ENERGY, PROTEIN AND METHIONINE INTERRELATIONSHIPS IN GROWING PIG RATIONS

by

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INTRODUCTION

Less than ten years ago, the recommendations for growing swine rations included a mixture of animal and plant proteins. However, with the discovery and identification of vitamin B_{12}, it became evident that the need for animal protein in the diet for weanling pigs was primarily a problem of amino acid balance and/or economics. Also, simultaneously with the identification of B_{12} as the animal protein factor (APF), the quality of the feed grade animal protein products was deteriorating, due largely to the fact that more and more of the higher quality trim and organs were going into the edible by-products for human consumption or for high quality special products for animal pets. Meanwhile, with improvement in processing methods, the quality of plant proteins was improving. With these changes taking place, it became obvious that the protein sources going into swine feeds needed a careful reevaluation.

Catron and Wallace (1949) reported studies in which they observed gains on all plant protein rations supplemented with APF equal to those on rations containing 15 percent meat and bone scraps. Later, the supplement was believed to have included an antibiotic in addition to vitamin B_{12}. Also, Burnside _et al_. (1949) observed that APF increased the feeding value of peanut meal and soybean oil meal to the extent that these plant proteins were similar in feeding value to fish meal. More recent studies at the Iowa Station (Diaz et al., 1956, 1957), involving both levels and sources of meat and bone scraps, reconfirm these observations that corn-soybean oil meal rations, adequately supplemented with vitamins, minerals and antibiotics, are equal to or superior to rations containing meat and bone scraps.

On the other hand work reported by Lewis et al. (1955), Peo (1956), and Hudman (1956) demonstrated that the performance of baby pigs on all plant protein rations does not approach that of pigs fed milk protein. Lewis et al. (1955) attributed the poor utilization of soya protein to an inadequately developed digestive enzyme system in the young pig. This theory is supported by enzyme assays of the baby pigs' digestive organs presented by Lewis et al. (1957) and Hudman et al. (1957) and also by digestibility studies reported by Lloyd and co-workers (1957) in which they demonstrated the baby pig's ability to digest various nutrients improved as the pig progressed from three to seven weeks of age.

With these reports available, it seemed advisable to study various factors which might improve the performance of a corn-soybean oil meal ration with both the weanling pig 25 to 75 pounds body weight and the baby pig one to seven weeks of age.

A comparison of the pig's calculated amino acid requirements and the calculated amino acid content of corn-soybean oil meal rations, Table 1, indicates that the amino acid most likely to be deficient is methionine. Thus, the effects of methionine supplementation of corn-soybean oil meal rations are the basis for the major portion of the research to be reported herein.

Studies with the baby pig involve the effect of methionine supplementation and various levels of soya protein on gains and feed conversion, and also the effects of methionine and arginine supplementation of soya protein and arginine supplementation of milk protein on the apparent digestibility, retention and biological value of soya and milk protein. With the weanling pig (25 to 75 pounds body weight), experiments were conducted to study the effect of protein level and energy level on the response to methionine supplementation of corn-soybean oil meal type rations. Also, studies involving the comparison of protein depletion-repletion and conventional growth trials are included.

Table 1. Comparison of amino acid requirements of pigs and the calculated quantities of the essential amino acids found in 20 percent protein rations

Amino acid	Requirement[a] % of diet	20% protein ration[b] Exp. 732	Percent of requirement met	Percent of requirement met by all soya protein Exp. 798	Percent of requirement met by all milk protein Exp. 798
Arginine	0.36	1.11	308	333	167
Histidine	0.30	0.43	143	153	200
Isoleucine	0.70	1.21	173	183	214
Leucine	0.92	1.50	163	139	222
Lysine	1.00	1.19	119	132	174
Methionine + cystine	0.66	0.54	82	73	106
Phenylalanine	0.50	0.91	182	192	204
Tryptophan	0.16	0.23	144	150	150
Threonine	0.70	0.87	124	108	123
Valine	0.62	0.99	160	161	239

[a]Requirements based on values reported in literature, Table 2.

[b]Amino acid content of feedstuffs taken from Lyman et al. (1956) and Block and Weis (1956).

REVIEW OF LITERATURE

Protein Requirements of Growing Swine

Increasing knowledge in protein nutrition indicates more and more that the emphasis should be placed on levels and ratios of amino acids in the diet and less on the crude protein level. However, due to the complications of ration formulation and the impractibility of extensive supplementation with purified amino acids, the workers in the animal nutrition field are continuously re-evaluating the various combinations of practical ingredients and attempting to establish a so called optimum protein level.

Earlier work on the protein requirements of swine, Carrol and Burroughs (1939), Keith and Miller (1939), and others, indicated the optimum protein level to be between 20 and 27 percent of the diet for the young growing pig (40 to 75 pounds body weight).

Crampton and Ashton (1942) working with a Canadian type ration concluded that 15 to 18 percent protein was adequate for pigs 30 to 100 pounds body weight and that 14 to 16 percent was adequate for pigs 100 to 200 pounds body weight.

It appears that work in England may have been overlooked by workers in this country, as a series of papers published by Woodman et al. (1939, 1940, 1941, 1945 and 1948) reported that 15, 14 and 12 percent protein rations were adequate for the weight groups, weaning to 90 pounds, 90 to 150 pounds and 150 to 200 pounds body weight, respectively. With these levels of crude protein in the ration, rate of gain equaled that on higher protein rations and the quality of their bacon type carcass was not seriously affected.

The rations used by Woodman and co-workers were similar to those used by the Canadian workers, consisting primarily of barley, wheatings, alfalfa and fish meal, whereas workers here in the mid-western section of the United States fed rations consisting mainly of corn, meat and bone scraps and soybean oil meal. The nutritional inadequacy of the corn-type diet without adequate vitamin supplementation can explain to a large extent the indicated need for higher levels of protein.

Advances in vitamin nutrition stimulated more work in this field and Cunha et al. (1949) working with Lederle's APF (animal protein factor) and vitamin B_{12} suggested that established requirements were possibly too high.

Cunha et al. (1950) compared 12.1, 15.9, 17.9 and 19.6 percent protein rations, with and without the APF supplement which contained the antibiotic, chlortetracycline. Initial weight of the pigs ranged from 18 to 20 pounds. The pigs fed the 12.1 percent protein rations with adequate vitamin and APF supplementation gained as rapidly as pigs on the 19.6 percent protein ration without supplementation. Also, the

17.9 percent ration was equal to the 19.6 percent protein ration when both were adequately supplemented.

Workers at the Iowa Station, while studying the effects of antibiotics on the performance of swine, realized the need for re-evaluating the protein requirements in the presence of antibiotic and adequate vitamin and mineral supplementation. Catron et al. (1952) reported 16, 13, and 10 percent protein rations to be adequate for the weight groups, weaning to 75 pounds, 75 to 150 pounds and 150 to 200 pounds body weight, respectively. They also concluded that in the presence of antibiotics these levels could be lowered two percentage units for each of the weight groups.

Hoefer et al. (1952) compared 18 and 15 percent protein rations for pigs from weaning to 100 pounds body weight and 15 and 12 percent from 100 to 200 pounds. The lower protein rations, 15 percent reduced to 12 percent at 100 pounds body weight, produced gains and feed efficiency equal to the higher levels. Walhstrom (1953) reported similar results using the same weight groups. Fourteen percent protein rations reduced to 12 percent at 100 pounds were equal to 18 or 16 percent rations reduced to 14 and 12 percent at 100 pounds body weight. No significant differences were detected between protein levels. These latter tests were also conducted with rations adequately supplemented with the B-complex vitamins and antibiotics.

The work of Robison *et al*. (1953) lends further support to that of Catron *et al*. (1952), in that 12.5 and 10.5 percent protein rations produced results equal to 18.5 and 16.5 percent for the weight groups, weaning to 120 pounds and 120 to 200 pounds, respectively. Also Wallace *et al*. (1954) reported that 14.3 percent protein rations were equal to 17.6 or 20.9 percent rations for pigs weighing 40 to 100 pounds body weight whether or not antibiotics were present.

Jensen *et al*. (1955) in comparing rations containing 10, 12, 14, 16, 18 and 20 percent protein continuously fed from weaning to market weight (30 to 200 pounds), reported that daily gains reached a maximum between 16 and 18 percent in the absence of an antibiotic, and between 14 and 16 percent in the presence of an antibiotic. Feed conversion data paralleled that of daily gain.

A review of the work cited herein indicates that the optimum level of protein varies with the weight of the pig, type of ration and level of fortification of the ration; however, one can readily see that extensive work has been done in this field and that further research needs to be directed toward factors affecting the requirement. The optimum level of protein for the growing pig (25 to 75 pounds body weight), apparently lies in the range of 14 to 16 percent for the corn-soybean oil meal type rations used in the studies to be reported herein.

The work on the protein requirement of baby pigs (one to five weeks of age) is not nearly so extensive as that for the older pig. Early work along this line was conducted with liquid synthetic milk diets, which have provided basic nutritional information but have proved impractical for common usage in the field. However, levels established with liquid diets have served as the basis for later work with dry rations for young pigs. Reber _et al_. (1953) fed levels of protein up to 45 percent and reported the best gains and feed conversion at about the 35 percent protein level for pigs two days of age to four weeks and approximately 25 percent for pigs older than four weeks of age.

