THE SUPPLEMENTATION OF MILO-SOY CREEP RATIONS WITH METHIONINE AND/OR LYSINE

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INTRODUCTION

Since the nutritional importance of amino acids and proteins was recognized over fifty years ago by Wilcock and Hopkins (1906) and Osborn and Mendel (1914), the supplementation of the diets of swine with amino acids has been increasingly used as a means of determining amino acid deficiencies. Progress in animal nutrition research, in amino acid synthesis, and in feed processing has given to the modern swine industry a new concept in formulating the protein portion of swine rations.

In young pigs, the primary functions of dietary protein are to furnish indispensable amino acids and to supply additional nitrogen needed for the growth of new tissues, the maintenance of established cellular constituents, and other metabolic requirements. During the early period of life, the growth rate is most rapid and the proportion of lean tissue contributing to the weight gain is higher than during the finishing stages. Therefore, dietary protein must be supplied adequately in proper combinations of essential amino acids in order to meet these requirements.

The experiments here reported were conducted to determine whether any difference existed between growth performance of baby pigs fed a complete starter ration (milo, soybean meal, fish meal, buttermilk and whey) and a milo-soy-whey starter supplemented with methionine and/or lysine.

REVIEW OF LITERATURE

Methionine

History and Chemical Nature

In 1922 Mueller discovered methionine in the course of efforts to elucidate the growth factors for a hemolytic streptococcus. This led to the isolation of the amino acid from casein hydrolysate. In 1928 the correct structure of methionine was established by Barger and Coyne through synthesis.

$$H_3C - S - CH_2 - CH_2 - \underset{\displaystyle H}{\overset{\displaystyle NH_2}{\underset{|}{\overset{|}{C}}}} - COOH$$

Methionine (α-amino-γ-methylthiobutyric acid)

In contrast to the sulfur of cystine and cysteine, that of methionine is stable to alkali. Although methionine is widely distributed among proteins, it represents a very small proportion of the total amino acids formed on hydrolysis. In its pure state, crystals of methionine are white, hexagonal plates, soluble in water and in alcohol.

Aside from its role as an essential constituent of tissue proteins, methionine, probably more than any other amino acid, enters into a number of metabolic pathways which can affect directly or indirectly the rate of tissue protein catabolism and anabolism. In the biosynthesis of many substances in the body, methyl groups play a very important role, and methionine is an excellent methyl donor (du Vigneaud et al., 1940 and 1941). As a methylating agent, methionine provides the N-methyl group for the hormone adrenalin (Keller et al., 1950), the methyl group to convert guanido-acetic acid into creatine (Borsook et al., 1947a and 1947b), and that needed to convert nicotinamide into N'-methyl nicotinamide (Perlzweig et al., 1943 and Cantoni, 1951).

By adding glycine and arginine with the DL-methionine, the toxicity of the excess methionine is reduced or eliminated and the synthesis of the tissue proteins is enhanced (Brown and Allison, 1948; Roth and Allison, 1949; Roth et al., 1950).

Deficiency Symptoms

Methionine is a precursor of cystine, hence a ration deficient in methionine will produce the deficiency symptoms of both amino acids in the rat. In the presence of adequate cystine, when methionine deficiency is initiated, fatty liver results unless choline is added to the diet (Follis, 1958). In the presence of choline, methionine deficiency results in poor formation of hemoglobin and plasma protein. With suboptimal amounts of methionine in the diet and no added cystine, acute necrosis of the liver occurs (Glynn et al., 1945).

Because of the high content of cystine in hair, it is not surprising to find derangements of hair growth in animals deficient in methionine (Smuts, 1935).

Requirement

Since Mitchell and Block (1949) reported that methionine is the limiting amino acid in soybean protein, varying results have been obtained concerning the effects of supplementation with methionine. Using an all-plant diet containing 17.5 percent protein, Ferrin (1946) showed that the addition of 0.1 percent methionine did not alter the average daily gain or feed consumption of growing pigs. However, Dyer _et al._ (1949) demonstrated that methionine supplementation of corn-soybean diets significantly improved the growth rate of growing pigs.

Depending on the level of cystine present in the ration, the methionine requirement of swine may vary greatly. Shelton _et al._ (1950a) by feeding purified diets containing 12.0 percent casein, 10.0 percent gelatin plus 0.3 percent tryptophan to weanling pigs for 28 days, reported that 0.6 percent methionine or 0.3 percent methionine plus 0.3 percent cystine was adequate to support optimum growth. This work is substantiated by Shelton _et al._ (1951) and Curtin _et al._ (1952b). In a later study, using a 21.0 percent protein purified diet containing oxidized casein and gelatin, Shelton _et al._ (1951) observed methionine deficiency in weanling pigs. When 0.1 percent cystine was present in the ration, the level of methionine which supported the best rate of gain and feed efficiency was 0.6 percent of the diet. If adequate cystine was present in the ration, 0.3 percent methionine provided a rate of gain and feed efficiency which was equivalent to that obtained when 0.6 percent methionine was fed. Tentatively, the methionine requirement for weanling pigs was set at 0.6 percent of the ration in the absence of cystine, or 0.3 percent methionine in the presence of 0.3 percent dietary cystine.

A higher methionine level was recommended by Curtin et al. (1952a). Weanling pigs were fed 22.0 percent protein diets containing various levels of methionine. When the ration was supplemented with 0.3 percent methionine to make 0.56 percent total dietary methionine, there was a 20.0 percent improvement in growth rate and 12.0 percent less feed was required. However, in the second experiment, when 0.27 percent cystine was present, the optimum level of methionine was set at 0.45 percent of the ration. Under the conditions of this experiment, the methionine plus cystine requirement was approximately 0.7 percent of the ration.

Maner et al.(1961) fed three-day old pigs 28.0 percent soybean protein basal diets containing 0.28 percent methionine and 0.17 percent cystine. DL-methionine was added at levels of 0.23 and 0.45 percent to provide a total of 0.45, 0.68 and 0.90 percent dietary methionine-cystine or 1.6, 2.4 and 3.2 percent of the protein. The methionine supplementation improved gain, nitrogen retention and feed conversion.

Although cystine can replace approximately 50.0 percent of methionine in the diet (Maynard and Loosli, 1962) the addition of 0.1 or 0.2 percent cystine alone to a ration deficient in methionine failed to improve the growth of weanling pigs fed a synthetic diet containing 12.6 percent isolated soybean protein and 0.15 percent methionine plus 0.17 percent cystine (Becker et al., 1955). However, a level of 0.25 percent methionine in the presence of 0.17 percent cystine supported a satisfactory rate and efficiency of gain. Expressed as a percentage of protein, the combined methionine-cystine requirement was 3.33 percent. It was estimated that cystine can apparently provide about 40.0 percent of the need for sulfur-bearing amino acids. In addition, the young pig was capable of utilizing DL-α-hydroxy-γ-methylmercapto butyric acid to at least partially satisfy the methionine requirement for normal growth.

