ESTROGENIC-ANDROGENIC EFFECTS OF WHEAT GERM OIL AND OCTACOSANOL

by

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INTRODUCTION AND LITERATURE REVIEW

Wheat germ oil has attracted a great deal of attention for many years. It has been employed for many different purposes and has generally been considered successful when it has been used as a growth or metabolic stimulant. The oil is deep brown in color and is prepared from the wheat germ either by cold-pressing or by solvent extraction. The solvent used now commercially for the extraction process is ethylene dichloride.

Tracy, Hoskisson, and Trimble (1944) discovered that wheat germ oil when incorporated into dairy products would prevent rancidity and thus it has been extensively tested for possible use as an antioxidant.

Another use for which it has been extensively tested is its possible applications as a curative agent for growth deficiency. Blumberg (1935) used rats, both male and female, which had been put on a deficient basal diet. Different supplements were then added to the diet and the growth of these rats was checked. Supplements of known vitamins failed to cure the disease. Wheat germ, and wheat germ oil produced a resumption of growth. The factor suspected was vitamin E although it had not yet been isolated at this time.

Hobson (1935) found that a fat-soluble factor was required for growth of blowfly larvae. Wheat germ oil contained this factor. Some sort of a sterol was suggested as the factor.

Wheat germ oil was again implicated as containing a growth factor when Martin (1937) and Emerson and Evans (1937), using rats placed on a basal diet of casein, yeast, salt mixture and sucrose, stimulated with wheat germ oil a resumption of growth after the rats had reached a plateau. Cotton seed oil was also administered but it did not show the potency that wheat germ oil had.

More recently, Carver and Johnson (1953) found unidentified growth factors for the chick in vegetable oils and fatty acid concentrates. They found that wheat germ oil and oleic acid appear to contain large amounts of this factor as they were superior to the others tested. Apparently alpha-tocopherol is not the factor since no response was observed when it was used by itself.

Wheat germ oil has also been tested in the therapy of muscular dystrophy and related dysfunctions. Evans and Burr (1928) implicated vitamin E in wheat germ oil with experiments on paralytic development in young rats. Pregnant rats placed on a simplified diet deficient in vitamin E gave birth to young which developed paralysis a day or two before weaning. This could be prevented if wheat germ oil was fed to the female during the gestational period. Also mothers on the "simplified diet" shifted on the day of birth to a "natural diet" reared normal young past the weaning age. It thus appeared that this substance is passed in the milk rather than through the placenta.

These results were reproduced by Goettsch and Ritzman (1939) in the therapy of induced muscular dystrophy in young rats. Rats given wheat germ oil, wheat germ oil treated with ferric chloride, or alpha-tocopherol were all protected from muscular dystrophy by these supplements. Since ferric chloride destroys vitamin E the results lend confusion to the picture. The suggestion was made that the alpha-tocopherol may have had impurities that were in themselves able to prevent the muscle disease or that the young rat may be able to utilize possible precursors for vitamin synthesis.

Wheat germ oil has also been employed on humans. Stone (1949) administered the oil over a period of ten years to children who had a variety of neuromuscular disturbances and other disturbances of the nervous system. It was found to be effective in retarding muscular dystrophy in a number of

patients. In anterior polioneuritis, it seemed to hasten the regeneration of atrophied musculature. In cases of mongolism and cretinism, it produced improvement in muscle tone and some increase in mental alertness.

Milhorat, Toscani, and Bartels (1945) reported that when wheat germ was given to patients with dermatomyositis, creatinuria decreased. However when the wheat germ was incubated in vitro with normal human gastric juice, creatinuria was increased. Wheat germ defatted by extraction with ethylene dichloride was entirely without effect on creatinuria.

A wheat germ preparation was found by Coons and Coons (1935) to improve iron and nitrogen retention during human pregnancy. The results were traced to the ash rather than to the organic portion.

Probably the use for which wheat germ oil has been studied the most and with which this study is concerned is its effect on reproduction.

Among the first to recognize that wheat germ oil did indeed contain a factor essential for reproduction were Evans and Bishop (1923). Female rats maintained on a diet of fat, carbohydrate, and protein to which had been added minerals and vitamins A and B, continued normal growth and health but practically all such animals were sterile. They also found that the female usually exhibited normal estrus, ovulation, fertilization and implantation, but invariably resorption of the embryo followed. Certain supplements to the diet were found to cure this sterility disease. Fresh green lettuce leaves, whole wheat, oats and especially wheat embryo showed remarkable potency to cure the sterility. The assumption was then made that the active factor was an undiscovered vitamin. This factor was successfully extracted from foodstuffs and was especially concentrated in a deep brown oil obtained by ether extraction from wheat germ.