Sewell _et al_. (1953b) obtained best results at the highest level fed (32 percent) for pigs two to 35 days of age; however, 24 and 28 percent protein diets produced results which were quite satisfactory. Becker _et al_. (1954), when comparing 10 to 30 percent protein diets for pigs one to four weeks of age and 10 to 25 percent for pigs five to nine weeks, concluded that 22 and 12 percent protein diets for the two age groups were equal to higher levels. Dried skimmilk provided the primary source of protein for these studies. Recent work with dry rations by Crampton and Ness (1954) comparing 30 and 26 percent protein rations, indicated the higher level to be more adequate.

Workers at the Iowa Station (Speer _et al_., 1954) in the development of their dry ration for weaning pigs at one week of age or five pounds body weight, were able to produce 50 pound pigs at eight weeks of age which indicates that their 24 percent protein prestarter for one to two weeks of age, 18 percent protein starter for two to five weeks of age and 16 percent protein grower from five to eight weeks of age were quite adequate for rapid gains. Again dried skimmilk served as the major source of protein for the very young pig.

Peo et al. (1954) in a comparison of 15, 20, 25 and 30 percent protein rations, concluded that the best overall performance, based on feed conversion and rate of gain was obtained with the 20 percent protein diet. A 50-50 mixture of dried skimmilk and solvent processed soybean oil meal (50 percent protein) served as the source of protein for this study. Studies reported by the Illinois workers, Jensen et al. (1957) indicated similar results with rations containing corn and crude casein as the source of protein. They suggested a level of 17 percent protein for the two to eight week period. Further work by Peo (1956) in which three sources of protein were compared at varying levels indicated the optimum level of milk protein to be at 25 percent. Results with soya protein, (from solvent processed soybean oil meal or Drackett Assay protein) were variable and inconclusive; however, 16 to 19 percent was comparable in performance to levels up to 34 percent.

Lewis _et al_. (1955) reported that the very young pig was unable to grow normally on corn-soybean oil meal diets and suggested that the young pig is not fully developed in its digestive enzyme systems, and for that reason can not adeqautely digest soya protein. These workers also reported a response to pepsin supplementation of the baby pigs' diet. Further organ assays by Lewis _et al_. (1957) indicates the pig to be very deficient in pepsin at birth and that the pepsin content of the stomach mucosa increases as the pig grows older and attains a quite high concentration by five weeks of age. This is in agreement with previous observations that the pig could not adequately utilize soya protein during the period one to five weeks of age but did very well on soya-type diets after about five weeks of age.

Digestibility studies by Lloyd _et al_. (1957) support the findings of Lewis _et al_. (1957). They studied the change in digestibility of various ration nutrients in three week old pigs and again when the same animals were seven weeks of age. These studies demonstrated that the very young pig was unable to digest the protein, fat and dry matter portions of the diet as efficiently as could the older pig.

It is recognized that the decreased digestibility could account for a relatively large portion of the difference in performance of milk- and soya-protein diets, however there are undoubtedly other factors involved. The logical starting place in a study of these factors is to compare the relative amino acid balance of the two proteins in question and to also compare them to the pig's requirement for each of the essential amino acids.

Amino Acid Requirements of Growing Swine

The extreme monetary cost of feeding purified amino acid mixtures to animals as large as the pig has handicapped the researchers in this field. However, a considerable amount of work has been conducted using diets made up of proteins which are limited in one or more of the amino acids in question.

The Purdue workers pioneered the work in this field by studying the tryptophan requirement of the pig (Beeson _et al_., 1948) and later (Mertz _et al_., 1952a) established that the same ten amino acids, that are essential to the rat (Rose _et al_., 1948), are likewise essential to the pig. They are, namely: arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine. The requirements for these ten amino acids are discussed briefly in the following pages and summarized in Table 2.

Arginine

Mertz _et al_. (1952a) concluded that the weanling pig can synthesize approximately 60 percent of the arginine required for normal growth. Pigs fed a purified diet containing 0.2 percent arginine gained 1.21 pounds daily, while those receiving no arginine gained 0.72 pounds per day. Tissue analysis by Williams _et al_. (1954) indicates that 0.9 percent is the approximate requirement; however from the previously mentioned feeding trial this would not appear to be the dietary requirement. The level of 0.2 percent presented by Mertz _et al_. does not represent the minimum requirement, however it would appear to be quite adequate.

Histidine

Likewise the histidine requirements are not clearly defined. Brinegar (1952) suggested that the pig's requirement does not exceed 1.9 percent of the protein in a 22 percent protein ration or approximately 0.42 percent of the total diet. More recently Beeson (1954) reported the requirement to be between 0.2 and 0.4 percent of a diet containing 13 percent protein. These figures agree quite favorably with the 0.34 percent calculated by Williams _et al_. (1954) using tissue analysis as their criteria.

Isoleucine

Brinegar _et al_. (1950a) reported a study on the pig's requirement for isoleucine. Their study was conducted using a mixture of DL-isoleucine and allo-isoleucine as the source of supplemental isoleucine. From this study they estimated the L-isoleucine requirement to be 0.7 percent of the diet or 3.2 percent of the dietary protein.

Becker _et al_. (1957), using a semi-synthetic diet similar to that used by Brinegar _et al_., conducted further studies on the isoleucine requirement of the pig at two stages of growth. Their studies involved a mixture of L-isoleucine and D-allo-isoleucine. Maximum rate of gain and efficiency of gain was obtained with a level of 0.46 percent L-isoleucine equal to 3.4 percent of the dietary (13.35 percent) protein. This was very similar to the 3.2 percent of the dietary protein reported earlier by Brinegar and co-workers. In the same study, using a 26.7 percent protein diet, maximum rate of gain was obtained at a level of 0.65 percent L-isoleucine equal to 2.4 percent of the dietary protein. This was observed for the pig weighing 30 to 35 pounds body weight. The same workers reported an optimum level of 0.76 percent L-isoleucine for 10 pound pigs on a 22 percent protein ration. These results are not entirely in agreement, however, considering that 26.7 percent protein is appreciably above the protein needs of the 30 to 35 pound pig, it is logical to assume that 3.2 to 3.5 percent of the dietary protein is a very good estimate of the isoleucine requirement.

Leucine

Beeson (1954) concluded that 6.0 percent of the dietary protein as L-leucine was adequate for normal growth of weanling pigs. Eggert *et al*. (1953 and 1954) reported that 4.0 to 5.0 percent of the dietary protein or 1.0 to 1.25 percent of the total diet as L-leucine was adequate to support normal growth of two day old suckling pigs. These workers employed a 25 percent protein synthetic milk diet.

Curtin *et al*. (1952a) and Williams *et al*. (1954) estimated the leucine requirement to be 4.6 percent of the dietary protein. Each were employing carcass analysis data and basing the estimate on the ratio of isoleucine to lysine, assuming that the lysine requirement was 5.5 percent of the dietary protein.

Lysine

Brinegar *et al*. (1950b) quantitatively determined the lysine requirement of pigs on a 10.6 percent protein diet containing linseed oil meal as the source of protein. The diet was supplemented with histidine and methionine to offset deficiencies of these amino acids. Also, the requirement was determined for pigs on a 22 percent protein ration containing sesame oil meal as the source of protein. In these two studies, maximum gains were obtained at 0.58 and 1.17 percent total lysine, respectively. A second 22 percent protein ration, based on meat and bone scraps, zein and wheat, gave maximal results at a lysine level between 1.0 and 1.2 percent total lysine. The reported lysine content of the third diet is considered by Almquist (1954) to be about 0.2 percent higher than would be expected from average analysis of the ingredients used. After making this assumption and then plotting the gain-feed ratio against the logarithm of the total lysine in the diet, Almquist obtained a curvilinear relationship, which was approximately linear until plateauing, as the limiting effect of protein level was reached. This manipulation of the data presented by Brinegar *et al*. has served as the basis for the calculation of the amino acids requirements from tissue analysis as presented by Curtin *et al*. (1952a) and Williams *et al*. (1954). This estimates the lysine requirement to be 5.5 percent of the dietary protein.

More recent studies at the Purdue Station by Shelton *et al*. (1951b) indicates the optimum level to more nearly approach 4.2 percent of the protein. However, this work employed a 23.8 percent protein ration which may have attained the plateau level mentioned by Almquist. The work of Jensen *et al*. (1952) and Jensen (1953) indicated that 0.7 percent lysine was the optimum level for the practical type ration fed. This represented 5.8 percent of the dietary protein fed which is in close agreement with the report by

Brinegar et al. Further studies by the Illinois workers. Hutchinson et al. (1956, 1957) estimates the optimum level of lysine for the 30 to 35 pound pig consuming a 11.69 percent protein ration to be 0.52 percent or less and for the very young pig (9.5 pounds body weight) to be 0.925 percent of a ration containing 14.25 percent protein.