The variation in methionine requirement for pigs is also due to the level of protein in the ration. Bell et al. (1950) reported that for growing pigs, methionine is the most limiting amino acid in a 10.0 percent soybean protein semi-purified diet. Compared to a control group which was fed a whole egg protein diet, the nitrogen in the unsupplemented soybean meal diet was less efficiently retained and had a significantly lower biological value; however, no nutritional difference was observed between the two diets when 0.27 percent methionine was added to the soybean protein diet to equal that of the whole egg diet. The authors suggested that, on a diet containing 10.0 percent protein, the methionine requirement for growing pigs is between 0.07 and 0.27 percent of the ration. The cystine content of the ration was not mentioned.

Curtin et al. (1952b) fed weanling pigs a 22.0 percent protein ration which contained 0.38 percent cystine. Good quality soybean meal was the only high protein feed. These workers found that the addition of methionine did not improve growth rate of the pigs. They suggested that the methionine requirement does not exceed 0.31 percent at this level of protein and cystine.

Nitrogen balance studies have indicated that a methionine level of 0.27 percent of the ration is adequate to promote acceptable nitrogen retention in growing pigs (Meade, 1956b). In a further study, Meade (1956c) fed growing pigs corn-soybean meal rations containing 11.8, 13.8 and 15.8 percent protein. The author demonstrated that in the presence of 0.04 percent supplemental tryptophan, the addition of 0.1 percent DL-methionine to a 11.8 percent protein diet resulted in marked improvement in nitrogen retention. It was concluded that a 13.8 percent protein diet supplemented with a 0.025 percent methionine provided satisfactory amino acid nutrition for growing swine.

Variation in the methionine requirements of pigs can be largely eliminated by expressing levels on the basis of dietary protein. Becker et al. (1954a) demonstrated that optimum performance of baby pigs from one to four weeks of age can be obtained by feeding a ration containing 22.0 percent protein with 0.58 percent methionine and 0.25 percent cystine, or 2.64 and 1.14 percent (a total of 3.78 percent) of the dietary protein. For pigs from five to nine weeks of age, the requirement for methionine and cystine was set at 0.32 and 0.14 percent, on a 12.0 percent protein ration, or 2.67 and 1.17 percent (3.84 percent), respectively, of the dietary protein.

Lysine

History and Chemical Nature

Lysine was first isolated from a casein hydrolysate by Drechsel in 1889. Although Drechsel suggested that lysine was pentamethylenediamine, the structure remained to be proven in 1902 by Fischer and Weigert who synthesized lysine and demonstrated the identity of the synthetic product and the racemized natural material

$$H_2N - CH_2 - CH_2 - CH_2 - CH_2 - \underset{\displaystyle H}{\overset{\displaystyle NH_2}{C}} - COOH$$

Lysine (α-ε-diamino-caproic acid)

Proteins vary considerably in lysine content. Although moderately large quantities of lysine are found in most animal proteins, it may be present in only small amounts in proteins of grains, which make up the largest portion of swine rations.

Lysine was one of the first indispensable amino acids recognized. Osborn and Mendel (1914) showed that growth of rats can be much improved when lysine was added to a tryptophan-zein mixture.

The nutritional requirement for lysine is extremely specific since only the L-isomer and a few derivatives support growth of rats (Neuberger and Sanger, 1944). Experiments in which lysine was labeled in the α-amino group with N^{15} and in the carbon chain with deuterium have demonstrated conclusively that all the lysine in the body proteins is derived directly from the lysine in the diet; thus, lysine does not undergo reversible deamination at the α-position in vivo, nor is the carbon skeleton of the D-amino acid used for the biosynthesis of the L-form (Fruton and Simmonds, 1961).

Lysine is converted via acetate to glutamic acid. It may also be a precursor of heme (Altman et al., 1952) and a source of arginine (Miller and Bale, 1954).

Deficiency Symptoms

Deprivation leads to two specific changes in the rat: chromotrichia or graying of hair (Vohra and Kratzer, 1956) and fatty liver (Singal et al., 1953). The pathogenesis of the alteration in hair color is not known. The fatty liver was of the periportal type.

Mertz et al. (1949) reported that lysine deficiency symptoms in the weanling pig, such as cessation of growth, retarded and depraved appetite, rough hair coat and emaciation, were alleviated when 2.0 percent DL-lysine HCl was added to the diet.

Requirement

Similar to methionine, there is considerable variation in the lysine requirement for swine reported by several workers. This variation can be explained by differences in experimental conditions, type and level of protein in the diet, age of pig and environment.

The amount of lysine needed in the ration depends on the level of protein fed. Brinegar et al. (1950a, 1950b) observed that 0.6 percent L-lysine was needed for optimum growth when a ration containing 10.6 percent protein was fed to pigs with an initial weight of 30 to 43 lb. However, 1.2 percent L-lysine was required when the dietary protein was increased to 22.0 percent. The authors showed that the difference in these requirements can be largely eliminated when lysine levels are expressed as a percentage of the protein in the ration. A similar relationship was also reported by McWard et al. (1959) who found that the rate of gain on two levels of protein was similar, but the lysine requirement varied with the level of protein fed. At 12.8 percent protein, the pig required 0.71 percent lysine in the diet, or 5.55 percent of the dietary protein; and at 21.7 percent protein, the lysine need was 0.95 percent of the diet or 4.38 percent of the dietary protein. These results compared favorably with those of Pfander and Tribble (1955).

At certain levels of dietary protein, the addition of excessive lysine will not improve rate of gain or feed efficiency. Brinegar et al.

(1949), by feeding a 10.6 percent protein basal diet supplemented with L-lysine to provide total levels of 0.34, 0.42, 0.50, 0.58 and 0.74 percent dietary L-lysine, found that each level of lysine up to 0.58 percent increased the protein efficiency and the biological value for growing pigs, but the 0.74 percent level caused no further improvement. They concluded that with a diet containing 10.6 percent protein, growing pigs required approximately 0.6 percent of L-lysine in the ration.

Shelton et al. (1950b), using a basal diet containing 16.0 percent zein, 10.0 percent gelatin, 0.3 percent tryptophan, 0.5 methionine and 0.2 percent histidine, set the optimum L-lysine requirement for weanling pigs at 1.0 percent of the diet. The addition of L-lysine up to 1.0 percent of the basal diet markedly improved growth rate and feed efficiency. However, no further improvement was observed when higher levels of lysine were added.

