#### THE INFLUENCE OF RIBOFLAVIN INTAKE ON THE RIBOFLAVIN CONTENT OF SOW'S MILK AND THE PERFORMANCE OF SOWS AND THEIR PIGS

By

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Thesis Approved: Thesis Adviser

Dean of the Graduate School

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#### INTRODUCTION

In mammals the new-born depends, for varying periods of time, almost entirely on the secretion from the mammary gland for food. Colostrum is of utmost importance for rapid growing, new born-animals because it represents the sole source of nutrients for the first few days of life and milk is a principal source of nutrients during the remainder of the lactation period.

The major constituents of colostrum and milk from swine have been determined. However, these investigations have provided little information concerning the B-complex vitamin content of colostrum and milk. A more complete knowledge of sow's milk is needed to give a fuller understanding of the nutritional requirements of the young suckling pig and to aid in formulating suitable milk replacer rations. Despite this need, only fragmentary data have been published concerning the effect of varying dietary levels of riboflavin upon colostrum and milk riboflavin and the manifestations of these levels in the performance of the offspring. It is important to establish this relationship in order to measure the adequacy of a given gestation-lactation ration. This is of even greater importance today as greater emphasis is placed on confinement rearing of gilts and sows. Lush pasture is an excellent source of riboflavin. but as swine producers change more and more toward total confinement, less advantage is taken of this nutrient source. Concentrates commonly used such as milo, corn, barley and certain of their by-products are

relatively low in riboflavin and thus gestation-lactation rations based on these feeds could be border line or low in relation to requirements.

Since the specific-pathogen-free (SPF) pig program requires the removal of the newborn pig from the dam immediately following delivery, a problem of raising the pig without benefit of colostrum has been created. Information on the B-vitamins in colostrum and milk affords a basis for the preparation of milk substitutes in non-maternal rearing of baby pigs, especially for the critical first few days of life.

This investigation was designed to study the reproduction and lactation performances of gilts confined to concrete pens and fed a plant protein ration containing two levels of riboflavin and to correlate the riboflavin content of colostrum and milk with the performance of the nursing pigs.

#### REVIEW OF LITERATURE

#### History and Chemical Nature

The chemical nature of the water-soluble, yellow-green fluroescent pigment of whey now referred to as riboflavin (synonymous with lactoflavin, vitamin G and vitamin  $B_2$ ) commanded the attention of chemists in the 19th century (Blyth, 1879). No unusual significance was associated with this pigment by early workers, who apparently regarded it only as one of the minor constituents of milk, the chemical nature of which was quite obscure. The nutritional studies of Osborne and Mendel (1913) showed that milk contained a water-soluble accessory factor necessary for growth. The recognition of the paramount biological importance of this substance did not come until Warburg and Christian discovered the first flavo-protein in 1932. This was followed almost immediately by the isolation of riboflavin and the recognition of its growth promoting properties.

In 1935 the structure of riboflavin was proven to be 6,7-dimethyl-9 (d-l'-ribityl)-isoalloxazine.



In its pure state, riboflavin is a bitter-tasting, orange-yellow, odorless compound in which the crystals are needle-shaped. This vitamin is quickly decomposed by ultraviolet rays and visible light. It is stable to heat, to oxidizing agents, and to acids; but it is sensitive to the effects of alkali. It dissolves sparingly in water to give a characteristic yellow-green fluorescence when exposed to ultraviolet light (Maynard and Loosli, 1962). This typical fluoresence is dependent upon the presence of a free 3-imino group, and neither 3-substituted riboflavin nor flavin-adenin-dinucleotide (FAD) will fluoresce.

#### Functions

Riboflavin is very widely distributed throughout the entire animal and plant kingdom and is present in all tissue cells. The highest body concentrations occur in the liver and kidneys (Scheid <u>et al.</u>, 1953).

Riboflavin participates in many oxidation-reduction reactions within the cell, its active forms being the phosphorylated, derivatives riboflavin-5'-phosphate (FMN, flavin mononucleotide) and flavin-adeninedinucleotide (FAD, FMN linked to adenylic acid through their respective phosphoryl groups). Each enzyme system contains a specific protein (apoenzyme) and a prosthetic group (coenzyme) with riboflavin, FMN or FAD, as a component of the latter (Best and Taylor, 1961). By combining with different proteins, the two coenzymes form a variety of enzyme systems, each capable of carrying out a specific reaction. These enzymes are called flavoproteins and serve as bridges over which hydrogen atoms pass from one molecule to another (Giese, 1963). Some important enzyme

#### TABLE I

#### SOME RIBOFLAVIN (FLAVOPROTEIN) ENZYME SYSTEMS

Name of riboflavin enzyme system

Aldehyde oxidase D-Amino acid oxidase L-Amino acid oxidase Diaphorase Cytochrome reductase Xanthine oxidase Succinic dehydrogenase

#### Substrate acted upon

Aldehyde D-Amino acid L-Amino acid Nicotinamide co-enzyme Cytochrome Hypoxanthine Succinic

Thus, riboflavin is an essential link in the metabolism of amino acids, fatty acids, and carbohydrates; it serves in the utilization of food for energy and in the synthesis of body substances. The unusual and serious nature of the manifestations of severe riboflavin deficiency in animals may well be due to the key position occupied by these enzyme systems in life processes.

#### Assay

The riboflavin content of feeds may be determined by chick or rat assay methods, but the more rapid and accurate microbiological or chemical methods are now used extensively. The most commonly used microbiological method is based on the essential nature of riboflavin for the growth of Lactobacillus casei.

The chemical assay of riboflavin relies upon the fact that the fluorescence of this vitamin is proportional to its concentration under certain controlled conditions. Riboflavin is decomposed by exposure to light, both visible and ultraviolet. Since fluorescence disappears in solutions exposed to sunlight (Elvehjem and Koehn, 1935), both collection of samples and riboflavin determinations should be performed under controlled conditions of light.

Riboflavin of heated milk is higher than that of raw milk, probably due to riboflavin-protein complexes and FAD which break down on heating since it has been shown that there occurs a large increase in fluorescence when FAD is split to FMN or to free riboflavin (Bessey <u>et al</u>., 1949). For this reason, in fluorometric determination of total riboflavin, FAD must be hydrolyzed to FMN.

Modi and Owen (1956), using paper chromatography, demonstrated that practically all the riboflavin in sow's milk is in the form of FAD. In contrast, Nagasawa <u>et al</u>. (1962) stated that only 18.1 percent of the total riboflavin in cow's milk was FAD.

#### Adequacy of Rations

Rapid advancements in the vitamin field have been made during the last 15-20 years. It is well recognized that vitamins are as important as amino acids, carbohydrates, minerals and other nutrients. Thus, unless a ration contains the proper amounts of the various necessary vitamins, it is inadequate. Riboflavin is apparently needed by all animals and certain micro-organisms. Some animals apparently need no dietary riboflavin or only a very small amount in their food. Micro-organisms which synthesize this vitamin live in the rumen of sheep (McElroy and Goss, 1939) and of cattle (McElroy and Goss, 1940<sub>b</sub>) and make the vitamin available to the animal. Omnivores like the pig must be provided with regular dietary supplies. There may be some synthesis of riboflavin in the gastro-intestinal tract of the pig, but the extent to which

absorption and utilization of the synthesized vitamin takes place is not known.

Riboflavin is one of the known vitamins which is most apt to be lacking in many common swine rations, particularly in legume-free diets. Rations fed in dry-lot need to be more nutritionally complete than those fed on pasture. It is a common recommendation to include ten percent or more of some legume in dry-lot breeding and gestation rations for swine. In view of the known vitamin content of legumes (alfalfa) and the common recommendation that it be included in dry-lot rations, most swine diets provide at least the minimum amounts of riboflavin needed for growth and reproduction. The riboflavin requirements of young pigs and lactating sows can apparently be met by allowing them good pasture or by the addition of ten percent alfalfa meal to dry-lot rations. The increased performance when alfalfa is included in the diet has been repeatedly demonstrated (Freeman, 1938; Hogan and Johnson, 1941; Cunha <u>et al.</u>, 1944; Fairbanks <u>et al.</u>, 1945; Teague, 1955; Seerley and Wahlstrom, 1965).

Riboflavin is present in the free state in feeds, or in combination with phosphate, or with protein and phosphate. It is utilized in all these forms. The riboflavin content of several commonly used swine ration ingredients is shown in Table II. Riboflavin is abundant in milk, especially the skim milk and whey fractions. The value of dairy byproducts such as whey and buttermilk in creep feeds is probably due in part to their high riboflavin content. Alfalfa hay, pasture and other green leafy forages are also important sources. Such commonly used concentrates as corn and milo and certain of their by-products are relatively low in riboflavin. Oil meals are better sources, as are certain meat byproducts. Fats and oils are practically devoid of this vitamin.

#### TABLE II

Milligrams Riboflavin per Pound of Feed Feedstuff 0 1 2 3 4 5 6 7 8 9 10 11 12 12.0 Buttermilk (dried) 19.5 Whey Skim Milk 9.1 Alfalfa Meal (17%) 7.0 Fish Meal (60%) 2.2 Meat and Bone Scraps (50%) 12.0 S.B.O.M. (50%) 14.4 Tankage 1.0 ]0.B Barley Oats 10.B Corn 10.6 Milo 70.6 ]0.5 Wheat Recommended allowance for gilts and sows (1.5 mgs./lb. ration).

### RIBOFLAVIN CONTENT OF CERTAIN FEEDSTUFFS(a)

(a) Sievert, 1960.

#### Deficiency Symptoms

Riboflavin, like most vitamins, is needed for growth, and a deficiency affects tissues that are of ectodermal origin. A rather wide variety of manifestations of riboflavin deficiency has been reported in experimental animals. In view of the basic biochemical roles of the vitamin in the release of food energy and the assimulation of food nutrienty, it is understandable why a deficiency is reflected in a wide variety of symptoms. However, a deficiency causes one general symptom common in all animal species, retardation of growth of all young. Reproduction and lactation failures also result. Lack of riboflavin in the young arrests growth and finally results in death in both young and old animals.

Riboflavin is known to be a nutritional essential for growth and normal health of the pig (Hughes, 1939; Patek <u>et al.</u>, 1941). Wintrobe <u>et al.</u> (1938) learned that symptioms of a vitamin B-complex deficiency in suckling pigs could be temporarily relieved by the administration of crystalline riboflavin. Hughes (1938, 1939) and Terrill <u>et al.</u> (1955) made similar observations on pigs of a more advanced age. Wintrobe (1939) reported some growth response when suckling pigs on a vitamin deficient ration were given riboflavin.

Using a diet which was probably inadequate in other factors as well as riboflavin, Hughes (1939) noted impaired growth, diarrhea, and an abnormal gait. Patek <u>et al.</u> (1941) observed impaired growth, a decline of body temperature, cloudiness of the cornea, changes in the skin, hair and hoofs, and a terminal collapse associated with hypoglycemia. The cloudiness of the cornea cleared partially after administration of riboflavin. A dense central opacity in the optical media was observed

following clearing of the cornea. Its location was not definitely defined as being in the lens, although Patek and his associates referred to this opacity as resembling a cataract. A "flat-footed" gait was noted which was attributed to muscle weakness or tendon relaxation. There was no loss of appetite or diarrhea, and there were no neurological symptoms until the onset of collapse.