Work reported by Pfander and Tribble (1954) indicates that the methionine and tryptophan requirements are independent of the protein level, however this same report supports the previous indications of the lysine requirement being directly proportional to protein levels. These and other reports suggest that a certain amount of skepticism must be held for calculated requirements based on percentage of total protein in the diet. Obviously the calculation based on tissue analysis must be viewed with caution since requirements for the index amino acid (lysine) may vary indirectly with protein levels and other dietary factors. Although Almquist (1954), after having reviewed large amounts of more thorough work with the chick, concluded that the amino acid requirements obey the law of diminishing returns, and that a plot of gain in body weight against the logarithm of the dietary essential amino acid usually results in a straight line, terminating in a well defined plateau when the limit of response is attained. The large amounts of data available indicates that the proportional requirement for amino acids remains essentially the same at varying protein levels.

Even though there are some discrepancies in the literature it is evident that these calculations can serve as guides in balancing rations for protein levels other than those tested.

Methionine and cystine

Shelton et al. (1951c) concluded that a ration containing 21 percent protein required 0.3 percent methionine plus 0.38 percent or more cystine to meet the needs of the pig. In the absence of cystine the methionine requirement appeared to be 0.6 percent. Curtin et al. (1952c) verified the above report and concluded that approximately half of the sulfur amino acid requirement can be met by the non-essential amino acid cystine.

Curtin et al. (1952b) reported further studies with methionine and estimated the weanling pig's requirement to be approximately 0.7 percent of a ration containing 22 percent protein. This corresponds to 3.2 percent of the dietary protein of which an estimated 1.7 percent can be replaced by cystine.

Calculated analysis by Curtin et al. (1952a) and Williams et al. (1954) indicates the requirement to be 1.2 percent of the protein or 0.23 percent of a 20 percent protein diet. This calculated requirement agrees quite well with the previous observations; however, Pfander and Tribble (1954) concluded that the methionine requirement is independent of the protein level, thus questions the validity of calculated requirement based on percent of protein.

More recently, Becker et al. (1955a) report that 0.25 percent methionine in the presence of 0.17 percent cystine is adequate for maximum growth and efficiency of feed conversion. Higher levels of methionine (0.35 and 0.45 percent) appeared to inhibit growth.

Phenylalanine

Studies on the phenylalanine requirements of swine were reported by Beeson et al. (1953b). Utilizing a ration containing 13 percent protein, these workers concluded that a pig required 0.46 percent phenylalanine. In assessing this value, they assumed that 0.14 percent tyrosine substituted for phenylalanine in meeting the pig's total needs for aromatic amino acids.

Threonine

Shelton et al. (1950) demonstrated threonine to be an essential amino acid, however did not assess a dietary level. Mertz et al. (1952b) estimated the requirement to be approximately 0.4 percent of a 13.2 percent protein diet. These workers used a racemic mixture for supplementation, however the estimated figure given assumes the D-threonine to be biologically inactive.

Sewell *et al*. (1953a) reported the results of a study using 25 percent protein rations and concluded that 0.92 percent was the dietary requirement under the conditions of their experiment. This value is considerably higher when expressed as percent of diet or percent of protein, than the previous report by Shelton *et al*. (1950). The calculated requirement (0.48 percent) from tissue analysis, Williams *et al*. (1954), more closely agrees with that of Shelton *et al*.

Tryptophan

Tryptophan is one of the amino acids more likely to be deficient in practical swine rations. The essentiality of this amino acid in swine rations was confirmed by Beeson *et al*. (1949). Shelton *et al*. (1951a) estimated the DL-tryptophan requirement to be 0.23 percent of a zein, gelatin and amino acid type ration. Thompson *et al*. (1952) concluded that the D-form was partially active in meeting the pig's requirement. However, the question concerning biological activity of the D-form of tryptophan and other amino acids is a subject that invites differences of opinion among workers studying amino acid nutrition.

Morrison *et al*. (1956) made a thorough study on the utilization of D-tryptophan by the chick and arrived at the conclusion that the D-isomer is biologically active, however the chick is limited in its ability to absorb the D-isomer resulting in very inefficient utilization. Thus, it appears from a practical point of view that one can assume the D-isomer to be essentially inactive for the chick and the pig, if one can also assume the pig to be similar to the chick in this respect.

Recent studies by Becker et al. (1955b) indicate that the tryptophan requirement is somewhat lower than the earlier reports, which also supports the belief that the D-isomer is inactive. These latter reports indicate that a level of 0.155 percent tryptophan was adequate for a diet containing 15.4 percent protein or 0.195 percent for a diet containing 19.6 percent protein, when feed conversion was the criteria of response. In these studies, maximum rates of gains were obtained on 0.115 and 0.155 for the two protein levels (15.4 and 19.6 percent), respectively.

Valine

There has been little work reported on the valine requirement of the pig. Jackson et al. (1953) reported the requirement to be approximately 0.4 percent of a corn-amino acid-diammonium citrate type diet containing 12.8 percent total crude protein. This represents 3.1 percent of the total protein in the ration. However, their results indicated that a clear cut minimum was not established. Rate of gain plateaued at the level of 0.4 percent L-valine, but the best feed conversion was obtained with the highest level fed, 0.7 percent. This latter observation is in line with the calculated requirement from tissue analysis, Williams *et al*. (1954).

This review of the amino acid requirements can best be summarized in tabular form and is presented in Table 2. The calculated values for the 16 and 20 percent protein rations are based on the assumption that the requirements are relatively proportional for the varying protein levels fed. These two levels of protein appear adequate for the age and weight of pigs used in the studies reported herein, thus these calculations have served as the criteria of adequacy for the various amino acids in question.

Methionine Supplementation of Practical Swine Rations

The swine rations of the mid-western section of the United States are based primarily on two major crops, corn and soybeans. Corn provides the major source of energy and soybean oil meal supplements the corn in providing the source of protein for the swine feeds. A review of the amino acid make-up of corn and soya protein compared to the pig's requirements indicates that deficiency of one of two

Table 2. Summary of studies on the amino acid requirements of swine

Amino acid	Initial wt. (lb.)	Protein level fed (%)	Requirement: % of total ration	Requirement: % of protein	Calculated requirement[a] for total ration: 16%	Calculated requirement[a] for total ration: 20%	Reference
Arginine	31	11.3	0.2[b]	1.8	0.29	0.36	Mertz et al. (1952a)
Histidine	30	13	0.2	1.5	0.24	0.30	Mertz et al. (1955)
Isoleucine	32	13.3	0.46	3.5	0.56	0.70	Becker et al. (1956)
	32	26.7	0.65	2.4	0.38	0.48	
	10	22	0.76	3.5	0.56	0.70	
	40	21	0.70	3.3	0.53	0.66	Brinegar et al. (1950a)
Leucine	6	25	1.0-1.25	4.0-5.0	0.64-0.80	0.80-1.00	Eggert et al. (1954)
	30	13	0.6	4.6	0.74	0.92	Mertz et al. (1955)
Lysine	33	10.6	0.6	5.7	0.91	1.14	Brinegar et al. (1950b)
	38	22	1.1	5.0	0.80	1.00	
	34	23.8	1.0	4.2	0.67	0.84	Shelton et al. (1951b)
	35	11.7	0.52	4.4	0.70	0.88	Hutchinson et al. (1956)
	35	14.2	0.52	3.7	0.59	0.74	
	10	14.2	0.92	6.5	1.04	1.30	

[a]Assuming the requirement to be directly proportional to protein content of ration.

[b]Minimum requirement not established.

Table 2. (Continued)

Amino acid	Initial wt. (lbs.)	Protein level fed (%)	Requirement: % of total ration	Requirement: % of protein	Calculated requirement for total ration: 16%	Calculated requirement for total ration: 20%	Reference
DL-Methionine	43	21	0.3	1.4	0.22	0.28	Shelton <u>et</u> <u>al</u>. (1951c)
	34	22	0.45	2.0	0.32	0.40	Curtin <u>et</u> <u>al</u>. (1952c)
	28	12.6	0.25	2.0	0.32	0.40	Becker <u>et</u> <u>al</u>. (1955a)
DL-Methionine +cystine	43	21	0.6	2.9	0.46	0.58	Shelton <u>et</u> <u>al</u>. (1951c)
	34	22	0.72	3.3	0.53	0.66	Curtin <u>et</u> <u>al</u>. (1952c)
	28	12.6	0.42	3.3	0.53	0.66	Becker <u>et</u> <u>al</u>. (1955a)
Phenylalanine	25	12.6	0.32	2.5	0.40	0.50	Mertz <u>et</u> <u>al</u>. (1954)
Phenylalanine + tyrosine	25	12.6	0.46	3.7	0.59	0.74	Mertz <u>et</u> <u>al</u>. (1954)
Threonine	4.2	25	0.9	3.6	0.58	0.72	Sewell <u>et</u> <u>al</u>. (1953a)
	24	13.2	0.4	3.0	0.48	0.60	Beeson <u>et</u> <u>al</u>. (1953a)
Tryptophan	31	24.5	0.1	0.41	0.07	0.08	Shelton <u>et</u> <u>al</u>. (1951a)
	30	15.4	0.12-0.16	0.8-1.0	0.16	0.16-0.20	Becker <u>et</u> <u>al</u>. (1955b)
		19.6	0.16	0.8	0.13	0.16	
		15.3	0.10	0.6	0.10	0.12	
Valine	28.1	12.8	0.4	3.1	0.50	0.62	Jackson <u>et</u> <u>al</u>. (1953)

amino acids is likely to occur. Rations relatively low in protein are border line in lysine adequacy, whereas rations relatively high in protein are likely to be deficient in methionine.