Hutchinson et al. (1957b) fed semi-purified rations containing 0.32, 0.42, 0.52, 0.62, 0.72 and 0.82 percent L-lysine to weanling pigs averaging 35 lb. and demonstrated that 0.32 and 0.42 percent lysine levels were inadequate for optimum performance. Diets containing 0.52 to 0.82 percent lysine all gave essentially the same rate and efficiency of gain. They concluded that the optimum requirement for lysine for pigs of this age was 0.52 percent in a 11.69 percent protein ration.

In a similar study, Germann et al. (1958) reported that the level of utilizable L-lysine which supported maximum gains and feed efficiency was 0.62 percent in a 13.4 percent protein semi-purified diet, or 4.7 percent of the dietary protein.

Magruder et al. (1961) showed that there was no difference in growth rate of baby pigs fed starter rations containing 16.0 percent protein and 14.0 percent protein supplemented with 0.1 percent lysine. Supplemental lysine improved growth rate and feed efficiency of growing-finishing pigs when it was added to the 14.0 percent ration, but not when it was added to the 16.0 percent ration.

Hale et al. (1961) fed sorghum grain-cottonseed meal rations containing 16.0 percent protein supplemented with various levels of lysine to growing pigs. The pigs in all groups receiving the ration with added lysine gained significantly faster and more efficiently, especially at the 0.3 percent supplemental level, than pigs without supplemental lysine. However, amounts of added lysine above 0.3 percent gave only small increments of increased growth rate.

As the amino acid balance of most natural pig rations is not ideal, it is probable that total protein intake can be lowered if amino acid deficiencies are corrected.

Based on growth rate and feed efficiency of growing pigs, Bowland (1962) demonstrated that there was no difference between a 16.0 percent protein ration and a 13.0 percent protein ration supplemented with 0.2 percent lysine. The pigs received the lower protein ration without supplemental lysine gained 10.0 percent more slowly and required 13.0 percent more feed per pound of gain as compared to those fed the higher protein ration.

In addition to the effect of dietary protein level, the lysine requirement of swine is also dependent on age and weight of the pig (Becker et al., 1954b). To support satisfactory performance, a lysine level of 0.63 percent of the diet was required for pigs from 40 to 100 lb., and 0.52 percent for pigs from 100 to 200 lb. This is substantiated by the work of Evans (1960) who fed growing pigs of different weights an all-vegetable basal diet distinctly low in protein, especially lysine. For maximum gain, growing pigs from 80 to 120 lb. required a lysine level of 0.65 percent of the ration. Hutchinson et al. (1957a), using a low protein diet, concluded that the lysine requirement does not exceed 0.935 percent of a ration containing 14.25 percent protein for two-week-old pigs.

Nitrogen balance studies in growing pigs showed varying results. Meade (1956a) reported that the supplementation of a 15.9 percent protein corn-soybean meal diet with lysine failed to improve nitrogen retention. The author concluded that a lysine level of 0.69 percent for this ration was adequate to promote acceptable nitrogen retention by growing pigs.

Soldevila and Meade (1964), using a 14.0 percent protein barley-soybean meal diet supplemented with lysine, demonstrated that there was no improvement in nitrogen retention when growing pigs were fed at the rate of 5.5 percent of $W^{.09}{}_{kg}$ daily. However, when a similar diet was fed at the rate of 6.5 percent of $W^{.09}{}_{kg}$ daily, the addition of 0.2 and 0.3 percent of L-lysine significantly increased nitrogen retention over that obtained with the basal diet.

Using an all-plant diet where 10.0 percent dietary protein was supplied by various ratios of soybean meal and corn, Clawson et al. (1963) demonstrated that increasing the proportion of the protein supplied by soybean meal resulted in significant increases in feed intake and daily gain. Carcass leanness as measured by the area of longissimus dorsi muscle was improved. These results were substantiated by Nielsen et al. (1963) who found that supplementation of 10.0 and 12.0 percent protein corn- or barley-soybean meal rations with lysine resulted in faster gains, more efficient feed conversion and leaner carcasses in growing-finishing pigs.

The Time Factor in Amino Acid Supplementation

The efficiency and extent of utilization of dietary protein is governed by the adequacy and completeness of the amino acids and also by the frequency of administration. Incomplete mixtures of amino acids are subjected to be catabolized rather than being held in the tissues for the arrival of the missing amino acids.

Geiger (1947) demonstrated that weanling rats grew satisfactorily when they were fed a tryptophan-deficient diet supplemented with tryptophan incorporated into the feed. However, those fed the added tryptophan at alternating 12-hour intervals with the rest of the ration showed no increase in weight. Similar results were obtained when animals were fed lysine-deficient rations containing treated casein and/or zein protein and a methionine-free casein ration. The author concluded that for efficient protein synthesis, all essential components must be present at the same time. These results were substantiated by Cannon et al. (1947) who observed a weight loss in rats by feeding a ration deficient in several amino acids. However, this loss in weight was regained steadily when an adequate repletion ration was used. The ration, deficient in amino acids, was divided into two parts. To the first part were added arginine, histidine, leucine, lysine and threonine. The second part contained added isoleucine, methionine, phenylalanine, tryptophan and valine. When these two supplemented parts were fed alternately, the rats had a slow recovery of lost weight. Furthermore, if both incomplete rations were fed simultaneously, free choice, rats still lost weight presumably because the time lapse between consumption of the two rations by rats was too great for effective protein synthesis. However, if the two incomplete rations were mixed and fed with similar intervals, rats recovered weight rapidly.

Studying the influence of administration of tryptophan in weanling rats, Berg and Rose (1929) showed that there was a linear correlation between the total gain and the frequency of tryptophan administration up to a 12-hour interval. Thus, those that received half of the daily allowance at 12-hour intervals made greater gain than those which received a single dose every 24 hours. A similar trend was observed when rats were fed a double dose every two days. However, feeding the amino acid more frequently than twice daily appears to produce little, if any, further improvement.

There is experimental evidence that the findings with rats apply to other species. In case of pigs, Eggert et al. (1953) were unable to demonstrate an effect by a 24-hour interval between feedings of protein supplements to a corn diet, but there was a decrease in nitrogen retention with a 36-hour interval.