Lehrer and Wiese (1952) reported that signs of a nutritional deficiency became apparent within one to two weeks following placing of two day old pigs on a synthetic milk containing no riboflavin. They observed growth impairment; rough, dry and thin hair coats; skin eruptions together with scaling and ulceration; lens opacities; and light sensitivity. As the trials progressed, diarrhea and scours, prevalent in all deficient animals, became very severe and in some cases ultimately resulted in rectal hemorraging and inflammation of the anus. After four weeks on the experimental diet, the gaits of the pigs appeared stiff, mincing, and hesitant; at times they walked on the tips of the hoofs. These findings coincide with the report of Wintrobe et al. (1944).

Miller <u>et al</u>. (1954), using a synthetic milk diet low in riboflavin, presented detailed studies of deficiency signs in the suckling pig. These included poor growth, loss of appetite, a heavy sebaceous exudate about the eyes and ears, cataracts in the lenses and various histologic changes.

A total of 65 growing pigs fed semi-synthetic diets in three separate experiments were used in riboflavin studies by Terrill <u>et al</u>. (1955). The pigs were reared on wire screen-bottomed cages and started on experiment after a one- or two-week depletion period. Signs of riboflavin deficiency, which appeared within a few weeks, included poor growth,

lowered feed consumption, coarse hair coats, rough skins and increased irritability and hyperactivity, confirming earlier findings. In addition, the deficient pigs had a considerable degree of scours after the first two weeks of the test. No differences were seen in the blood picture of the deficient pigs as compared with the positive control pigs, confirming the studies of Forbes and Haines (1952).

Wintrobe <u>et al</u>. (1942) failed to produce a riboflavin deficiency in the pig. This can be accounted for by the presence of riboflavin in the crude casein which formed 26.1 percent of the basal diet. The pigs in the above mentioned experiment probably received as much as 40 mcg. of riboflavin per kg. body weight through this medium. Wintrobe <u>et al</u>. (1944) repeated the above experiment, using vitamin-free casein. Pigs, started at 20 days of age on a diet lacking in riboflavin, showed signs of nutritional deficiency within two to three weeks. Appetites were maintained quite well, however. The failure to gain weight at a normal rate, in spite of good consumption of a diet which they had found to support good growth when supplemented with riboflavin, led Wintrobe and associates to postulate peer food utilization in riboflavin deficiency.

Lower efficiency of food utilization in riboflavin deficient rats has been attributed in part to incomplete oxidation of nutrients (Sure, 1941; Sure and Dichek, 1941).

Burch <u>et al</u>. (1960) suggested partial uncoupling of oxidative phosphorylation as a possible explanation of the 15-20 percent reduction in efficiency of food utilization exhibited by riboflavin deprived rats. They found that liver succinic dehydrogenase was decreased to approximately one-fourth of normal.

Lutz <u>et al.</u> (1956) reported there was some indication that nitrogen retention may be directly related to serum riboflavin levels in human subjects that were partially depleted of riboflavin.

Miller <u>et al</u>. (1964) reported that baby pigs reared on riboflavin deficient synthetic milk diets produced significantly lower body weight gains than pigs reared on nutritionally complete diets. Reduced actual weights of the thyroid, adrenals, kidneys, spleen, liver and heart resulted. Significant increases in relative thyroid weights were produced.

Scheid <u>et al</u>. (1953), working with swine, stated that the variations in tissue riboflavin potency appeared to be more markedly influenced by the vitamin intake than by the age of the animal.

A number of workers have reported significant decreases in the riboflavin content of tissues from experimental animals maintained on a riboflavin deficient diet (Axelrod <u>et al.</u>, 1940; Carlsson and Sherman, 1938). However, very little is known of the manner in which riboflavin deficiency produces the pathologic changes observed in the pig. The sudden collapse which occurs in severe deficiency in the pig is probably dependent on failure of important enzyme systems and hence failure of cellular respiration.

In a comprehensive study of the effect of injection of riboflavin into deficient rats, Burch and Combs (1956) observed a rapid synthesis of FAD in the liver. There was a large increase in glycolate and d-amino acid oxidase activity. Oxidases for glycine, xanthine and l-amino acids were regenerated less rapidly.

The speed with which riboflavin supplementation returns pigs to normal in severe deficiencies is striking. Lehrer and Wiese (1952) stated that supplementation of 1 to 1.5 mgs. of riboflavin per day for

16 days cured all external symptoms previously exhibited by 31 day old pigs raised on a synthetic milk diet devoid of riboflavin. However, a few days after discontinuing supplementation pigs exhibited a reoccurrence of deficiency symptoms.

It should be emphasized that a borderline deficiency may exist without the pig showing any of the symptoms mentioned above.

In summary, riboflavin deficient swine assume an unthrifty appearance and fail to gain weight rapidly. No well defined deficiency syndrome or disease with a long history, such as scurvy or beriberi is associated with a lack of riboflavin. At present the only apparent method of identifying a riboflavin deficiency is to cause a remission of the symptoms in question by riboflavin supplementation. In brief, the signs lack true specificity and can not be accepted as definite signs of a riboflavin shortage.

#### Requirement

In the reported studies on riboflavin requirements there has not been agreement in the interpretation of the results or in the values obtained for the riboflavin requirement. Most of these discrepancies may be explained by differences in experimental conditions, including age and breed of pig, types of ration and environment. In general, the riboflavin requirement of animals is related to body size and weight, to the amount of food ingested and to the ambient temperature.

Replacing the sow's milk in the diet of the baby pig has opened new areas of research in the evaluation of the riboflavin requirement of the newborn piglet. McRoberts and Hogan (1942) initiated feeding of an artificial milk, containing 3.2 mcg. riboflavin per ml., to piglets

at 36-48 hours post farrowing. In two of three trials the pigs survived only five or six weeks. However, these researchers did not speculate as to the reason for deaths.

Lehrer <u>et al</u>. (1949) stated that the addition of 0.65 mg. riboflavin per kg. of synthetic milk was successful in preventing deficiency symptoms in young suckling pigs.

Forbes and Haines (1952), using semi-synthetic milk diets, concluded that the riboflavin requirement of the baby pig in a warm environment (85° F.) lies between 1.5 and 2.0 mcg. per gm. of dry matter (0.68 and 0.91 mg. per lb. of feed dry matter).

In a similar study at Michigan State, Miller <u>et al.</u> (1954) reported that at an environmental temperature of 70° F., a level of 1.36 mg. of riboflavin per 1b. of feed gave optimum performance while a 0.43 mg. level was completely inadequate for growth. The authors stated that genetic and other experimental factors might explain the higher riboflavin requirement as compared with that reported by Forbes and Haines (1952).

Lehrer and Wiese (1952), in a study with eight baby pigs, suggested that the riboflavin requirement was near 3.0 mg. daily per 100 pounds of body weight (equivalent to about 1 mg. per pound of feed).

Studies with the growing pig indicate a lower riboflavin requirement than that for the younger suckling pig. Miller and Ellis (1951) used practical diets containing low levels of riboflavin. They concluded that the level of riboflavin required was between 0.55 and 0.83 mg. per pound of diet.

Hughes (1940) concluded that the minimum daily requirement for riboflavin in the young growing pig lies between 1 and 3 mg. per 100

pound body weight (22 and 66 mcg. per kg. body weight). Assuming that the 40 pound pig consumes 6.0 pounds of feed per 100 pounds of body weight daily, this would be equivalent to 0.17 to 0.5 mg. per pound of diet.

Van Poucke and Krider (1946) reported more rapid and efficient gains of weanling pigs fed 1.5 mg. riboflavin per pound of purified ration than when 0.5 or 0.75 mg. were fed.

Krider <u>et al</u>. (1949) reported the results of adding 0.5, 0.75 and 1.5 mg. of riboflavin per pound of a purified ration fed to 30 lb. pigs, and of adding 0.25, 0.5 and 1.0 mg. of riboflavin per pound of a ration of natural ingredients fed to 38 lb. pigs, so as to result in rations containing 1.43, 1.68 and 2.18 mg. of riboflavin per pound of complete diet. These workers concluded that 1.4 mg. of riboflavin per pound of ration appeared to be the practical minimum level for weanling pigs in dry-lot.

Mitchell <u>et al</u>. (1950) have reported on the effect of change in environmental temperature on the riboflavin requirement of growing pigs. They demonstrated rather conclusively that the riboflavin requirement is inversely proportional to the environmental temperature. A level of 0.55 mg. per pound of feed was optimum in a warm environment ( $85^{\circ}$  F.) while a 1.59 mg. per pound level was needed in a cold environment ( $42^{\circ}$  F.).

Terrill <u>et al</u>. (1955), using purified diets, showed that the riboflavin requirement of the growing pig (29 to 39 lbs.) was between 0.40 and 0.65 mg. per pound of the diet when the mean environmental temperature was 53° F. The next lowest level of riboflavin tested was 0.4 mg., which was clearly suboptimal. Tests with vitamin  $B_{12}$  and chlortetracycline showed that these substances had no significant effect on the riboflavin requirement.

It has been assumed that gilts fed in dry-lot require all of the vitamins known to be needed by the pig during earlier growth. The ration the sow receives during gestation, as well as during lactation, will definitely affect the ability of the baby pig to survive and grow.

The necessity for riboflavin in the ration of pregnant sows has been demonstrated (Ensminger <u>et al.</u>, 1947). These workers observed that sows fed a riboflavin-deficient purified ration had poor appetites, made slow gains and farrowed abnormal pigs four to sixteen days prematurely. All pigs were either dead at birth or died within 48 hours thereafter. Abnormalities present were enlarged front legs, generalized edema and hairlessness.

Krider <u>et al</u>. (1948) offered evidence that the addition of fermentation solubles to the basal rations for sows, with an increase in the riboflavin content of the ration from 1.0 to 2.3 mg. per pound, significantly improved the gestation performance, although it did not eliminate the farrowing of weak or dead pigs.

The National Research Council Committee on Animal Nutrition (Hughes et al., 1950) recommended the riboflavin allowance of 0.80 mg. per pound of feed for both market and breeding stock.

The dietary riboflavin requirement for reproduction of swine has been studied by Miller <u>et al.</u> (1953). A total of 26 sows was fed practical rations containing four different levels of riboflavin (0.55, 0.83, 1.25 and 1.65 mg. per pound of feed). The lowest level, 0.55 mg., proved inadequate for the reproduction, resulting in death of three out of seven sows and in lack of ability to conceive in three other sows. The one sow which conceived farrowed an apparently normal litter, but the pigs survived less than 48 hours. Sows receiving the 0.83 mg. level of the

riboflavin showed symptoms of deficiency after the second litters were produced (reduction in birth weight of pigs, reduced viability and poor growth of suckling pigs). Normal reproduction was obtained with the addition of 1.25 mg. of riboflavin per pound of diet. No significant differences were observed in the condition of the sows during either gestation or lactation. It appeared that a riboflavin level that was critical for optimum reproduction did not adversely affect body weight and condition of the dams. These authors concluded that the 1.25 mg. level was near optimum for gestation and lactation performance.

The current recommended riboflavin allowance for breeding stock is 1.5 mg. per pound of feed (N.R.C. 1964).