Preliminary steps by Evans and Burr (1924) showed this factor was in the unsaponifiable fraction of wheat germ oil representing about 0.3% of the original oil. Evans and Bishop (1924) found the factor to be stable and that the mammal body can store this so-called compound X for some time. When animals were shifted to diets deficient in X, they lost fertility after some time. Tissues of animals which had been fed compound X retained this compound for such tissues were in themselves curative agents for the above named deficiency disease. The term vitamin "E" for this compound X was first proposed by Sure (1924a) and has been universally adopted since.

Mattill, Carman, and Clayton (1924) and Evans and Burr (1925) confirmed that in cases of vitamin E deficiency ovulation occurs but implantation is unsuccessful and that male rats placed on a vitamin E deficient diet retain normal procreative power to about 100 days of age, then there is progressive degeneration of the testes with final destruction of the entire seminiferous epithelium. The organ is reduced to 30-60% of its normal weight and shows a dark, glassy appearance.

Sure published a series of experiments dealing with the reproductive dietary complex. In the third publication (1924b), he showed that this complex existed in ether extracts of yellow corn, wheat embryo and hemp seed. In the fifth (1926a), he showed that ether, acetone and benzene extracts of wheat germ added to the diet resulted not only in the birth of healthy young but also in successful lactation. Cottonseed oil, corn oil, and palm oil also stimulated lactation and possess anti-sterility properties but are not as potent as wheat germ oil.

In the seventh of the series, (1926b), he suggested the possibility that wheat germ oil contains two fat-soluble dietary factors, one thermostable possessing lactation promoting properties. Sure (1927) compared the effect

of vegetable oils on fertility. They were, in the order of decreasing potency, wheat oil, crude corn, cottonseed oil, cocoa butter and finally peanut oil which showed no potency at all.

Overdoses of vitamin E in the wheat germ oil (Bodenheimer and Lasch, 1950) result in no decisive changes in the male. In the female however, overdoses produce sterility which after a few months becomes irreversible. Histologically the changes occurring are disappearance of primordial follicles, the change of greater follicles into pseudocorpora lutea, hypertrophy of interstitial and luteum tissues, and luxuriant growth of thick-walled blood vessels with a narrow lamina in the central part of the ovary. In the final stages, large parts of the germinal epithelium of the ovary disappear.

Wheat germ oil has not only been tested on small laboratory animals but on farm animals as well. Card (1929) added benzene extracted wheat germ oil at the rate of 2% to a mixed grain ration and found that it did not improve either fertility or hatching power of eggs laid by White Leghorn hens.

Semen production in cockerels was checked by Titus and Burrows (1940) when wheat germ oil was added to the ration. The opposite of what was expected resulted. Those cockerels which were fed the wheat germ oil had a pronounced decrease in semen production. Those cockerels which were fed wheat germ oil treated with ferric chloride to destroy the vitamin E, exhibited no decrease in semen production.

Much work and testing has been done on cows in the treatment of sterility and various related diseases. Vogt-Moller and Bay (1931) were probably among the first to try wheat germ oil for the sterility dysfunction. Cows that had been checked by rectal palpation for possible pathological conditions and cows that had failed to conceive were injected intramuscularly with wheat germ oil.

Out of 11 cows treated, only two failed to conceive. Similar results were reported by Tutt (1933) and Bay and Vogt-Moller (1934). Lentz (1938) performed similar experiments but did not report success equal to that of the previous workers. Only 60% of the cases on which he reported conceived after treatment.

The "saturation" method reported by Pacini (1938), intended to supply the animal with the physiological amount of vitamin E to insure breeding success. The animal was fed a single dose of four ounces of cold-pressed wheat germ oil, followed by a tablespoonful every third day for a time equivalent to one estrous cycle, 21 days. Thereafter, the animal was furnished with grain to which four ounces of wheat germ oil has been added to each ton of feed. Successful breeding followed in 85% of the cases. Pacini (1938) also reported that this treatment had been used effectively with bulls, cows, stallions, mares, boars, sows, rams, and ewes. He suggested that wheat germ oil might contain factors separate from vitamin E which are responsible for the curative action: an anti-abortive factor, preventive toxemia of pregnancy factor, and a factor which exerts galactogogue action. He then suggested that less emphasis be placed on vitamin E and that the therapeutic use not be considered synonymous with vitamin therapy.