Soya protein served as the major source of protein for the majority of studies reviewed dealing with the methionine requirement of the pig. In addition to these, numerous reports have appeared on the methionine supplementation of practical swine rations.

Dyer et al. (1949) reported a growth response when methionine was added to a 10 percent protein soybean oil meal ration. This response to methionine was not observed in the presence of adequate choline which in this case indicates the response to be from a methyl-group donor rather than a response to methionine as an amino acid.

Bell et al. (1950) studied the effects of methionine supplementation of a soybean oil meal purified ration and found that the supplemented ration resulted in efficiency of gain and biological value equal to a similar ration containing whole egg as the source of protein.

Robison (1951) reported that synthetic DL-methionine did not improve a corn-soybean oil meal type diet containing 15 percent protein. Also, Curtin et al. (1952d) reported no statistically significant improvements of a 22 percent protein semi-purified ration containing soybean oil meal as the source of protein. However, in this report the observed data indicated an improvement in apparent nitrogen retention, feed conversion and daily gain. Possibly the high level of protein fed (22 percent) to weanling pigs (25 pounds body weight) could explain the variability in response to supplemental methionine.

Acker (1953) reported a response to methionine supplementation of a corn-soybean oil meal ration provided that the ration was also supplemented with lysine. This indicates that each of the amino acids were limiting; however, in the 12 and 14 percent protein rations fed, it appeared that lysine was the first limiting and that a response to methionine could not be realized unless the lysine deficiency was first corrected.

Pfander and Tribble (1955) concluded from their studies that supplemental methionine added to a corn-soybean oil meal-tankage ration improved growth rate and feed conversion, but did not improve performance when added to a corn-soybean oil meal type diet. These conclusions were based on single lots of pigs and one would question the methods used in the statistical analysis of the data and therefore question the soundness of conclusions drawn.

Meade (1956b) using nitrogen balance as the criteria of response found supplemental methionine did not improve a corn-soybean oil meal type diet containing 15.9 percent protein.

In these studies daily feed allowance was restricted to 4.25 percent of body weight. Later the same year, Meade (1956a) reported studies with corn-soybean oil meal diets containing 12, 14 and 16 percent protein and again observed no improvement in nitrogen balance that could be attirubted to supplementation with methionine.

From the reports cited herein, one would conclude that little or no improvement of a standard corn-soybean oil meal ration could be expected from supplemental methionine; however, the research conducted concerning the methionine requirements of the pig has to a large extent made use of soya protein thus demonstrating that this protein is deficient in methionine. Evidently corn and soya protein complement each other to the extent that rations based on these two ingredients are quite adequate in methionine.

On the other hand, baby pig rations which contain a higher level of protein and in which simple sugars are substituted for a portion of the corn as a source of carbohydrate, contain a relatively higher percentage of soya protein. With these shifts in the ration formula, a level of methionine is established which is definitely border line in adequacy based on the reported requirements.

Energy, Protein and Methionine Interrelationships

The increasing of the energy content of a corn-soybean oil meal diet by the addition of fats or oils is also another situation in which a methionine deficiency might exist. As fat replaces corn in the ration, in order to maintain a constant protein level, it becomes necessary to further decrease the percent of corn and increase the amount of soybean oil meal. This adjustment brings about a lowering of the methionine content, the extent of which depends on the quantity of fat added. Furthermore, research indicates that the percentage protein (or amino acid) requirement increases as the energy content of the diet increases. This theory is not new as many reports are present in the literature concerning the interrelationships of carbohydrates, fats and proteins in the diet. Much of this work is summarized in a comprehensive review by Munro (1951). Further interest developed in this field with the introduction of high energy broiler diets by Scott et al. (1947).

Hill and Dansky (1950) observed that growth of chicks was reduced when a high energy-low protein ration was fed, and that reduction of the energy level restored rate of growth. Also, Sunde (1956) observed a reduced rate of growth on a ration high in protein and low in energy. The addition of fat improved rate of growth. Donaldson et al. (1956) attempted to establish optimum calorie to protein ratios for chick rations. They observed a high correlation between carcass composition and the calorie-protein ratio but the effects on gains and feed conversion were not nearly so clear cut. The increase of protein content of the diet improved feed conversion on each level of energy and also the increase of energy content improved feed conversion on each level of protein, indicating no apparent interaction between level of protein and level of energy, although the design of the experiments reported did not permit statistical confirmation of these observations. Recently Balloun (1957) reported an improvement in feed conversion with increased energy levels independent of the protein level of the ration.

Work with baby pigs (Peo, 1956) dealing with the protein and fat requirements did not show an interaction of level of protein and level of fat in the ration on gains or feed conversion. The protein levels of 15, 20, 25 and 30 percent and fat levels of 0, 2.5, 5.0 and 10.0 percent would appear to cover such a range that interreactions should be detectable.

Rosenberg et al. (1955b) working with broilers reported an interaction of added methionine and added fish meal on rate of gain. They observed a greater response to methionine in the presence of fish meal than in the absence. Also, Rosenberg et al. (1955a), Baldini and Rosenberg (1955) and Rosenberg and Baldini (1956) reported an interaction of increased energy and added methionine on gains and suggested that in the earlier reports the energy level may have been responsible for the increased response to methionine in presence of added fish meal.

These reports indicated that there was a need for further studies on the interrelationships of protein, amino acids and energy levels in swine rations.

Protein Depletion-Repletion

In previous work conducted at this station a considerable amount of variation has been noted among individual pigs receiving the same ration treatment. This variation has frequently masked any treatment differences that may have been present or increased the error term in the analysis of variance to such an extent that observed treatment differences would not stand up statistically. Peo *et al.* (1957a, 1957b) reported studies in which they made use of a modification of the protein depletion-repletion technique of Cannon *et al.* (1944), in an attempt to overcome a portion of this variability.

The depletion-repletion technique with adult animals has proven very satisfactory as a method for assessing biological value of proteins, however previous to the work of Peo *et al.*, there had been little work reported in which young growing animals were used. Also the literature contains very little information concerning direct comparisons of the deplete-replete technique with other acceptable methods. Although a comprehensive study sponsored by the

Bureau of Biological Research, Rutgers University (1946-50) concerning the cooperative determination of the nutritive value of six selected proteins has demonstrated a close correlation between results obtained with repletion of adult animals and growth of young animals.

Benditt _et al_. (1948) made an indirect comparison of the rat-repletion technique and growth trial as methods of measuring protein utilization. They made use of growth data obtained by Barnes and co-workers (1946) and compared the results to their data obtained with the rat-repletion method. The results obtained by the two groups using different methods were remarkably similar; however, one would question the validity of such a comparison on the basis of the complicating factors such as differences in environment, time, genetic make-up of the animals, etc., which would undoubtedly affect the final results. They also question the applicability of their results to other species, specifically human beings.

Recently Beaty _et al_. (1957) reported work conducted with young growing chicks and concluded that the protein depletion-repletion technique resulted in valid answers. In a comparison of three sources of protein by depletion-repletion and also by conventional growth tests, they found that the three sources maintained similar positions with respect to nutritive value in each of the two methods. This again was an indirect comparison as the two tests were not conducted simultaneously.

It would appear that a young growing animal depleted of its protein stores would be more sensitive to small changes in the quality of the dietary protein and thus would be a useful tool in studying the advantages of amino acid supplementation of baby pig rations. The work of Peo et al. (1957a) indicates that repletion gains are less variable than gains observed in conventional trials, as coefficients of variation of less than 3.0 percent were observed.

The technique was used in a portion of the experiments reported herein to further study the validity of the results and to also determine whether or not the protein depleted animal is more sensitive to slight changes in protein quality such as would be brought about by amino acid supplementation.

INVESTIGATIONS

The research reported herein was conducted as a series of six baby pig experiments and three growing pig experiments and are on file at the Animal Husbandry Section of the Iowa Agricultural Experiment Station as Swine Nutrition Experiments 732, 746, 754, 768, 772, 798, 715, 774 and 774a. Certain features of the experiments were similar and will be described briefly to avoid unnecessary repetition in the subsequent detailed discussion of individual experiments.

All animals used were crossbred pigs resulting from the mating of Farmer's Hybrid boars to three-way crossbred sows (Poland China, Landrace and Duroc). The male pigs were castrated during their first week of life and all pigs were treated for anemia with an iron-copper paste at one and seven days of age. The baby pigs were also injected with 10 cubic centimeters of cholera-erysipelas anti-serum prior to going on experiment. The heavier pigs used in experiments 715, 774 and 774a were previously immunized against cholera and erysipelas and sprayed with a benzene-hexachloride solution to control external parasites.

The buildings were steam cleaned and thoroughly disinfected prior to the initiation of each experiment. Care was taken throughout the experiments to avoid contaminating diseases by minimizing number of visitors, providing disinfectant pans for boots of workers and visitors, cleaning the pens daily and by avoiding the transfer of other animals to and from the units.