Riboflavin thus has a profound effect on the animal organism, making possible growth in all its phases, e.g. tissue repair, adolescent growth, reproduction and lactation.

#### Colostrum and Milk Riboflavin

The secretion of the mammary gland which follows parturition is known as colostrum and it is several days before what is considered to be "normal" milk is secreted. It is difficult to say exactly when the transition occurs.

The importance of colostrum to the survival and well-being of young mammals has long been recognized. The first post partum secretion of the mammary gland differs markedly in chemical composition and biological properties from mature milk. The yellowish, slightly viscid fluid has a higher content of total solids, the components of which are not exactly the same as those of milk. Colostrum has nutritive values that are of particular importance for the newborn because of its rich content of

certain vitamins. Riboflavin is one of these vitamins. With respect to this vitamin the sow resembles the cow, goat and ewe, whose colostrum contains appreciably more riboflavin than does the milk content of colostrum.

Houston <u>et al</u>. (1940) reported cow colostrum to be three to four times higher in riboflavin than later milk. This agrees with the data presented by Pearson and Darnell (1946). Nagasawa <u>et al</u>. (1962) stated that cow colostrum contained from two to three times as much riboflavin as did mid-lactation milk.

The high riboflavin content of colostrum signifies that one of the functions of colostrum is to supply riboflavin to the newborn pig.

It has been adequately demonstrated in several species that riboflavin is secreted in colostrum and milk, increasing with increase dietary intake (Pearson and Darnell, 1946; Pratt and Hamil, 1951; Roderuck <u>et al.</u>, 1945; Catron <u>et al.</u>, 1948; Belavady, 1962). Everett (1948) reported that approximately 20 percent of riboflavin ingested by lactating animals appeared in the milk.

Pearson and Schweigert (1947) stated that oral administration of 2 gm. of riboflavin daily to goats and sheep increased the riboflavin content of the milk by approximately 26 percent.

McElroy and Goss  $(1940_a)$  observed that the output of riboflavin in cow's milk was frequently up to ten times greater than the intake in the food, indicating the formation of riboflavin in the digestive tract of the ruminant.

Riboflavin concentration in cow's milk shows considerable seasonal variation. Evidence indicates that the variation is due to diet (pasture). In winter it tends to fall because autumn grass and hay are

relatively poor sources of the vitamin. Hand and Sharp (1939) found that summer milk contained approximately 20 percent more riboflavin than winter milk.

Kon (1940) in a review article stated that fluorimetric assays show that there is a seasonal variation in the riboflavin content of milk which in summer contains some 20-50 percent more of the vitamin than in winter.

Johnson <u>et al</u>. (1941) found a decrease in the riboflavin content of milk from 0.18 to 0.13 mg. per 100 ml. when cows were transferred from pasture to artificial feeds.

Cows having access in winter to phosphoric acid grass silage maintained a high milk riboflavin level throughout the year (Hand and Sharp, 1939).

Kramer <u>et al</u>. (1939) in a study of the riboflavin content of milk of several dairy breeds maintained on a very dry pasture during a drought found that after subsequent rains the same pasture, which then supplied green grass, enabled the cows to produce milk 25 percent higher in riboflavin. The beneficial effect of green pasture on milk riboflavin has been noted by other workers (Houston <u>et al.</u>, 1940 and Kiermeier and Haisch, 1961).

Holmes and Holmes (1943) utilized five breeds of dairy cattle in determining the uniformity of riboflavin content of milk produced during the winter months of December through March, inclusive. Observed differences were very small and nonsignificant.

The riboflavin content of colostrum and milk from several farm animals has been studied and the results of a number of these investigations are shown in Table III. It should be emphasized that the figures for individual animals may vary widely from these values. In compiling

#### TABLE III

THE RIBOFLAVIN CONTENT OF COLOSTRUM AND MILK OF DIFFERENT SPECIES

# ,

Spectro	[] []	Riboflavin Concer	Boforonce		
Phectes	Average	Range	Average	Range	Vet el ence
Ewe	20.08	<u>/#CR.7945,777.046</u> ,077.000,000,000,000,000,000,000,000,000	4.36		Pearson & Darnell (1946)
Ewe			-	1.53-2.74	Oracova & Corner (1960)
Ewe	· 4		5.55	5.40-5.70	Pearson & Schweigert (1947)
Ewe			7.25	6.70-7.80	Pearson & Schweigert (1947)
Goat			3.17	2.40-3.70	Pearson & Schweigert (1947)
Goat			3.83	3.50-4.30	Pearson & Schweigert (1947)
Cow					1. J. B. B.
Jersey	5.74	3.20-8.01			Luecke et al. (1947)
Holstein	6.00	2.87-9.00			Luecke et al. (1947)
Beef	4.98	3.22-6.75			Luecke et al. (1947)
Cow	5.10	4.20-6.70	2.10	1.70-2.40	Kramer et al. (1938)
Cow		3.50-7.80		1.10-1.50	Houston et al. (1940)
Cow		2.10-8.80		1.45-3.25	Kiermier & Haisch (1961)
Corr			1.47	1.13-1.75	Holmes & Holmes (1943)
Cour			2.04	1.21-2.54	Causeret (1959)
Cow			2.20	0.60-3.42	Hand & Sharp (1939)
Cow			1 00	0.73-1.04	Modi <u>et al</u> . (1959)
Cow	6.10		1.70		Nagasawa <u>et al</u> . (1962)
<b>v</b> , <b>v</b> n			1.7		Fearson & Darnell (1946)

a table showing riboflavin concentration of such variable biological products as colostrum and milk, values should be presented together with information regarding such factors as diet, stage of lactation, breed, number of lactations and assay method. The data presented are useful, nevertheless, to point out differences which exist within and between the species.

#### Sow Colostrum and Milk

From the standpoint of baby pig nutrition, the first few days of life are the most critical because this is the period when the newborn pig receives colostrum. Until fairly recently it has not been possible to obtain satisfactory milk samples from sows for chemical analysis. The stimulatory effect of injections of the oxytocic principle of the pituitary, however, has provided a means whereby representative samples of the whole milk can readily be obtained. Using such a technique, Braude <u>et al</u>. (1947) were able to obtain samples adequate for analysis, and the results of their investigations constitute the first truly reliable information on the composition of the milk of this species following a complete milking.

Milk production in swine has since been studied extensively both from the standpoint of chemical composition and yield. The composition of sow colostrum has been studied by Heidebrecht <u>et al.</u> (1950), Sheffy <u>et al</u>. (1952) and Braude <u>et al</u>. (1947) and is shown in Table IV.

The composition of sow milk has been studied by Braude <u>et al.</u> (1947) and Heidebrechet <u>et al.</u> (1950) and is shown in Table 5.

#### TABLE IV

Component	Percent
Total solids	25.76
Solids-not-fat	21.33
Fat	4.43
Protein	17.77
Lactose	3.46
Ash	0.63
Calcium	0.05
Phosphorous	0.08

#### COMPOSITION OF SOW COLOSTRUM

#### TABLE V

#### COMPOSITION OF SOW MILK

Composition	Percent
Total solids	19.89
Fat	8.25
Solids-not-fat	11.64
Protein	5.79
Lactose	4.81
Ash	0.94
Calcium	0.25
Phosphorous	0.16
Vitamin A (mcg./100 ml.)	22.00
Vitamin C (mg./100 ml.)	11.00
Thiamine (mcg./ml.)	0.60
Niacin (mcg./ml.)	7.00
Panothenic acid (mcg./ml.)	2.90
Riboflavin (mcg./ml.)	2.20

Only fragmentary data concerning the riboflavin composition of swine colostrum and milk were available prior to the extensive investigations of Braude <u>et al.</u> (1945-46). These workers stated that the colostrum riboflavin content of Large White sows averaged 45.6 mcg. per 100 ml., with extremes of 21 to 78 mcg. per 100 ml. They were admittedly surprised by such a relatively low concentration in the milk of an animal whose young grow at a very rapid rate. Their work showed no benefit was derived by suckling young from the addition of riboflavin to their milk diet. However, it should be pointed out that all sows were on pasture and probably the riboflavin content of the milk was adequate to meet the pig's requirement. Also, pigs were taking solid feed one week after riboflavin supplementation was initiated.

Braude <u>et al.</u> (1947) compared the riboflavin content of colostrum from sows farrowing in winter versus summer. Values of 27.1  $\pm$  3.1 and 62.6  $\pm$  13.7 mcg. riboflavin per 100 ml. of colostrum were reported for winter and summer, respectively. All colostrum samples (both winter and summer) averaged 44.9  $\pm$  5.7 mcg. per 100 ml. Riboflavin analysis of the milk of these same sows revealed values of 43.1  $\pm$  2.5 mcg. and 48.2  $\pm$  2.3 mcg. for winter and summer, respectively. All milk samples averaged 45.7  $\pm$  1.7. The observed deviation between summer and winter values was termed a seasonal difference. This difference, however, is apparently due to supplementation as summer sows were confined. This method of supplementation had a much greater effect on colostrum than on milk.

Davis <u>et al</u>. (1950) obtained milk samples from 34 sows of Hampshire, Duroc and Chester White breeding during spring and autumn farrowing periods. The sows were maintained in concrete pens on plant protein rations termed nutritionally adequate for reproduction and lactation. Microbiological tests revealed the following colostrum and milk riboflavin concentrations:

		Riboflavin C	(mcg./ml.)	
		sow spring farrow	sow autumn farrow	gilts autumn farrow
Colostrum Milk 5th 15th 55th	day day day	6.5 2.7 2.2 3.3	4.7 1.6 2.2 3.2	3.4 1.5 2.2 3.0

These riboflavin concentrations are much higher than those originally reported by the British group. In an attempt to resolve this obvious conflict in absolute riboflavin values, collaboration between the two groups of workers was undertaken. It was concluded that the extraction method used by Braude was unsuitable for sow's milk and that absolute values quoted by them were much too low.

Davis <u>et al.</u> (1951), using Hampshire, Berkshire and Chester White sows, stated that average colostrum riboflavin was  $5.0 \pm 2.7$  mcg. per ml., while values of  $2.1 \pm 0.9$ ,  $2.2 \pm 0.9$  and  $3.2 \pm 0.7$  mcg. per ml. were found for 5, 15 and 55 day post partum milk samples.

Luccke <u>et al</u>. (1947), using equal numbers of Chester White, Hampshire and Berkshire sows, observed an average sow colostrum riboflavin concentration of 4.00 mcg. per ml., with a range of 1.65 to 6.25 mcg. per ml. These sows were on rye pasture or a ten percent alfalfa hay diet. No information was presented on milk riboflavin values.

Catron <u>et al</u>. (1948) reported that the riboflavin content of sow's milk could be influenced by ration during either gestation or lactation.

