# GROWTH, REPRODUCTION AND LACTATION STUDIES WITH RATS FED CORN-SOYBEAN OIL MEAL RATIONS.

# GROWTH, REPRODUCTION, AND LACTATION STUDIES WITH RATS FED CORN-SOYBEAN OIL MEAL RATIONS

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INTRODUCTION

It has long been known that the growth rate of the pig is directly dependent upon the nutrients fed. More recently it has been demonstrated that the poor reproduction and lactation performance of the sow can be corrected by feeding certain feeds. Reproduction and lactation failures continue to be a major problem in the swine industry. This problem has been studied extensively but the length of the reproduction cycle of the sow has been responsible for the slow progress made in determining the specific nutrient(s) contained in some feeds which will correct the poor reproduction and lactation performance. Fortunately, the performance of the rat has been found to closely parallel that of swine, which has provided a laboratory animal which is useful in studying this problem.

Due to the difficulties encountered in the assay procedures for this factor(s) it has been difficult to prove that the unidentified factor(s) required for optimum growth is identical to the factor(s) required for normal reproduction and lactation. Properties of the unidentified factor(s) required by the rat suggest that it may be the same as the factor(s) required for growth of chicks and hatchability of eggs. However, some feeds seem to have a greater relative potency for normal growth of chicks and hatchability of eggs than for normal growth, reproduction and lactation of swine.and the rat. This has suggested there may be a combination of related factors rather than a single factor.

Much of the early work which has been reported on this subject has shown that various vitamins of the B-complex are necessary for normal growth and optimum reproduction and lactation in both swine and rats. However, many rations which supply all of the known requirements of both swine and rats have been found inadequate for growth and reproduction and lactation. This has suggested that all of the nutrient requirements are not known and have not been isolated in a purified state. The unidentified factor(s) required has been associated with animal protein sources although recent work has indicated that it may occur in limited amounts in plant products. The purpose of this work was to determine the specific nutrient or nutrients required by the rat for normal growth, reproduction and lactation when fed common swine rations.

## REVIEW OF LITERATURE

For many years, a ration of corn supplemented with animal by-products, principally tankage, was considered a standard swine ration in most sections of the United States. The shortage of these animal by-products and the increased acreage of soybeans grown primarily for their oil content has made available a large quantity of soybean oil meal which has been used extensively in swine rations. A corn-soybean oil meal ration has been found inadequate for optimum reproduction and lactation of the sow when confined to dry lot by many workers and attempts have been made to isolate the factor(s) which would improve such a ration. Ross (1942a) demonstrated that the reproduction and lactation performance of rats fed a corn-soybean oil meal ration closely parallels that of swine and that the rat could be used in pilot experiments to study this problem of swine nutrition. The early work on this subject has been summarized by Ross (1942a) and Cunha (1944a).

During the past several years considerable data have been published suggesting that an unidentified factor(s) is required for optimum growth, reproduction and lactation of the rat. It has been found in some natural feeds and several by-products.

Liver and liver extracts have been consistently shown by many workers to contain an unidentified factor necessary for optimum growth, reproduction and lactation in the rat. In many cases an attempt has been made to isolate the factor(s) in liver and to determine its properties. Ross (1942a) showed that supplementing a basal ration composed of corn 76.35%, soybean oil meal 17.50%, alfalfa meal 5.00%, calcium carbonate 0.65%, and salt 0.50% with 2.00% of Wilson's 1:20 liver concentrate powder would give optimum growth and normal reproduction and lactation. Cunha (1944a) obtained similar results with the liver powder.

McIntire, et al (1943) found that superior growth was obtained when synthetic diets containing various levels of thiamine were supplemented with sulphited liver extract. They suggested that the increased growth from liver may have been due to a growth stimulating substance or that it provided a favorable condition for intestinal flora which synthesized growth-stimulating factors such as those produced when the rat is receiving a normal diet. Their work also suggested that p-aminobenzoic acid and inositol probably produced the same effect as the liver extract.

Jaffe' (1946) found that an alcoholic extract of fresh liver produced a growth response in rats fed a natural diet. Sporn, et al (1947) using a synthetic ration composed of sucrose 73.00%, casein 18.00%, corn oil 5.00%, salts IV 4.00% supplemented with thiamine, niacin, pyridoxine, riboflavin, pantothenic acid, folic acid, biotin, inositol, choline, p-aminobenzoic acid and vitamins A and D, obtained a definite increase in growth with the addition of 1.00% of 1:20 liver powder. Using this same basal ration, they obtained a slightly greater growth response with 3.00% lyophilized liver (Squibb). A similar increase in growth was also obtained with the addition of 0.50 grams of fresh liver daily to such a diet. These workers, using a natural diet with the same added vitamins, obtained a greater growth response with the 1.00% of 1:20 liver powder than with the 3.00% lyophilized liver. Also the growth rates which they obtained on the natural diets were much higher than those on the synthetic diets. Continuing these experiments through the reproduction and lactation period, they obtained a significant increase in the percentage of young weaned by the addition of the 0.50 grams of fresh liver per day to the synthetic ration. They suggested that the growth factor was produced within the animal body but not in sufficient amounts during the first few weeks on the ration. They also suggested that natural rations should be used in order to devise more suitable assay procedures.

Bosshardt, et al (1946) found that Wilson's 1:20 liver powder and a butyl extract of this same liver powder contained a factor or factors that increased the growth rate and also the growth utilization of dietary protein in rats.

Spitzer (1947) found that supplementing a basal ration composed of corn 76.35%, soybean oil meal 17.50%, alfalfa meal 5.00%, minerals 1.15% and the known B-complex vitamins with additional alfalfa meal, certain liver preparations, a combination of casein and choline or fish meal improved reproduction and lactation. Using only the basal ration, more than 35.00% of the females were sterile. Many of the young which were alive at birth apparently died of starvation. This worker also suggested that under certain dietary regimes there appeared to be a storage of dietary essentials needed for reproduction and lactation in the rat. His work also suggested that the factors necessary to adequately supplement the basal ration may be easily destroyed during the processing and storage of the materials. The active factor was contained in fresh beef liver, lyophilized pork liver and the alcohol insoluble fraction of lyophilized pork liver. Supplementation with additional soybean oil meal was without effect. A lactogenic hormone was variable as a supplemental source of the factor or factors.

Van Landingham and Lyon (1947) found that a natural ration containing 20.00% soybean oil meal and 2.00% alfalfa meal was inadequate for good lactation in the female and growth of her young. This diet was adequate if supplemented with 2.00% dried pig's liver but was not adequate if supplemented with an additional 5.00% alfalfa meal or 5.00% dried brewer's yeast. This would confirm the results obtained by other workers.

Many of the experiments which have been conducted in the laboratory with rats have been closely paralleled with swine. Ross (1942a) has summarized the early work which has been done on this subject. Miller, Keith, Thorp and McCarty (1943) observed growth abnormalities of weanling pigs on a yellow corn, low-protein tankage, alfalfa and salt ration. These pigs grew well but there were unexplained death losses, lameness and dermatitis. These symptoms were largely overcome by the addition of vacuum-dried liver to the ration. Satisfactory results were also obtained when the basal ration was supplemented with soybean oil meal, additional alfalfa meal or carotene and yeast. However, a combination of purified vitamin B factors was virtually ineffective.

Krider, Fairbanks and Carroll (1944b) feeding a corn-soybean meal-wheat flour middlings ration under drylot conditions to swine, obtained increased growth with the addition of six synthetic vitamins and 1.50% liver extract. Their results indicated that the all plant ration was deficient in riboflavin. Fairbanks, Krider and Carroll (1944a) feeding a ration of yellow corn, wheat flour middlings, soybean meal, tankage, fish meal, minerals and fortified cod-liver oil found that supplementing the basal ration with dried corn distillers solubles increased the rate of gain, reduced the death loss and improved the appearance of the pigs. Dried corn distillers solubles was superior to corn distillers dried grains with solubles, but both of these feeds improved the performance of the basal ration. It might be noted that both of these feeds are sources of the water-soluble vitamins.

Cunha, Ross, Phillips and Bohstedt (1943a) found that sows fed a corn-soybean meal ration supplemented with irradiated yeast and shark liver oil failed to reproduce normally. Three of the four sows were sterile and the fourth gave birth to a very poor litter. Supplementing the basal ration with crystalline riboflavin and choline gave no improvement but the addition of 15% alfalfa meal resulted in normal reproduction. Ross, et al (1944), working with a similar basal ration, found that there was defective reproduction as shown by embryological abnormalities and that lactation was extremely poor. Supplementing the milk diet of these pigs with various supplements, including liver extract and 1:20 liver powder, failed to improve the appearance of the pigs or improve the rate of growth. The addition of 10% alfalfa meal to the basal ration which contained 5% alfalfa meal, improved the reproduction and lactation performance of the sows but the ration still did not give optimum results. It was concluded that alfalfa meals varied greatly in their potency of the unknown factor. Somewhat similar results were observed by Cunha, Ross, Phillips and Bohstedt (1944b). They also presented evidence to show that the ration of the sow during the growing period has a direct influence upon her reproduction and lactation performance. Those sows given 15.00% alfalfa meal during the growing period gave birth to more normal litters and resulted in more pigs weaned and in heavier weaning weights than similar sows not fed 15.00% alfalfa meal during the growing period. Krider, Fairbanks and Carroll (1944a) also observed this same residual effect of the ration of the sow prior to weaning and the performance of the pigs after weaning.

One of the most extensive fractionations of the growth-stimulating factor in liver was done by Jaffe' and Elvehjem (1947). Their assay ration was made up of grains and other crude products and was used because growth was faster on such rations than on purified rations. They found that the growth factor was present in an extract of fresh liver. They also found that the factor in an alcoholic extract of liver could be adsorbed on and eluted from norit. It was soluble in ethanol but not in butanol and was stable to heat in a boiling water bath for ten minutes. They compared two anti-pernicious anemia liver extracts and found that they contained a growth promoting substance. Fish press water also stimulated growth in rats in a manner similar to that observed in the rats receiving liver.

Krider and co-workers (1946b) using a corn-soybean meal ration supplemented with all the known required B-vitamins obtained significantly improved gestation and lactation results with swine with the addition of alfalfa meal or liver extract to the ration. The addition of folic acid concentrate did not give a significant increase in the weaning weights, but the pigs from the sows receiving the folic acid appeared more thrifty and vigorous than those pigs from the sows on the basal ration. Cunha, et al (1946) using a purified ration obtained no significant increase in growth of pigs with the addition of folic acid. He reported, however, that the folic acid may have helped slightly with hemoglobin formation. These same workers reported (1947) that the addition of folic acid to a natural ration containing tankage "caused some stimulation of growth during the first four weeks and caused the pigs to be cleaner in appearance and have bigger appetites at the end of the experimental period". They further suggested that this may "mean that even though folic acid does not help growth with a purified ration, it may under certain conditions promote growth with natural grain rations."

