statistical significance all of the time. The only S. foetida fed group that showed a significant loss in weight was group 6 which did not receive any hormone injections. It lost 50 grams the second week and 60 grams the last four days of this 18-day period. These losses are not readily explained. Since group 6 was not being injected, hormones and their solvents could not have been the cause. The five groups of S. foetida oil fed birds which were injected with hormones all experienced variable but statistically nonsignificant gains and losses during the course of injections. One might speculate that some gains and losses may have resulted from metabolic adjustments to the hormone injections and even to the increased amounts of S. foetida oil.

In these experiments weight losses are not likely to be due to the hormones administered. The function of LH is one of promoting rupture of the ovarian follicle. One might expect that estrogen would cause a gain in weight

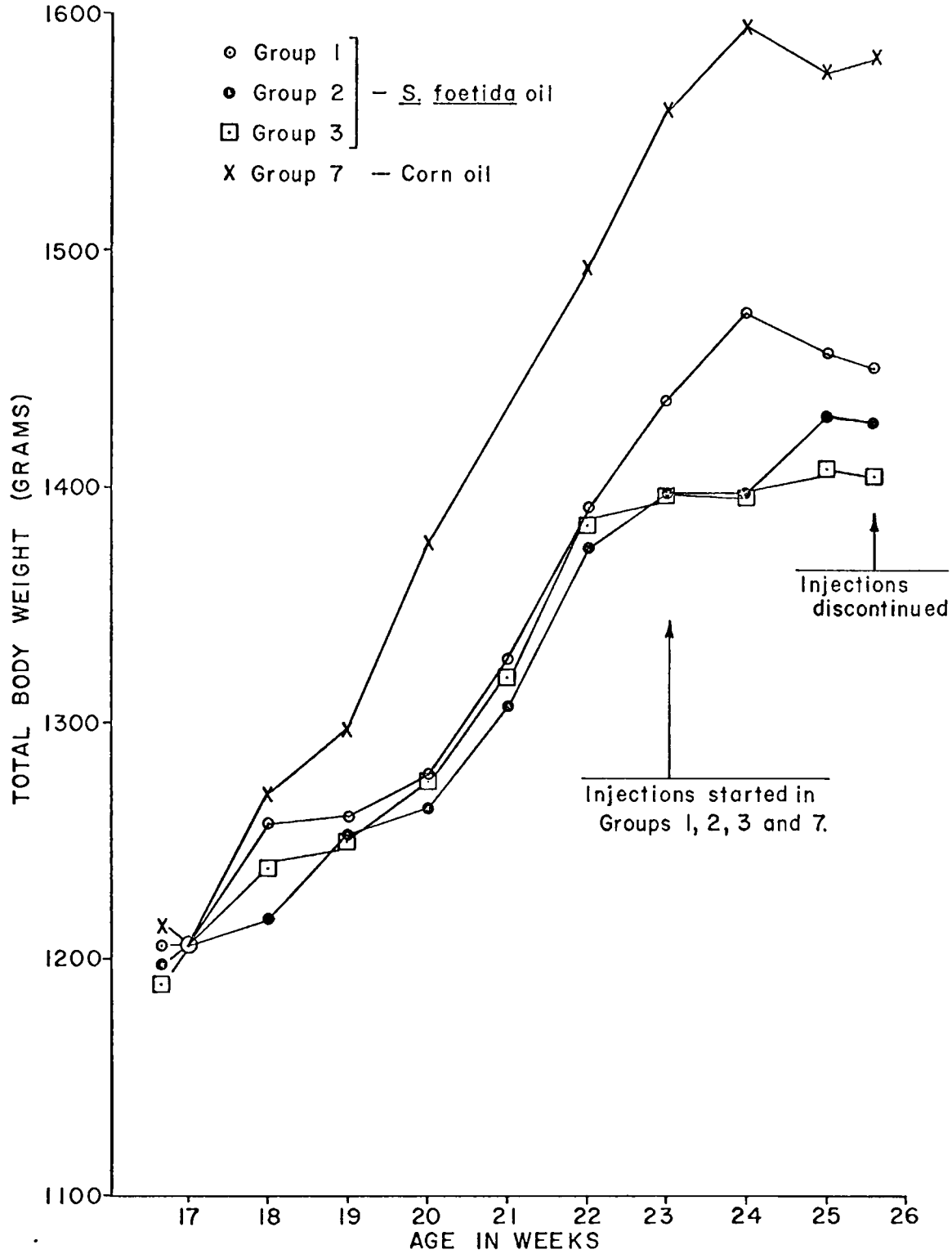

Figure 1. – Comparison of growth of S. foetida oil fed pullets injected with FSH, LH and Progesterone.