Barnhart <u>et al</u>. (1954) found that the riboflavin content of Duroc sow's milk was increased when ten percent dehydrated alfalfa meal was added to a basal ration to increase dietary riboflavin from 0.73 to 2.23 mg. per pound. Average milk riboflavin from the control and supplemented group as determined by microbiological assay were: 2.70 and 2.15; 1.45 and 2.30; 1.08 and 1.60; and 1.45 and 2.20 mcg. per ml. for 2, 7, 21 and 56 day post partum samples. Supplementation increased the average riboflavin from all sampling periods by 23 percent. An elevation of dietary riboflavin from 0.84 to 2.34 mg. per pound of ration increased the average riboflavin from all sampling periods by 45.0 percent. All above increases, however, were no statistically significant. No differences were observed in the performance of the offspring. In another experiment, the addition of dehydrated alfalfa meal and meat and bone scraps to a basal ration, with a corresponding increase in dietary riboflavin from 1.35 to 3.89 mg. per pound, significantly increased the riboflavin content of the milk. Average riboflavin from all sampling periods was increased by 34.0 percent. No mention was made of litter performance.

Catron <u>et al</u>. (1948) could find no definite relationship between the riboflavin in the milk and the liveability or growth of the pigs when a basal ration was supplemented with alfalfa meal.

Barnhart <u>et al</u>. (1954) collected separate samples from each gland from each of four sows on the 54th and 56th days of lactation in an attempt to determine if there were any differences in the riboflavin content of milk from different glands of the same sow. Observed differences were small and nonsignificant. This is in agreement with the findings of Smith (1952).

#### Lactation Curve

It is important to review the lactation curve of swine. During the course of lactation, milk secretion increases to a maximum and then

declines; the maximum daily yield is usually about 30-40 percent greater than the average daily yield.

The capacity of sows to produce milk is apparently at its peak in two to three year old sows (Wells <u>et al.</u>, 1940), while within a given lactation the peak of production occurs between the third and fifth week (Pond <u>et al.</u>, 1962<sub>b</sub>; Smith, 1952; 1959; Lodge, 1959; Hartman and Pond, 1960; Hughes and Hart, 1935; Kovacs, 1955; and Lalevic, 1954). Allen and Lasley (1960) observed breed differences in the time at which this peak was reached. The peak was reached at the third week by Duroc and Landrace X Poland sows, the fourth week by Poland sows and in the fifth week by Landrace sows. Using equal numbers of Yorkshire and Berkshire sows, Pond <u>et al.</u> (1962<sub>a</sub>) found that the largest average milk yield from individual glands was obtained at three weeks after farrowing (74.6 gm.) and the second largest at four weeks after farrowing (59.0 gm.).

Hand and Sharp (1939) found a definite inverse relationship between milk riboflavin content and milk production in the cow. Their data yielded a correlation of -0.83. Since the square of the correlation ratio is 0.69 it may be concluded that approximated two-thirds of the factors other than feed which determine the riboflavin content of milk may be accounted for by changes in volume of milk produced. Thus, we might visualize a riboflavin concentration secretion curve as the inverse of total milk produced. This point is of particular importance when applied to swine, as there is a positive correlation between litter size and total milk production.

Johnson <u>et al</u>. (1941), using both cows and goats, observed an inverse relationship between the milk yield and the riboflavin concentration of the milk.

Pearson and Darnell <u>et al</u>. (1946) observed a sharp decrease in cow colostrum riboflavin by the first day after parturition. A slower decrease was apparent to day four, followed by a very slight drop through the last sampling period (day 30).

Kramer <u>et al</u>. (1938), working with dairy cattle, stated that in contrast to the drop in riboflavin content per gram of milk as a given lactation progressed, the total daily output of the vitamin per cow increased due to the increase in milk yield.

The literature indicates that there are also characteristic changes in sow milk riboflavin concentration over the course of the lactation period. The data of Davis <u>et al.</u> (1950) and Davis <u>et al.</u> (1951) indicate that riboflavin concentration decreased very markedly from colostrum to day five milk, decreased at a slower rate between day five and 15, then rose so that the 55 day value was approximately that observed for day five. The same general riboflavin secretion pattern was evident in the work of Barnhart <u>et al.</u> (1954). Minimum observed milk riboflavin concentrations occurred at day 21, with 56 day values equaling the values for day seven. It is thus obvious that difficulties exist when one uses only one sampling period in attempting to state the absolute milk riboflavin concentration.

#### General

In composition, the normal milk of the sow is more comparable to that of the ewe than of other mammals. In comparison with that of other farm animals, sow's colostrum and milk are apparently low in riboflavin. This low level of riboflavin in the milk of an animal whose young grow at a very rapid rate, doubling their weight in approximately ten days,

is puzzling. This tends to indicate that the relationship between the concentration of certain essential nutrients in the milk and the growth rate of the young as suggested by Pearson and Darnell (1946) may not always hold true. Although the newborn pig doubles its weight in a shorter time than does the newborn lamb, the concentration of riboflavin is apparently lower in the colostrum of the sow. This apparent anomaly could be accounted for by the relatively smaller size of the newborn pig in relation to the larger newborn lamb.

#### Summary

Despite the many gaps and the clear need for more systematic comparative studies, a general trend in colostrum and milk riboflavin content stands out. The work cited on milk riboflavin content indicates that riboflavin concentration, expressed as mcg. per ml. of milk, changes over the course of the lactation period so that apparent milk riboflavin values are inversely related to total milk production. The colostral secretion is high in riboflavin. This high level diminishes considerably during the first three to five days of lactation. During the next 20 to 30 days there is a small but steady fall, followed by a rise as the lactation comes to an end. With the normal decline in milk yield the concentration of riboflavin rises so that at weaning (56 days) the riboflavin values are similar to seven day determinations.

#### EXPERIMENTAL PROCEDURE

Twenty primary specific-pathogen\_free (SPF) gilts averaging approximately 302 lbs. and 10.2 months of age were obtained from the Oklahoma State University purebred Yorkshire herd. The gilts were allotted to either (1) a low riboflavin ration (basal) or (2) a high riboflavin ration (basal plus 2.51 mg. of riboflavin per pound of ration). The chemical composition of the feeds utilized in these rations is presented in Table VI. The composition of the dry-lot rations is shown in Table VII. The two diets fed were 15 percent crude protein, milo-soybean cil meal rations containing 0.94 versus 3.45 mg. riboflavin per pound of feed. The rations met National Research Council allowances for all other nutrients. The calculated average daily nutrient intake for each treatment group is shown in Table VIII. The levels of riboflavin were furnished at a given concentration in the dist rather than on an individual body weight basis. The treatment groups were balanced as nearly as possible with respect to litter, body weight and general appearance. Gilts that reoccurred in heat were rebred and allowed to remain in their treatment group.

The two treatment groups were maintained under similar conditions throughout the gestation and lactation periods. Each group of gilts was restricted to a pen (three gilts per pen) with concrete floors and was not on pasture at any time. The confinement pens had a 6 ft. by 15 ft. area under roof and an adjoining 6 ft. by 19 ft. area outside
# TABLE VI

# CHEMICAL ANALYSIS OF FEEDS USED IN ALL LACTATION RATIONS

	Percent Composition						Riboflavin
Feed	Dry Matter	Ash	Protein <sup>a</sup>	Fat	Crude Fiber	NFE	Content (mg./lb.) <sup>b</sup>
Milo, Western Yellow	89.31	1.63	7.70	3.17	2.00	75.07	0.84
Soybean Meal	91.01	6.71	50.25	3.11	2.07	28.87	1:55
Riboflavin Supplement		€0000000000000000000000000000000000000				<b>a</b> aa	17,500

30

<sup>a</sup>Calculated as N x 6.25.

<sup>b</sup>Riboflavin assays by Wisconsin Alumni Research Foundation, Madison, Wisconsin.

#### TABLE VII

Riboflav: High <sup>a</sup>	in Level Low <sup>b</sup>
К	<b>%</b>
80.07 17.68 0.30 1.30 0.50 37 gms. 12 gms. 0.22 gms. 0.09 gms. 6.5 gms.	80.07 17.68 0.30 1.30 0.50 37 gms. 12 gms. 0.22 gms. 0.09 gms.
lions	
15.01 77.30 0.60 0.40 6.00 17.80 5.10	15.01 77.30 0.60 0.40 6.00 17.80 5.10
	Riboflav: High <sup>a</sup> 80.07 17.68 0.30 1.30 0.50 37 gms. 12 gms. 0.22 gms. 0.09 gms. 6.5 gms. 100.00 ions 15.01 77.30 0.60 0.40 6.00 17.80 5.10 2.45

## COMPOSITION OF RIBOFLAVIN RATIONS

<sup>a</sup>This ration was formulated to contain 233 percent of N.R.C. riboflavin recommendation.

<sup>b</sup>This ration was formulated to contain 62.7 percent of N.R.C. riboflavin recommendation.

cProtein content chemically determined.

dContains 6 mg. of B12 activity per pound.

Contains 10,000 U.S.P. of A activity per gram.

<sup>f</sup>Contains 27,000 U.S.P. of D activity per gram.

SContains 95% pantothenic acid activity.

hContains 17.5 gm. riboflavin activity per pound, analysis by Wisconsin Alumni Research Foundation, Madison, Wisconsin.

#### TABLE VIII

#### CALCULATED AVERAGE DAILY NUTRIENT INTAKE DURING GESTATION

	Riboflav	vin Level
	High (3.45 mg./lb.)	Low ) (0.94 mg./lb.)
Av. amount fed (lbs.)	4.8	4.8
Protein (1bs.)	0.72	0.72
T.D.N. (1bs.)	3.71	3.71
Calcium (lbs.)	0.03	0.03
Phosphorus (lbs.)	0.02	0.02
Riboflavin (mg.)	16.56	4.51

the barn. Each pen was equipped with a mist type sprinkler which was turned on during days of hot weather. In winter a section under the roofed area was bedded. Water was available at all times from an automatic fountain in each pen. The gilts were housed and fed in pens of three or four and received approximately 4.8 lbs. of feed per head per day, fed in equal portions twice daily (a.m. and p.m.).

As each gilt reached 110 days of gestation she was weighed, taken to a central farrowing barn, placed in a farrowing crate and prepared for farrowing. Al gilts received routine management during partuition. Pigs were weighed, to the nearest one-tenth of a pound, at birth, at seven days and at weekly intervals for five additional weeks. At two and twentyone days of age all pigs received 100 mg. of iron in the form of a 1 ml. intra-muscular injection if iron-dextran. During the period from 110 days of gestation to 14 days post farrowing the gilts were hand fed twice daily on an individual basis in relation to their appetite and condition. The gilts were fed the same levels of riboflavin during lactation that were used for gestation. When the litter was 14 days old, mother and young were transferred to another pen where gilts were individually self-fed their respective rations until the pigs were weaned. The concrete pen area was 10 ft. by 10 ft. in the barn with a 10 ft. by 15 ft. concrete lot outside the barn where the self-feeder was located. Every effort was made to accurately measure the feed consumed. Each individual litter was permitted to run with its mother and allowed to suckle normally until weaning. No creep feed was allowed the pigs, however, they had access to the sow's ration. The litters were weaned at six weeks of age and the sows weighed and returned to their respective gestation regimen.

During the course of the lactation period, colostrum and milk samples were obtained for chemical analysis at parturition, day three and seven, and each subsequent week during lactation. Colostrum was obtained during or immediately following parturition by manual expression of the udder. To facilitate later milkings, pigs were removed from the sows approximately one to two hours prior to milking. No particular precautions were taken to standardize the length of time between milking and previous suckling. The sew was restrained by placing a running noose on the upper jaw, and approximately 20 U.S.P. units of pituitary exytocic principle<sup>1</sup> were injected into an ear vein to accomplish milk let-down. After a few seconds, milk was obtained from several teats by manual expression of the udder. No attempt was made to milk out the entire udder. After through stirring, a suitable aliquot (20-25 ml.) was taken from the 100-180 ml. sample, quick frozen, and maintained at -15° C. until analyzed for riboflavin. Precautions were taken to protect the samples against photochemical destruction of riboflavin.