To study the influence of the time factor, Geiger (1948) fed rats rations containing two proteins each lacking at least one amino acid but complementary to each other when mixed. The combinations used were wheat gluten (low in lysine) plus blood meal (low in isoleucine); yeast (low in methionine) plus blood meal; and wheat gluten plus yeast. Each group of animals was allotted into four treatments: two were fed a semi-purified diet containing 9.0 percent of one of the protein combinations, the third lot was fed a mixture of 4.5 percent from each protein type to provide a 9.0 percent protein ration, and the fourth lot was fed alternately a diet containing 9.0 percent of one protein combination described with a time lapse between feedings. Results showed that, when fed individually at a 9.0 percent level, each protein did not promote growth. Satisfactory growth of rats was obtained when pairs of protein combinations were mixed at a 4.5 percent level each, but if fed separately as in lot four, poor growth resulted. It was concluded that delayed provision of missing essential amino acids is ineffective, not only when fed as amino acids themselves, but also when supplied as proteins.

Amino Acid Adequacy of Various Feedstuffs

The level of dietary protein essential to insure an adequate intake of amino acids is of paramount importance in the nutrition of the young pig. Several workers have shown that sow's milk normally provides approximately 33.0 percent protein on a dry matter basis.

Although belonging to the same type of feedstuff, an ingredient may vary in protein adequacy. Heller et al. (1951) found considerable variation in the protein content of different varieties of grain sorghum (from 10.5 to 15.0 percent). Variation among varieties was no greater than that within a single variety grown under widely different soil and climatic conditions. Under drouth conditions, sorghum grain showed consistently higher protein content than samples grown under normal conditions. However the overall grain yield was decreased.

Amino acid adequacy studies of milo have shown that lysine is the first limiting amino acid (Shelton et al., 1951; Hillier et al., 1954). These results were substantiated by Pond et al. (1958) who fed weanling albino rats a basal ration containing 8.5 percent crude protein, 0.19

percent lysine and 0.28 percent threonine and two control rations containing 11.0 and 21.0 percent casein. The addition of 0.5 percent of L-lysine and 0.2 percent of DL-threonine to the basal ration produced growth approximately equal to that obtained with the 11.0 percent casein control ration, but inferior to the 21.0 percent casein ration. The liver fat content of rats receiving the basal diet was significantly reduced by the addition of 0.5 percent of L-lysine and 0.2 percent of DL-threonine. It was postulated that lysine and threonine are probably the most limiting amino acids in milo for growth of rats.

Smith (1930) in comparing the nutritive value of corn with that of various grain sorghums, concluded that sorghums are, in general, higher in protein and lower in fat than corn. Shelton et al. (1951) demonstrated that, for a short feeding trial, sorghum gluten meal produced little or no gains in growing pigs. This is due to the fact that sorghum gluten meal is extremely low in lysine and is also a poor source of some other essential amino acids. When sorghum gluten meal made up not more than 30.0 percent of the dietary protein or not more than 4.0 percent of the total ration, satisfactory gains were obtained. Based on nutritive value, sorghum gluten feed was inferior to sorghum gluten meal in that an extra 100 lb. of feed was needed for each 100 lb. gain.

Mitchell and Block (1946) using rats, and Bell et al. (1950) using pigs, demonstrated that methionine is the most limiting amino acid in soybean meal. These works were substantiated by Berry et al. (1962) who suggested that methionine is the most limiting acid in soybean protein, followed by threonine and lysine.

Comparative studies have shown that for weanling pigs in dry-lot, blended soybean meal protein was superior to that of extracted soybean meal and meat and bone scrap (Terrill et al., 1954). The efficiency of meat and bone scrap protein was significantly improved by the addition of 0.1 percent DL-tryptophan. These workers found that dried skim milk was superior to solvent extracted soybean meal and blended soybean meal was superior to meat and bone scrap.

The superiority in nutritive value of skim milk protein over that of soybean meal was confirmed by Hays et al. (1959) who further demonstrated that the addition of arginine to the soybean meal diet had no significant effect on rate of gain and feed conversion of baby pigs. However, marked improvement was observed when this basal diet was supplemented with DL-methionine.

The apparent digestibility of the dry matter and protein of the milk rations was high at two weeks of age and changed very little as the pigs reached five weeks. The digestibility of the dry matter and protein of the soybean diets, however, increased as the pigs increased in age from two to five weeks. It was concluded that age was a significant factor in the digestion and utilization of plant proteins in the young pig.

Using young pigs and rats, Maner et al. (1961) studied the effect of proteolytic enzymes, amino acids and age on the utilization of isolated soybean protein. When pepsin and/or trypsin were added to a basal soybean protein diet, no improvement was observed in growth rate, feed efficiency, and protein digestibility. Addition of DL-methionine to the same basal diet improved rate of gain, nitrogen retention and feed conversion. The improvement was significantly greater when casein was used as the source of protein. The difference in the digestibility and retention of absorbed nitrogen of the ration components between soybean protein and casein occurred at three weeks but not at ten weeks of age.

In contrast to the results obtained with pigs, supplementation of the basal soybean protein diet of rats with DL-methionine produced gain equal to those obtained with casein. In the absence of added methionine, the addition of L-lysine and/or DL-tryptophan had no significant effect on growth.

Among the protein sources for swine rations, fish meal is one of the most commonly used due to its high protein content and superior amino acid quality. In comparative studies with whole egg, Sure and Easterling (1952) demonstrated that all fish meals have relatively high biological value. The biological value and net utilization of various fish meals are:

	B.V. %	Net Utilization
Dried whole egg	96.5	94.5
Sardine meal	86.4	84.5
Menhaden fish meal	86.9	77.5
Red fish meal	87.3	75.8
Herring meal with fish solubles	82.2	73.6
Alaska herring meal	79.7	69.5
Anchovies meal	82.6	63.1
Crab meal	85.9	60.2

Although powdered mackerel had a higher nutritive value than casein, it contained no detectable quantity of vitamin A (Deuel et al., 1946). When two rations, one containing powdered mackerel and the other casein, were supplemented with 1.2 and 3.5 I.U. of vitamin A per rat, per day, the growth of rats receiving the fish protein exceeded that of the rats on the standard casein diet by 31.0 percent and 43.3 percent, respectively.

EXPERIMENTAL PROCEDURE

Two experiments were conducted continuously at the Oklahoma Agricultural Experiment Station at Stillwater from November 1964 until November 1965. The experimental procedure was the same for both experiments. In the first experiment a complete creep ration containing soybean meal, fish meal, buttermilk and whey as sources of protein (Ration 1) was compared with a ration containing only soybean meal and whey supplemented with methionine (Ration 2).

In the second experiment Ration 1 was compared to a soybean meal-whey ration supplemented with methionine and lysine (Ration 3). Rations 1 and 3 contained equal amounts of methionine but lysine was 0.06 percent higher in Ration 3. All comparisons were based on the growth performance of the pigs.