All experiments in which wheat germ oil was used have not showed success as was evidenced by the work of Card (1929) and Titus and Burrows (1940). Salisbury (1944) also reported failure in experiments in which wheat germ oil was fed to bulls used for artificial insemination. He failed to find an increase in the volume of semen produced, an increase in the sperm concentration in the semen, improved mobility of the sperm, or improvement of the fertility of the bulls to which it was fed. Gwatkin and MacLeod (1938) reported that

wheat germ oil did not prevent or suppress Brucella abortus infection in either cattle or guinea pigs.

Cromer (1938) found that in seven cases of repeated spontaneous abortion in humans, abortion was prevented by administration of large amounts of wheat germ oil before conception occurred and continued throughout pregnancy. He also found that threatened abortion in humans was prevented in the same manner. Watson and Tew (1936) also found that wheat germ oil was of significant value in treatment of habitual abortion in humans.

Levin (1945) offered an explanation for the inability of various workers to repeat certain work and to account for the difference of opinion regarding the value of vitamin E in animal breeding. He speculated that the method of preparing the oil, whether by cold-pressing or by solvent extraction, and the subsequent treatment of the oil may affect its chemical stability. He also suggested the possibility that other nutritional factors beside vitamin E were present in wheat germ oil.

Enlarging upon the idea that some factor other than vitamin E is the reproductive factor, Levin, Silbernagel, and Nichols (1950) found wheat germ oil favorably influences conception, litter size, birth weights and weaning weights of rats when it was incorporated into the diet. Failure of the control group to conceive cannot be due to the lack of vitamin E since a sufficient amount of the tocopherols was incorporated in the diets of both the control and experimental groups.

Using wheat germ oil produced by cold-pressing, Saphir (1936) failed to demonstrate any estrogenic, gonadotropic or luteinizing effects when wheat germ oil was tested on immature, normal, and castrated female rats. Levin and Buras (1950) however, demonstrated androgenic activity of wheat germ oil by

demonstrating increase in the weight of seminal vesicles of castrate rats, increase in combs of full grown capons, and increase in weight of combs on 1-5 day old chicks with its use. A progestational factor was also demonstrated by endometrial proliferation in the rabbit. Activity was absent in rabbits given alpha-tocopherol.

Levin, Burns, and Collins (1951) advanced these experiments by using: (1) immature female rats, 21 days old, (2) adult spayed female rats, and, (3) immature hypophysectomized female rats, 21 days old. Immature and spayed adult rats came into estrus when fed wheat germ oil while the others did not. The hypophysectomized females showed uteri that weighed double the weight of control uteri. The calculated potency of the wheat germ oil was 9 I.U. of estrone per cc. Androgenic effects were tested by the chick comb growth method. Again they reported positive results with the calculated potency 2.5 μg of testosterone propionate per cc. The rat seminal vesicle assay was also used and undiluted wheat germ oil stimulated growth comparable to 5 μg per cc testosterone propionate. Gonadotropic activity was also demonstrated by: (1) the repair of the "deficiency cells" in the ovaries of hypophysectomized rats, (2) the formation of corpora hemorrhagica in the rabbit, (3) the increased weight of ovaries, and, (4) progestational proliferation in the uterus of hypophysectomized rats. The active principle was thought to be a ketonic compound, not vitamin E.

Since wheat germ oil seemed to exhibit some estrogenic activity, Booth, Rickoff, and Kohler (1960) decided to compare various vegetable oils. The list, in the order of decreasing potency, included rice bran oil, coconut oil, soybean oil, olive oil, peanut oil, wheat germ oil, linseed oil, corn oil, cod-liver oil, refined wheat germ oil, safflower oil, cottonseed oil,

and castor oil. When cereal fractions were fed, wheat germ showed the greatest potency, then rice polish, rice bran and finally wheat bran.

Extracts from various other plants have been shown to elicit ovarian or uterine response. Friedman and Friedman (1934) reported an ovarian response when an extract from alfalfa meal was injected into the rabbit, with production of corpora lutea and corpora hemorrhagica in unmated rabbits. Extracts from grasses, hay, and clover (Bartlett, et al. 1948) stimulated uterine growth significantly.

Paula (1943) identified several substances from coffee oil including sitosterol, tocopherol and a variety of other hydrocarbons. The residue, insoluble in hot petroleum ether, had estrogenic action on castrated guinea pigs. It had a melting point of 145-148° and was apparently related to isoprene.