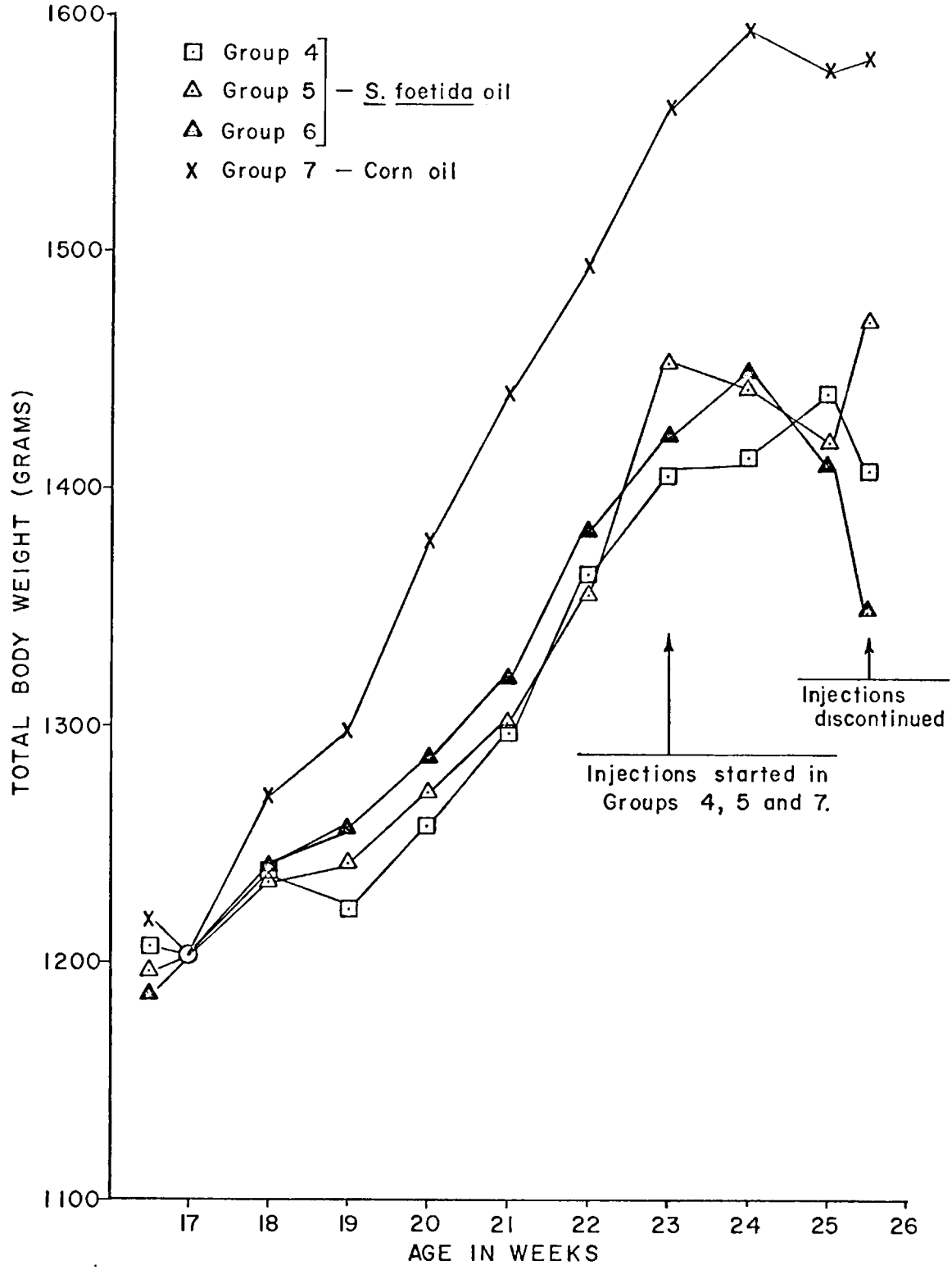

Figure 2.- Comparison of growth of S. foetida oil fed pullets injected with Estrogen and Chorionic Gonadotropin.