<sup>1</sup>P.O.P., Armour Pharmaceutical Company, Kankakee, Illinois.

The above animals were maintained on their respective treatment levels and carried through two additional lactations. Only those sows which developed defects of unsoundnesses were culled. The sows were rebred at first heat after weaning. In this way, the cumulative effect of the riboflavin levels, including possible depletion of body reserves caused by lactation stress, was measured. The sows were cared for in the same manner as described above. The lactation periods, involving a total of 34 lactations, began in the summer of 1963 and terminated in the fall of 1964.

Response criteria employed to measure sow performance were the number of pigs farrowed, average birth weight of live pigs, number of pigs weaned, survival and growth rate of offspring during the six week suckling period and condition of gilts and sows during gestation and lactation as measured by body weight changes. Riboflavin concentration of colostrum and milk at various stages of lactation was determined.

Total riboflavin was determined fluorimetrically by the method of Bessey <u>et al.</u> (1947), with minor modifications. Standard errors and the "F" test were calculated according to the procedures set forth by Steel and Torrie (1960). Comparisons among means were analyzed by the LSD multiple range test.

#### RESULTS

The results section is divided into three parts according to lactation period.

#### First Lactation

The results of the colostrum and milk riboflavin analyses are summarized in Table IX. The riboflavin content of the mammary secretion on day zero and day three was higher (P $\lt$ .01) for the high level sows (10.92 vs. 7.44 mcg. per ml.). No significant difference was evident at day seven, but the high level sows were again higher (P $\lt$ .10) at days 14 and 21, and remained higher through the remainder of the lactation period.

Within riboflavin levels, there were characteristic colostrum and milk riboflavin changes over the course of the lactation period (Table IX). Maximum colostrum riboflavin values were observed at day three and day zero for high and low treatment groups, respectively. Regardless of the treatment imposed, day zero did not differ significantly from day three, but did differ (P < .01) from all other sampling periods. Lowest riboflavin concentrations were noted at day 28 for both treatments.

The colostrum and milk riboflavin data were subjected to analyses of variance. The mean squares for dietary riboflavin level and stage of lactation were both highly significant ( $P \lt.005$ ), indicating important differences in colostrum and milk riboflavin concentration between ration riboflavin levels and between the sampling periods examined in this study.

# TABLE IX

### RIBOFLAVIN CONTENT OF COLOSTRUM AND MILK OF SOWS FED TWO LEVELS OF RIBOFLAVIN: FIRST LACTATION

Riboflavin			Riboflav: Sta	in Concen <sup>®</sup> ge of Lac	tration (1 tation (d	ncg./ml.) avs)		· .	Significant
Levela	0	3	7	14	21	28	35	42	<b>Differences</b> <sup>0</sup>
High (H)b	10.92 (0.98)d	11.44 (1.29)	6.50 (0.22)	5.43 (0.22)	4.74 (0.17)	4.38 (0.09)	4.74 (0.14)	5.60 (0.29)	0>all higher than 3** 3>all others** 7>(21, 28, 35)** 28<42
Low (L) <sup>b</sup>	7.44 (0.62)	7.00 (0.36)	5.68 (0.24)	4.21 (0.25)	3.56 (0.23)	2.48 (0.03)	3.20 (0.14)	3.67 (0.19)	0>all higher than 3** 3>7*, all others** 7>14*, all others** 14>28** 21>28 28<42
Percent increase <sup>c</sup>	46.8	63.4	14.4	29.0	33.1	76.6	48.1	52.6	
Significant differences <sup>e</sup>	H>L**	H>L**	NS	H>L	H>L	H>L**	H>L*	H≯L*	

<sup>a</sup>See the Experimental Procedure for amount of riboflavin represented by each level.

bValues are an average of eight sows for High and nine for Low.

<sup>C</sup>Calculated as High minus Low, divided by Low.

dStandard error.

eP <.10; \*P <.05; \*\*P <.01; NS = nonsignificant.

Differences in riboflavin level and stage of lactation (days) accounted for 60.0 and 36.5 percent of the total variance, respectively. There was also a highly significant (P<.005) interaction of dietary riboflavin level and stage of lactation, indicating differences in response to treatments were not of the same order of magnitude from one sampling period to another.

The results of the colostrum and milk riboflavin analyses are plotted in Figure 1. A significant ( $P \lt.005$ ) deviation from linearity was apparent for both treatments. This indicates that a curved line fits the data better than a straight line; in other words, the change in riboflavin concentration for each additional increment of lactation is not constant. The average riboflavin content of colostrum and milk from all sows at a particular stage of lactation can be determined by fitting a second-degree polynomial. Regression equations were calculated for each of the two dietary riboflavin levels. The regression equations were as follows:

 $\hat{Y} = 0.4596X^2 - 3.644X + 11.243$  (High riboflavin)  $\hat{Y} = 0.2640X^2 - 2.251X + 7.6781$  (Low riboflavin)

where  $\widehat{\mathbf{Y}}$  is the colostrum or milk riboflavin level in mcg. per ml.,  $X^2$  is the square of X and X is the stage of lactation in weeks.

A common pattern is readily apparent for both treatment groups. These data indicate that milk riboflavin activity decreases markedly from day three to day seven, decreases at a slower rate between day 14 and day 28, then with the normal decline in milk yield rises so that the 42 day value is approximately that observed for day 14 in the case of high riboflavin and day 21 for the low dietary riboflavin level.





The performance of the sows is presented in Table X. The dietary riboflavin levels fed did not significantly affect conception rate, however, there was a tendency for the high riboflavin ration to promote a conception rate superior to that obtained with the basal diet. All sows on supplemental riboflavin conceived at first service while onethird of the low riboflavin sows required at least two services. No abnormal breeding behavior was observed in either treatment group.

No difference in incidence of farrowing difficulty between treatment groups was apparent. Vigor of the two groups of pigs at birth was very similar. Newborn pigs displayed no visible signs of a riboflavin deficiency.

There was no significant difference, due to dietary riboflavin level, in length of gestation, number of live and stillborn pigs farrowed per litter and average pig birth weight.

Pigs nursing high level sows were heavier at every weigh period, except at birth, up to weaning. The weaning weight of pigs nursing supplemented sows was 22.2 lbs., while pigs born to low riboflavin sows averaged 20.1 lbs.

The cumulative and by period growth rate of pigs from high and low riboflavin treated sows is summarized in Table XI. Pigs from high riboflavin sows had a significantly (P $\lt$ .10) higher average daily gain (0.38 vs. 0.27 lbs.) during the first week. This suggests that the higher colostrum riboflavin was beneficial and/or body stores of riboflavin in the low riboflavin pigs were inadequate in producing maximum growth response. Although growth rates during the remainder of the nursing period were not significantly different, higher rates of gain did occur in those pigs nursing sows receiving supplemental riboflavin, suggesting that the

## TABLE X

#### PERFORMANCE OF SOWS MAINTAINED ON TWO LEVELS OF DIETARY RIBOFLAVIN: FIRST LACTATION

	Hi	Riboflavi gh	n Level	a. DW
Av. days on treatment prior to farrowing No. sows bred No. sows retained on treatment	125.5 10 8 <sup>b</sup> ,	c	136.0 10 9b	
Av. no. services per litter farrowed Av. gestation length. days	1.0	$(0.0)^{d}$ (0.6)	1.67	(0.37) (0.7)
Av. no. pigs farrowed per litter Av. no. stillborn per litter	9.6	(0.96) (0.5)	8.7	(0.68) (0.67)
Av. litter birth wt., lbs. Av. no. weaned per litter	23.8	(3.10) (1.15)	24.3	(4.70) (0.83)
Av. pig wt., lbs. Birth	2.7	(0.08)	2.8	(0.05)
7 days 14 days	5.4 8.8	(0.15) (0.42)	4.7	(0.09) (0.15)
21 days 28 days	11.2	(0.44) (0.59)	9.8 12.9	(0.16)
35 days 42 days	18.4	(0.81) (0.93)	15.9	(0.38) (0.45)
Av. litter weaning wt., lbs. Av. pig survival, %	138.9 78.2	(17.16) (7.04)	140.2 85.6	(13.29 (10.93

<sup>a</sup>See the Experimental Procedure for amount of riboflavin.

<sup>b</sup>One sow failed to produce milk after farrowing and was not included in the results.

COne sow failed to conceive.

dStandard error.

eCalculated as pigs weaned of those born alive.

# TABLE XI

GROWTH	RATE	OF	PIGS	NURS:	ING	SOWS	FED	TWO	LEVELS
	OF	RIB	OFLA	VIN:	FIR	ST I	LACTAT	ION	

		Riboflavi High	n Level <sup>a</sup> Low	Difference High - Low
Av.	daily gain, lb. (Cumul 7 days 14 days 21 days 28 days 35 days 42 days	ative) .38 (.043) <sup>b</sup> .43 (.069) .40 (.048) .45 (.055) .45 (.048) .46 (.055)	.27 (.023) .34 (.025) .33 (.019) .36 (.022) .38 (.027) .41 (.029)	.11 <sup>c</sup> .09 .07 .09 .07 .05
Av.	daily gain, lb. (By per Birth - 7 days 8 - 14 days 15 - 21 days 22 - 28 days 29 - 35 days 36 - 42 days	riods) .38 (.043) .48 (.110) .34 (.052) .57 (.080) .46 (.087) .51 (.062)	.27 (.023) .41 (.037) .31 (.033) .45 (.067) .46 (.059) .56 (.057)	.11 <sup>€</sup> .07 .03 .12 .00 05

<sup>a</sup>See the Experimental Procedure for amount of riboflavin represented by each level. <sup>b</sup>Standard error.

CP4.10.

milk from the supplemented sows was more nutritionally adequate for the suckling pigs. When average daily gain by weekly periods is studied it is noted that by five weeks the growth rates of the pigs from both treatment groups were identical and that during the last week of lactation the low level pigs were outgaining the pigs on the high riboflavin The disappearance of the early advantage in daily gain of pigs SOWS. nursing supplemented sows may indicate a change in riboflavin requirement. Perhaps during the early growing stages the colostrum and milk riboflavin level of the low riboflavin gilts did not meet the requirements of the pig but as the pig continued to grow its riboflavin requirement decreased and thus the lower level of riboflavin appeared to be adequate for the latter stages of growth. This increased growth rate may be termed "compensatory growth" as the pigs tended to make up for the lowered growth rate exhibited during the earlier periods. Also, the overall superior weight gain of pigs from high riboflavin sows as compared to those from low riboflavin sows may also indicate that the body storage of riboflavin in the latter group was inadequate for maximum growth rate.

There was a greater incidence of scours and other digestive disturbances noted among the pigs in the first lactation than in either of the subsequent lactations. These scours were observed to occur mainly during the first two weeks of nursing. Litters with rough hair coats were noted in both treatment groups.