Pacini (1938) and Levin (1945) suggested that possibly factors in wheat germ oil other than vitamin E might be capable of exhibiting certain of the described responses. One of the substances suspected is 1-octacosanol. This compound is a straight chain alcohol, $C_{28}H_{57}OH$, with a molecular weight of 410.75 and a melting point of 83.2-83.4°. Not only is it found in wheat germ oil but various workers have reported it in other substances. Pollard, Chibnall, and Piper (1933) found the principal component of wheat wax to be octacosanol. It has been isolated from carnauba wax in states of 95% purity or better by Koonce and Brown (1944). Candelilla wax, obtained from certain desert plants, also contains octacosanol according to Schuette and Baldinus (1949). Blair, Mitchell, and Silker (1953) and Hirai, Naganawa, and Matsumoto (1956) have shown it to be present in alfalfa wax and alfalfa leaves. It was discovered in wool wax by Murry and Schoenfield (1955) and Horn and

Matic (1957) found it to be the main hydrocarbon component of sugar cane cuticular wax. Ageta (1959) extracted it from the leaves of Ginkgo biloba and Ephedra gerardina.

The purpose of this project[1] was to study the androgenic and estrogenic effects of wheat germ oil and octacosanol[2] on common laboratory animals. The melting point and infrared spectrum for the octacosanol had been determined to verify its purity.

METHODS AND MATERIALS

Androgenic Effects

General Procedure:

Five separate experiments were run to determine the androgenic effects of wheat germ oil and octacosanol using one day old cockerels. Small and large chicks were discarded and the remaining chicks were sorted at random into the separate groups. The limiting weights for discards varied slightly from one group to another. Water and a commerical chick starter were provided continuously to all groups. The groups were rotated in the cages each day to eliminate compounding environmental factors.

The test material was applied topically on the comb or injected intraperitoneally with a 2cc Tuberculin glass syringe at the rate of .05 cc per day. At the end of the test period the chicks were killed by placing them in a gallon jar with a pad of ether-soaked cotton. The combs were removed,

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[1]Kansas State Agriculture Experiment Station Project 5-689: Reproductive Physiology, under the direction of Dr. H.T. Gier and Dr. G.B. Marion.

[2]VioBin Laboratories, Monticello, Ill.

placed in petri dishes, and weighed on a Mettler Gram-atic balance to the nearest tenth of a milligram. Body weights and comb weights were recorded in order, so it was possible to correlate comb weights with the body weight of the chick from which it was removed. The ratio was calculated according to the following formula:

$$\text{Ratio} = \frac{\text{comb wt. (mg.)} \times 100}{\text{body wt. (g.)}}$$

Hy-line cockerels from hatcheries in Topeka and Wichita, Kans. were used throughout the experiments except for a portion of the fifth experiment when Hy-line and White Leghorns from Honegger Hatcheries of Forrest, Ill. were compared.

All chicks were sexed by experts who guaranteed 96% accuracy. They arrived in good condition and losses in experimental chicks were negligible. The feed appeared to be good and the chicks thrived on it.

Procedure for specific experiments:

Experiment No. 1

Six groups of chicks were treated for five days and killed on the sixth.

1. Controls treated with Mazola corn oil, applied topically.
2. Controls treated with Mazola corn oil, injected intraperitoneally.
3. Wheat germ oil topically.
4. Wheat germ oil intraperitoneally.
5. Octacosanol .11% in corn oil applied topically.
6. Octacosanol .11% in corn oil injected intraperitoneally.

Experiment No. 2

Five groups of 35 chicks each were treated for seven days and killed on the eighth. Application was topical in all groups. Wesson oil with 5% of a commercial lecithin as the carrier for the octacosanol was used.

1. Controls treated with Wesson oil with 5% lecithin.
2. Wheat germ oil.
3. .22% octacosanol.
4. .11% octacosanol.
5. .022% octacosanol.

Experiment No. 3

Forty chicks were used in each group for this experiment with one-half from each group being killed on the day after the fourth treatment and the remainder being killed on the day after the seventh treatment. The feed was removed approximately five hours before the chicks were killed to eliminate undigested food in the crop as a factor in body weight. Application was topical in all groups.

1. Controls treated with Wesson oil with 8% lecithin added.
2. Wheat germ oil.
3. .11% octacosanol.
4. .011% octacosanol.

Experiment No. 4

This series was a duplicate of No. 3 except all chicks were injected intraperitoneally instead of having a topical application.

Experiment No. 5

This experiment was designed to compare the effects of wheat germ oil and octacosanol on two different strains of chicks: Hy-lines, from the same source

as those used in the previous experiments, and White Leghorns. Approximately 30 birds from each strain were placed into each of six different groups. Number tags were placed on the wings of all birds. Application was topical in all cases. The wheat germ oil and octacosanol was diluted considerably. The methods of dilution were as follows:

Stock solution of octacosanol - 110 mg. octacosanol dissolved in 20 cc of Wesson oil-lecithin solution (8 parts egg lecithin + 92 parts Wesson oil), then made up to total volume of 100 cc. This makes the octacosanol equivalent to that which is found in wheat germ oil; .11% by weight and is referred to as 1:1 ratio.