The plan for the experiments is shown in Table I. The chemical composition of the feeds utilized in these rations is presented in Table II. The ingredient makeup of rations 1, 2 and 3 is shown in Table III. The calculated nutrient composition for these rations is shown in Table IV.

Thirty-seven purebred Yorkshire and Hampshire sows were assigned to each experiment. Based on age, they were equally allotted to either treatment 1 or 2 so that each treatment had a similar number of sows of the same age. All sows received the same ration containing 14.0 percent protein throughout the course of the study.

As each sow reached 110 days of gestation, she was weighed, taken into a central farrowing barn and placed in a farrowing crate. All

TABLE I

PLAN OF EXPERIMENTS

	Experiment I		Experiment II	
	Comparison: Ration 1 vs. Ration 2		Comparison: Ration 1 vs. Ration 3	
	Treatment 1	Treatment 2	Treatment 1	Treatment 2
Number of sows	18	17	18	17
Number of pigs	141	137	151	154
Date started	November 17, 1964		April 28, 1965	
Date ended	April 13, 1965		November 9, 1965	

sows received routine management during parturition. During the period from 110 days of gestation to 16 days post-farrowing, the sows were hand-fed twice daily on an individual basis in relation to their appetite and condition. When the litter reached 16 days, the sow and pigs were transferred to the nursery barn. The concrete pen area under the roof was six feet by twelve feet, connected to a six foot by fifteen foot concrete lot outside the barn where the self-feeder for the sow was located. From the sixteenth day post-farrowing, all sows were self-fed the same ration until the pigs were weaned at 42 days of age, at which time the weight back of each sow's feed was measured. Water was available to the litter at all times. Each individual litter was permitted to run with its mother and allowed to suckle normally until weaning.

Pigs were weighed individually, to the nearest 0.1 pound, at 16, 42 and 56 days of age. At two and 21 days of age, all pigs received 100.0 mg. of iron in the form of a 1.0 ml. intra-muscular injection of

TABLE II

CHEMICAL COMPOSITION OF FEEDS UTILIZED IN ALL RATIONS

Feeds	Percent Composition					
	Dry Matter	Ash	Protein[a]	Fat	Crude Fiber	NFE
Milo, ground	89.25	2.05	9.00	2.78	2.26	73.05
Soybean meal	91.06	5.67	49.85	0.68	2.78	32.08
Buttermilk	93.50	6.70	32.20	5.75	0.28	47.57
Fish meal	93.27	17.80	60.75	8.40	0.68	5.64
Whey	93.70	9.75	12.30	0.85	----	70.09

[a]Calculated as 6.25 x N.

TABLE III

INGREDIENT MAKEUP OF CREEP RATIONS

Ingredient	Ration 1 %	Ration 2 %	Ration 3 %
Milo, ground (9.0 %)[a]	63.95	63.695	63.653
Soybean meal (50.0 %)[a]	17.52	23.07	23.07
Buttermilk (32.0 %)[a]	3.00	---	---
Fish meal (60.0 %)[a]	3.00	---	---
Whey (12.0 %)[a]	5.00	5.00	5.00
Molasses	5.00	5.00	5.00
Hygromix[b]	0.25	0.25	0.25
SP-250[c]	0.25	0.25	0.25
Calcium carbonate (38.0 % Ca)	0.56	0.70	0.70
Dicalcium phosphate (28.0 % Ca - 18.0 % P)	0.91	1.34	1.34
Trace mineralized salt	0.50	0.50	0.50
Zinc sulfate	0.02	0.02	0.02
Vitamin supplement[d]	0.04	0.04	0.04
Methionine[e]	---	0.135	0.044
Lysine[f]	---	---	0.133
Total	100.00	100.00	100.00

[a]Protein content chemically determined.

[b]Contains 12 gm. hygromycin B per ton of feed.

[c]Contains 20 gm. chlortetracycline, 20 gm. sulfamethazine and 10 gm. penicillin per pound.

[d]Composed of 3 gm. riboflavin, 12 gm. pantothenic acid, 20 gm. niacin, 20 mg. vitamin B_{12}, 2,000,000 U.S.P. vitamin A and 200,000 U.S.P. vitamin D per ton of feed.

[e]The 1964 N.R.C. requirement is 0.85 percent for pigs from 5 to 10 lb. and 0.55 percent for pigs from 25 to 75 lb.

[f]The 1964 N.R.C. requirement is 1.40 percent for pigs from 5 to 10 lb. and 0.75 percent for pigs from 25 to 75 lb.

TABLE IV

CALCULATED NUTRIENT COMPOSITION OF CREEP RATIONS

Nutrient	Ration 1	Ration 2	Ration 3
Protein, %	18.02	18.01	18.01
T.D.N., %	75.51	74.86	74.87
Calcium, %	0.803	0.804	0.804
Phosphorus, %	0.602	0.606	0.606
Methionine[a], %	0.24	0.331	0.24
Lysine[b], %	0.94	0.886	1.00
Riboflavin, mg./lb.	3.10	2.68	2.68
Pantothenic acid, mg./lb.	12.70	11.67	11.67
Niacin, mg./lb.	26.40	15.30	15.30

[a]Contains 95 percent of pure DL-methionine.
[b]Contains 95 percent of pure L-lysine.

iron dextran. When the pigs were 16 days old, creep rations were introduced in a small self-feeder and contimued post weaning until the pigs reached 56 days of age. The data were analyzed statistically by analysis of covariance and t-test (Steel and Torrie, 1960).

RESULTS AND DISCUSSION

Experiment I

The results of the first experiment are presented in Table V. The average total gain per pig during 40 days on test was 33.79 and 32.98 lb. for Treatment 1 and 2, respectively. The difference of 0.81 lb. in favor of the pigs fed creep Ration 1 was not statistically significant ($P > .10$). The superiority in weight gain occurred during the first four weeks, after the creep rations were introduced. This early advantage in weight gain may have been due to the milk supply from the mother since average litter size for Treatment 1 was 0.26 pig less than that for Treatment 2. It should be noted that from day 42, when the pigs were weaned, until day 56, the rate of gain in both treatments was identical, i.e., 1.077 lb. per pig per day. This increase in growth rate after six weeks of age of pigs fed Ration 2 was probably due to higher digestibility of soy protein. Hays et al. (1959) reported that as pigs increased in age from two to five weeks, the digestibility of soy protein increased from 78.0 to 82.0 percent, whereas no change was observed for milk protein. Similar results were reported by Maner et al. (1961).