Zucker and Zucker (1948) have tentatively given the name "zoopherin" to the unknown factor(s) necessary for reproduction and lactation. The deficiency which they have reported differs somewhat from that observed by other investigators in that the highest mortality of young occurred after the 28th day. The young, which they weaned on the 28th day, were about 15% below normal in weight. At about 30-31 days of age, very high mortality was observed. The weight of rats which survived on the deficient diets were 40% below normal at 20 weeks of age. There was a tendency for the litters to either all survive or all die indicating differences in storage, requirement, or possibly synthesis of the missing factor. They concluded that the probable cause of death was a hemorrhage in the fundic portion of the stomach. The walls of the small intestine were very thin and the blood showed high urea values. The kidneys were enlarged but the water content found in the kidneys was normal. The livers were also enlarged but no fatty livers were observed. In their preventative studies, these same workers found that the factor was present in crude casein, Wilson's 1:20 liver powder and fish solubles. It was not present in purified casein or in dried brewers yeast. Since the brewers yeast did not contain the factor, they concluded that it could not have been a deficiency of folic acid. They also found that it was very soluble in water, dilute acid and alkali, and dilute alcohol, moderately soluble in 95% alcohol and insoluble in petroleum ether and ethyl ether. It was relatively quite stable to heat, light, air, dilute acid and dilute alkali and the activity was not lost during the concentration procedures. The factor could be salted out with ammonium sulfate and was precipitated by basic lead acetate but not by trichloro acetic acid or picric acid.

Other workers have found that a similar unidentified nutrient is present in a wide variety of feeds. Hartman and co-workers (1946) have shown that it was present in milk, skimmilk powders, cheese, beef and pork muscle, egg yolk, liver extracts and leafy foods and feeds such as lettuce, timothy, alfalfa and blue grass. It was not present in white, enriched or whole wheat flour or in wheat bran, corn meal, soybean or linseed meals or in yeasts or egg whites. They have designated this unidentified nutrient as factor X. They observed that in those rations deficient in factor X, the growth rates were considerably lowered, feed intakes were reduced, kidneys were hypertrophied, gestation failures were frequent and the number of young and percentage weaned greatly reduced. Also, those animals on a deficient ration frequently stopped growing at markedly subnormal weights.

Bowland, Ensminger and Cunha (1948) conducted work to study the supplemental effect of biotin, p-aminobenzoic acid, inositol, folic acid, a 95% alcohol soluble liver fraction, and 15% dehydrated alfalfa meal to a purified basal ration. Thiamine, riboflavin, choline, pyridoxine, pantothenic acid, niacin and vitamins K, C, A, D and alpha tocopherol were added to the basal ration. When the alfalfa meal was added to the ration, the protein content was kept constant by deleting part of the casein and substituting the remainder of the alfalfa meal at the expense of the sucrose in the basal ration. They found that the addition of the liver fraction at the 1.00% level or the 4 B-complex vitamins had no supplemental effect on growth and that the addition of folic acid alone depressed the growth of weanling rats. These workers suggested that this may have been due to a vitamin imbalance. The addition of the 15.00% alfalfa meal more than doubled the rate of growth. They also found that the addition of the 4 B-complex vitamins or alfalfa meal increased the rate of conception but that the liver paste or folic acid had no effect. All the supplements were significantly helpful in increasing gains during gestation but alfalfa meal gave the greatest response. These supplements also increased the liveability of the young and were helpful in improving the ability of the females to lactate. The addition of alfalfa meal to the basal ration was found to give best results. Reproduction was normal and an improvement in lactation was observed when alfalfa meal was added as a supplement. However, the lactation results obtained with the alfalfa meal were still not optimal. They suggested that the alfalfa meal supplied an unknown factor but not in large enough quantities for optimum lactation in the rat.

Novak and Hauge (1948a) found that a purified ration containing extracted casein and all the known vitamins was not satisfactory for rat growth. However, they were able to obtain a significant increase in growth with distillers' dried solubles. They found that the factor was stable to heat, acid and alkali. It was soluble in ether, ethanol and water at a pH range of 3.0-8.5. It was also precipitated by phosphotungstic acid and lead acetate. It was not adsorbed on fullers' earth or Darco but was adsorbed from an acid solution of Florisil, Lloyds' reagent, Norit and Decalso. More recently these same workers (1948b) have isolated this factor in a non-crystalline but highly purified state. They have also found that it was present in a rice polishing concentrate and liver extract. They found that 2 micrograms of this substance daily would give a definite growth stimulation while 10 micrograms per day would give the maximum effect. In addition, it was found to be soluble in acetone, chloroform and benzene. They have tentatively called it vitamin $B_{13}$ because it is distinct from all the known vitamins.

Distillers' by-products have been shown to possess a growth-stimulating factor when fed to swine. Fairbanks, Krider and Carroll (1945a) continuing growth and fattening work with distillers' by-products and using a basal ration of ground yellow corn, wheat flour middlings, soybean meal, fish meal, tankage, minerals and fortified cod-liver oil found that a supplement of 6.00% dried corn distillers' solubles increased the gains 16% and decreased the death losses when compared to pigs not receiving the solubles. When the basal ration was supplemented with thiamine hydrochloride, riboflavin, nicotinic acid, calcium pantothenate, pyridoxine and choline chloride, they were able to increase the gains and decrease the death loss from 31 to 9 per cent. By supplementing the basal ration with 10.00% alfalfa meal, there was an increase in gain and the feet, legs, gait, hair coats and skins were more nearly normal than in any of the other lots. From a practical standpoint, the alfalfa meal supplement was the most effective, most economical and most complete nutritionally of any of the supplements used in this test. Mention was also made of the fact that those pigs weaned from sows given the dried corn distillers' solubles during the gestation period had a much lower death rate during the fattening period than those pigs whose dams had been on the basal ration alone. This again suggests a residual effect of the ration of the dam upon the later performance of the weanling pigs.

Fairbanks and co-workers (1945b) feeding sows the same basal ration as they fed in their growth experiments found that the addition of dried corn distillers' solubles and alfalfa meal, either alone or in combination or crystalline B-complex vitamins improved the breeding efficiency, fertility and the strength of the pigs farrowed. They attributed the value of the dried solubles and the alfalfa meal as supplements to the basal ration to their content of water-soluble vitamins. The beneficial effects which they obtained from the crystalline B vitamins were in disagreement with the findings of Cunha, et al (1944b) who found that feeding certain crystalline B-vitamins to sows was detrimental. Cunha suggested that this may have been due to a vitamin imbalance, however. In their second experiment, Fairbanks and co-workers (1945b) again demonstrated the residual effect of the ration of the sow during the gestation period upon the performance of the pigs. Those sows fed additional alfalfa meal during the gestation period and the basal ration during the lactation period weaned an average of 82.5% of their pigs whereas those sows on the basal ration during the entire period weaned only 10% of their pigs. They also found that there was not as great a residual effect when the crystalline vitamins were fed as when the natural vitamin carriers were fed. They suggested that this indicated either a greater storage of the known factors or a higher concentration of the unknown factors in the natural vitamin carriers.

Krider, et al (1946a) found that supplementing a corn-soybean oil meal ration with either three or six per cent of sardine condensed fish solubles (fresh basis) gave normal reproduction and lactation performance for swine in drylot. Those sows getting the three per cent fish solubles weaned 92% of their pigs with an average weaning weight of 31.1 pounds while those on six percent fish solubles weaned 71% with a weaning weight of 33.7 pounds. The pigs in both lots appeared normal and healthy. Only 26% of the pigs farrowed on the basal ration survived even though they all appeared normal and healthy at birth. Between 2 and 5 days of age, these pigs became listless, wobbly and weak and 30% were dead at the end of three days. At weaning time, those remaining averaged only 17 pounds per head and were emaciated, had rough haircoats, were listless and had wobbly, unsteady gaits. Autopsy of the pigs revealed that their stomachs were full of milk, their livers were yellowish, enlarged and "fatty-like" and the kidneys were hypertrophied. Supplementing the basal ration with rye pasture proved as effective as the fish solubles. This experiment again demonstrated the residual effect of the ration of the sow during the gestation period on their subsequent performance during lactation. In this same connection, Ferrin (1946) found that fish meal was a more efficient supplement to corn than was tankage or soybean oil meal for growing-fattening pigs.

Krider, Becker and Carroll (1946c), continuing their work with a corn-soybean oil meal basal ration, found that the deficiencies of the basal ration were corrected by supplementing it with rye pasture, 10% alfalfa meal and either 2 or 4% condensed fish solubles (fresh basis). They also found in this experiment that the menhaden solubles were almost as effective as the sardine solubles in supplementing the basal ration. These results were essentially in confirmation of those reported previously.

Krider, et al (1947) using a corn-soybean meal-meat scrap basal ration found that a supplement of 4% alfalfa meal increased the rate of gain and decreased the feed required per 100 pounds of gain. The addition of either two or four per cent of fish solubles to the ration produced equally as good results as did 10% alfalfa meal. A further experiment was conducted using a corn-soybean oil meal ration to compare semi-solid fish and fish solubles. They stated that, "the addition of 3% or 5% of semi-solid fish from Cod and Haddock, 3% of semi-solid Red Fish, or 2% sardine fish solubles to the basal ration increased gains slightly. The results with various fish by-products were similar."

Cunha, Warwick, Ensminger and Hart (1948) working with swine compared cull peas, meat meal and soybean oil meal as a protein supplement for growing-fattening pigs fed in dry lot. The alfalfa content of the rations were varied between 5 and 15%. They reported that the cull peas compared very favorably with the meat meal and soybean oil meal as a protein supplement for growth but that the gains made by pigs in all lots receiving 15% alfalfa instead of 5% alfalfa were lower. At the end of the growth experiment, representative gilts were taken from the several lots and carried through the reproduction and lactation experiment. When only 5% alfalfa was included in the ration, the gilts took much longer to conceive, some of the gilts were sterile and a very small percentage of the pigs were weaned. When 15% alfalfa was included in the ration, there was no sterility, the majority of the gilts conceived at the first heat period and a high percentage of the pigs were weaned. These results indicated, "That the ration fed gilts during growth has a very important bearing on results obtained later during conception, reproduction and lactation. This means that prospective herd gilts need to be fed much better rations, especially higher in alfalfa during growth than animals being fattened out for market."