0.5 cc of 1:1 + 29.5 cc lecithin-Wesson oil = 1:60

1 cc of 1:60 + 9cc lecithin-Wesson oil = 1:600

2 cc of 1:600 + 18 cc lecithin-Wesson oil = 1:6000

2 cc of 1:6000 + 18 cc lecithin-Wesson oil = 1:60,000

The wheat germ oil was diluted with lecithin-Wesson oil = 1:60.

The group treatments were:

1. Negative control-Wesson oil-lecithin solution
2. Positive control - Testosterone propionate (20 gamma per cc of Wesson oil-lecithin diluent).
3. Wheat germ oil 1:60
4. Octacosanol 1:60
5. Octacosanol 1:6000
6. Octacosanol 1:60,000

Estrogenic Effects

General Procedure:

A. At 18-20 days of age, female mice were weighed individually, placed at random into the various groups and maintained on the experimental rations for ten days. They were then sacrificed by placing them in a jar containing an ether soaked pad of cotton and the body weight was then recorded. The uterus was removed and carefully trimmed of the remaining fat and mesentery, placed in a petri dish to prevent drying, then individually weighed on a Mettler Gram-atic balance to the nearest tenth of a milligram. The ovaries were checked under a stereoscopic microscope for developing follicles.

B. Mice were ovariectomized at 35-40 days. They were allowed nine days for recovery after which they were placed on the experiment and processed as those in A.

The animals were marked with alcoholic picric acid on various places on the body as a numbering system. The beginning weight could then be correlated with the terminal body weight.

Commercial dog kibbles were put through a food grinder to insure a more homogenous mixture with the wheat germ oil. Feeders were two ounce short style glass jars with one inch holes drilled in their plastic covers so the mouse could enter and consume the diet.

Procedure for specific experiments:

Experiment A

1. Control - 5% Wesson oil-lecithin added to the dry meal.
2. 10% wheat germ oil.
3. 5% wheat germ oil.

The greatest weight increase occurred in the group with octacosanol by topical application. The same pattern exists for weight gains as was seen when comparing comb weights: wheat germ oil was more effective when injected, octacosanol was more effective when applied topically.

In experiment 2, a few of the conditions were changed: length of treatment, medium for the suspension of octacosanol and the addition of lecithin to the medium. In addition, different levels of octacosanol were tried. None of the groups showed an increase over the controls (Table II). Weight increase in this group did, however, produce an interesting pattern. The highest level of octacosanol produced the greatest increase and subsequent values decreased as the levels were decreased. A close correlation exists between the increase in weight of those given wheat germ oil and those given .11% octacosanol. Since octacosanol occurs at about the level of .11% in wheat germ oil, it would be assumed that these two groups would produce approximately the same increase and this is exactly what happened.

To determine what effect the length of the test period had, in the next experiment one-half of each group was killed on the fourth day, and the remainder on the seventh day. None of the groups showed a significant increase over the controls (Table III). Test chicks killed on the seventh day showed essentially no gain over controls except a 1.6 gram increase for the wheat germ oil.

Experiment 4 was a duplicate of experiment 3 except application was by injection instead of topical. As was the case in experiment 3, no significant results were produced either on the comb weights or increase in body weight (Table IV). The one possible exception might be .11% octacosanol on the seven day treatment in which a one gram increase was recorded.

Table I: Androgenic Effects: Comparison of Octacosanol and Wheat Germ Oil by Intraperitoneal and Topical Application.

GROUP	Initial Body Weight (g.)			Terminal Body Weight (g.)			Comb Weight (mg.)			Ave. wt. Increase (g.)	Ratio*	t	Level of Significance
	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.				
Control Topically	27.0	29.9	36.0	52.0	56.9	66.0	11.0	14.5	18.5	27.0	26.00	-	-
Control Intraperitoneally	27.0	29.9	36.0	52.0	56.0	64.5	11.2	14.6	18.8	26.1	25.95	-	-
Wheat Germ Oil Topically	28.0	30.4	36.0	50.0	59.2	69.0	10.4	16.6	22.6	28.8	28.08	1.857	.1
Wheat Germ Oil Intraperitoneally	28.0	30.5	36.0	49.5	59.1	67.5	12.9	16.9	20.9	28.6	28.71	2.793	.01
Octacosanol Topically	28.0	30.6	38.0	53.0	62.1	67.5	14.1	18.6	23.5	31.5	30.11	3.736	.001
Octacosanol Intraperitoneally	28.0	31.1	40.0	51.0	60.3	73.0	13.0	17.3	27.2	29.2	28.78	2.339	.02

* Comb weight per 100 grams body weight.