Animals that died during the experiments, or were removed from the experiments because of illness, were taken to the Iowa State College Veterinary Diagnostic Laboratory for necropsy and bacteriological examinations. In group feeding experiments, when an animal was removed the average gain for the remaining pigs in the pen was used as the gain observation and the feed required per pound of gain was adjusted for any gains made by the pig prior to removal, assuming the animal removed was as efficient in feed conversion as the other pigs in the same pen. It is realized that this method of adjustment usually penalizes the pen in question, however it also appears impossible to accurately estimate the feed consumption by other means. When a pig was removed from an experiment employing individual feeding the missing data were calculated by appropriate methods as described by Snedecor (1956). The observations were treated as calculated values in the analysis of variance and the corresponding degrees of freedom removed from the error term.

In each of the experiments, the pen was considered the experimental unit and the data were analyzed on that basis, according to methods described by Snedecor (1956) and Cochran and Cox (1957). Statements concerning statistical significance pertain to the probability level of 5 percent or less.

Methionine Supplementation of Baby Pig Rations

Experiment 732 - Methionine levels for baby pig rations

Objective. Previous work at this station by Peo *et al.* (1957a) indicated that the depletion-repletion technique could be applied to the young growing pig; however direct comparisons of the method with conventional growth trials were not reported. Thus, this experiment was conducted to directly compare the two methods and to study the effect of supplemental methionine to corn-soybean oil meal diets for baby pigs.

Experimental

Animals. Thirty-two crossbred pigs averaging 8.4 pounds body weight and 11.5 days of age were selected and randomly assigned by initial weight to the eight treatments. The restriction was placed on allotment that no two litter mate pigs would receive the same treatment. The animals were confined to individual metabolism cages; however the screen flooring was removed so that the pigs were on concrete with wood shavings provided as bedding. Feed and water were provided *ad libitum* by individual self-feeders and continuous flow type waterers.

The pigs were housed in a controlled temperature, well ventilated baby pig nursery (Unit E) equipped with germicidal lamps around the room, at the entrance and at the air inlet. The concrete floor was radiant heated by thermostatically controlled circulating water and the room temperature was controlled by a forced air heating and air conditioning system. Initially, the room temperature was maintained at 80 degrees Fahrenheit and the floor temperature at 85 degrees Fahrenheit. The temperatures were reduced four degrees each week to 68 degrees and 72 degrees, respectively, and maintained at those temperatures for the remainder of the experiment.

Weight gains and feed intake data were collected weekly and the experiment terminated after 28 days including the preliminary feeding period.

Rations. During the first week all animals were fed a common pre-starter ration (Table 12) similar to that described by Speer et al. (1954) for early weaned pigs. This preliminary feeding period was used to avoid placing the pigs directly on a protein-free ration and also to allow the pigs to adjust to the new environment.

After the end of the preliminary feeding period, half of the pigs were fed the four experimental repletion diets for three weeks and the other half were fed a protein-free depletion ration for one week, then repleted with the experimental rations for two weeks.

The protein-free depletion ration was composed primarily of cane sugar and lactose and was similar to the depletion ration used by Peo et al. (1957a). The four experimental repletion rations were 20 percent protein pig starter rations supplemented with 0.00, 0.025, 0.05 or 0.10 percent DL-methionine. The rations were composed primarily of practical ingredients commonly used in swine feeds. Solvent processed soybean oil meal (50 percent protein) provided the major source of protein with lesser amounts contributed by sweet dried whey (13 percent protein) and ground yellow corn (8.9 percent protein). The whey was used as an economical source of lactose, which Hudman (1956) demonstrated to be the carbohydrate of choice for baby pigs. The whey (at a level of 15 percent) contributed 9.8 percent of the total protein fed. However, whey protein is also low in methionine (approximately 1.2 percent) thus the calculated analysis of the basal ration (Table 1) indicated that methionine was the first limiting amino acid. The composition and calculated analysis of the protein-free and basal rations are presented in Tables 3 and 13.

The rations were supplemented with 0.25 percent pepsin (activity 1:3000 N.F.) in view of the report by Lewis et al. (1955) that pepsin improved the performance of baby pigs fed plant protein diets. Supplemental vitamins, antibiotics and minerals were added at the levels indicated in Tables 3, 14 and 15. Similar rations were used in subsequent

Table 3. Experiment 732 - Composition of 20 percent protein repletion and depletion ration

Ingredients	Proportion	
	Repletion basal[a]	Depletion
Ground yellow corn (8.9% protein)	32.46	
Cane sugar	15.00	15.00
Lactose		72.10
Solvent soybean oil meal (50.0% protein)	28.06	
Dried whey (sweet) (13.0% protein)	15.00	
Stabilized lard	2.50	2.50
Solka flock		2.00
Dried beet pulp	2.00	
Pepsin (1: 3000 N.F.)	0.25	
Vitamin-antibiotic premix[b]	2.00	2.00
Calcium carbonate	0.33	
Dicalcium phosphate	1.75	3.90
Iodized salt	0.50	0.50
Trace mineral mix	0.15[c]	2.00[d]
Total	100.00	100.00

[a]Ration analyzed; 1.273 percent lysine and 0.318 percent methionine.

[b]Composition given in Table 15.

[c]Trace mineral mixture 35-C-41, composition given in Table 14.

[d]Trace mineral mixture 35-D-10A, composition given in Table 14.

experiments 746, 754 and 772.

Analysis of data. The data for total gain and feed required per pound of gain were analyzed according to the analysis of variance plan presented in Table 17 in the Appendix. The two week repletion gains and feed required per pound of gain and the three week conventional gains and feed required per pound of gain were the observations used in the analysis.

Results and discussion. Summaries of the average total gains and feed required per pound of gain are presented in Table 4.

The general overall performance of the pigs in this experiment was quite variable and relatively poor. Moderately severe scouring was observed during the second week of the experiment, however the intensity or persistency of scouring could not be associated with rate of gain or feed conversion. One pig, receiving 0.05 percent supplemental methionine in the conventional growth group, died during the course of the experiment. Necropsy examination revealed a non-specific enteritis, which may have contributed to the causes of scouring in the other animals.

Due to the variability of response and small number of animals involved, no definite conclusions can be drawn concerning the effects of supplemental methionine on gains or feed conversion. However, the performance appeared less

Table 4. Experiment 732 - Summary of gains and feed required per pound of gain

	Added methionine levels							
	0.00	0.025	0.05	0.10	0.00	0.025	0.05	0.10
Rep.	2 week repletion gains (lbs.)				3 week conventional growth (lbs.)			
1	10.6	14.7	5.9	8.4	15.3	19.2	15.9	18.2
2	13.1	15.1	16.8	16.1	18.0	13.4	17.7[a]	13.2
3	14.0	11.3	13.1	15.9	16.7	12.4	18.7	6.7
4	12.9	14.7	11.7	14.1	6.3	10.0	10.4	4.6
Average	12.6	14.0	11.9	13.6	14.1	13.8	15.0	10.7
Rep.	Feed/gain (lbs.)							
1	1.63	1.44	1.80	2.57	1.58	1.61	1.92	1.52
2	1.33	1.45	1.36	1.42	1.43	1.62	1.54[a]	1.57
3	1.46	1.40	1.63	1.47	1.48	1.58	1.36	2.82
4	1.43	1.51	1.23	1.29	1.62	2.00	2.16	2.13
Average	1.46	1.45	1.50	1.69	1.53	1.70	1.81	2.01

[a]Calculated value (Snedecor, 1956, p. 310)

variable when measured by the depletion-repletion technique than when measured by conventional growth. The coefficients of variation for repletion gains and feed required per pound of gain were 19.0 and 17.9 percent, respectively, as compared to 24.4 and 22.2 percent for the gains and feed conversion on the conventional growth study. This is in agreement with the observation of Peo *et al*. (1957a), further suggesting that the depletion-repletion technique is more sensitive than conventional feeding trials.

Experiment 746 - Methionine levels for baby pig rations

Objective. The results of experiment 732 did not show a beneficial effect that could be attributed to supplemental methionine; however they did indicate that the depletion-repletion technique is more sensitive than conventional growth trials, thus should be useful in detecting small differences in protein quality that would be brought about by amino acid supplementation. It was the purpose of this experiment to further study the effects of supplemental methionine on the quality of corn-soybean oil meal diets as measured by rate of gain and efficiency of feed conversion, making use of the depletion-repletion technique.

Experimental

Animals. Eighty crossbred pigs averaging 7.1 pounds body weight and 10.5 days of age were selected and randomly assigned by weight within litter to a randomized block design. Four pens (replications) of four pigs per pen were subjected to each of the five ration treatments. The animals were housed in a baby pig nursery (Unit C) equipped with a forced air ventilating system. The experiment was conducted during the summer months of July and August, 1956, thus the temperature was allowed to fluctuate with outside temperatures.

Feed and water were provided *ad libitum* by self feeders and automatic float controlled water fountains. Weight gains and feed intake data were determined weekly and the experiment was terminated after 35 days including the seven day preliminary feeding period.

Rations. The rations used in this experiment were identical to those used in experiment 732 (Table 3) except for an additional level (0.20 percent) of supplemental methionine. All pigs were fed a common pre-starter ration for one week. They were fed the protein-free depletion ration for a week and then repleted with their respective replete rations which contained 0.0, 0.025, 0.05, 0.10 and 0.20 percent supplemental methionine. After the repletion period, the depletion-repletion cycle was repeated.