as it functions to maintain the ovaries, oviduct, and comb, yet group 4 showed an overall loss of 25 grams for the 18 days of estrogen injections. It may be that the dose of estrogen was too high and caused an increased rate of lipolysis which might have lead to a loss in weight if estrogen was not overcoming the weight loss caused by S. foetida oil. However, the pituitary gland of the hen is very resistant to estrogenic inhibition (McLaren, 1966). Thus it is unlikely that the levels of estrogen given were high enough to prevent the release of FSH and thus the maturation of the follicles. Chorionic gonadotropin is often used as a substitute for the pituitary fractions because it mimics the pituitary actions. It is not the same as the pituitary fractions biochemically even though both are glycoproteins. As chorionic gonadotropin would be expected to have an action similar to a mixture of FSH and LH it too should be eliminated as a cause of any weight loss. It is quite certain that progesterone is secreted by the ovary and regulates the ovulatory cycle in the chicken. Its action is mainly upon LH but it also exerts some effect on FSH. The level of progesterone determines whether it stimulates or inhibits. Ralph and Fraps (1960) obtained premature ovulation in the laying hen nine times out of 10 with 50 micrograms of progesterone injected intramusculary and five times out of six with 100 micrograms. In

view of this data it seems unlikely that too high a dose was given and that either FSH or LH was inhibited causing a delay in weight gains.

These results suggest that the hormones used are unable to overcome the classical weight losses observed when S. foetida oil is ingested. It was also found that the propylene glycol had no specific effect on weight in the control group. The possibility that the hormones or their carriers were responsible for the weight gains or losses in the S. foetida oil fed groups is unlikely.

Effect of Hormones on Comb Development

The hormones caused no apparent changes in the comb developments of the test groups. Group 4 was watched very closely for changes as maintenance of the hens' comb is a function of estrogen, but no growth was noted.

Effect of Hormones on Egg Production

Table 4 and Figs. 3 and 4 contain the data collected on egg production during the last 26 days of the experiment. Egg production among the six S. foetida oil fed groups only varied slightly, but great differences as expected were noted between the corn oil fed and the S. foetida oil fed groups ($P < .05$). Group 1 (FSH injected) showed the highest percentage increase in egg production. FSH may have been responsible by increasing the clutch

Table 4. Comparison of egg production of S. foetida oil fed pullets injected with hormones.

	GROUPS						
	1	2	3	4	5	6	7
Oil Fed	S.f.[a]	S.f.	S.f.	S.f.	S.f.	S.f.	Corn Fed
Hormone and Dose Injected in ug	FSH 125 & 250µg	LH 125 & 250µg	Proges-terone 50 & 100µg	Estro-gen 80 & 160µg	Chor-ionic Gonado-tropin 300 & 600µg	none	Propyl-ene Glycol 0.2ml
No. of Birds	18	18	18	18	18	18	18
Age in Weeks	Average Daily Egg Production in Percent						
23	6.3	8.6	6.3	6.3	6.3	8.6	70.6
	Hormone Injections Started						
24	12.6	13.6	7.9(17[b])	11.9	4.9(17)	15.9	65.0
25	19.0	13.5(17)	9.2	9.5	10.4(16)	12.3(17)	72.9

Table 4. (Continued).

No. of Birds	18	18	18	18	18	18	18
	13.9[c]	12.5(16)	7.3	2.7(17)	10.9	12.5(16)	77.7
	Hormone Injections Stopped						

[a]S.f. - Sterculia foetida.

[b]Number in parenthesis denotes the number of surviving birds.

[c]Averages for four days.