Table II: Androgenic Effects: Comparison of Wheat Germ Oil with Different Levels of Octacosanol by Topical Application.

GROUP	Initial Body Weight (g.)			Terminal Body Weight (g.)			Comb Weight (mg.)			Ave. wt. Increase (g.)	Ratio*	t	Level of Significance
	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.				
Control	28.0	30.0	33.0	55.0	63.2	70.5	12.5	19.2	27.6	33.2	30.39	-	-
Wheat Germ Oil	28.0	29.9	33.0	50.0	65.2	77.0	9.0	18.9	30.1	35.3	28.98	0	0
.22% Octacosanol	28.0	29.9	32.0	61.0	68.4	77.0	12.6	19.7	26.4	38.5	28.80	0	0
0.11% Octacosanol	28.0	29.9	32.0	59.0	65.3	79.0	13.9	19.4	24.8	35.4	29.71	0	0
.022% Octacosanol	28.0	29.7	32.0	58.0	63.0	71.0	11.8	18.9	27.0	33.3	30.00	0	0

Table III: Androgenic Effects: A Comparison of the Length of the Test Period (Topical Application).

GROUP	Beginning Body Weight (g.)			Test Period	Terminal Body Weight (g.)			Comb wt. (mg.)			Av. Wt. Increase (g.)	Ratio	t	Level of Significance
	Min.	Av.	Max.	(days)	Min.	Av.	Max.	Min.	Av.	Max.				
Control	28.0	31.2	34.0	4	33.0	39.5	44.0	7.8	12.1	15.2	8.3	30.63	-	-
				7	43.0	50.9	60.0	11.7	15.8	21.1	18.7	31.04	-	-
Wheat Germ Oil	28.0	30.8	34.0	4	31.5	37.7	41.5	9.1	11.3	13.5	6.9	29.97	0	0
				7	41.0	51.1	59.5	10.0	14.5	19.5	20.3	28.38	0	0
.11% Octacosanol	28.0	31.5	34.0	4	34.0	38.3	42.5	9.0	11.7	16.2	7.2	30.55	0	0
				7	44.0	50.4	57.5	10.4	15.7	22.3	18.9	31.15	.0494	0
.011% Octacosanol	28.0	31.4	34.0	4	33.0	37.5	43.5	8.3	11.1	14.2	6.1	29.60	0	0
				7	44.0	50.2	57.0	9.5	14.5	18.8	18.8	26.88	0	0

Table IV: Androgenic Effects: A Comparison of the Length of the Test Period (Intraperitoneal Application).

GROUP	Beginning Body Weight (g.)			Test Period	Terminal Body Weight (g.)			Comb wt. (mg.)			Av. Wt. Increase (g.)	Ratio	t	Level of Significance
	Min.	Av.	Max.	(days)	Min.	Av.	Max.	Min.	Av.	Max.				
Control	28.0	30.9	33.0	4	36.0	41.7	47.5	9.6	12.0	15.4	10.8	28.77	-	-
				7	46.0	59.3	65.0	10.6	17.7	25.2	28.4	29.88	-	-
Wheat Germ Oil	28.0	31.0	33.0	4	32.0	40.2	47.5	8.4	11.5	15.4	9.2	28.61	0	0
				7	52.0	59.5	67.0	11.9	16.3	22.2	28.5	27.39	0	0
.11% Octacosanol	28.0	30.4	33.0	4	35.0	41.4	46.5	8.8	11.4	14.7	11.0	27.54	0	0
				7	54.0	59.8	66.0	14.4	16.9	21.4	29.4	28.26	0	0
.011% Octacosanol	28.0	30.3	33.0	4	38.0	41.4	45.5	9.3	10.6	12.3	11.1	25.60	0	0
				7	46.0	57.3	63.5	8.8	14.3	18.2	27.0	24.96	0	0

At this point the suggestion was offered that possibly strain differences might exist among these chicks. In an effort to resolve this question, two strains of birds, Hy-lines and White Leghorns, were subjected to the test. Greater dilutions of both wheat germ oil and octacosanol than had been used before were tried. Those birds of the Hy-line strain showed no significant response whatsoever when evaluated (Table V). Two groups produced a value for t which was insignificant for all practical purposes. Even those birds tested with testosterone propionate failed to produce much of a t value.