Assuming that a linear relationship exists between litter size and rate of gain of the pigs, the regression plots from Figure 1 demonstrate that as litter size increases, gain per pig decreases. This is probably due to greater competition for the sow's milk. The regression equations were as follows:

TABLE V

GROWTH PERFORMANCE OF PIGS ON BASAL AND METHIONINE SUPPLEMENTED CREEP RATIONS (Experiment I)

	Days	Treatment 1[a]		Treatment 2[b]	
Avg. no. of pigs per litter	16	8.11	(0.587)[c]	8.51	(0.486)
	42	7.94	(0.574)	8.21	(0.479)
	56	7.83	(0.573)	8.09	(0.433)
Avg. litter weight[d], lb.	16	70.41	(4.497)	72.90	(5.236)
	42	215.31	(12.290)	214.41	(13.804)
	56	329.13	(20.538)	329.51	(19.128)
Avg. pig weight, lb.	16	8.83	(0.244)	8.98	(0.452)
	42	27.54	(0.729)	26.87	(0.992)
	56	42.62	(1.151)	41.96	(1.447)
Avg. total gain per pig, lb.	16-42	18.71	(0.581)	17.89	(0.634)
	16-56	33.79	(1.015)	32.98	(1.082)
	42-56	15.08	(0.706)	15.09	(0.764)
Avg. daily gain/pig, lb.	16-42	0.719	(0.022)	0.688	(0.024)
	16-56	0.844	(0.025)	0.824	(0.027)
	42-56	1.077	(0.050)	1.077	(0.054)
Avg. daily feed intake/litter, lb.		8.80	(0.541)	8.97	(0.537)
Avg. daily feed intake/pig, lb.		1.17	(0.071)	1.07	(0.049)
Lb. of feed per lb. of gain		1.386	(0.054)	1.298	(0.074)
Avg. daily feed intake per sow[e], lb.		18.47	(0.831)	17.76	(1.328)

[a]Starter Ration 1 contained 0.24 percent dietary methionine and 0.94 percent dietary L-lysine.

[b]Starter Ration 2 contained 0.331 percent dietary methionine and 0.886 percent L-lysine.

[c]Standard error.

[d]Only pigs that reached 56 days of age were included.

[e]All sows in both treatments received the same 14.0 percent protein ration.

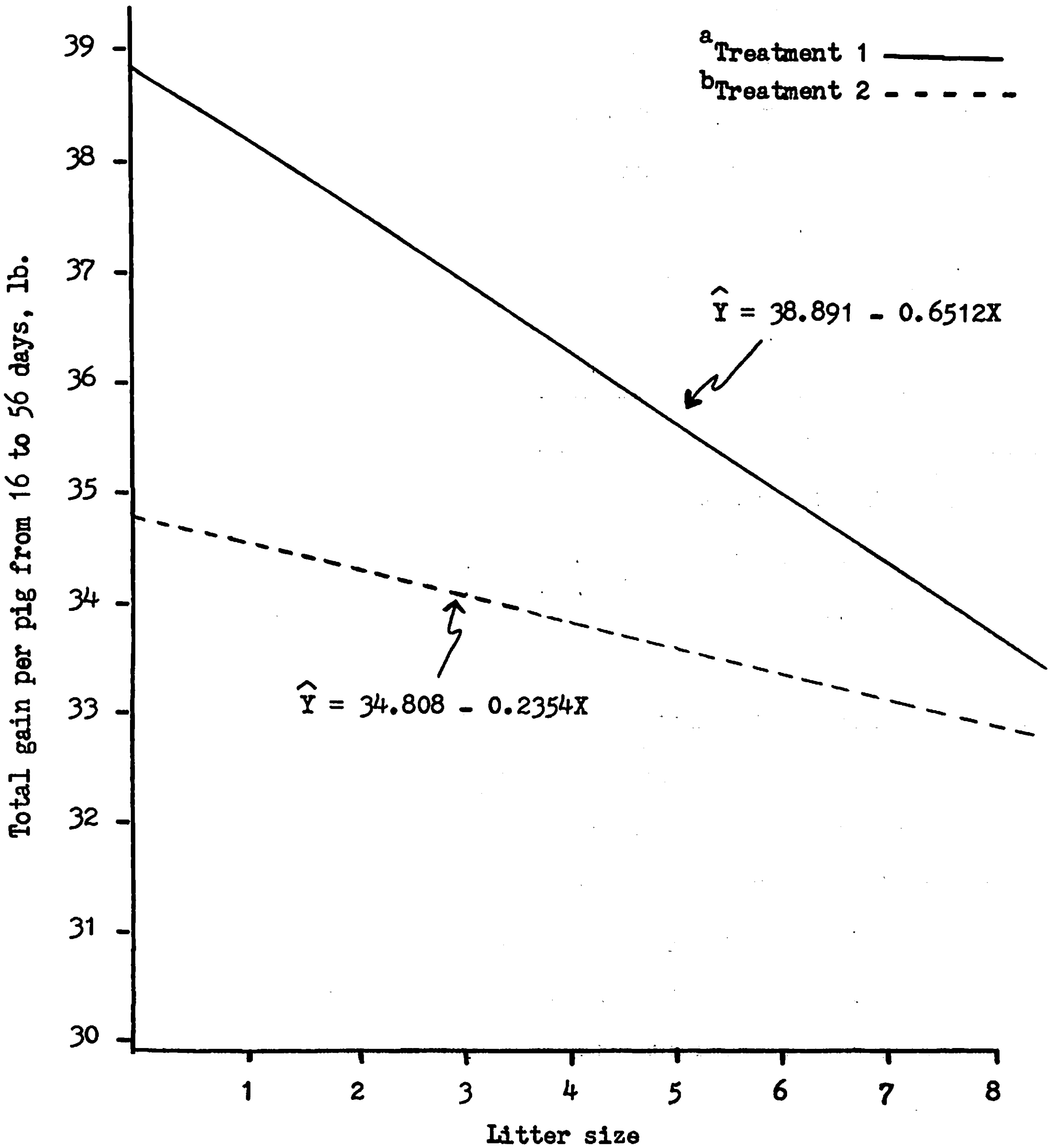

Figure 1. The Influence of Litter Size on Total Gain Per Pig from 16 to 56 Days of Age.

[a]Pigs on Treatment 1 received Ration 1 which contained 0.24 percent methionine and 0.94 percent lysine.