Analysis of data. The gain and feed data for the two repletion periods were pooled and analyzed according to the analysis of variance plan in Table 19. The pen of four pigs was considered the experimental unit and the average total repletion gain and feed required per pound of gain were the observations used in the analysis.

Results and discussion. Summaries of the gain and feed required per pound of gain data are presented in Figure 1 and Table 18.

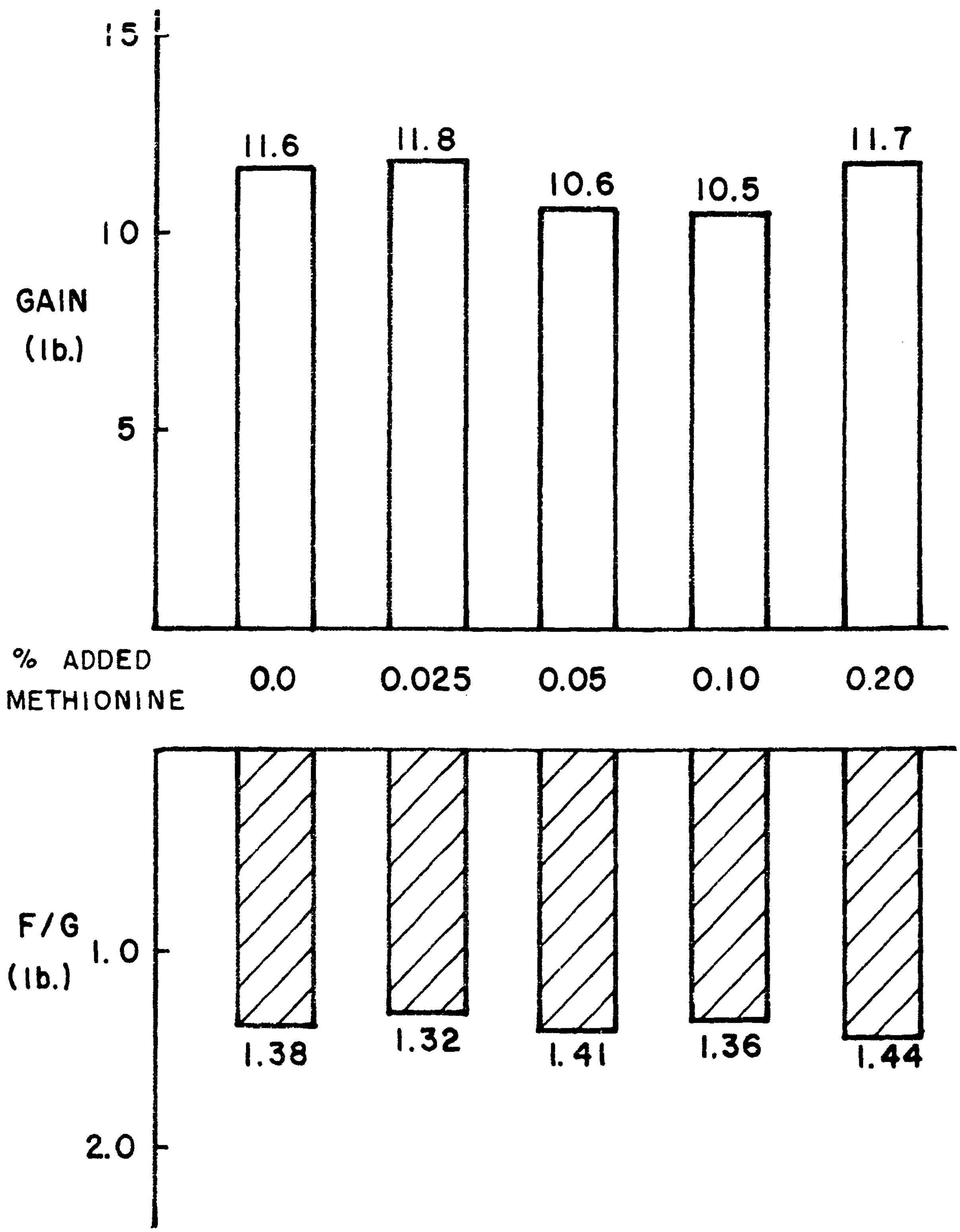


Figure 1. Experiment 746 - Effect of supplemental DL-methionine on repletion gains and feed required per pound of gain

The general performance of the pigs in this experiment was quite satisfactory with an average repletion gain of 11.2 pounds after an average summed depletion loss of 1.38 pounds. The within treatment variability was relatively low with an observed coefficient of variation of 8.3 percent for repletion gains and 4.2 percent for feed required per pound of gain.

The maximum repletion gains and most efficient feed conversion were observed for the pigs receiving the ration containing 0.025 percent supplemental methionine, however there was no consistent trends in repletion gains or feed conversion that could be associated with levels of supplemental methionine. Thus the slight improvements observed for the 0.025 percent added methionine were probably a result of random variation.

These findings support the results of experiment 732 in that supplemental methionine did not consistently improve the repletion performance of protein depleted pigs fed a 20 percent protein ration. Evidently the corn and whey proteins complemented the soya protein to the extent that methionine was not the first limiting nutrient in the rations fed.

Experiment 754 - Protein levels for baby pig rations, with and without methionine supplementation

Objective. Two previous experiments failed to demonstrate a beneficial effect of supplemental methionine to a

20 percent protein ration. Thus, this experiment was conducted to study the effects of increasing levels (19, 22, 25, 28 and 31 percent) of protein on feed conversion and repletion gains and to also compare the response to each of these levels of protein in the presence and absence of 0.05 percent supplemental DL-methionine.

Experimental

Animals. A total of 120 crossbred pigs averaging 8.9 days of age and 6.9 pounds body weight were selected and assigned to the ten ration treatments. Three replications (four pigs per pen) of a randomized block design were employed using initial weight as the basis for allotment with the restriction that no two litter mates appear on the same treatment. The pigs were maintained in Unit C (same as used in experiment 746) in concrete floored pens with wood shavings provided for bedding. The room temperature was held above a minimum of 72 degrees Fahrenheit and the maximum temperature allowed to fluctuate with the prevailing outside temperatures during the months of September and October in which the experiment was conducted. Also, heat lamps were used during the first week of the experiment to provide additional restricted space heating. Feed and water were provided *ad libitum* by self feeders and float controlled water fountains. Weight gains and amounts of feed consumed were determined weekly and the experiment was terminated after 35 days.

Rations. The common pre-starter ration used in experiment 732 was fed to all pigs for a seven day preliminary period after which time they were fed a protein-free depletion ration for one week, then repleted for one week and finally the depletion-repletion cycle repeated. The management of the pigs was essentially the same as that for experiment 746.

The repletion rations consisted of five levels of protein (19, 22, 25, 28 and 31 percent) with and without 0.05 percent supplemental methionine to make up the ten ration treatments. The composition and calculated analysis of the 19 percent protein ration are presented in Tables 5 and 13. The rations were similar to those fed in experiments 732 and 746 except that the corn and soybean oil meal were adjusted accordingly to give the desired protein levels; also the dicalcium phosphate and calcium carbonate were adjusted to give constant calculated levels of calcium and phosphorus. Vitamins and trace elements were added at the rates indicated in an attempt to insure adequate intake of these nutrients.

Analysis of data. The data for the two repletion periods were pooled and analyzed according to the analysis of variance plan presented in Table 21. The average total repletion gains and feed required per pound of gain were used as the observations in the analysis. Statements concerning

Table 5. Experiment 754 - Composition of 19 percent protein basal ration

Ingredients	Proportion
Ground yellow corn (9.4% protein)	35.35
Cane sugar	15.00
Solvent soybean oil meal (50.0% protein)	25.00
Dried whey (sweet) (13.0% protein)	15.00
Stabilized lard	2.50
Dried beet pulp	2.00
Pepsin (1:3000 N.F.)	0.25
Vitamin-antibiotic premix[a]	2.00
Calcium carbonate	0.25
Dicalcium phosphate	2.00
Iodized salt	0.50
Trace mineral mixture (35-C-41)[b]	0.15
Total	100.00