All groups of the Leghorn breed produced a highly significant value. The remarkable part of this evaluation was that the greatest dilution of octacosanol, 1:60,000, produced the highest value for t.

The results of this experiment seem to confirm, then, this idea of a strain difference in response to these test materials. No ready explanation can be offered for the results of the previous four experiments except possibly that the first group turned out to be Leghorns and thus the positive results for this first experiment. This difference of response between breeds then brings out genetic implications and the metabolic efficiency for on site utilization.

In the experiment utilizing immature female mice to determine estrogenic activity, wheat germ oil in the feed at the 5% level showed no increment (Table VI). However, wheat germ oil at the 10% level produced a significant value for uterine stimulation. Both groups showed a significant value for uterine stimulation. Both groups showed a significant weight gain over that of the controls, and those fed wheat germ oil at the 10% level showed more gain than did those fed wheat germ oil at the 5% level.

Table V: Androgenic Effects: Comparison of the Effects Produced Between White Leghorn and Hy-Line Chicks.

GROUP	Terminal Body Weight (g.)			Comb wt. (mg.)			Ratio	t	Level of Significance
	Min.	Av.	Max.	Min.	Av.	Max.			
Hy-Lines, Negative Control	44.0	52.0	63.0	10.7	16.9	27.4	32.35	-	-
Leghorns, Negative Control	46.0	55.3	62.5	10.9	14.7	20.2	26.57	-	-
Hy-Lines, Testosterone Propionate	44.0	51.0	57.0	12.2	17.4	28.8	33.96	.9096	.4
Leghorns, Testosterone Propionate	42.0	53.5	62.0	10.8	17.4	26.8	32.38	4.469	.001
Hy-Lines, Wheat Germ Oil 1:60	48.0	53.5	60.0	12.0	16.7	22.0	30.85	0	0
Leghorns, Wheat Germ Oil 1:60	49.0	57.5	67.0	13.1	18.4	29.3	31.92	3.849	.001
Hy-Lines, Octacosanol 1:60	44.5	55.0	64.0	12.6	18.3	28.0	33.08	.4078	.7
Leghorns, Octacosanol 1:60	44.0	54.5	68.5	10.6	18.0	26.1	32.89	5.496	.001
Hy-Lines, Octacosanol 1:6000	46.5	55.6	64.5	14.2	18.0	24.0	32.34	0	0
Leghorns, Octacosanol 1:6000	47.5	59.1	68.5	11.0	19.3	28.1	32.59	4.426	.001
Hy-Lines, Octacosanol 1:60,000	47.0	57.2	63.5	10.8	17.2	28.2	29.80	0	0
Leghorns, Octacosanol 1:60,000	44.0	56.7	81.0	14.2	19.1	28.2	33.67	5.680	.001

Table VI: Estrogenic Effects on Immature Female Mice.

GROUP	Beginning Body Weight (g.)			Terminal Body Weight (g.)			Uterine Weight (mg.)			Av. Wt. Increase	Ratio	t	Level of Significance
	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.				
Control	6.00	8.44	11.50	9.50	13.42	18.00	6.10	13.17	24.90	4.98	92.48	-	-
Wheat Germ Oil 10%	6.00	8.88	12.00	12.00	14.63	19.00	4.60	17.36	40.50	5.75	116.85	2.069	.05
Wheat Germ Oil 5%	6.00	8.23	10.50	10.00	13.60	18.50	3.10	11.38	23.30	5.37	82.81	0	0

Table VII: Estrogenic Effects on Ovariectomized Mature Female Mice.

GROUP	Beginning Body Weight (g.)			Terminal Body Weight (g.)			Uterine Weight (mg.)			Av. Wt. Increase	Ratio	t	Level of Significance
	Min.	Av.	Max.	Min.	Av.	Max.	Min.	Av.	Max.				
Control	21.00	22.40	26.50	21.00	23.60	30.00	4.50	8.34	12.80	1.2	35.20	-	-
Wheat Germ Oil 10%	21.00	22.85	25.00	21.50	24.25	27.50	4.40	7.57	10.50	1.4	31.35	0	0
Wheat Germ Oil 5%	21.50	23.33	25.50	22.00	24.44	27.00	4.50	8.50	12.40	1.11	34.84	0	0

Ovariectomized mature females gave no indication of estrogenic stimulation from wheat germ oil at either the 10% level or 5% level.