A somewhat similar factor is involved in growth of chicks and hatchability of eggs from hens fed corn-soybean meal rations. Various workers have reported the occurrence of an unidentified chick growth factor(s) in crude casein, liver meal, dried skimmilk, whey solubles, distillers' dried solubles, meat scraps, fish by-products, cow manure and hen feces. In attempting to identify the nature of this factor, Combs, Heuser and Norris (1948) fed chicks a basal ration containing soybean oil meal as the chief source of protein and one in which 2% white fish meal was added. They were able to get a marked increase in growth and efficiency of gain up to eight weeks of age when the basal ration was supplemented with fish meal. Both of these rations were then completely analyzed for all the known B-complex vitamins and the amino acids. They found that the basal ration was complete in all the vitamins with the exception of choline but that the increased gain and feed utilization obtained with the fish meal ration could not have been due to the increase in the choline content because the addition of fish meal decreased the choline content of the ration slightly. Adequate amounts of the essential amino acids were found in the basal ration with the exception of cystine and methionine and perhaps arginine. The addition of the fish meal to the basal ration, increased the arginine and methionine content of the ration slightly but decreased the level of cystine. The fish meal ration supplied slightly less than the estimated requirements of both cystine and methionine. They further supplemented the basal ration with an additional 10% soybean oil meal and this increased the content of methionine and arginine to the estimated requirement but the ration was still somewhat low in cystine. The growth of chicks receiving this ration was lower than those observed on the basal ration. From this work they concluded that fish meal contains a growth factor(s) which is not identical with any of the known B-complex vitamins or amino acids required by the chick.

Roblee, et al (1948) working with condensed fish solubles have attempted to determine some of the properties of the unidentified chick growth factor. They found the factor to be soluble in water, 70% methyl alcohol, 70% ethyl alcohol, somewhat soluble in absolute methyl alcohol and very slightly soluble in 95% ethyl alcohol. It was insoluble in ether and acetone. It was dialyzable through a cellophane membrane and no loss in activity was produced by enzymatic digestion. It was also heat-stable (100° for 2 hours) over a range of pH 3 to 9 but was destroyed by autoclaving with one tenth normal hydrochloric acid or one tenth normal sodium hydroxide. These properties differ somewhat from the properties of the growth factor in cow manure as reported by Rubin and Bird (1946a). They found that the factor is stable to heat in the dry state at 100° for 1 hour and to autoclaving in solution for fifteen minutes. They also found that it would not dialyze through a cellophane membrane. Their factor was only moderately soluble in water, 50% ethyl alcohol and 95% ethyl alcohol and was insoluble in chloroform and ether. They also found that the factor could be transmitted from the hen through the egg to the chick.

Rubin and Bird (1946b) have summarized the characteristics of their chick factor from cow manure. They demonstrated that it was not identical with the L. casei factors from liver, yeast or fermentation residues, Factors U, R or S, vitamins $B_{10}$ or $B_{11}$, synthetic folic acid or pyracin lactone.

Heuser, Norris, Lucas and Combs (1946) in comparing soybean meals found that in general the expeller meals were superior to the solvent meals. The hatchability was poorest on the basal soybean meal rations during the winter and early spring but improved markedly during the summer. They are of the opinion that there is a possible synthesis of the unknown factor by the hen or by microorganisms. They also obtained a growth response from 2-3% fish meal, condensed fish solubles, liver paste, liver meal, choline and folic acid.

Kennard and Chamberlin (1948) using a corn-soybean oil meal ration found that built-up floor litter served as a potent source of the nutritional factors, including the unidentified animal protein or vitamin factor(s) which was necessary to supplement the incomplete ration to secure optimum growth. This would be in confirmation of the earlier work done by Rubin, Bird and Rothchild (1946a) when they observed a growth promoting factor in hen feces.

Groschke, Rubin and Bird (1948) studying the effect of this unidentified dietary factor upon the seasonal variation in hatchability found that the addition of dried cow manure to a corn-soybean oil meal ration improved hatchability and eliminated the seasonal variation. They also observed that there was an increase in hatchability of eggs from hens fed the basal ration during the summer months. They are of the opinion that this was due to coprophagy. McGinnis, et al (1947) had previously shown that the synthesis of the growth factor occurred after voiding of the feces and not to any extent in the digestive tract. They found that frozen feces contained little or none of the factor. Therefore, the decreased hatchability during the winter months may have been due to the less favorable environmental conditions for bacterial synthesis during this period.

Bird, Rubin, Groschke (1942) have further identified some of the properties of the growth factor in cow manure. They found that it was soluble in water at a pH 3.0 if the protein had previously been removed. It was soluble in 80% acetone and could be extracted to a slight extent by neutral ethanol and completely by ammoniacal ethanol. It was stable when autoclaved for 2 hours in a neutral solution but easily destroyed when autoclaved for 1 hour with 2N hydrochloric acid. It appeared that the factor was destroyed when allowed to stand in a slightly alkaline solution or when the solution was dried.

There is some disagreement in the literature as to whether a specific factor is required for lactation but not required for growth or reproduction. Nelson and Evans (1947b) have stated that additional dietary substances are required for lactation over and above the requirement for growth or reproduction. However, the observations made by Sica and Cerecedo (1948) have lead them "to believe that reproduction has dietary requirements as great qualitatively as lactation and that there are no specific dietary factors required for lactation only." They also found that "regardless of the diet, the survival of newborn rats is dependent largely upon their weight at birth." They fed a purified ration supplemented with thiamine, riboflavin, pyridoxine, calcium pantothenate, alpha tocopherol, choline and vitamins A and D. Other experimental diets fed contained such supplements as folic acid, biotin, xanthopterin and milk. Their observations were based on 117 litters divided into two groups. Group I was composed of the young in the surviving litters and Group II of the young that failed to survive. They found that the average birth weight of the surviving group was 16.30% higher than that of the non-surviving group. They also noted that the critical birth weight seemed to be between 5.0 and 5.4 grams. Seventy-four per cent of the surviving young weighed over 5.4 gms. while only 4.1% weighed under 5 grams. Of the young in the litters that failed to survive, only 9.5% weighed over 5.4 grams. They proposed that in studies with rats, the weights of the young at birth be used as the first indication of the adequacy of a diet, and further, that lactation success be calculated on the basis of those young in litters surviving on the third day of lactation. Their basis of this theory was that of 46 litters which failed to survive, 29 of them failed to survive the third day of lactation.

Many workers, using synthetic rations, have been able to show the beneficial effects of various vitamins of the B-complex on growth, reproduction and lactation in the rat. Zucker and Zucker (1944) found that the unidentified factor required for growth is not always present in the usual concentrated sources of the vitamin B-complex but rather that it is associated, whether as an impurity or a new essential amino acid, with protein of good quality.

Sure (1943) demonstrated that p-aminobenzoic acid had a favorable influence on lactation but apparently gave no response in growth experiments. Inositol, however, seemed to have a pronounced injurious effect when fed with his rations which was counteracted by the p-aminobenzoic acid. Inositol likewise showed no response in growth studies.

Warkany and Schraffenberger (1943) observed congenital malformations in the offspring of female rats fed a purified diet lacking riboflavin. These same workers (1944), supplementing their basal ration with viosterol, found that these congenital malformations could be prevented by the addition of liver to the diet. Kennedy and Palmer (1945) found that biotin was essential for successful gestation and probably for lactation in rats. Nelson and Evans (1947c) have presented evidence to show that folic acid is required by the rat for successful lactation. Nelson, et al (1946) had previously reported that supplementation of a purified diet with low levels of folic acid did not produce beneficial effects on lactation.

Krehl, et al (1946) found that the addition of corn or corn grits to a synthetic ration low in nicotinic acid, retarded growth. This condition could be completely corrected by adding nicotinic acid or tryptophane to the ration. They reported that the kind of carbohydrate influenced the extent of the growth depressing action of corn. They also suggested that the addition of corn to the diet may result in the development of unfavorable intestinal flora which may destroy nicotinic acid or that corn may contain a substance or substances which combine with nicotinic acid or in some way make it unavailable.

Salmon (1947) presented evidence which was in disagreement with that of Krehl, et al (1946) concerning the harmful effects of corn. He found that the amount and nature of protein and the amount of fat in the diet determined the requirement for dietary nicotinic acid. He found that growth was greater with 86.5% cornmeal than with 40.0% cornmeal in a ration containing 9.0% casein, either with or without nicotinic acid. The inclusion of 15.0% lard in a 9.0% casein diet containing 40.0% cornmeal but no added nicotinic acid caused a significant increase in growth. This author believed that the problem with corn diets is a deficiency problem and not due to the harmful effects of corn.

Wintrobe, et al (1945) using a purified ration supplemented with thiamine, riboflavin, pyridoxine, choline, pantothenic acid, inositol and p-aminobenzoic acid found that there was a definite relationship between the level of protein and the nicotinic acid requirement for weanling pigs. When the ration contained 26.1% crude casein and supplemented with thiamine, riboflavin, pyridoxine, choline and pantothenic acid, and without nicotinic acid, no nutritional deficiency was noted except a slightly lower rate of gain. However, when the ration only contained 10.0% crude casein, the omission of nicotinic acid resulted in slower growth, rough hair coat, diarrhea, poor appetite and severe anemia. This would suggest that there is a close nutritional relationship between protein and nicotinic acid.

Powick, Ellis, Madsen and Dale (1947) using a purified basal diet containing 25.0% casein supplemented with accessory factors other than nicotinic acid observed a highly significant depression of growth, conspicuous impairment of appetite, a high incidence of diarrhea, high mortality and a high incidence of necrotic lesions of the colon and cecum. The addition of nicotinic acid seemed to correct these symptoms. They found that a level between 0.6 and 1.0 mg. of nicotinic acid per kilogram of live weight per day appeared to give optimal growth for growing pigs between three and nine weeks of age.

Further studies on the nicotinic acid requirement of pigs have been made by Leucke, et al (1946). They used a corn-soybean oil meal ration plus casein, minerals and liberal amounts of thiamine, riboflavin, pantothenic acid and pyridoxine for weanling pigs. The deficiency symptoms of nicotinic acid developed in four weeks. By feeding d,l-tryptophane to these pigs, good growth resulted. The addition of nicotinic acid to these pigs resulted in a smaller increase in gain and a less healthy appearance than when supplemented with tryptophane. They concluded from their results that both tryptophane and nicotinic acid are needed in the ration which was used and that tryptophane is the more critical nutrient of the two.

Powick, Ellis and Dale (1948) using a modified purified diet containing corn and casein or gelatin were able to correct the symptoms of a nicotinic acid deficient diet by the addition of crystalline nicotinic acid. A similar diet but of a lower tryptophane content produced deficiency symptoms not entirely corrected by nicotinic acid. Their results suggested that in general tryptophane would act as a substitute for nicotinic acid but that nicotinic acid was not completely effective as a substitute for tryptophane.

Other workers have also shown the need for vitamins of the B-complex when feeding natural rations. Cunha, et al (1943b) found that supplementing a natural ration with inositol gave a growth response and also cured and prevented a widespread loss of hair in the rat. These workers suggested that the phytin present in their ration was either inadequate to supply the required inositol or that it was unavailable.