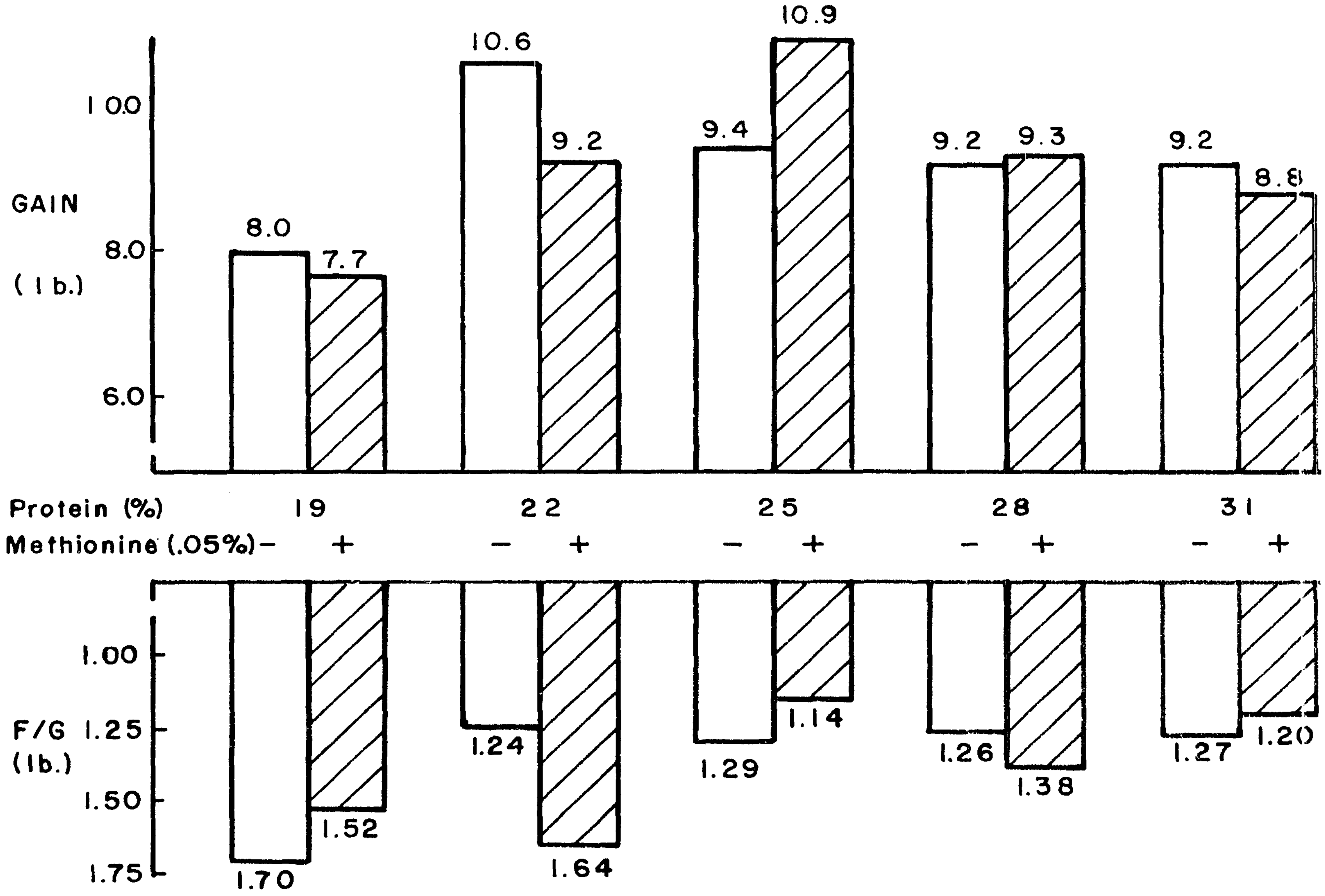
[a]Composition given in Table 15.

[b]Composition given in Table 14.

significance pertain to a probability level of 5 percent or less.

Results and discussion. Summaries of the repletion gains and feed required per pound of repletion gain are presented in Figure 2 and Table 20.

Figure 2. Experiment 754 - Effects of supplemental DL-methionine and level of protein in the diet on repletion gains and feed required per pound of gain


GAIN
(l b.)
10.0
8.0
6.0
8.0
7.7
10.6
9.2
9.4
10.9
9.2
9.3
9.2
8.8
Protein (%)
19
22
25
28
31
Methionine (.05%)
-
+
-
+
-
+
-
+
-
+
F/G
(lb.)
1.00
1.25
1.50
1.75
1.70
1.52
1.24
1.64
1.29
1.14
1.26
1.38
1.27
1.20

The overall performance of the pigs was mediocre to poor with an average final weight of 16.0 pounds. A moderately severe outbreak of scours moved through the building and could have been partially responsible for the slow rate of gain. A daily index of the degree of scouring was recorded; however the intensity or persistency of scouring could not be associated with ration treatment, rate of gain or feed conversion.

One pig died during the last repletion phase and six pigs died within three days following the termination of the experiment. Necropsy examination of the first pig showed hyperemia of the mesenteric vessels and diffuse hemorrhage of the myocardium. Examination of the other pigs revealed mandibular absesses in two pigs, edema of the eyelids and gastric submucosa, congested somatic lymph nodes, and gross lesions of bronchitis in one pig, excessive sero-sanguiness fluid in thoracic and abdominal cavities in one pig, severe anemia in one pig, and finally no macroscopic lesions in the sixth pig. These findings could not be associated with the repletion diets; however, the protein depletion-repletion cycles used in this experiment could have been contributing stress factors in bringing on the described conditions, although in the two previous experiments, 96 pigs were exposed to similar depletion-repletion treatments without resulting in any deaths. Also in similar studies, Peo _et al_.

(1957a, 1957b) did not encounter such death losses.

Rate of gain increased with increasing protein levels up to 25 percent and then declined as the protein level was increased further to 31 percent. The gains of the pigs on 22 percent protein was essentially equal to that on 25 percent (9.9 versus 10.2 pounds) indicating that the peak of the curve may have been between the 22 and 25 percent protein level. This quadratic regression of gains on protein levels was significant. Methionine supplementation had no consistent effect on rate of gain. Supplementation of the two protein levels giving best performance resulted in a slight improvement on the 25 percent protein ration and a slight depression on the 22 percent protein ration. This slight improvement on the 25 percent protein level did not prevail as the protein level was increased, thus it would appear that the differences in performance on supplemented and unsupplemented diets were largely due to random variation and not associated with the level of protein in the ration.

The feed required per pound of gain decreased from 1.61 pounds on the 19 percent protein ration to 1.22 pounds on the 25 percent protein ration and leveled off as the protein level increased to 31 percent. There was a definite improvement in feed conversion with 25 percent protein as compared to 22 percent, thus suggesting 25 percent protein as the optimum level of protein for the conditions of this experiment. The quadratic regression of feed required per pound of gain on protein levels was significant. Also the protein level x supplemental methionine interaction was significant but it is difficult to explain on the basis of the observed data. Had methionine also improved feed conversion at the 22 percent protein level, one could propose that the rations were deficient in methionine and this deficiency could be overcome by over feeding of protein. However, with this apparent reversal at the 22 percent level, one would suspect that this was a one in 20 chance where the statistical analysis erroneously indicated a significant effect.

Experiment 772 - Protein levels for baby pig rations, with and without methionine supplementation

Objective. The results of experiment 754 indicated that 22 to 25 percent protein in a corn-soybean oil meal-whey type starter diet resulted in maximum repletion gains and feed conversion. This experiment was conducted to compare performance on levels of protein ranging from 16 to 25 percent using similar rations to those used in experiment 754 but using a conventional growth trial rather than depletion-repletion technique. This would provide additional information on the protein requirement and also, with the results of experiment 754, would give an indirect measure of the validity of the depletion-repletion method.

As in experiment 754 each level of protein was fed with and without 0.05 percent DL-methionine supplementation.

Experimental

Animals. At the initiation of the experiment, the intentions were to test three levels of protein (19, 22 and 25 percent) with and without supplemental methionine. However after having completed two replications (pens) of four pigs per pen per treatment, it was deemed necessary to also include an additional level of protein (16 percent) with and without methionine, thus three replications of these two ration treatments were conducted simultaneously with a third replication of the other six ration treatments. For testing 19, 22 and 25 percent protein with and without methionine, 12 litters of six pigs each were selected and assigned by weight within litter to the six ration treatments. Eight litters were selected for the first two replications of four pigs per pen then later four more litters were selected for the third replication. Simultaneously with the selection of the third replication, other litters were selected to provide three pens of four pigs per pen for each of the two 16 percent protein rations.

The pigs were housed in a concrete floored baby pig nursery (Unit C, same as used in experiment 746 and 754) with wood shavings provided as bedding. The room temperature was maintained at 72 degrees Fahrenheit by a thermostatically controlled forced air heating system, also heat lamps were used for the first two weeks of the experiment to provide additional restricted space heating. The experiment was conducted during the months of January, February and March 1957. Feed and water were provided ad libitum by self feeders and automatic float controlled water fountains. Initially, the pigs averaged 8.2 pounds body weight and 10.6 days of age. Weight gains and feed intake data were recorded weekly and the experiment terminated after 35 days including the seven day preliminary feeding period.

Rations. The eight ration treatments used in this experiment were identical to the 19, 22 and 25 percent protein rations fed in experiment 754 plus two additional rations of 16 percent protein with and without supplemental methionine. The composition and calculated analysis of the 19 percent protein ration were presented in the discussion of experiment 754, Tables 5 and 13.