The response to octacosanol and wheat germ oil by a weight increase was found to be true in several experiments. However, in some cases the gain was negligible. Similar results are reported in the literature. Blumberg (1935) and Emerson and Evans, (1937) produced added gains to those animals fed "vitamin deficient" diets. Carver and Johnson (1953) and Martin (1937) however, showed that animals gained on wheat germ oil after reaching a plateau on a basal diet.

Androgenic and estrogenic effects were obvious in some experiments while in others no effects were produced. However, the results seem to indicate that both wheat germ oil and octacosanol possess activity in some cases. While Saphir (1936) failed to demonstrate estrogenic activity of wheat germ oil, others (Booth, et al. 1960 and Levin, et al. 1951) were apparently successful. Androgenic activity was reported also by Levin, et al. (1951) and by Levin and Burns (1950).

On the basis of past work as reported in the literature and this report, it seems likely that wheat germ oil contains a substance with mysterious capabilities and that this substance is probably octacosanol. The idea that compounds other than hormones may have estrogenic or androgenic activity is not new. Friedman and Friedman (1934) produced ovarian response by an extract from alfalfa meal. Extracts from herbage taken from pastures were shown to possess estrogenic activity by Bartlett, et al. (1948). Paula (1943) reported estrogenic activity of an extract from coffee oil with the compound apparently being related to isoprene.

Why wheat germ oil at the 10% level on the immature mice should produce some uterine growth and none on the ovariectomized mice is not known. It could perhaps be explained on the basis that wheat germ oil exerts an indirect effect upon the uterus, possibly by way of the pituitary and ovary.

SUMMARY

This study deals with the androgenic and estrogenic effects of wheat germ oil and octacosanol. Immature day old chicks were used for the androgenic study. The chicks were separated into groups and maintained under constant controlled conditions. Various levels of wheat germ oil and octacosanol was applied either topically to the comb or by intraperitoneal injection.

The first experiment showed both wheat germ oil and octacosanol producing significant results. Octacosanol was more effective when applied topically than when injected intraperitoneally. Wheat germ oil was more effective when injected intraperitoneally. The average increase in weight followed the pattern of comb weights, the greatest occurring when the octacosanol was applied topically.

The next three experiments, with a slight variation in methods, produced no significant results for an increase in comb weight. Experiment 2, however, again produced an increase in body weight with the highest level of octacosanol, .22%, producing the greatest increase. The weight increase decreased with subsequent decreases in levels of octacosanol, with .11% octacosanol producing approximately the same increase as wheat germ oil.

In comparing two strains of birds, Hy-lines and White Leghorns, a strain specificity was observed. While the White Leghorns showed highly

significant results with all levels of wheat germ oil and octacosanol, the Hy-line strain showed no response and only a slight response to testosterone propionate.

Immature and ovariectomized female mice were used in testing for the estrogenic activity of wheat germ oil. The oil was incorporated into the meal at the 5% and 10% levels. Wheat germ oil at the 5% level produced no stimulation but produced stimulation at the 10% level resulting in increased uterine growth significantly greater than the controls.

Neither wheat germ oil at the 5% or 10% level produced any uterine growth on the ovariectomized females.

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ESTROGENIC-ANDROGENIC EFFECTS OF WHEAT GERM OIL AND OCTACOSANOL

by

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Using immature cockerel chicks, immature female mice and mature ovariectomized mice, tests were made to determine the androgenic and estrogenic effects of wheat germ oil and octacosanol.

The comb growth method with immature cockerels was employed exclusively in testing for androgenic effects. Weights of chicks were recorded to determine if any increase in weight resulted from the application of these test materials. The results varied with some groups responding to the treatment and other groups failing to respond.

In three test runs, neither wheat germ oil nor octacosanol effected any response. In one test run, they did effect a response with octacosanol showing more potency when applied topically and wheat germ oil when injected intraperitoneally. However, topical application of octacosanol provided a higher value than intraperitoneal injection of wheat germ oil.

To account for the variation of results among groups, strain differences in response were tested. White Leghorn cockerels responded to all levels of octacosanol and wheat germ oil and the greatest response occurred with the greatest dilution of octacosanol. In contrast, the Hy-Line strain failed to respond to the same levels and showed only a slight response to testosterone propionate.

Response by an increase in body weight was found to vary also with some groups responding and others not.

The uterine response method for determining estrogenic effects was employed using immature female mice and mature ovariectomized female mice.

Only wheat germ oil when incorporated into the food at the 10% level was found to elicit a response in immature mice. The level of significance here was not high. No response was observed in the ovariectomized female mice.

A slight response by an increase in body weight in some immature mice was of doubtful significance.