Another vitamin which has been found to be beneficial for successful reproduction in the rat is vitamin K. Brown, et al (1947) observed that those females which were raised to maturity on a synthetic diet deficient in vitamin K, then fed a diet deficient in lard and vitamin K, gave birth to offspring that had a high incidence of brain hemorrhages. Most of these young were either dead at birth or died during the first twenty-four hours. Some, however, developed hemorrhages between the eleventh and twenty-first day. This condition could be prevented by the addition of either lard or vitamin K.

Various other workers have demonstrated the vitamin requirements of swine for growth, reproduction and lactation. Van Foucke and Krider (1946), using a basal purified diet deficient in riboflavin, observed several deficiency symptoms among which were extremely thickened skin, enlarged spleen, anemia and a light grey discoloration of the kidneys. Those pigs receiving the highest supplement of riboflavin daily, 3.96 mg. per 100 pounds live weight, were the most thrifty, had the highest daily rate of gain and had the lowest feed consumption per pound of gain. These results indicated that riboflavin is associated with the utilization of feed for growth. Ferrin (1946) found that the addition of choline to a corn-soybean oil meal ration had a depressing effect upon rate of gain and daily feed consumption.

In order to demonstrate the vitamin requirements for reproduction and lactation, Ensminger, Bowland and Cunha (1946) fed gilts a purified ration supplemented by vitamins A, D, E, K and the B-complex vitamins, niacin, pantothenic acid, pyridoxine, thiamine, riboflavin and choline, the latter three being deleted individually to create deficient rations. They found that the control ration was evidently deficient in some unknown factor. The thiamine deficient sows had a high mortality of pigs at birth and many leg abnormalities. The sows fed the ration deficient in riboflavin exhibited a loss of appetite, an abnormally early parturition date and some resorption of the developing embryos. All live pigs at birth died within forty eight hours and exhibited many abnormalities. There was a heavy mortality of young on the riboflavin deficient diet and the pregnancy gains of the sows were somewhat less than the gains of the sows on other rations. The young that were farrowed were weak, had "spraddled legs" and showed muscular incoordination.

Fairbanks and co-workers (1944b) using a basal ration of yellow corn, beef meal, soybean meal, alfalfa meal and salt found that the addition of hulled oats would improve the growth rate of weanling pigs in drylot. Their results indicated that the better performance of the pigs receiving hulled oats may have been due to a higher caloric intake of this ration or that the hulled oats supplied an unknown missing factor to the ration.

Some investigators, in studying the problem of deficiencies of a corn-soybean oil meal ration for successful reproduction and lactation in swine, are of the opinion that there was some essential nutrient lacking in the milk. Therefore, there have been rather recent attempts at raising pigs on a synthetic milk diet immediately after farrowing. The results have been somewhat variable. Bustad, Ham and Cunha (1947) placed pigs on a synthetic milk diet immediately after birth. None of their pigs survived more than 22 days. They observed a severe diarrhea, an unthrifty condition and the presence of a dark exudate around the eyes of the pigs. Supplementing the ration with crude casein, lactose, 1:20 liver powder or anti-pernicious anemia liver extract gave no apparent results. Anderson and Hogan (1947) however, were able to obtain good results with a synthetic milk diet supplemented with all the known vitamins. These workers did not begin their experiment until after the third day so that these pigs had received colostrum. Johnson, James and Krider (1947) successfully raised pigs on a synthetic milk ration starting at 4 days of age and two weeks of age. From those results, it would appear that it was necessary for the pigs to receive the colostrum milk of their mothers before they could be successfully raised on a synthetic ration.

In studying the problem of lactation in rats, Nelson and Evans (1947c) have proposed short-term studies in preference to those taking a longer period of time. They placed the females on the experimental diets at the time of parturition and the mother and young were weighed every five days. The young were then weaned on the twenty-first day after parturition. The criteria which they used to determine the adequacy of the ration were: (1) average weight of young at weaning and (2) weight change of lactating mothers during the twenty-one day period, including only those females weaning five or six young. They found that the deficiencies observed using the short-term procedure were almost as severe as in the long-term experiments. By using this procedure, the time necessary for conducting an experiment was greatly decreased.

More recently, Rickes, et al (1948) have isolated a crystalline compound from liver which they have designated as vitamin $B_{12}$. Shorb (1948) has shown that this vitamin $B_{12}$ is either wholly or partially responsible for the LLD (Lactobacillus lactis Dorner) growth activity observed from the liver extracts. This same worker has also tested many of the source materials and fractions derived therefrom which have been reported to contain unidentified factors for poultry nutrition. She stated that:

> "The LLD factor activity occurs in fairly high amounts, in approximately decreasing order, in a papain digest of acid precipitate from cow manure, the acid precipitate from cow manure, fish meal, pancreatin, papain, egg white, egg yolk, and in lower amounts in alcoholic extract of whey, potassium permanganate-oxidized alcoholic extract of whey, soybean oil meal, gelatin, zein and Mylase P. enzyme. The TJ factor activity is also found, in approximately decreasing order, in the papain digest of acid precipitate from cow manure, the acid precipitate of cow manure, egg yolk, papain, and pancreatin, but in much lower amounts in fish meal, alcoholic extract of whey, soybean oil meal, crude casein, egg white, zein, Mylase P. enzyme, potassium permanganate-oxidized alcoholic extract of whey, and gelatin. The distribution of the LLD and TJ factor activities in these materials suggests that they may be involved in chicken nutrition."

## OBJECTIVES OF THE EXPERIMENT

To determine the specific nutrient or nutrients needed by the rat to produce normal growth, reproduction and lactation when fed common swine rations.

## EXPERIMENT I

**Procedure:**

The female rats used in Experiment I were of the Sprague-Dawley Strain. At the time of weaning (21 days), they were placed in cages with wire bottoms, five animals per cage, and fed the following depleting ration (Basal Ration #2):

Basal Ration #2

| | |
|---|---|
| Ground yellow corn | 68.85% |
| Expeller process soybean oil meal | 25.00 |
| Alfalfa leaf meal | 5.00 |
| Calcium carbonate | 0.65 |
| Iodized salt | 0.50 |

Each female received two drops of cod-liver oil plus the equivalent of 1 mg. of alpha tocopherol daily fed weekly by dropper. Each gram of the cod-liver oil contained 20,000 U.S.P. units of vitamin A and 4,000 U.S.P. units of vitamin D.

The following amounts of crystalline vitamins were added to each kilogram of ration: thiamine 4 mg., riboflavin 6 mg., pyridoxine hydrochloride 3 mg., calcium pantothenate 20 mg., nicotinic acid 20 mg., choline chloride 1 mg., folic acid 1 mg., inositol 20 mg., and p-aminobenzoic acid 20 mg.

The rats were continued on this ration until they were fourteen weeks of age, at which time they were divided into eight lots of eight females each. The rations fed each respective lot are shown below. These rations were fed throughout the reproduction and lactation periods.

| Lot No. | Ration |
|---|---|
| 1. | Basal #2. |
| 2. | Basal #2 ≠ 1:20 liver powder residue (Fraction 1). |
| 3. | Basal #2 ≠ 1:20 liver powder insoluble in 95% ethyl alcohol (Fraction 2). |
| 4. | Basal #2 ≠ 1:20 liver powder soluble in 95% ethyl alcohol (Fraction 3). |

5. Basal #2 ≠ Norit filtrate of 1:20 liver powder soluble in 95% ethyl alcohol (Fraction 4).
6. Basal #2 ≠ Norit eluate of 1:20 liver powder soluble in 95% ethyl alcohol (Fraction 5).
7. Basal #2 ≠ concentrated injectable liver extract (Lederle).
8. Basal #2 ≠ 2% 1:20 liver powder.

The liver fractions fed Lots 2, 3, 4, 5 and 6 were added at a rate of 2-1/2% equivalent of 1:20 liver powder. Lot 7 was fed the basal ration #2 and each female was given a 0.2 cc. intramuscular injection twice weekly of a commercial anti-pernicious anemia liver extract (Lederle). This extract contained 1.5 U.S.P. injectable units per cc. It was injected on the inside fleshy portion of the thigh with a 1 cc. tuberculin syringe. Lot 8 females were fed the basal ration plus 2.0% 1:20 liver powder. All supplements were added at the expense of the whole ration.

Males from the stock colony were placed with the females at the time they were placed on the different rations. The males were rotated among the different lots every four days so as to reduce the failure of conception due to possible sterility of the males. They males were left with the females for two weeks.

The liver fractions were all prepared from Wilson's 1:20 liver concentrate powder. One part of this liver powder is equivalent to twenty parts of fresh liver. The liver fractions were prepared as follows: Five kilograms of the liver powder were dissolved in approximately six liters of cold 70% ethyl alcohol. The mixture was heated on a steam bath to a temperature of 50-60° C. The material not fully dissolved was put through a fine wire mesh screen until it was homogeneous. The material was filtered with suction and the precipitate was repeatedly extracted with 70% ethyl alcohol at 50-60° C. The extraction was repeated four times using three liters of alcohol for each extraction. This gave a total volume of soluble material of between sixteen and eighteen liters. The combined extracts were refiltered to remove a small amount of precipitate and the filtrate concentrated under reduced pressure.

That material which was insoluble in the 70% ethyl alcohol was combined and was dried in a vacuum oven. It was finally dried at a temperature of 100° C. for three days. It was then ground through a food chopper and further dried for twenty-four hours. The residue was finally ground to pass through a 40 mesh screen with a Wiley mill. This fraction was Fraction 1.

The fraction which was soluble in 70% ethyl alcohol was concentrated to a very thick syrup, then extracted repeatedly with 95% ethyl alcohol. The first two portions of alcohol were about two liters each and successive portions were about 500 cc. each. When the material became the consistency of taffy, absolute alcohol was substituted for the 95% ethyl alcohol. These alcohol solutions were combined and concentrated under reduced pressure. A second precipitation with ethanol was carried out as before and the insoluble fractions combined. A portion of the 95% alcohol soluble fraction was fed as Fraction 3. The remainder was used for a further fractionation.