[b]Pigs in Treatment 2 received Ration 2 which contained 0.331 percent methionine and 0.866 percent lysine.

$$\hat{Y} = 38.891 - 0.6512\ X \quad \text{(Treatment 1)}$$

$$\hat{Y} = 34.808 - 0.2354\ X \quad \text{(Treatment 2)}$$

where $\hat{Y}$ is the estimated total gain in lb. per pig during the period from 16 to 56 days of age and X is the number of pigs per litter. The slope of -.6512 of Treatment 1 is greater than all other treatments in this study. It may have been a result of lack of uniformity in litter size of the sows in Treatment 1.

Treatments did not have any significant effects upon average litter weight, average pig weight, average daily gain, daily feed intake and feed efficiency. However, it appears that as the rate of gain of the pigs increases, the feed required for each pound of gain also increases. There was no significant difference in feed intake of the sows.

It should be noted that both rations contained 18.0 percent protein but the methionine level for Rations 1 and 2 are 0.24 and 0.331 percent of the diet or 1.33 and 1.83 percent of the dietary protein, respectively. These levels are much lower than a level of 0.55 percent (or 3.05 percent of the protein) recommended by the National Research Council (1964). Cystine can replace approximately 50.0 percent of the methionine requirement in the diet (Maynard and Loosli, 1962). The satisfactory performance of the pigs in both treatments suggests that the cystine content of the rations used in this experiment may have provided adequately the additional methionine needed.

Methionine levels utilized in this study are similar to those of Curtin et al. (1952b) who suggested that the methionine requirement does not exceed 0.31 percent of the ration (or 1.41 percent of dietary protein) for weanling pigs fed a 22.0 percent protein ration containing 0.38

percent cystine (1.72 percent of dietary protein). This also compared favorably with the methionine level recommended by Shelton *et al.* (1950a, 1951), Curtin *et al.* (1952a) and Meade *et al.* (1956a).

There were a few incidences of mild scours which occurred mainly during the first four weeks of age of the pigs.

EXPERIMENT II

The results of the second experiment are summarized in Table VI. The difference in average total gain per pig of 2.73 lb. during 40 days on test in favor of the pigs fed Ration 3 was statistically significant ($P < .10$). The possible factors that caused the difference in weight gain included: treatment effect, effect of litter size, age of the dam and seasonal effect. As stated in the procedure, the treatments were conducted simultaneously, and the dams were equally divided into each treatment according to age. Therefore, these two factors were minimized. The main effects contributing to the difference in growth performance of the pigs, then, are level of lysine and litter size.

Assuming a linear relationship exists between litter size and weight gain of the pigs, the regression plots are presented in Figure 2. The regression equations were as follows:

$$\hat{Y} = 27.4045 - 0.1892X \quad \text{(Treatment 1)}$$

$$\hat{Y} = 30.2402 - 0.1995X \quad \text{(Treatment 2)}$$

where $\hat{Y}$ is the estimated total gain in lb. per pig during the period from 16 to 56 days of age and X is the number of pigs per litter. The similarity of slopes of two treatments reflects the uniformity of litter size. This is expected since in this second experiment, only litters with eight pigs or more were used.

TABLE VI

GROWTH PERFORMANCE OF PIGS ON BASAL AND LYSINE SUPPLEMENTED CREEP RATIONS (Experiment II)

	Days	Treatment 1[a]			Treatment 2[b]	
Avg. no. of pigs per litter	16	9.33	(0.511)[c]		9.68	(0.461)
	42	8.55	(0.452)		9.12	(0.320)
	56	8.38	(0.421)	**	9.07	(0.338)
Avg. litter weight[d], lb.	16	64.83	(4.648)		70.26	(4.417)
	42	179.80	(9.732)	***	210.22	(8.906)
	56	277.21	(16.798)	***	326.87	(15.066)
Avg. pig weight, lb.	16	7.50	(0.397)		7.45	(0.416)
	42	21.65	(0.896)		22.91	(0.706)
	56	33.32	(1.259)	*	36.00	(1.084)
Avg. total gain per pig, lb.	16-42	14.15	(0.637)	*	15.46	(0.682)
	16-56	25.82	(1.066)	*	28.55	(0.986)
	42-56	11.67	(0.620)	*	13.09	(0.495)
Avg. daily gain per pig, lb.	16-42	0.545	(0.025)	*	0.594	(0.026)
	16-56	0.645	(0.026)	*	0.713	(0.024)
	42-56	0.834	(0.044)	*	0.935	(0.036)
Avg. daily feed intake/litter, lb.		6.30	(0.483)	***	7.87	(0.479)
Avg. daily feed intake/pig, lb.		0.747	(0.041)	***	0.860	(0.037)
Lb. of feed per lb. of gain		1.158	(0.040)		1.205	(0.039)

[a]Starter Ration 1 contained 0.24 percent dietary methionine and 0.94 percent dietary L-lysine.

[b]Starter Ration 3 contained 0.24 percent dietary methionine and 1.00 percent dietary L-lysine.

[c]Standard error.

[d]Only pigs that reached 56 days of age were included.

[e]All sows in both treatments received the same 14.0 percent protein ration.

*Significantly different ($P < .10$).