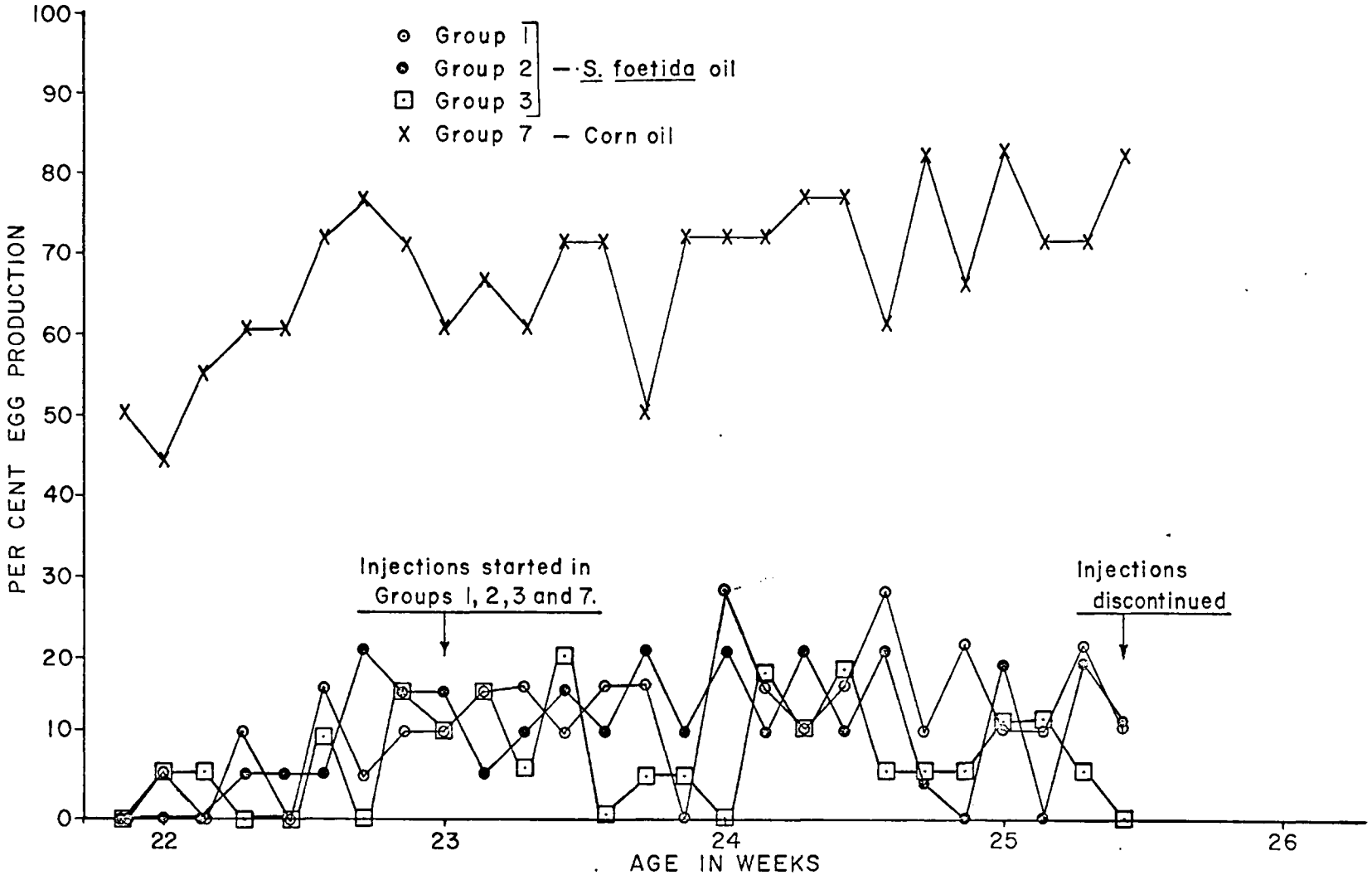

Figure 3. – Comparison of egg production from S. foetida oil fed pullets injected with FSH, LH and Progesterone.