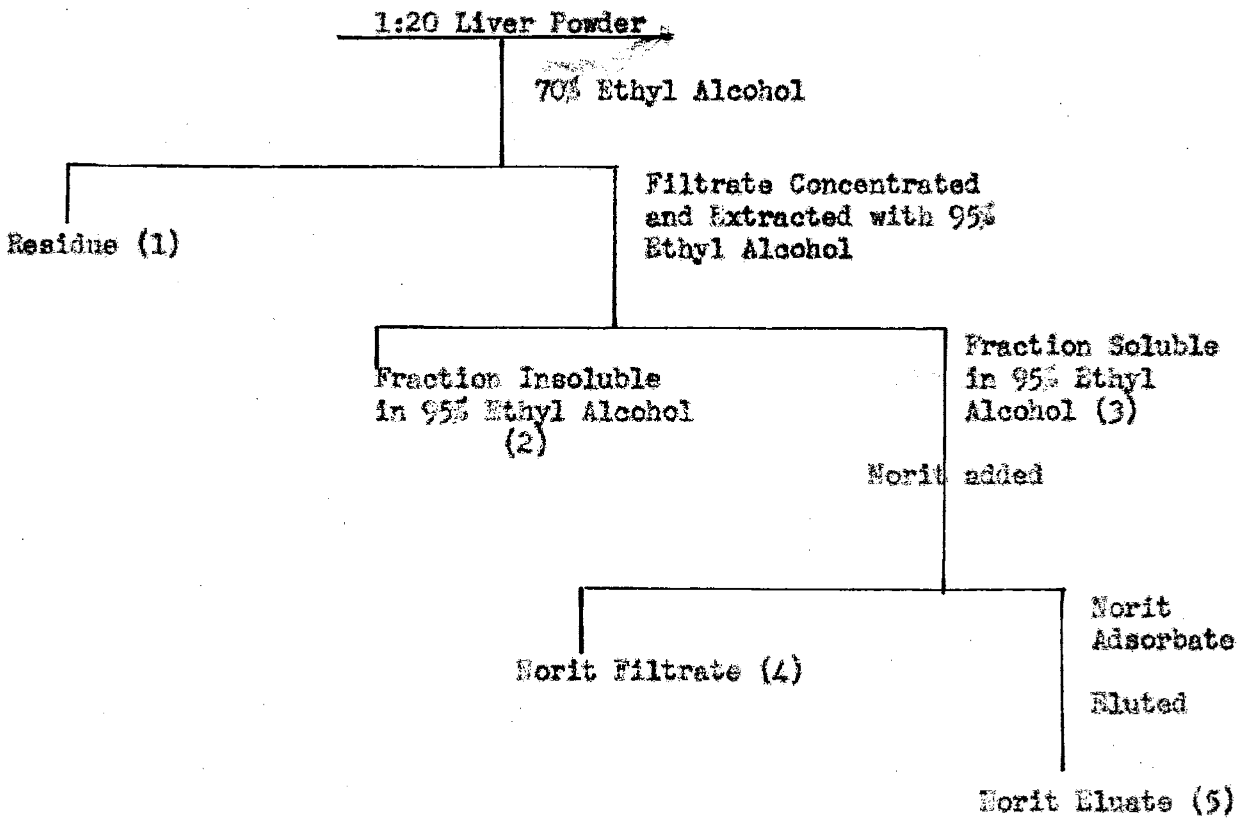
The residue from the extractions with 95% alcohol was triturated with absolute alcohol until it could be filtered. Approximately one-tenth of this or roughly 100 grams was dissolved in 300 cc. of 70% ethyl alcohol. This was Fraction 2.

Approximately 800 cc. of the concentrated fraction soluble in 95% ethyl alcohol was adjusted to a pH of 2.5 with concentrated HCl. This portion was extracted five times with 15 gram portions of Norit A (Pfansteihl Chemical Co.), the carbon residues being saved after each extraction. At the end of the five extractions very little decolorization of the 95% ethyl alcohol soluble fraction was observed. The volume was reduced under pressure to 500 cc. This Norit filtrate was Fraction 4.

All of the carbon residues were combined and eluted six times with 250 cc. portions of 5% $NH_4OH$ in 95% ethyl alcohol. The eluate was very dark in color.

This eluate was Fraction 5. A graphic summary of the fractionations of the liver powder is given in Figure I.

FIGURE I.




The females were weighed weekly and in addition, they were weighed immediately after parturition and at the time of weaning the young. This was done in order to obtain the gain or loss in weight during the lactation period.

At least two days prior to parturition, the females were placed in individual cages. The bottoms of the cages were covered with one inch of wood shavings to serve as litter. The feed and water was supplied ad libitum in individual separate glass containers. As soon after parturition as practicable, a record was made of the total number of young and the number of live young remaining. This count would not be correct in all cases since it was observed that some of the young which were dead at birth were eaten by the mother before they had been counted.

All litters containing more than six young the second day after parturition were reduced to six. When the number of young was reduced to six, an attempt was made to keep the litters as uniform as possible from the standpoint of sex. In those litters of six live young or less, the females were expected to raise all of those young. If some of the young died during the first forty-eight hours in such litters, they were still included in the calculation of the data of the number of young given the female to raise. However, those young which died during the first forty-eight hours after birth in litters having more than six live young were not included in the number given the female to raise, providing the number remaining was six or more. For example, a female having eight live young at birth and losing two during the first two days was considered as being given six young to raise. A female having eight young at birth and losing three during the first forty-eight hours was still expected to raise six young.

At approximately one week after parturition, the female and her young were removed from the cages containing the wood shavings and placed in individual wire bottom cages. Food and water continued to be supplied <u>ad libitum</u> in individual containers.

The young were weaned at 21 days of age and the total weight of the litter was obtained. After weaning the females were continued on the same rations as during the reproduction and lactation period and the young were placed on an adequate stock ration.

Results:

The summary of the results of Experiment I are given in Table I. These data were analyzed statistically by the analysis of variance method of Snedecor (1946). There were no resorption or toxemia deaths encountered in this experiment. Some sterility was encountered in each lot but there was no significant difference between lots in this regard. Lot 6, receiving the Norit eluate, had the smallest litters at birth and also weaned the fewest number of litters. Lot 7, receiving the injectable liver extract, had the highest percentage of dead young at birth but weaned the largest percentage of young. The basal ration had the highest mortality of young during the first forty-eight hours and also weaned the smallest percentage of young.. Many of the young which died had milk in their stomachs indicating that milk was available. The young from this lot were lighter in weight at weaning than the average of the rest of the lots in the experiment and this difference was significant at the 1% probability level. Lot 8, receiving Wilson's 1:20 liver concentrate powder, and Lot 3, receiving Fraction 2, had the highest average weaning weights. There was no significant difference between the weaning weights of the young in Lots 7 and 8.

In comparing the weaning weights of the lot fed the fraction insoluble in 95% ethyl alcohol (Lot 3) with the one fed the fraction soluble in 95% alcohol (Lot 4), it was found that the weaning weights of the young of Lot 3 were higher with significance at the 1% level. This would suggest that the active factor of the liver powder was readily soluble in 70% ethyl alcohol, but less soluble in 95% ethyl alcohol. The lot fed the Norit eluate (Lot 6) produced heavier rats at weaning than did the lot receiving the Norit filtrate (Lot 5). This difference was significant at the 1% level. However, only 56.6% of the young given the females to raise were weaned in the Norit eluate lot, whereas, 70.27% of the young were weaned in the lot receiving the Norit filtrate. Two of the litters born of those receiving the Norit eluate had only one young each and they failed to survive until weaning.

TABLE I.

Experiment I — Reproduction and Lactation Data

| Lot No. | Rations | No. Females | % Conception | Resorption and Toxemia Deaths | No. of Litters Born | No. of Litters Weaned | No. of Live Young at Birth | No. of Dead Young at Birth | No. of Young Dead or Eaten First 48 Hours after Birth | No. of Young Given Females to Raise | No. of Young Weaned of Those Given Females to Raise | % Young Weaned of those Given Females to Raise | Av. Weaning Weight (grams) of Young at 21 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Basal #2 | 8 | 75.0 | 0 | 6 | 4 | 39 | 5 | 16 | 34 | 19 | 55.88 | 27.47 |
| 2. | Basal #2 + 1:20 L.P. Residue (Fraction 1) | 7 | 100.0 | 0 | 7 | 5 | 56 | 3 | 7 | 40 | 28 | 70.00 | 31.46 |
| 3. | Basal #2 + 1:20 L.P. Ins. in 95% ETOH (Fraction 2) | 8 | 87.5 | 0 | 7 | 6 | 58 | 4 | 3 | 42 | 35 | 83.33 | 36.63 |
| 4. | Basal #2 + 1:20 L.P. Sol.in 95% ETOH (Fraction 3) | 7 | 85.7 | 0 | 6 | 5 | 48 | 2 | 3 | 32 | 23 | 71.88 | 32.96 |
| 5. | Basal #2 + Norit Filtrate (Fraction 4) | 8 | 87.5 | 0 | 7 | 5 | 50 | 3 | 6 | 37 | 26 | 70.27 | 27.08 |
| 6. | Basal #2 + Norit Eluate (Fraction 5) | 8 | 75.0 | 0 | 6 | 3 | 30 | 2 | 8 | 30 | 17 | 56.67 | 34.53 |
| 7. | Basal #2 + Conc. Inj. Liver Extract | 8 | 87.5 | 0 | 7 | 5 | 43 | 9 | 2 | 30 | 30 | 100.00 | 34.23 |
| 8. | Basal #2 + 2% 1:20 L.P. | 8 | 87.5 | 0 | 7 | 6 | 59 | 1 | 2 | 39 | 33 | 84.62 | 36.48 |

## EXPERIMENT IA

Procedure:

The females used in Experiment IA were the same as those of Experiment I. These females were continued on the same rations as those used in Experiment I. Those which were sterile in the first experiment were destroyed. Also, those which developed any unthriftiness or respiratory disorders were removed from the experiment. This accounts for the reduced number of females in the second trial. These females were mated at about 28 weeks of age to males from the stock colony, in the same manner as in Experiment I.

The females were weighed weekly and in addition, the weight of the female was taken immediately after parturition and at the time of weaning the young. The procedure which was followed at the time of parturition and during the lactation period was the same as that followed in Experiment I.

The young were weaned when they were 21 days of age. In addition to the litter weight, individual weaning weights of the young were obtained. After the young were weaned, the females were destroyed. Due to the high incidence of colds and respiratory disorders encountered in the second trial, this was the only experiment in which the females were carried through a second reproduction and lactation period. It was decided that each experiment should be conducted with a new group of females, thus making the experiments more nearly comparable as to age and previous history of the experimental animals.

Results:

The results of Experiment IA are summarized in Table II. These data were analyzed statistically by the analysis of variance method of Snedecor (1946). Lot 1 lost the largest number of young during the first forty-eight hours and also weaned the lowest percentage of young. However, due to the small number of females in many of the lots and somewhat more uniform weaning weights, no statistically significant difference between the weaning weights of Lot 1 and those of the other lots of the experiment were found. Lots 3 and 8 again had the highest weaning weights of young followed quite closely by those of Lot 7. There was no significant difference between the weaning weights of Lots 7 and 8. Lot 8 had the highest number of young dead at birth. The young from Lot 3 were heavier at weaning time than those from Lot 2. This provided additional evidence that the active factor was soluble in 70% ethyl alcohol. However, in this experiment the slight difference in weaning weights between Lots 3 and 4 was not statistically significant.