Body weight changes and feed consumption of the sows is summarized in Table XII. As is evident from the data presented therein, level of dietary riboflavin did not significantly affect average daily gain of the sows. However, high riboflavin sows were heavier at both farrowing and weaning.

#### TABLE XII

#### BODY WEIGHT CHANGES AND FEED CONSUMPTION OF SOWS FED TWO LEVELS OF RIBOFLAVIN: FIRST LACTATION

		Di Hi	ietary Ribof igh	lavin Le	evel <sup>a</sup> Low
Sow we: Ar Ar Ar Ar Ar Ar Ar Ar	ights, lbs. v. initial (I) v. at farrowing (F) v. gain, (I) to (F) v. daily gain, (I) to (F) v. at weaning (W) v. loss, (F) to (W) v. daily loss, (F) to (W) v. gain, (I) to (W)	307.7 423.7 116.1 1.0 361.7 62.7 1.50 54.0	(19.53) <sup>b</sup> (11.51) (13.18) (0.13) (8.87) (11.20) (0.84)	297.7 413.3 115.7 0.97 342.9 71.1 1.68 45.2	(14.25) (17.20) (19.07) (0.15) (15.48) (7.32) (0.57)
Feed Co A A A A A	onsumption, lbs. v. per sow, (I) to (F) v. per lb. of gain, (I) to (F) v. per sow, lactation <sup>C</sup> v. per sow daily, lactation	596.0 5.2 518.7 12.3	(86.77) (0.48) (38.01) <sup>d</sup> (0.91) <sup>d</sup>	652.8 6.3 611.5 14.5	(112.57) (1.10) (24.60) (0.59)

<sup>a</sup>See the Experimental Procedure for amount of riboflavin represented by each level.

<sup>b</sup>Standard error.

<sup>C</sup>Includes feed intake from 110 days of gestation until 42 days post farrowing.

dp**<**.10.

Feed efficiency was greater on the riboflavin supplemented ration, though not significantly so. Feed intake during lactation was significantly (P<.10) less for the high riboflavin diet (12.35 vs. 14.54 lbs.). Daily feed consumption during lactation was 15.06 percent higher for the low riboflavin sows. This elevation in feed consumption resulting from the deletion of supplemental riboflavin suggests that the somewhat lower performance observed on the low riboflavin ration cannot be attributed to palatability of the diet.

#### Second Lactation

The results of the colostrum and milk riboflavin analyses are presented in Table XIII. There were highly significant (P $\lt$ .005) differences in colostrum and milk riboflavin concentration between dietary riboflavin levels and between the sampling periods. Differences in riboflavin level and stage of lactation (days) accounted for 67.3 and 24.0 percent of the total variance, respectively. A highly significant (P $\lt$ .005) interaction between dietary riboflavin level and stage of lactation was observed. Therefore, these factors did not act independently of one another.

Riboflavin content of the mammary secretion on day zero and day three was higher (P $\lt$ .01) for the high level sows. Riboflavin content was also higher (P $\lt$ .05) at day seven and 21. The difference at day 14 and 28 was not significant, although high level sows were secreting a greater concentration of riboflavin. A highly significant (P $\lt$ .01) difference in milk riboflavin activity was again apparent at day 35 and day 42.

Within the high level, days zero and three were higher  $(P \leqslant .01)$  than all other sampling periods. Day seven was higher  $(P \leqslant .01)$  than

## TABLE XIII

## RIBOFLAVIN CONTENT OF COLOSTRUM AND MILK OF SOWS FED TWO LEVELS OF RIBOFLAVIN: SECOND LACTATION

Riboflavin			Riboflavi	Stage of Lectation (mcg./ml.)					Significant
Levela	0	3	7	14	21	28	35	42	Differencese
High (H)b	14.86 (0.83)d	8.64 (0.12)	6.69 (0.42)	5.07 (0.51)	4.82 (0.31)	4.21 (0.13)	5.56 (0.44)	5.92 (0.55)	0>all others** 3>all others** 7>(14, 21, 28)**, 35* 21<42* 28<35*, 42**
Low (L) <sup>b</sup>	6.61 (0.38)	6.08 (0.33)	5.30 (0.37)	4.17 (0.31)	3.58 (0.37)	3.40 (0.23)	3.40 (0.18)	3.66 (0.17)	0>7, 14*, all others** 3>all higher than 7** 7>14*, all others **
Percent increase <sup>c</sup>	124.8	42.1	26.2	21.6	34.6	23.8	63.5	61.7	
Significant differences <sup>e</sup>	H>L**	H>L**	H>L*	NS	H>L*	NS	H>L**	H>L**	

aSee the Experimental Procedure for amount of riboflavin represented by each level.

bValues are an average of four sows for High and eight for Low.

CCalculated as High minus Low, divided by Low.

dStandard error.

ep<.10; \*P<.05; \*\*P<.01; NS = nonsignificant.

days 14, 21, 35 and higher (P $\lt$ .05) than day 35. Within the low level, little difference was observed between day zero and day seven, however day zero was higher (P $\lt$ .01) than all sampling periods past day 14.

The colostrum and milk riboflavin results are plotted in Figure 2. In both treatment groups there was a significant ( $P \lt.005$ ) curvilinearity in the regression. The regression equations were as follows:

> $\hat{\mathbf{Y}} = 0.6199 X^2 - 4.6247 X + 12.2931$  (High riboflavin)  $\hat{\mathbf{Y}} = 0.1730 X^2 - 1.5232 X + 6.6333$  (Low riboflavin)

where  $\hat{\mathbf{Y}}$  is the colostrum or milk riboflavin level in mcg. per ml.,  $X^2$  is the square of X, and X is the stage of lactation in weeks. Maximum riboflavin concentration values were observed at day zero for both treatment groups. These data indicate that the riboflavin activity of colostrum from the high level sows decreased greatly during the first three days, less markedly during the next two sampling periods and reached a minimum at day 28. From that point, the riboflavin concentration increased.

In obvious contrast to the sharp decrease in colostrum riboflavin observed in the high level, low level concentrations decreased very slowly, reaching a minimum at days 28 and 35, then rising slightly so that the day 42 value was about that observed for day 21. Absolute riboflavin concentration differences between treatments tended to be greater during early and late lactation with smaller differences occurring in midlactation.

The performance of the sows is presented in Table XIV. Although the differences were not significant, low level sows farrowed more pigs, had heavier litters at birth and weaned more pigs per litter. Pigs farrowed by high level sows were heavier at every weight period. High level



Figure 2. Relationship between stage of lactation and riboflavin concentration in sow colostrum and milk: second lactation.

#### TABLE XIV

#### PERFORMANCE OF SOWS MAINTAINED ON TWO LEVELS OF DIETARY RIBOFLAVIN: SECOND LACTATION

		R: Higl	iboflavin 1	n Level <sup>a</sup> Lo	e DW
No. No. Av. Av. Av. Av. Av. Av.	sows bred sows retained on treatment no. services per litter farrowed gestation length, days no. pigs farrowed per litter no. stillborn per litter litter birth wt., lbs. no. weaned per litter pig wt., lbs.	4 4 1.75 112.0 8.5 0 25.0 7.75 <sup>c</sup>	(0.48) <sup>b</sup> (0.67) (1.85) (0) (4.4) (1.31)	8 1.33 113.4 11.0 0.25 29.9 8.63	(0.33) (0.60) (0.73) (0.11) (1.5) (0.60)
Av. Av.	Birth 7 days 14 days 21 days 28 days 35 days 42 days litter weaning wt., lbs. pig survival, %d	3.0 5.4 8.2 11.0 14.9 18.4 24.6 219.2 100	(0.13) (0.29) (0.31) (0.42) (0.63) (0.74) (1.00) (9.1)* (0)**	2.8 4.7 7.3 10.0 13.2 16.2 20.1 169.7 79.6	(0.06) (0.13) (0.23) (0.35) (0.44) (0.58) (0.74) (14.5) (3.21)

aSee the Experimental Procedure for amount of riboflavin represented by each level.

bStandard error.

<sup>C</sup>Three pigs were transferred from one sow and were not included in this value.

dCalculated as pigs weaned of those born alive.

\*P **<**.05.

\*\*P**< .**005.

pigs were 4.5 lbs. heavier at weaning (24.6 vs. 20.1 lbs., a 22.4 percent advantage in body weight). High level sows produced significantly (P $\lt$ .05) heavier litters at weaning and had more (P $\lt$ .005) pigs surviving to weaning.

No riboflavin deficiency symptoms were apparent in the newborn of either treatment group. Vigor of the two groups of pigs at birth was very similar.

Table XV presents the cumulative and by period growth rate of pigs from high and low riboflavin sows. Although no significant differences occurred in cumulative average daily gains, high riboflavin pigs had superior gains at all weigh periods. When the average daily gains by weigh periods are investigated, high level pigs gained faster (P $\lt$ .05) during the last week of nursing.

Body weight changes and feed consumption of the sows is summarized in Table XVI. High level sows made slightly faster and more efficient gains during gestation, were heavier at farrowing and lost more weight during lactation than low level sows.

As in the first lactation, daily feed intake during lactation was less for the high riboflavin diet (12.5 vs. 13.2 lbs.).

#### Third Lactation

Table XVII presents the results of the colostrum and milk riboflavin analyses. There were highly significant ( $P \lt.005$ ) differences in colostrum and milk riboflavin concentration between dietary riboflavin levels and between the sampling periods. Sixty-two and 31.3 percent of the total variance was accounted for by differences in riboflavin level and by stage of lactation, respectively. There was a highly significant

# TABLE XV

## GROWTH RATE OF PIGS NURSING SOWS FED TWO LEVELS OF RIBOFLAVIN: SECOND LACTATION

			Riboflavin High	Level <sup>a</sup> Low	Difference High - Low
Av.	daily gain, 7 days 14 days 21 days 28 days 35 days 42 days	lb. (Cumulative .3 .3 .4 .4 .4	e) 95 (.079) <sup>b</sup> 97 (.035) 98 (.036) 92 (.036) 94 (.027) 11 (.038)	.27 (.034) .33 (.036) .35 (.037) .37 (.037) .38 (.042) .41 (.046)	.08 .04 .03 .05 .06 .10
Av.	daily gain, Birth = 7 8 = 14 15 = 21 22 = 28 29 = 35 36 = 42	lb. (By periods days	) 5 (.079) 99 (.048) 99 (.031) 56 (.062) 50 (.023) 58 (.110)	.27 (.034) .37 (.044) .39 (.051) .46 (.046) .43 (.074) .54 (.073)	• 08 • 02 • 00 • 10 • 07 • 34*

<sup>a</sup>See the Experimental Procedure for amount of riboflavin represented by each level.

<sup>b</sup>Standard error.

\*P**<.**05.

## TABLE XVI

#### BODY WEIGHT CHANGES AND FEED CONSUMPTION OF SOWS FED TWO LEVELS OF RIBOFLAVIN: SECOND LACTATION

	Di H	etary Ribo igh	flavin Le	vel <sup>a</sup> Low
Sow weights, lbs. Av. at farrowing (F) Av. gain during gestation Av. daily gain during gestation Av. at weaning (W) Av. loss, (F) to (W) Av. daily loss, (f) to (W)	504.5 145.0 0.91 408.5 96.0 2.27	(3.42) <sup>b</sup> (4.45) (0.05) (8.21) (8.39) (0.21)	487.5 133.7 0.83 407.6 80.0 1.90	(21.73) (14.53) (0.11) (18.34) (8.30) (0.20)
Feed consumption, lbs. Av. per sow, lactation <sup>C</sup> Av. per sow daily, lactation	527.3 12.5	(50.5) (3.8)	556.1 13.2	(37.3) (2.8)

<sup>a</sup>See the Experimental Procedure for amount of riboflavin represented by each level.

bStandard error.