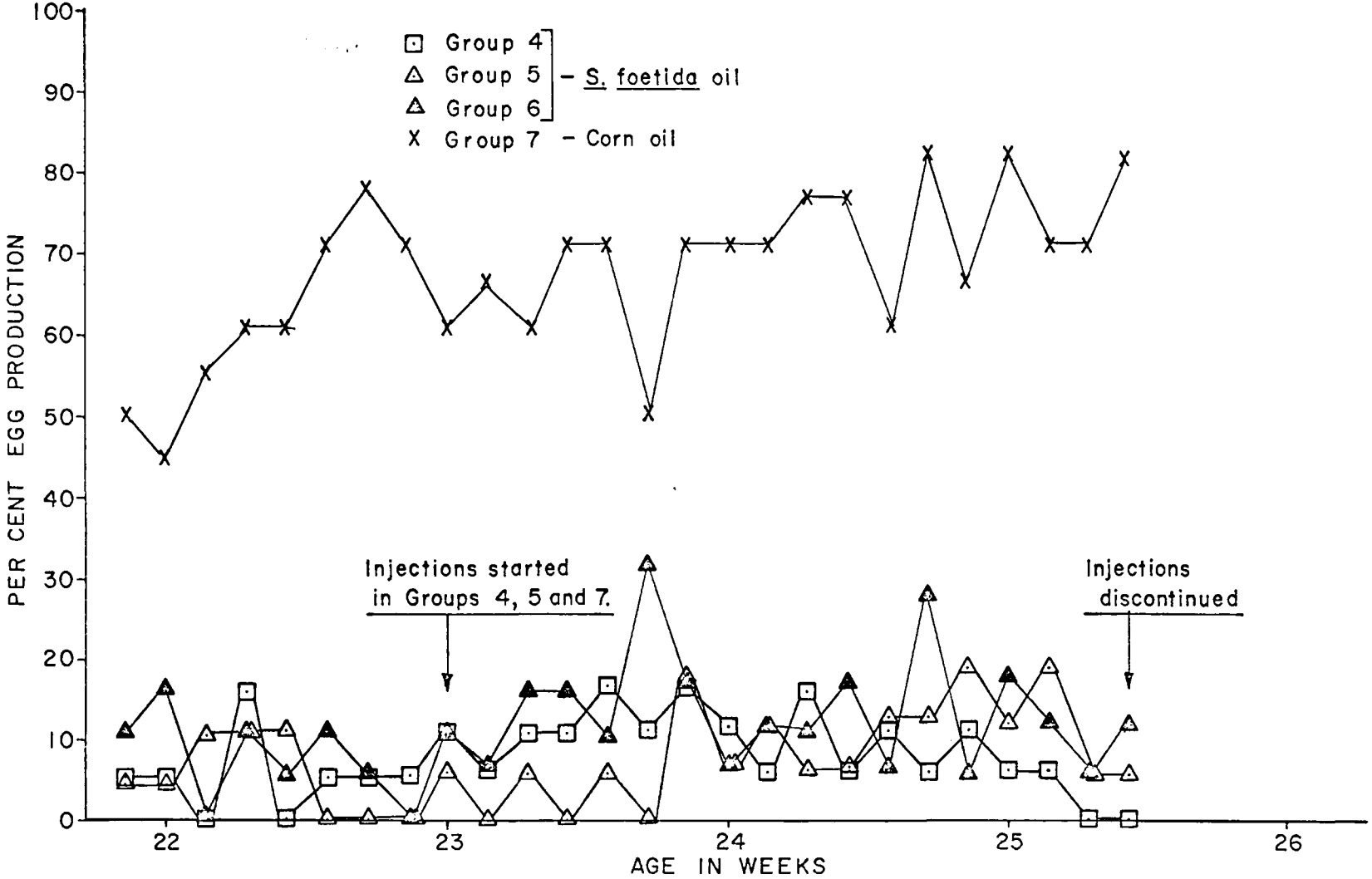

Figure 4. Comparison of egg production from S. foetida oil fed pullets injected with Estrogen and Chorionic Gonadotropin.

length of those hens that were laying. Nalbandov (1964) states that there is a continuous flow of FSH from the pituitary of the hen, and the level of FSH controls the clutch size. An analysis of variance shows that the control group (corn oil fed) had a significantly higher percentage egg production ($P < .05$) than the six S. foetida oil fed groups, and that none of the S. foetida oil fed groups were significantly different from each other. This data indicates that the hormones as administered in these experiments failed to alleviate the reduced egg production caused by S. foetida oil. Egg production is a better indication of the action of S. foetida oil on sexual maturity than is weight. The higher percentage of egg production by the control group was always statistically significant while the variations in weight were not always meaningful.

Eggs layed by the S. foetida oil fed pullets had putty-like yolks after one day of refrigeration at 13°C. Pink whites were also noted after 27 days of refrigeration at 13°C. These effects were reported by Sherwood in 1928 and are indicative of ingestion of cyclopropenoid fatty acids by the laying hen.

Comparisons of Ovary-Oviduct Weights

Following the 18-day period of hormone injections three birds from each dosage level were sacrificed from

each of the seven groups and their ovaries and oviducts removed and weighed (Table 5).