**Significantly different ($P < .05$).

***Significantly different ($P < .025$).

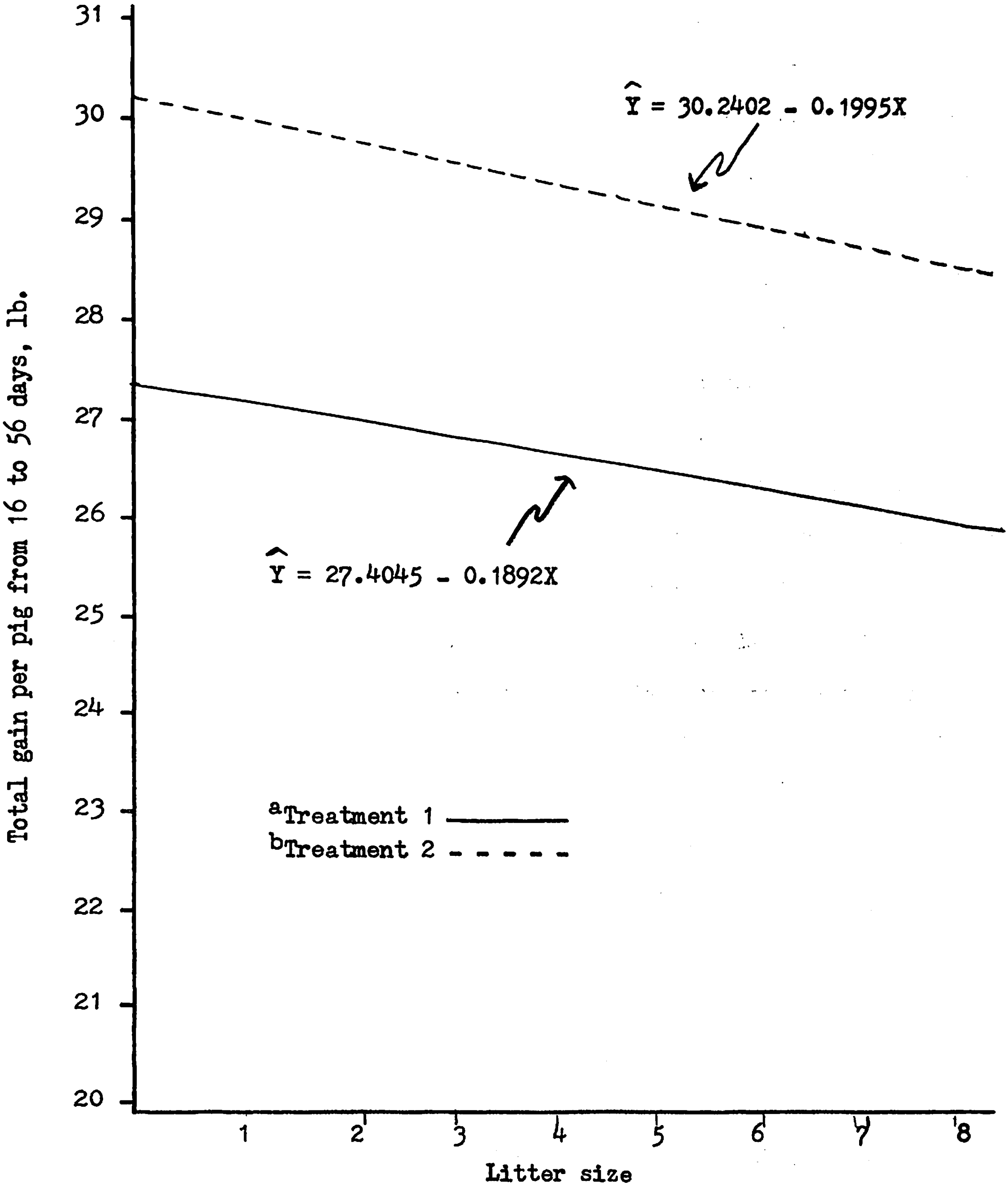

Figure 2. The Influence of Litter Size on Total Gain Per Pig from 16 to 56 Days of Age.

[a]Pigs in Treatment 1 received Ration 1 which contained 0.24 percent methionine and 0.94 percent lysine.

[b]Pigs in Treatment 2 received Ration 3 which contained 0.24 percent methionine and 1.0 percent lysine.

In mice, the favorable effect of reduction of litter size on the growth rate of the surviving members of a litter has been demonstrated by Parkes (1926), McDowell _et al._ (1930) and McCance (1962). The average litter size of Treatment 1 and 2 was 8.38 and 9.07, respectively. The difference of 0.70 in favor of Treatment 2 approached significance ($P < .05$). Regardless of this fact, the pigs fed Ration 3 (Treatment 2) gained significantly faster than those fed Ration 1 (Treatment 1) ($P < .10$), especially during the last two weeks on test.

There was no significant difference in the initial average litter weight at day 16. However, the difference in litter weight at day 42 and 56 was significant ($P < .025$). This may have been a result of larger litter size of the sows in Treatment 2 and treatment effect.

The average daily gains per pig from 16 to 42 days and from 42 to 56 days of age were 0.545 and 0.834 lb. for Treatment 1 as compared to 0.594 and 0.935 for Treatment 2. The fact that there was less advantage in weight gain for the pigs in Treatment 2 during the 16-42 day suckling period than during the 42-56 day period may have been due to larger litter size, greater competition for milk and lower digestibility of soy protein at an early age of the pig. It should be noted that both Rations 1 and 3 contained 18.0 percent protein. However, while soybean meal made up only 48.6 percent of the protein in Ration 1, it was increased to 64.0 percent in Ration 3.

The methionine content was the same for both rations: 0.24 percent of the ration or 1.33 percent of the dietary protein. This level is substantially lower than a level of 0.55 percent (or 3.05 percent of the protein) recommended by National Research Council (1964) for pigs from 25 to 75 lb. However, since the pigs in this study performed satisfactorily, it was assumed that cystine may have replaced part of the methionine needed.

The lysine content of Rations 1 and 3 was 0.94 and 1.0 percent, respectively, or 5.22 and 5.55 percent of the dietary protein. Under the conditions of this experiment, the favorable effect of lysine supplementation upon growth rate of the pigs suggests that a ration containing 18.0 percent protein with 0.94 percent lysine can be improved by adding supplemental lysine.

The lysine level utilized in this experiment is similar to that recommended by Brinegar _et al._ (1949, 1950b, 1950c) and McWard _et al._ (1959). The levels used by these workers were 5.66, 5.45 and 5.54 percent of the dietary protein, respectively. Shelton _et al._ (1950b), however, suggested a lower lysine requirement for weanling pigs. They set the optimum level at 1.0 percent or 4.2 percent of the dietary protein in a 23.8 percent protein ration. This is also in agreement with the recomendations of Hutchinson _et al._ (1957b) and Germann _et al._ (1958).

The pigs in Treatment 2 consumed significantly more feed than those in Treatment 1 ($P < .025$), but there was no significant difference in feed efficiency. Since this experiment was conducted during the summer months, heat stress and more exposure to diseases and parasites may have been major factors contributing to the higher occurrence of scours and lighter weaning weight.

SUMMARY

Two experiments involving 74 Yorkshire and Hampshire sows and 583 pigs were conducted to determine the influence of the supplementation of milo-soybean meal creep rations with methionine and/or lysine. Response comparisons were based on weight gains of the pigs.

In the first experiment there were no significant differences in growth rate, feed intake and feed efficiency observed between pigs fed a ration containing milo, soybean meal, fish meal, buttermilk and whey and pigs fed a milo-soybean meal-whey ration supplemented with 0.135 percent methionine.

In the second experiment a comparison was made between two rations of equal methionine level but the milo-soybean-whey ration contained 0.06 percent more lysine than the unsupplemented milo-soy-fish meal-buttermilk-whey ration. Pigs receiving the supplemented creep ration made significantly higher gains ($P < .10$) than those on the low lysine ration. Feed intake was significantly higher ($P < .025$) for the pigs on the lysine supplemented diet but feed efficiency was not significantly different.

There was a tendency for the pigs raised in winter to have a higher weaning weight and less disease disturbance than those raised in summer.

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