One striking difference between this experiment and the previous ones was the difference between the lots receiving the Norit filtrate and the Norit eluate. Those young from the lot receiving the Norit filtrate were much heavier at weaning than were those receiving the Norit eluate. This difference was significant at the 1% level. These results were in direct contradiction to those observed in Experiment I. Only two litters were weaned, however, from the females receiving the Norit eluate. The percentage of young weaned of those given the females to raise was approximately the same in both of these lots. It appears from these data that more experiments need to be conducted before definite conclusions can be drawn as to the behavior of the active factor when treated with Norit. With the exception of Lot 7, the percentage of young weaned of those given the females to raise increased in Experiment IA as compared to the results of the first reproduction and lactation Experiment I.

TABLE II

Experiment IA

Reproduction and Lactation Data

| Lot No. | Rations | No. Females | % Conception | Resorption and Toxemia Deaths | No. of Litters Born | No. of Litters Weaned | No. of Live Young at Birth | No. of Dead Young at Birth | No. of Young Dead or Eaten First 48 Hours after Birth | No. of Young Given Females to Raise | No. of Young Weaned of those Given Females to Raise | % Young Weaned of those Given Females to Raise | Av. Weaning Weight (grams) of Young at 21 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Basal #2 | 6 | 83.33 | 0 | 5 | 4 | 33 | 6 | 9 | 27 | 21 | 77.78 | 34.90 |
| 2. | Basal #2 ≁ 1:20 L.P. Residue (Fraction 1) | 3 | 100.00 | 0 | 3 | 3 | 22 | 0 | 1 | 14 | 14 | 100.00 | 34.60 |
| 3. | Basal #2 ≁ 1:20 L.P. Ins. in 95% ETOH (Fraction 2) | 6 | 100.00 | 0 | 6 | 6 | 34 | 2 | 0 | 26 | 26 | 100.00 | 35.65 |
| 4. | Basal #2 ≁ 1:20 L.P. Sol. in 95% ETOH (Fraction 3) | 3 | 66.67 | 0 | 2 | 2 | 21 | 0 | 0 | 12 | 12 | 100.00 | 37.00 |
| 5. | Basal #2 ≁ Norit Filtrate (Fraction 4) | 7 | 85.71 | 0 | 6 | 6 | 39 | 3 | 2 | 32 | 27 | 84.38 | 35.56 |
| 6. | Basal #2 ≁ Norit Eluate (Fraction 5) | 3 | 100.00 | 0 | 3 | 2 | 18 | 1 | 0 | 14 | 12 | 85.71 | 28.25 |
| 7. | Basal #2 ≁ Conc. Inj. Liver Extract | 5 | 80.00 | 0 | 4 | 4 | 32 | 1 | 4 | 22 | 20 | 90.91 | 37.75 |
| 8. | Basal #2 ≁ 2% 1:20 L.P. | 6 | 83.33 | 0 | 5 | 4 | 45 | 10 | 0 | 24 | 23 | 95.83 | 38.13 |

## EXPERIMENT II

Procedure:

As was brought out in the Review of Literature, there is some disagreement as to whether there is a factor required for reproduction and lactation in addition to that required for growth of the rat. This experiment was designed to test whether two factors existed and to demonstrate possible differences, if any, between them.

Female weanling rats of the Sprague-Dawley Strain weighing between 50-60 grams were equally allotted according to weight and placed on the experimental rations shown in Table III. There were nine rats in each of the first six lots and eight rats in the other three lots. They were fed in wire cages, two rats per cage. Due to a respiratory disorder, one rat had to be removed from each of Lots 2, 4 and 8. The feed and water was supplied <u>ad libitum</u> and no attempt was made to measure the quantity of food eaten daily. All the rats received two drops of cod-liver oil plus an equivalent of 1 mg. of alpha tocopherol daily fed weekly by dropper. The rations of Lots 4 and 5 were supplemented with crystalline B-complex vitamins as shown in Table III. The ration of Lot 9 was supplemented with the Norit eluate of that fraction of Wilson's 1:20 liver powder soluble in 95% ethyl alcohol (Fraction 5). This was fed at a level equivalent to 2% 1:20 liver concentrate powder in the ration.

The females on this growth experiment were weighed weekly for a period of five weeks. An attempt was made to weigh them under as nearly the same conditions as possible each week. In every case they were weighed immediately after feeding and the weights recorded.

At the completion of the five week growth period, these females were placed on reproduction and lactation Experiment II. Those females in Lots 1, 2, 3 and 4 were fed the basal ration #2 used in Experiment I and IA. The Lot 5 females were eliminated from the experiment. Lot 6 was placed on basal ration #2 plus 2% 1:20 liver concentrate powder. Lots 7 and 8 were fed the rations shown below:

Lot 7

| | |
|---|---|
| Ground yellow corn | 78.85% |
| Vitamin test casein(GBI) | 15.00 |
| Alfalfa leaf meal | 5.00 |
| Calcium carbonate | 0.65 |
| Iodized salt | 0.50 |

Lot 8

| | |
|---|---|
| Ground yellow corn | 76.35% |
| Expeller process soybean oil meal | 13.50 |
| Fish solubles(Borden) | 4.00 |
| Alfalfa leaf meal | 5.00 |
| Calcium carbonate | 0.65 |
| Iodized salt | 0.50 |

Lot 9 was provided basal ration #2 plus Fraction 5 fed in the previous experiments. All of these rations were supplemented with the same added vitamins as in Experiments I and IA.

TABLE III

Growth Experiment

| Lot No. | 1 % | 2 Basal #2 | 3 | 4** | 5** | 6 | 7 | 8 | 9*** |
|---|---|---|---|---|---|---|---|---|---|
| Gr. yellow corn | 76.35 | 68.85 | 63.85 | 63.85 | 63.85 | 76.35 | 86.10 | 76.35 | 76.35 |
| Soybean oil meal | 17.50 | 25.00 | 25.00 | 25.00 | 23.00 | 15.50 | | 13.50 | 17.50 |
| Alfalfa meal | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 | 5.00 |
| Casein (Vitamin free) | | | 5.00 | 5.00 | 5.00 | | 7.75 | | |
| 1:20 Liver Powder | | | | | 2.00 | 2.00 | | | |
| Fish solubles | | | | | | | | 4.00 | |
| Calcium carbonate | .65 | .65 | .65 | .65 | .65 | .65 | .65 | .65 | .65 |
| Iodized salt | .50 | .50 | .50 | .50 | .50 | .50 | .50 | .50 | .50 |

**Lots 4 and 5 were supplemented with crystalline B vitamins per kilogram of feed as follows:

Thiamine, 4 mg.; riboflavin, 6 mg.; pryidoxine, 3 mg.; Ca pantothenate, 20 mg.; nicotinic acid, 20 mg.; choline chloride, 1 gm.; folic acid, 1 mg.; inositol, 20 mg.; and para aminobenzoic acid, 20 mg.

***Lot 9 was supplemented with Norit eluate from 1:20 liver powder at the equivalent rate of 2% of 1:20 liver powder in the ration.

At 17 weeks of age, those females fed basal ration #2, Lots 1, 2, 3 and 4, were redivided into three lots and given the rations shown below. The rations fed the other four lots were those used from the conclusion of the growth trial to 17 weeks of age.

Ration

Lot No.
1. Basal #2.
2. Basal #2 ≠ Norit filtrate of 1:20 liver powder insoluble in 95% ethyl alcohol (Fraction 6).
3. Basal #2 ≠ Norit eluate of 1:20 liver powder insoluble in 95% ethyl alcohol (Fraction 7.)
4. Basal #2 ≠ 2% 1:20 liver powder.
5. Same as Lot 7 above.
6. Same as Lot 8 above.
7. Basal #2 ≠ Norit eluate of 1:20 liver powder soluble in 95% ethyl alcohol (Fraction 5).

All liver fractions were added at an equivalent rate of 2-1/2% 1:20 liver powder.

Since better results had been obtained in Experiment I and IA with the liver fraction soluble in 70% ethyl alcohol but insoluble in 95% alcohol an attempt was made to further test the properties of this fraction. A portion of this fraction was dissolved in 70% ethyl alcohol and the pH adjusted to 2.5 with concentrated HCl. It was extracted five times with 15 gram portions of Norit A and the carbon residues were saved after each extraction. The volume of the Norit filtrate was reduced to 800 cc. and this was fed as Fraction 6.

All the carbon residues were combined as before and eluted six times with 250 cc. portions of 5% $NH_4OH$ in 95% ethyl alcohol. The eluate was somewhat darker than the eluate from the 95% alcohol soluble fraction. This was Fraction 7.

These females were mated at 17 weeks of age to males from the stock colony which had been receiving the stock ration. The males were rotated between the different lots in the same manner as in Experiments I and IA. The females were weighed weekly, immediately after parturition and at the time of weaning the young.

On the second day after parturition the number of young in each litter was reduced to six. The manner in which the young were reduced and the calculation of the lactation performance was the same as in the previous experiments. All of the young were weaned at 21 days of age and the individual weaning weights taken.

Results: Growth

The results of the growth period are given in Table IV. These data were analyzed statistically by the analysis of variance method of Snedecor (1946). There was no statistically significant difference in growth rates of the females among the different lots. In general, all the lots made the greatest gain during the second week, then the gains decreased quite rapidly. The lowest gains were made during the fourth week. This was followed by a slight increase in gain during the fifth and final week. None of the females developed any abnormalities during the course of the experiment. Those lots which received Wilson's 1:20 liver powder had a more healthy appearance and had smoother haircoats but this was not reflected in the rate of gain. Lots 4 and 5 which received the vitamin supplement made a slightly higher rate of gain than did the other lots but this was not a statistically significant difference. The range in differences in gain in the individual lots was about the same and was apparently due to the individual differences that existed among the females. From the results obtained in this experiment, the corn-soybean oil meal rations produced a fair rate of growth but none of the growth rates obtained with these females would be considered optimum for this particular stage in their development.