In the six S. foetida oil fed groups the organ weights were varied. This was not totally unexpected as the weights of the hens were not uniform and their egg productions were irregular. The mean organ weights of the six S. foetida oil fed groups were 55 g as compared with 119 g for the controls. This is obviously significant, but no attempt was made to determine statistical significance of differences among the six test groups. Nor was there any distinct divergence between the first group of three birds and the second group in the five groups injected with two levels of hormone. One would have to conclude from these patterns that the hormones administered did not produce any notable change in the delayed development of the ovary-oviduct caused by feeding S. foetida oil.

Table 5. Comparison of combined ovary-oviduct weights of S. foetida oil fed pullets injected with FSH, LH, estrogen, progesterone, and chorionic gonadotropin.

Bird No.[b]	Ovary-oviduct weights (gm)	Range[a]	g per kg body wt.	Remarks
Group No. 1 (FSH)				
1	7.1		5.10	no noticeable development
2	1.9		2.51	no noticeable development
3	39.3	5.1-75.0	26.18	several small eggs forming
4	21.3		16.46	slight egg formation
5	119.7		75.47	complete development, egg in oviduct with shell
6	51.2		26.25	egg formation
Group No. 2 (LH)				
1	24.2		19.06	slight egg formation
2	87.2		62.85	3 to 4 eggs being formed
3	96.6	2.3-68.0	68.03	3 to 4 eggs being formed
4	77.9		56.18	2 to 3 eggs being formed
5	1.7		2.37	no noticeable development
6	96.6		58.51	5 to 6 eggs being formed

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

[b]The first three birds in each group have been injected with the lower concentrations while the last three have been injected with the higher levels.

Table 5. (Continued) Comparison of combined ovary-oviduct weights.

Bird No.[b]	Ovary-oviduct weights (gm)	Range[a]	g per kg body wt.	Remarks
Group No. 3 (Progesterone)				
1	33.4		25.16	slight egg formation
2	25.8		16.32	slight egg formation
3	2.4	2.3-68.7	2.36	no noticeable development
4	88.1		68.74	good egg formation
5	125.4		66.57	complete development, egg in oviduct with shell
6	2.9		3.38	no noticeable development
Group No. 4 (Estrogen)				
1	58.5		38.50	2 to 3 eggs being formed
2	24.6		18.36	no eggs fully developed
3	28.0	9.2-57.7	21.45	no eggs fully developed
4	17.8		13.00	no eggs fully developed
5	87.8		57.67	good egg formation
6	12.4		9.17	no noticeable development

Table 5. (Continued) Comparison of combined ovary-oviduct weights.

Bird No.[b]	Ovary-oviduct weights (gm)	Range[a]	g per kg body wt.	Remarks
Group No. 5 (C.G.)				
1	16.1		14.08	1 small egg formed
2	4.8		3.76	no noticeable development
3	77.9	3.8-93.3	54.51	2 to 3 eggs being formed
4	77.4		52.49	2 to 3 eggs being formed
5	9.6		7.40	no noticeable development
6	129.4		93.32	complete development, egg in oviduct with shell
Group No. 6 (No Injections)				
1	43.1		32.0	2 to 3 eggs being formed
2	7.6		6.41	1 small egg formed
3	2.5	3.3-89.0	3.30	no noticeable development
4	63.2		41.13	4 to 5 eggs being formed
5	67.5		46.63	5 to 6 eggs being formed
6	123.6		89.03	complete development, egg in oviduct with shell

Table 5. (Continued) Comparison of combined ovary-oviduct weights.

Bird No.[b]	Ovary-oviduct weights (gm)	Range[a]	g per kg body wt.	Remarks
Group No. 7 (Propylene Glycd.)				
1	101.7		67.31	complete development, egg in oviduct with shell
2	133.8		79.04	complete development, egg in oviduct with shell
3	138.0	62.6-109.1	109.10	complete development, egg in oviduct with shell
4	134.8		92.27	complete development, egg in oviduct with shell
5	106.0		74.00	complete development, egg in oviduct, no shell
6	97.8		62.57	complete development, no egg in oviduct

SUMMARY AND CONCLUSIONS

Feeding of S. foetida oil to female chickens before sexual maturity resulted in:

(1) lowered weight gains during the entire experiment;

(2) inhibited egg production;

(3) retarded comb development;

(4) retarded ovary and oviduct development.