<sup>C</sup>Includes feed intake from 110 days of gestation until 42 days post farrowing.

## TABLE XVII

#### RIBOFLAVIN CONTENT OF COLOSTRUM AND MILK OF SOWS FED TWO LEVELS OF RIBOFLAVIN: THIRD LACTATION

Riboflavin Level <sup>a</sup>	0	3	Riboflav: Stag 7	in Concen ge of Lac 14	tration (1 tation (da 21	ncg./ml.) ays) 28	35	42	Significant Differences <sup>e</sup>
High (H) <sup>b</sup>	14.24 (1.66) <sup>d</sup>	8.49 (0.11)	6.76 (0.15)	5.29 (0.27)	4.65 (0.35)	4.10 (0.10)	5.22 (0.86)	5.64 (0.95)	0>all others** 3>7*, all others** 7>all others** 14>28 28 <b>&lt;</b> 42*
Low (L)b	7.10 (0.11)	6.64 (0.19)	5.52 (0.64)	3.46 (0.16)	3.42 (0.21)	3.05 (0.20)	3.50 (0.20)	3.57 (0.24)	0>7*, all others** 3>all higher than 7** 7>all others**
Percent increase <sup>c</sup>	100.6	27.9	22.5	52.9	36.0	34.4	49.1	58.0	· · · · · · · · · · · · · · · · · · ·
Significant differences <sup>e</sup>	H>L**	H>L*	H>L	.H <b>&gt;L*</b>	H>L	NS	H <b>&gt;L*</b>	H>L**	•

Sec.

aSee the Experimental Procedure for amount of riboflavin represented by each level.

<sup>b</sup>Values are an average of four sows for High and eight for Low.

Calculated as High minus Low, divided by Low.

dStandard error.

<sup>e</sup>P<.10; \*P<.05; \*\*P<.01; NS = nonsignificant.

 $(P \lt.005)$  interaction between dietary riboflavin level and stage of lactation, indicating that the size of the difference between riboflavin levels was dependent upon the stage of lactation.

Between ration riboflavin levels, riboflavin content of the mammary secretion was significantly ( $P \lt. 01$ ) higher at days zero and 42. As in the previous two lactations, differences tended to be more significant at the two extremes of the nursing period, with less or no significance occurring in mid-lactation.

Within riboflavin levels, day zero differed significantly (P $\lt$ .01) from all other days of lactation, with the exception of day seven in the low level.

The colostrum and milk riboflavin results are plotted in Figure 3. A highly significant (P $\lt$ .005) curvilinearity in the regressions was found. The regression equations were as follows:

> $\hat{\mathbf{Y}} = 0.5741 \mathbf{X}^2 - 4.3628 \mathbf{X} + 11.9451$  (High Riboflavin)  $\hat{\mathbf{Y}} = 0.2477 \mathbf{X}^2 - 2.0490 \mathbf{X} + 7.1975$  (Low Riboflavin)

where  $\hat{\Upsilon}$  is the colostrum or milk riboflavin level in mcg. per ml.,  $X^2$  is the square of X and X is the stage of lactation in weeks.

Maximum riboflavin content was observed at day zero for both treatments. In the high riboflavin group, riboflavin content decreased rapidly during the first three days, less rapidly during the next four sampling periods, reaching a minimum at day 28, then rising during the remainder of lactation. Low riboflavin sows followed a similar, though less pronounced pattern.

The performance of the sows is presented in Table XVIII. No significant differences were observed in gestation length, number of live and stillborn pigs farrowed, litter weight at birth and weaning and average

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sow colostrum and milk: third lactation.

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# TABLE XVIII

# PERFORMANCE OF SOWS MAINTAINED ON TWO LEVELS OF DIETARY RIBOFLAVIN: THIRD LACTATION

		H	Riboflav igh	in Level L	a ow
No. No.	sows bred sows retained on treatment	2 2	(a)b	3	(0)
Av.	gestation length, days	114.5	(1.50)	115.3	(0.89)
Av.	no. pigs farrowed per litter	10.0	(1.0)	9.3	(1.87)
Av.	no. stillborn per litter	0	(0)	0	(0)
AV.	litter birth wt., 1bs.	27.4	(5.2)	23.5	(3.0)
Av.	no. weaned per litter pig wt., lbs.	0.5	(2.5)	0.07	(0.20)
-	Birth	2.7	(0.12)	2.6	(0.10)
	7 days	4.7	(0.22)	4.7	(0.21)
	14 days	6.9	(0.46)	8.3	(0.28)
	21 days	9.1	(0.51)	10.7	(0.40)
	28 days	12.1	(0.86)	14.1	(0.63)
	35 days	16.4	(1.09)	17.3	(0.90)
	42 days	21.6	(1.14)	22.1	(1.04)
Av. Av.	litter weaning wt., lbs. pig survival, % <sup>c</sup>	193.8 83.3	(88.5) (2.5)	147.7 76.3	(16.9) (13.4)

<sup>a</sup>See the Experimental Procedure for amount of riboflavin represented by each level.

bStandard error.

Calculated as pigs weaned of those born alive.

pig weight at each weight interval. However, high riboflavin sows did wean a heavier litter, reflecting the increase in number of pigs weaned. Survival rates were excellent. As in the previous lactations, no riboflavin deficiency symptoms were observed in any of the newborn pigs.

Table XIX exhibits the cumulative and weekly growth rate of pigs from the treatment groups. Differences in rate of gain were not significant during any part of the nursing period. Superior gains were made by low level pigs except during the two last growth periods.

Sow body weight changes and feed consumption have been summarized in Table XX. High riboflavin sows gained more weight during gestation and lost more weight during lactation. In contrast to the previous lactations, riboflavin supplemented sows ate slightly more feed during lactation.

## TABLE XIX

## GROWTH RATE OF PIGS NURSING SOWS FED TWO LEVELS OF RIBOFLAVIN: THIRD LACTATION

		Riboflavi High	n Level <sup>a</sup> Low	Difference High - Low
Av.	daily gain, lb. (Cumula 7 days 14 days 21 days 28 days 35 days 42 days	ative) .28 (.060) <sup>b</sup> .30 (.090) .30 (.075) .33 (.105) .39 (.110) .45 (.090)	.31 (.024) .41 (.025) .39 (.027) .41 (.041) .42 (.055) .46 (.049)	03 11 09 08 03 01
Av.	daily gain, lb. (By per Birth - 7 days 8 - 14 days 15 - 21 days 22 - 28 days 29 - 35 days 36 - 42 days	riods) .28 (.060) .31 (.125) .31 (.050) .42 (.175) .61 (.130) .73 (.010)	.31 (.024) .51 (.062) .35 (.055) .47 (.100) .45 (.110) .70 (.030)	03 20 04 05 .16 .03

<sup>a</sup>See the Experimental Procedure for amount of riboflavin represented by each level.

bStandard error.

#### TABLE XX

#### BODY WEIGHT CHANGES AND FEED CONSUMPTION OF SOWS FED TWO LEVELS OF RIBOFLAVIN: THIRD LACTATION

	Dietary Ribo High	lavin Level <sup>a</sup> Low	
Sow weights, lbs. Av. at farrowing (F) Av. gain during gestation Av. daily gain during gestation Av. at weaning (W) Av. loss, (F) to (W) Av. daily loss, (F) to (W)	538.0 (21.00) <sup>b</sup> 122.0 (15.00) 0.78 (0.08) 433.5 (35.50) 104.5 (14.50) 2.45 (0.35)	535.7 (27.60) 63.0 (18.52) 0.42 (0.15) 481.7 (20.61) 54.0 (20.01) 1.27 (0.49)	
Feed consumption, lgs. Av. per sow, lactation <sup>C</sup> Av. per sow daily, lactation	528.5 (28.50) 12.6 (0.70)	498.0 (49.61) 11.8 (1.18)	

<sup>a</sup>See the Experimental Procedure for amount of riboflavin represented by each level.

<sup>b</sup>Standard error.

CIncludes feed intake from 110 days of gestation until 42 days post farrowing.

#### DISCUSSION AND CONCLUSIONS

In the three lactation periods studied a total of 14 and 20 litters (131 and 194 pigs) were farrowed on the high and low dietary riboflavin levels, respectively.

Results from this experiment show that sow colostrum and milk riboflavin content can be significantly increased by feeding a higher level of riboflavin than recommended by the N.R.C. (1964).

In each lactation, the addition of a riboflavin supplement to a plant protein basal ration, with a corresponding increase in dietary riboflavin from 0.94 to 3.45 mg. per pound, resulted in a highly significant  $(P \lt .005)$  improvement in colostrum and milk riboflavin. This is in agreement with Catron <u>et al.</u> (1948) who stated that the riboflavin content of sow's milk could be significantly influenced by the ration. Diet is known to also affect the riboflavin content of the milk of the cow (Kiermeir and Haisch, 1961), of the sheep and goat (Pearson and Schweigert, 1947) and of the human (Pratt and Hamil, 1951 and Belavady, 1962).

A 45.5, 49.8 and 42.6 percent increase in the average riboflavin from all sampling periods was observed for first, second and third lactations, respectively. These observations are similar to Barnhart <u>et al</u>. (1954) who found a highly significant ( $P \lt.01$ ) increase in milk riboflavin when ration riboflavin was increased from 1.35 to 3.89 mg. per pound of feed. A 34.0 percent increase in average milk riboflavin was calculated from their data. These workers obtained a 45.0 percent increase

(not significant) when dietary riboflavin was elevated from 0.84 to 2.34 mg. per pound of ration.

Regardless of the treatment imposed or the lactation, stage of lactation had a highly significant (P $\lt.005$ ) affect on the observed milk riboflavin content. Also common to each lactation was a highly significant (P $\lt.005$ ) interaction of dietary riboflavin level and stage of lactation.

Colostrum and milk riboflavin concentrations were plotted against stage of lactation. A highly significant ( $P \lt.005$ ) deviation from linearity was observed for both treatments at all lactations and second-degree polynomials were fitted to the data. A similar quadratic response is evident in the data presented by Davis <u>et al.</u> (1951).

Non-linear correlation coefficients, which measure the goodness of fit of the quadratic equations were -.81, -.94; -.88, -.91; and -.89, -.95 for the first, second and third lactations, respectively. Coefficients of determination for the high and low riboflavin levels were .66, .88; .77, .83; and .80, .90 for the first, second and third lactations, respectively.

Regression equations were calculated for each of the two dietary riboflavin levels using the pooled data from all lactation periods. The regression equations were as follows:

> $\hat{\Upsilon} = 0.5218X^2 - 4.0270X + 11.6430$  (High riboflavin)  $\hat{\Upsilon} = 0.2252X^2 - 1.9296X + 7.2073$  (Low riboflavin)

where  $\hat{\mathbf{Y}}$  is the colostrum or milk riboflavin level in mcg. per ml.,  $X^2$ is the square of X and X is the stage of lactation in weeks. This clearly demonstrates the difficulties involved in attempting to use a single milk riboflavin value in nutritional studies.