Results: Reproduction and Lactation

The summary of the results of the reproduction and lactation period of Experiment II is given in Table V. These results were analyzed in the same manner as in Experiments I and IA. This was the first experiment in which any of the females died due to resorption or toxemia. The female in Lot 1 which

TABLE IV

AVERAGE WEEKLY GROWTH RATE IN GRAMS DURING THE 5 WEEK PERIOD

WEEKLY GAIN

| Lot No. | 1 | 2 | 3 | 4 | 5 | Av. |
|---|---|---|---|---|---|---|
| 1. Basal #2 - 7.5% S.O.M.[1] | 28.3 | 33.2 | 22.1 | 12.6 | 18.2 | 22.9 |
| 2. Basal #2 | 29.2 | 36.0 | 24.7 | 10.6 | 18.2 | 23.7 |
| 3. Basal #2 + 5% Casein[2] | 31.6 | 34.1 | 21.3 | 14.8 | 15.0 | 23.4 |
| 4. Basal #2 + 5% Casein[2] + B vitamin | 35.4 | 34.7 | 23.3 | 16.3 | 18.2 | 25.8 |
| 5. Basal #2 + 5% Casein[2] + 2% 1:20 L.P.[3] + B vitamin | 37.6 | 34.4 | 20.1 | 16.6 | 18.0 | 25.3 |
| 6. Basal #2 - 7.5% S.O.M.[1] + 2% 1:20 L.P.[3] | 27.5 | 34.9 | 29.6 | 13.7 | 12.9 | 23.7 |
| 7. Basal #2 - 17.25% S.O.M.[4] + 7.75% Casein[5] | 28.9 | 34.2 | 24.2 | 13.1 | 11.1 | 22.3 |
| 8. Basal #2 - 7.5% S.O.M.[1] + 4.0% Fish solubles[6] | 28.7 | 34.6 | 23.7 | 16.9 | 13.0 | 23.4 |
| 9. Basal #2 - 7.5% S.O.M.[1] + Norit eluate of 1:20 L.P. soluble in 95% ETOH (Fraction 5) | 29.5 | 35.5 | 27.1 | 12.7 | 17.6 | 24.5 |

[1]Corn replaced the 7.5% soybean oil meal.
[2]Casein added at the expense of corn.
[3]1:20 liver powder added at the expense of soybean oil meal.
[4]Corn replaced the 17.25% soybean oil meal.
[5]Casein added at the expense of soybean oil meal.
[6]Fish solubles added at the expense of soybean oil meal.

died, upon post mortem examination, had eight distinct placental sites but there was no evidence of resorbed young at the time of death. In this case there was no noticeable gain in weight during the gestation period indicating that resorption must have taken place quite early in the gestation period. The post mortem findings of the female which died in Lot 2 were very similar to those described above.

The female which died in Lot 6, upon examination, was found to have seven fully developed young. Advanced post mortem changes had occurred prior to examination, making it difficult to recognize any distinct abnormalities. The female in Lot 8 which died had ten fully developed young. In this case there was a considerable hemorrhage in the uterus as shown by blood in the vagina, and some inflammation of the lungs. There also appeared to be some fatty degeneration of the liver. In both of the latter cases, no abnormalities could be detected in the young and there were no indications of resorption of the young.

In this experiment, there was a higher degree of sterility of the females fed the basal ration #2 than in the previous experiments but there was a much lower mortality of the young during the first forty-eight hours and also a higher percentage of heavier young weaned. Many of the young which died were found to have no milk in their stomachs and apparently died of starvation. The ration which seemed to be least adequate for lactation was that ration in which vitamin test casein was substituted for the soybean oil meal. These females had large vigorous litters but a very high percentage of the young failed to survive the first forty-eight hours. Only 20.51% of the young given the females to raise survived until weaning time. This suggested that the active factor(s) necessary for optimum lactation was not present in purified casein, and that substituting purified casein for soybean oil meal aggravated the deficiency.

In this experiment, those females receiving fish solubles weaned young having the highest weaning weight of any of the lots. The young of this lot were much heavier at weaning than those in the lots receiving the liver powder and the liver powder fractions. This difference was significant at the 1% level. One striking feature of this experiment was the failure of the lot receiving the liver powder to perform as well as in the previous experiments. The performance of those females fed basal ration #2 was superior to the lot

TABLE V.

Experiment II

Reproduction and Lactation Data

| Lot No. | Rations | No. Females | % Conception | Resorption and Toxemia Deaths | No. of Litters Born | No. of Litters Weaned | No. of Live Young at Birth | No. of Dead Young at Birth | No. of Young Dead or Eaten First 48 Hours after Birth | No. of Young Given Females to Raise | No. of Young Weaned of Those Given Females to Raise | % Young Weaned of Those Given Females to Raise | Av. Weaning Weight (grams) of Young at 21 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Basal #2 | 10 | 70.0 | 1 | 6 | 6 | 51 | 1 | 2 | 36 | 33 | 91.67 | 33.16 |
| 2. | Basal #2 + Norit filtrate of L.P. Fraction ins. in 95% ETOH (Fraction 6) | 8 | 100.0 | 1 | 7 | 5 | 50 | 10 | 4 | 36 | 29 | 80.56 | 35.55 |
| 3. | Basal #2 + Norit Eluate of L.P. Fraction Ins. in 95% ETOH (Fraction 7) | 8 | 87.5 | 0 | 7 | 4 | 36 | 17 | 10 | 29 | 20 | 68.97 | 32.45 |
| 4. | Basal #2 + 2% 1:20 L.P. | 9 | 100.0 | 0 | 9 | 7 | 59 | 13 | 3 | 45 | 32 | 71.00 | 31.30 |
| 5. | Same as 1, except casein substituted for S.O.M. | 8 | 87.5 | 0 | 7 | 2 | 55 | 12 | 33 | 39 | 8 | 20.51 | 32.38 |
| 6. | Basal #2 + Fish solubles substituted for part of S.O.M. | 7 | 85.7 | 1 | 5 | 5 | 41 | 2 | 1 | 29 | 25 | 86.21 | 37.76 |
| 7. | Basal #2 + Norit eluate of 1:20 L.P. soluble in 95% ETOH(Fraction 5) | 6 | 100.0 | 1 | 5 | 4 | 41 | 1 | 2 | 27 | 22 | 81.48 | 30.86 |

fed liver powder. Also, those females receiving liver fractions 6 and 7, Lots 2 and 3, weaned young which were heavier than were those of females in Lot 4 receiving the 1:20 liver powder. This difference was significant at the 5% level. In comparing the performances of those lots receiving the Norit filtrate, Fraction 6, with the Norit eluate, Fraction 7, it was found that the performance of the lot receiving the Norit filtrate was much superior. This lot had less sterility, larger litters, a higher percentage of live young at birth, greater survival of young during the first forty-eight hours, a higher percentage of young weaned and heavier young at weaning. The difference in weaning weights was significant at the 5% level of probability. This would suggest that the active factor was not readily adsorbed on Norit under the particular conditions employed.

## EXPERIMENT III

Procedure:

Since Ross, et al (1942a) has demonstrated that the performance of the rat receiving corn-soybean oil meal rations closely parallels that of swine and Cunha, et al (1948) and others have shown that a ration containing 15% alfalfa leaf meal will give satisfactory reproduction and lactation performance in swine, the basal ration #2 was changed in order to test some of the properties of alfalfa meal.

The females used in Experiment III were weaned from the females in Experiment I and were fed a stock ration from weaning until they were approximately seven weeks of age. At this time they were divided into six lots of seven females each. The rations fed each respective lot are shown below. These rations were fed throughout the remainder of the growth period and the reproduction and lactation period.

| Lot No. | Ration |
|---|---|
| 1. | Basal #3. |
| 2. | Basal #3 + 15% alfalfa leaf meal. |
| 3. | Basal #3 + alfalfa fraction insoluble in 95% ethyl alcohol. |
| 4. | Basal #3 + alfalfa fraction soluble in 95% ethyl alcohol. |
| 5. | Basal #3 + 2% 1:20 liver powder. |
| 6. | Basal #2 + anthranilic acid. |

The composition of the basal ration #3 and the ration fed Lot 2 is shown below. The alfalfa leaf meal was added at the expense of the corn and soybean oil meal. The soybean oil meal was reduced slightly to provide a level of crude protein equivalent to that of basal ration #3.

| Basal Ration #3 | |
|---|---|
| Ground yellow corn | 73.50% |
| Expeller process soybean oil meal | 25.00 |
| Salt mixture | 1.50 |

| Lot 2 | |
|---|---|
| Ground yellow corn | 61.25% |
| Expeller process soybean oil meal | 22.25 |
| Alfalfa leaf meal | 15.00 |
| Salt mixture | 1.50 |

The salts mixture was that of Hegsted, et al (1941) and had the following composition: $CaCO_3$ 600, $K_2HPO_4$ 645, $CaHPO_4 \cdot 2H_2O$ 150, $MgSO_4 \cdot 7H_2O$ 204, NaCl 335, $Fe(C_6H_5O)_2 \cdot 6H_2O$ 55, KI 1.6, $MnSO_4 \cdot 4H_2O$ 10, $ZnCl_2$ 0.5, $CuSO_4 \cdot 5H_2O$ 0.6.

All of the rations were supplemented with the same crystalline vitamins, cod-liver oil and alpha tocopherol as in the preceding experiments. The alfalfa fractions fed Lots 3 and 4 were added to the basal ration #3 at a rate of 15% equivalent of alfalfa leaf meal. Lot 5 was given the basal ration #3 plus 2% 1:20 liver powder. The liver powder was included so as to give a positive control. Lot 6 was fed basal ration #2 plus anthranilic acid at the rate of 40 mg. per kilogram of ration. This was used at the suggestion of Green (1947) who supplied information to the effect that it had proven partially successful in stimulating human lactation.

The alfalfa leaf meal fractions were prepared as follows: 1200 grams of alfalfa leaf meal (Valdo Dehydrated) were extracted with 70% ethyl alcohol. The first extraction was done with 5700 cc. of alcohol and the second with 2400 cc. of alcohol. The material was then filtered completely by suction and extracted again. The mixture was heated almost to boiling before each extraction. A second 1300 gram portion of the alfalfa leaf meal was extracted in the same manner as the first and the two extracts were combined. The combined extracts were concentrated under reduced pressure on a steam bath until the material was a thick syrup. 2500 cc. of absolute alcohol was added and the mixture stirred repeatedly. It was then allowed to stand overnight in a refrigerator.

The alcohol soluble material was decanted from the residue. The residue was a thick material, similar in appearance and consistency of that observed from the liver extraction in the previous experiments. 500 cc. of absolute alcohol was added and it was allowed to stand overnight in a refrigerator. It was then extracted repeatedly with 500 cc. portions of 95% ethyl alcohol. Onehalf of this alcohol insoluble fraction was dissolved in two liters of water and was added to the basal ration of Lot 3.

The alcohol soluble material from the first extraction was reduced to a thick mass under reduced pressure. This material was then dissolved in 95% ethyl alcohol and was combined with the last extractions of the alcohol insoluble fraction. This solution was made to a volume of 2500 cc. so that each cc. would equal one gram of the alfalfa leaf meal. This fraction was then added to the basal ration of Lot 4.

Males from the stock colony were placed with the females when they were approximately 14 weeks of age. The males were rotated in the same manner as in the preceding experiments. The females were weighed weekly, at the time of parturition and at the time of weaning the young. The manner in which the females were handled during the reproduction and lactation period was the same as in Experiments I, IA and II, except that cane pomace was substituted for wood shavings for litter.

The young were weaned at 21 days of age and the individual weaning weights of the young recorded. Immediately after the young were weaned, the females were destroyed.