The injection of various levels of pituitary gonadotropins, estrogen, and progesterone did not reverse the above effects. This indicated that the delay of sexual maturity in the female chicken caused by S. foetida oil is not a result of lowered levels of the hormones and that the cyclopropenoid fatty acids are not inhibiting the desaturation step in the in vivo synthesis of estrogen.

LITERATURE CITED

Doberenz, A. R., D. L. Schneider, A. A. Kurnick, M. G. Vavich, and A. R. Kemmerer, 1960. Effect of Sterculia foetida oil on uptake of water by the avian egg yolk. Proc. Soc. Exptl. Biol. Med. 105:359-361.

Johnson, A. R., J. A. Pearson, F. S. Shenstone, and A. C. Fogerty, 1967. Inhibition of the desaturation of stearic to oleic acid by cyclopropene fatty acids. Nature. 214:1244-1245.

Kemmerer, A. R., B. W. Heywang, and B. W. Lowe, 1967. Effect of cottonseed oil on egg production and egg quality. Poultry Sci. XLVI:1165-1167 (No. 5).

Kircher, H. W., 1964. The addition of mercaptans to methyl sterculate and sterculene: An hypothesis concerning the nature of the biological activity exhibited by cyclopropene derivatives. J. Am. Oil Chemists Soc. 41:4-8.

Masson, J. C., M. G. Vavich, B. W. Heywang and A. R. Kemmerer, 1957. Pink discoloration in eggs caused by sterculic acid. Science, 126:751.

McLaren, Anne, 1966. Advances in Reproductive Physiology. Lagos Press (London).

Morgan, C. L., and R. C. Ringrose, 1939. Vegetable proteins in laying and breeding rations. Ann. Rept. South Carolina Agr. Expt. Sta., 52nd; 93-95.

Naber, E. C., and C. L. Morgan, 1957. Studies on a hatchability depressing factor in cottonseed oil. Poultry Sci. 36:429-431.

Nalbandov, A. V., 1958. Reproductive Physiology. W. H. Freeman and Company, San Francisco.

Nalbandov, A. V., 1964. Reproductive Physiology. 2nd ed. W. H. Freeman and Company, San Francisco.

Nunn, J. R., 1952. The structure of sterculic acid. J. Chem. Soc. 1952:313-318.

Ory, R. L., and A. M. Altschul, 1964. Cyclopropenyl compounds as sulfhydryl reagents. Biochem. Biophys. Res. Comm. 17:12-16.

Raju, P. K., and Raymond Reiser, 1966. Inhibition of fatty acyl desaturase by cyclopropene fatty acids. J. Biol. Chem. 379-384.

Ralph, C. L. and R. M. Fraps, 1960. Induction of ovulation in the hen by injection of progesterone into the brain. Endocrinology 66:269-272.

Rascop, A. M., E. T., Sheehan, and M. G. Vavich, 1966. Histomorphological changes in reproductive organs of rats fed cyclopropenoid fatty acids. Proc. Soc. Exptl. Biol. Med. 122:142-145.

Schneider, D. L., 1962. Some physiological and biochemical effects of Sterculia foetida oil on animal systems. Ph. D. thesis, Univ. of Ariz., Tucson, 70 p. and University Microfilms, Ann Arbor, Mich., 63-3463 CB.

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, 77:403-407.

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

Sheehan, E. T., 1964. Effects of ingestion of Sterculia foetida oil on the sexual development of the female rat. M.S. thesis, Univ. of Ariz., Tucson, 24 p.

Sheehan, E. T., and M. G. Vavich, 1965. Delay of sexual maturity of the female rat by Sterculia foetida oil. J. Nutrition 85:8-12.

Shenstone, F. S. and J. R. Vickery, 1959. Substances in plants of the order Malvales causing pink whites in stored eggs. Poultry Sci. 38:1055-1070.

Sherwood, R. M., 1928. The effect of various rations on the storage quality of eggs. Texas Agr. Expt. Sta. Bull. 376, 12 p.

Sherwood, R. M., 1931. The effect of cottonseed meal and other feeds on the storage quality of eggs. Texas Agr. Expt. Sta. Bull. 429, pp. 5-19.

Simpson, G. G., Anne Roe and Richard C. Lewontin, 1960. Quantitative Zoology. Revised Edition. Harcourt, Brace & World, Inc. New York.