The results herein lend support to the idea advanced by Hand and Sharp (1939) and Johnson <u>et al.</u> (1941) that there is a definite inverse relation between riboflavin content and cow milk production. If we apply this to the sow data, we might visualize a riboflavin (concentration) secretion curve the inverse of milk production. Hence, we would expect total milk production to be maximum at or near the time of lowest milk riboflavin concentrations. Minimum milk riboflavin values were observed at day 28. The peak of milk production occurs between the third and fifth week (Pond <u>et al.</u>, 1962<sub>b</sub>; Smith, 1952 and 1959; Lodge, 1959; Hartman and Pond, 1960; Hughes and Hart, 1935; Kovacs, 1955; and Lalevic, 1954).

With the exception of high level sows during the first lactation, maximum riboflavin concentrations, expressed as mcg. per ml., were observed at the first milking (colostrum). This agrees with Barnhart <u>et al</u>. (1954), Davis <u>et al</u>. (1950) and Davis <u>et al</u>. (1951), who found sow colostrum to be higher in riboflavin than later milk. On the other hand these results are contrary to those reported by Braude <u>et al</u>. (1947) who reported that colostrum and milk riboflavin content were about the same.

It has been adequately demonstrated in other species that colostrum is much higher in riboflavin activity than subsequent milk (Pearson and Darnell, 1946; Houston <u>et al.</u>, 1940; Kiermeier and Haisch, 1961; and Kramer <u>et al.</u>, 1939).

Colostrum riboflavin values ranged from 10:92 to 14.86 and 6.61 to 7.44 mcg. per ml. for the high and low level sows, respectively. These values are much greater than those reported by Braude <u>et al.</u> (1945-46), Davis <u>et al.</u> (1950), Davis <u>et al.</u> (1951) and Barnhart <u>et al.</u> (1954). However, the concentrations observed for low level colostrum were near the upper limit of the range in sow colostrum riboflavin reported by

Luecke <u>et al</u>. (1947) and were within the range (1.89 to 12.80 mcg. per ml.) observed by Heidebrecht (1955). High level concentrations are similar to those reported in the colostrum of the ewe (Pearson and Darnell, 1946).

Supplemental ration riboflavin elevated colostrum riboflavin levels by 46.8, 124.8 and 100.6 percent in the first, second and third lactation, respectively.

Scheid <u>et al</u>. (1953) have shown that the riboflavin potency of numerous body organs can be markedly infulenced by the vitamin intake. This suggests that the higher level of colostrum riboflavin were probably due to greater riboflavin storage during gestation as well as the higher average daily intake of riboflavin by the high level sows during lactation.

Colostrum from the high and low treatments contained 2.3 to 3.1 and 1.84 to 2.1, respectively, times as much riboflavin as mid-lactation milk. These observations are similar to the values of 2 to 3 and 3.4 obtained in cow's milk by Nagasawa <u>et al.(1962)</u>, and Pearson and Darnell (1946), respectively.

Within dietary riboflavin levels, characteristic changes in colostrum and milk riboflavin content were observed over the course of the lactation period. There was a large reduction in riboflavin activity during the transition from colostrum to milk, although this reduction was much less pronounced on the low riboflavin diet. Average percent decreases in riboflavin concentration, from the high and low level sows from farrowing to one week post-farrowing, were 50.3, 23.7; 55.0, 19.8; and 52.5, 22.2 for first, second and third lactations, respectively.

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A 67.1 percent decrease in cow milk riboflavin content during the initial week of lactation was calculated from the data of Pearson and Darnell (1946).

Since riboflavin is known to exist, in part, as riboflavin protein complexes, we might expect a parallel decrease in milk protein during early lactation. Brent <u>et al.</u> (1964) stated that the concentration of total protein, casein and heat coagulable proteins all decreased rapidly and in a linear fashion from farrowing to 12 hours post-farrowing. The calculated percentage decrease in casein nitrogen during the first week of lactation was 56.4, a value which is very close to the observed percentage decrease in riboflavin for the high level sows for the same time period.

Under the conditions of this study, milk riboflavin concentrations were at a minimum at day 28. With the normal decline in milk yield, this value rose so that the 42 day values were approximately the same as day 14 in the case of the high riboflavin and day 21 for the low dietary riboflavin level. Barnhart <u>et al.</u> (1954) obtained minimum riboflavin values at day 21, with day 56 values being greater than day 21. Davis <u>et al.</u> (1950) presented data to indicate minimum concentrations were reached at day 15, with day 55 values being higher than day five.

Milk riboflavin concentrations obtained in this study were much higher than reported by Barnhart <u>et al.</u> (1954) and Davis <u>et al.</u> (1951). Milk riboflavin from high riboflavin sows was similar to that reported in the milk of the ewe (Pearson and Schweigert, 1947).

There was little difference in conception rates between the two treatment groups. Although differences were not significant, high level sows had consistently shorter gestation lengths, averaging 1.1, 1.4 and

0.8 days less than low level sows for first, second and third lactations, respectively. No clear cut and consistent differences were apparent in the degree of farrowing difficulty, vigor of the newborn and number of live and stillborn pigs farrowed per litter. Instead of an increase in birth weight of pigs normally expected from consecutive litters over that of first litter gilts, there was a reduction in birth weight from the first to the second lactation in the low level sows. This effect was observed by Miller <u>et al</u>. (1953) when sows were fed 0.83 mg. of riboflavin per pound of ration.

Suckling pigs had access to the sow's self-feeders, and it was observed that the pigs consumed some of the feed fed the sows of the respective lots. It is possible that the heavier weight of the pigs may have been due in part to the consumption of feed containing supplemental riboflavin. The weaning weights of the pigs in both treatment groups were suboptimum. This was expected since the pigs were not allowed creep feed during the six week nursing period. Pigs nursing a sow will not grow at a maximum rate unless they are permitted to consume supplemental feed. Also, the frequent handling of the pigs could have reduced body gain. As the sows were on legume-free rations, the possibility exists that the milk was low or devoid in certain unidentified growth factors. Regardless of the riboflavin treatment imposed or the number of the lactation, average pig birth weights were higher and average number of stillborns per litter were lower than those summarized for Yorkshire sows by Pond <u>et al</u>. (1959-60).

No deficiency symptoms or abnormal pigs were observed in any of the offspring at any time.

With the exception of the third lactation, pigs nursing high level sows were heavier at each weigh period. There was a 10.4 and 22.4 percent increase in weaning weight in the first and second lactation, respectively, favoring the pigs consuming the high riboflavin milk.

Total litter weaning weight in the second and third lactation was in favor of the high level, with a very slight advantage favoring the low level in the initial lactation.

Within riboflavin levels, the cumulative and by period growth rate of the pigs was very similar during the first two lactations. During these lactations, superior gains were exhibited by the pigs nursing the high riboflavin sows. Baby pigs reared on riboflavin deficient synthetic milk diets will produce significantly lower body weight gains (Miller et al., 1964). This is in contrast to Braude et al. (1945-46) who stated that no benefit was derived by suckling pigs from the addition of riboflavin to their milk diet. Unlike Catron <u>et al</u>. (1948), who was unable to establish a definite relationship between the milk riboflavin content and the growth and livability of the offspring, McRoberts and Hogan (1942) reported that pigs receiving 3.2 mcg. riboflavin per ml. of milk appeared unthrifty and survived only five or six weeks. The reason for the failure of this level of dietary riboflavin to stimulate weight gains is not clear, but this may possibly reflect a difference in the ability of the pig to utilize riboflavin from dietary sources compared to that from body stores.

The superior weight gain of pigs from high riboflavin sows as compared to those from low riboflavin sows indicates that the body storage of riboflavin in the latter group was inadequate for maximum growth rate and/or the milk from the high level sows was more nutritionally adequate
for the suckling pigs. The former hypothesis agrees with the observation of Ensminger <u>et al</u>. (1947) that riboflavin deficient gilts give birth to pigs very low in riboflavin.

The improvement in gain due to additional milk riboflavin might possibly be explained on the basis of improvement of the biological value of the milk protein (Mayfield and Hedrich, 1949), more complete coupling of oxidative phosphorylation (Burch <u>et al.</u>, 1960), increased nitrogen retention due to elevated serum riboflavin levels (Lutz <u>et al.</u>, 1956), higher levels of several liver enzymes (Burch and Combs, 1956) and more complete oxidation of nutrients (Sure, 1941; Sure and Dichek, 1941).

There was a tendency for high level sows to gain more weight during gestation and to lose more weight during lactation. During the first and second lactation periods, low level sows ate substantially more feed (2.2 and 0.7 lbs. per day, respectively) than did the high level sows. Ensminger <u>et al.</u> (1947) reported poor appetites were exhibited by sows fed a riboflavin deficient purified ration.

In agreement with Miller <u>et al</u>. (1953), no significant differences were observed in the condition of the sows during gestation and lactation. It appeared that a riboflavin level which was critical for optimum reproduction did not adversely affect body weight and condition of the dams.

Continuous housing on concrete floors resulted in a few leg weaknesses in sows of both treatments but apparently this had little adverse affect on the performance of their offspring.

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## SUMMARY

Three lactation periods involving 34 Yorkshire litters (325 pigs) were studied to determine the influence of supplemental dietary riboflavin on the riboflavin content of sow colostrum and milk at various stages of lactation. Several response criteria were employed to measure sow performance. In addition, growth rates of the offspring were studied.

Fluorimetric riboflavin analyses showed that the addition of a riboflavin supplement to a plant protein basal ration, with a corresponding increase in dietary riboflavin from 0.94 to 3.45 mg. per pound, resulted in a highly significant (P<.005) improvement in both colostrum and milk riboflavin. Regardless of the dietary riboflavin level or the lactation, stage of lactation had a highly significant (P<.005) affect on the observed milk riboflavin content. Also common to each lactation was a highly significant (P<.005) interaction of dietary riboflavin level and stage of lactation.

Colostrum and milk riboflavin concentrations in each of the three lactation periods were plotted against stage of lactation. A highly significant (P $\langle .005 \rangle$ ) deviation from linearity was observed for both treatments at all lactations and second-degree polynomials were fitted to the data.

Within dietary riboflavin levels, characteristic changes in colostrum and milk riboflavin content were observed over the course of each lactation period. With the exception of high level sows during first lactation, maximum riboflavin concentrations were observed at the first

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milking (colostrum). There was a large reduction in riboflavin activity during the transition from colostrum to milk, although this reduction was much less pronounced on the low riboflavin diet. Milk riboflavin activity was at a minimum at day 28. With the normal decline in milk yield, the riboflavin activity rose so that the 42 day values were approximately the same as day 14 in the case of the high riboflavin and day 21 for the low dietary riboflavin level.

No clear cut and consistent differences were apparent in the performance of the sows.

With the exception of the third lactation, pigs nursing high level sows were heavier at each weigh period.

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Master of Science

Thesis: THE INFLUENCE OF RIBOFLAVIN INTAKE ON THE RIBOFLAVIN CONTENT OF SOW'S MILK AND THE PERFORMANCE OF SOWS AND THEIR PIGS

Major Field: Animal Science on

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