Results:

The results of Experiment III are summarized in Table VI. These data were analyzed in the same manner as in Experiments I, IA and II. In this experiment, there was a much higher rate of sterility among the females than was encountered in any of the preceding experiments. One noticeable feature, however, was the fact that of the number of litters born, there was an equal number of litters weaned in all lots. This showed that all of the females which gave birth to young were capable of weaning at least part of their litter. In this experiment there were very few young dead at birth and a very low mortality of young during the first forty-eight hours. Also, the much heavier weaning weights obtained in this experiment than in any of the others indicated that the weaning weights in the previous experiments were not optimum even when liver powder was included in the ration. The new basal ration also gave much superior results to those obtained previously when the ration included 5% alfalfa meal, calcium carbonate and salt. This might be explained on the basis of the more adequate salts mixture used in this experiment over those used in the previous experiments. There were no statistically significant differences between the weaning weights of the young of those females fed basal ration #3 and those of females which received various supplements to basal ration #3.

The young from the females in Lot 5 were much heavier at weaning than were any obtained previously and heavier than those of the lots receiving alfalfa meal or alcoholic fractions of alfalfa meal. This difference was significant at the 1% level. The performance of the lot receiving 15% alfalfa meal was the poorest of the whole experiment. There was a high rate of sterility in the females and the young were very small at weaning. It was observed that these females did not make as rapid growth as those in the other lots and were smaller at the time they reached breeding age. The females in the lots which received the fractions of alfalfa meal weaned young which were heavier than those receiving the 15% alfalfa meal. This difference was statistically significant at the 1% level of probability. However, there was no statistically significant difference between the weaning weights of the young of Lots 3 and 4. There was a much higher rate of sterility, however, in the lot receiving 95% alcohol soluble fraction (Lot 4). From this experiment no conclusions could be reached as to the solubility in alcohol of the active factor in alfalfa.

From the results of this experiment, it would appear that anthranilic acid was of no benefit for reproduction and lactation. There was a high per-

## TABLE VI

Experiment III — Reproduction and Lactation Data

| Lot No. | Rations | No. Females | % Conception | Resorption and Toxemia Deaths | No. of Litters Born | No. of Litters Weaned | No. of Live Young at Birth | No. of Dead Young at Birth | No. of Young Dead or Eaten First 48 Hours after Birth | No. of Young Given Females to Raise | No. of Young Weaned of Those Given Females to Raise | % Young Weaned of those Given Females to Raise | Av. Weaning Weight (Grams) of Young at 21 days |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | Basal #3 | 7 | 85.7 | 0 | 6 | 6 | 50 | 0 | 3 | 35 | 28 | 80.00 | 37.14 |
| 2. | Basal #3 + 15% alfalfa meal | 7 | 57.1 | 0 | 4 | 4 | 26 | 2 | 3 | 20 | 19 | 95.00 | 27.05 |
| 3. | Basal #3 + 15% equiv. alf. insol. fraction | 5 | 80.0 | 0 | 4 | 4 | 32 | 2 | 1 | 22 | 18 | 81.82 | 39.44 |
| 4. | Basal #3 + 15% equiv. alf. sol. fraction | 6 | 50.0 | 0 | 3 | 3 | 26 | 1 | 1 | 18 | 16 | 88.89 | 38.06 |
| 5. | Basal #3 + 2% 1:20 L.P. | 7 | 85.7 | 0 | 6 | 6 | 52 | 0 | 0 | 33 | 33 | 100.00 | 42.88 |
| 6. | Basal #2 + anthranilic acid | 7 | 57.1 | 0 | 4 | 4 | 33 | 0 | 1 | 22 | 21 | 95.45 | 31.76 |

centage of sterility and also rather low weaning weights of the young on the lot receiving anthranilic acid. The weight of the young at weaning was the same as that which would have been expected from females fed basal ration #2 without the addition of anthranilic acid.

## DISCUSSION

The results which were obtained in the experiments, using a corn-soybean oil meal ration and the known vitamins, confirm, in general, the observations of other workers. In Experiment I, there was a high mortality of young during the first forty-eight hours and a low percentage of young weaned from females fed an unsupplemented corn-soybean oil meal ration. Many of the young that died within forty-eight hours after parturition were found to have milk in their stomachs. The weaning weights were also subnormal. These results would confirm the work of Spitzer (1947) and others. In Experiment II, the liveability and weaning weights of the young of females fed such a ration were higher than in Experiment I, but the females were about 5 weeks older at the time of breeding. This would suggest that either the requirement of the active factor is less for the older females or that there was a greater storage of the factor by these females.

The addition of Wilson's 1:20 liver powder to rations containing corn-soybean oil meal gave the most consistently good results of any supplement added. In Experiments I and IA, alcoholic fractions of liver powder indicated that the active factor was readily soluble in 70% ethyl alcohol but less soluble in 95% ethyl alcohol. These findings are in agreement with those reported by Zucker and Zucker (1948). However, the deficiency symptoms which these workers observed occurred primarily after the 28th day of age. The greatest death loss of young observed in the experiments reported in this paper occurred during the first forty-eight hours.

The results of treating the alcoholic fraction of liver powder soluble in 70% ethyl alcohol but insoluble in 95% ethyl alcohol with Norit indicated that the active factor was not readily adsorbed on Norit. The results of treating the 95% alcohol soluble fraction with Norit was quite variable. Subsequent data indicated that only a small amount of the active factor was present in the liver fraction soluble in 95% ethyl alcohol.

The injection of liver extract (Lederle) into females fed the corn-soybean oil meal ration increased the liveability and weaning weights of their young. An increase in growth rate of weanling rats had been observed from similar preparations by Jaffe' and Elvehjem (1947). The addition of fish solubles to a corn-soybean oil meal ration also increased the weaning weights of the young and reduced the sterility of the females fed such a ration as compared to those receiving the unsupplemented corn-soybean oil meal basal ration. This would confirm the work of Zucker and Zucker (1948) and others.

It was also observed that the reproduction and lactation factor was not present in vitamin free test casein. The females had large litters but the liveability of the young was very poor. Most of the young appeared to die of starvation suggesting that the ration was inadequate for lactation. Zucker and Zucker (1948) observed that the active factor for reproduction and lactation was present in crude casein but not in the purified casein. Other workers, using purified casein in their synthetic rations, have observed similar results. In view of the fact that these data indicate that a corn-casein ration was grossly inadequate for normal lactation, this ration might be more suitable for a basal assay ration for studying this problem than a corn-soybean oil meal ration. The data from this experiment suggest that soybean oil meal contains small amounts of the active factor or that soybean oil meal provided conditions for a more favorable intestinal flora for the possible synthesis of the factor required for successful lactation. In addition the requirement of the factor by females might be higher when fed a corn-casein ration than when fed a corn-soybean oil meal ration or that casein inhibits the availability of the factor. Shorb (1948) found that soybean oil meal contained some LLD factor activity and she postulated that this feed may contain some vitamin

$B_{12}$ which may be involved in chicken nutrition.

In Experiment III, the addition of 15% alfalfa meal to a corn-soybean oil meal basal ration depressed the weaning weights of the young and percentage conception of the females but increased the liveability of the young. There was very little difference in the weaning weights and liveability of the young from females fed the basal ration and those in which the alcoholic fractions of alfalfa meal were added. However, there was a higher percent of sterile females in the lot receiving the alfalfa fraction soluble in 95% ethyl alcohol. The increased liveability of the young observed with the addition of 15% alfalfa meal to the basal ration would confirm the results of Bowland, et al (1948). The high incidence of sterility would be in direct disagreement with the results obtained by these same workers. Many other workers have reported decreased sterility and improved reproduction and lactation with alfalfa meal at this same level in both swine and rats. This failure may have been due to the quality of the alfalfa meal, but from all outward appearances, it was of the highest quality. Part of the cause for the depressing effect of alfalfa meal on weaning weight of young from females fed this supplement may have been due to a lower caloric intake than in the basal corn-soybean oil meal ration. Since these results disagreed with so much of the previous work that has been reported, both with swine and rats, it should be repeated under these same conditions by using a larger number of females.

There was no consistent gain or loss of weight by the females during the lactation period in any of the lots in any of the experiments. There was as much variation within lots as there was among lots. The results indicated that the gain or loss in weight of the lactating female is not a good measure of the ability of a ration to support normal lactation as reported by Nelson and Evans (1947b).

Several other properties of the active factor of 1:20 liver concentrate powder were noted. It is soluble in water because the original liver powder is a water soluble material. It was stable to heating at a temperature of 50-60° C. It was also stable to mild acid and alkaline conditions. It was stable to oxidation when exposed to air. These properties are the same as those reported by Novak and Hauge (1948a), Zucker and Zucker (1948) and Jaffe' and Elvehjem (1947). These properties are also in agreement with the properties of the chick growth factor from fish solubles reported by Roblee, et al, (1948) but are somewhat different from the properties of the chick growth factor in cow manure as reported by Rubin and Bird (1946c). This suggests that there may be a group of related factors rather than one single factor.

## SUMMARY

1. A basal ration composed of ground yellow corn 68.85%, soybean oil meal 25.00%, alfalfa meal 5.00%, $CaCO_3$ 0.65% and iodized salt 0.50% supplemented with cod-liver oil, alpha tocopherol and all the known B-complex vitamins failed to support normal reproduction and lactation in the rat in Experiment I but the performance of females fed this ration was improved in Experiment IA and II.

2. The addition of 2% 1:20 liver powder to a corn-soybean oil meal ration resulted in apparently normal reproduction and lactation in all experiments.

3. Of the alcoholic fractions of 1:20 liver powder which were tested, it appears that this unknown factor is readily soluble in 70% ethyl alcohol, less soluble in 95% ethyl alcohol and is not completely adsorbed on Norit.

4. Additional properties of the active factor in liver powder are that it is soluble in water, stable to moderate heating (50-60° C.), stable to mild acid and alkaline conditions and stable to oxidation when exposed to air.

5. The injection of liver extract (Lederle) increased the liveability and weaning weights of the young from females fed a corn-soybean oil meal basal ration.

6. Substituting vitamin free test casein for soybean oil meal in the basal ration supplemented with cod-liver oil, alpha tocopherol and all the known B-complex vitamins resulted in almost complete lactation failure. This ration may be a more suitable assay ration for studying this problem than a corn-soybean oil meal ration.

7. Substituting fish solubles for part of the soybean oil meal in the basal ration resulted in improved lactation performance as evidenced by increased weaning weights of the young.

8. The addition of 15% alfalfa meal to a basal ration composed of ground

yellow corn 73.50%, soybean oil meal 25.00%, minerals 1.50%, supplemented with cod-liver oil, alpha tocopherol and all the known B-complex vitamins failed to support normal reproduction and lactation.

9. Anthranilic acid was apparently not the active factor needed for optimum reproduction and lactation in the rat according to results of the work reported herein.

10. The data from these experiments indicate that the weight change of the lactating female is not a good measure of the adequacy of the ration for optimum lactation.

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