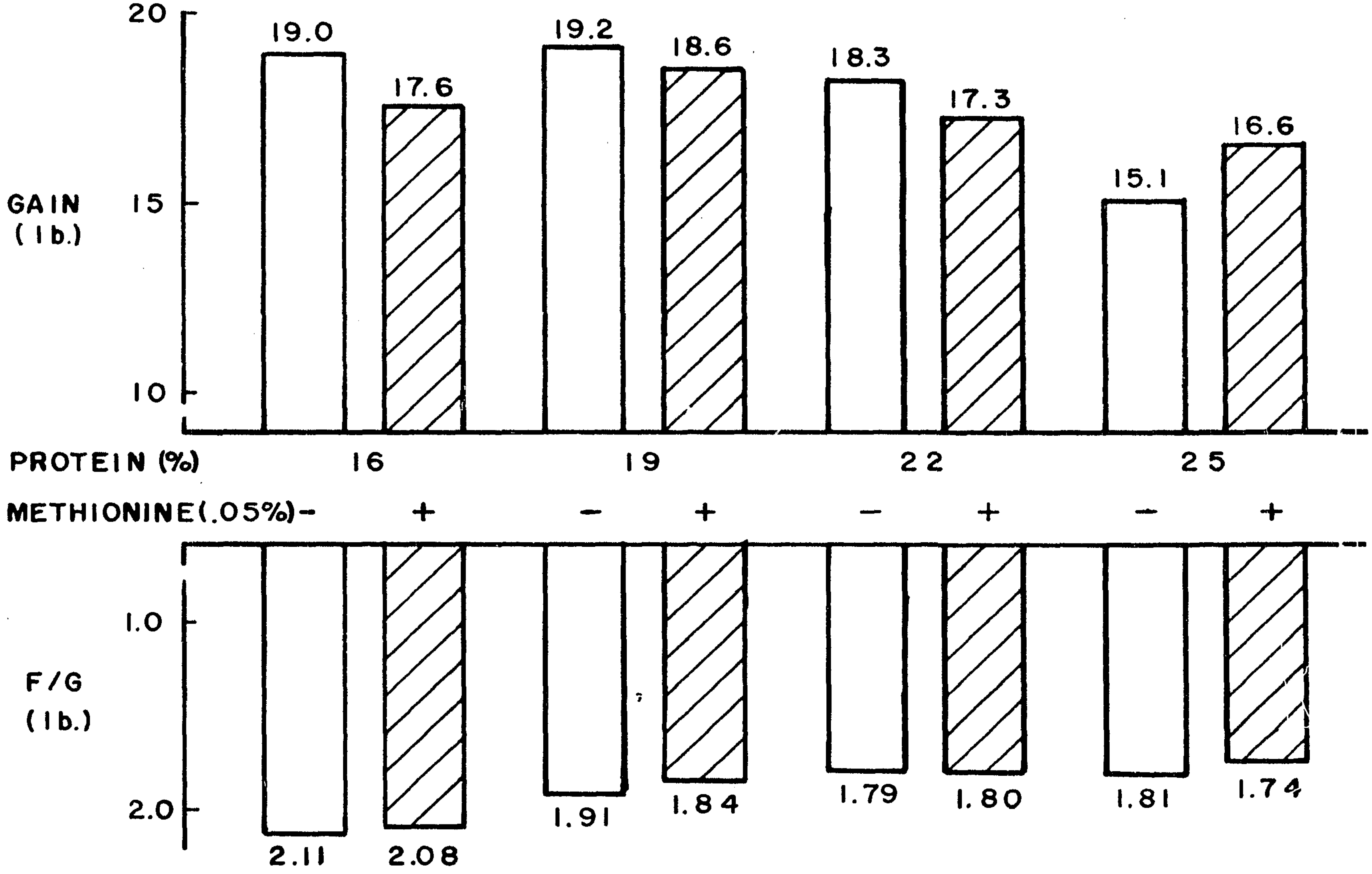
Analysis of data. The gain and feed required per pound of gain data for the 19, 22 and 25 percent protein rations were analyzed according to the analysis of variance plan presented in Table 23. The data for the 16 percent protein rations were not used in the analysis as all three replications of these two treatments were initiated simultaneously with the third replication of the other six ration treatments. The first two replications of the six ration treatments had been initiated at an earlier date. Also litter mates were controlled across the 19, 22 and 25 percent protein rations, thus the observed treatment differences for the two 16 percent protein rations are confounded with litter differences and time effects.

Results and discussion. Summaries of average total gains and feed required per pound of gain are presented in Figure 3 and Table 22.

The gains and feed conversions were quite satisfactory for pigs fed diets composed primarily of plant proteins. The average four week gain for all levels of protein was 17.7 pounds with an average of 1.88 pounds of feed required per pound of gain. One pig receiving the 25 percent protein ration without supplemental methionine died during the course of the experiment. Necropsy examination of the pig showed the presence of a ruptured liver with extensive abdominal hemorrhage.

On completion of two replications on the rations containing 19 to 25 percent protein, it was observed that 19 percent protein had produced maximum gains, thus it was deemed necessary to also check the performance on 16 percent protein rations. Three replications of four pigs per pen were carried on the 16 percent protein rations simultaneously with a third replication of four pigs per pen on the other rations. The average gains on the 16 percent protein rations

Figure 3. Experiment 772 - Effects of supplemental DL-methionine and level of protein in the diet on gains and feed required per pound of gain


GAIN
(lb.)
F/G
(lb.)
20
15
10
1.0
2.0
19.0
17.6
19.2
18.6
18.3
17.3
15.1
16.6
PROTEIN (%)
16
19
22
25
METHIONINE(.05%)
-
+
-
+
-
+
-
+
2.11
2.08
1.91
1.84
1.79
1.80
1.81
1.74

were greater than any of the other levels except 19 percent protein. However it should be noted, in Table 22, that the gains made by the third replication were considerably greater than those by the first two replications of the three higher protein levels. Also the average gain made by the three replications of the 16 percent protein rations was less than the average gain of the third replication of the other six ration treatments. These observations would indicate that the 16 percent protein rations were not adequate to promote maximum gains. Also the feed conversion data revealed that five of the pens on 16 percent protein required more than 2.0 pounds of feed per pound of gain whereas none of the pens on the other protein levels required as much feed per pound of gain. It was quite evident that 16 percent protein was inadequate and it was also evident that supplemental methionine did not improve the performance of the pigs receiving 16 percent protein rations.

The statistical analysis was computed on the gain and feed data for the six rations containing 19 to 25 percent protein and revealed that the linear regression of gain on protein levels was significant. Maximum gains were observed on 19 percent protein without supplemental methionine and the gains decreased as protein in the ration was increased to 22 and 25 percent. Supplementation of the 25 percent protein with 0.05 percent DL-methionine appeared to improve gains (16.6 pounds as compared to 15.1 for the unsupplemented group); however the performance on both the 25 percent protein rations was considerably below the average gain of 19.2 pounds on the unsupplemented 19 percent protein ration.

The linear regression of feed required per pound of gain was also significant. Feed conversion improved up to 25 percent protein and the most efficient ration was the 25 percent protein plus methionine. Supplemental methionine slightly improved feed conversion on both 19 and 25 percent protein, but did not on the 22 percent protein ration. The average effect of methionine or the methionine x protein level interaction did not approach statistical significance.

From the results of this experiment, it appears that approximately 19 percent protein is required for maximum rate of gain whereas 25 percent or higher is necessary for most efficient feed utilization. The 22 percent protein ration appeared to be near adequate as the greatest improvement in feed conversion resulted from increasing protein from 19 to 22 percent as evidenced by feed conversion figure of 1.88 reduced to 1.80 while further increasing the protein level to 25 percent only reduced the pounds of feed required per pound of gain to 1.78 pounds.

The results of this experiment agree quite well with those of experiment 754 in that most efficient feed conversion was observed on the 25 percent protein rations. Although the gain data differs somewhat in that most rapid gains were observed for the 22 percent protein ration in the earlier experiment, whereas 19 percent protein produced the most rapid gains in this experiment.

Experiment 798 - Effects of arginine and methionine supplementation on relative digestibility and retention of soya and milk protein

Objective. Previous experiments in which practical type diets were used failed to demonstrate a significant response to supplemental methionine. Thus, it was decided to study the influence of supplemental methionine on utilization of semi-purified rations containing soybean oil meal as the only source of protein, and at the same time to compare the relative performance of these pigs to that of pigs fed diets containing dried skimmilk as the only source of protein. It has been commonly accepted that baby pigs fed diets high in dried skimmilk gain faster than those on low dried skimmilk diets and the higher digestibility of the milk protein has been frequently suggested as the primary reason for this superior performance; however, to date a direct comparison of the digestibilities, using the baby pig as the test animal, has not been reported. Thus, nitrogen balance techniques were used to study the differences in digestibility of the two protein sources. These determinations were made at two and again at five weeks of age in order to compare the effect of age on utilization of the two proteins.

A re-check of the amino acid requirements and the quantity of each in these rations (Table 1) definitely indicated methionine to be the most limiting one in the soya diet. It also indicated that if any were there in excessive quantities, arginine would be one to immediately suspect as the soya ration contained more than three times the suggested requirement. Also arginine is the single essential amino acid that differs greatly in its concentration in milk and soya protein. Thus, it was decided to also study the effect of added arginine to the milk and soya protein diets on the utilization of protein by the young growing pig.

Experimental

Animals. Six litter mate groups of six pigs per group, averaging 10.2 days of age and 6.7 pounds body weight were selected for this experiment. The animals were assigned at random within litters to the six ration treatments. They were maintained in individual wire floored metabolism crates with feed provided *ad libitum* during the entire five week experimental period. Water was provided in flat pans of half gallon capacity, however the pans were filled a minimum of four times daily to essentially provide *ad libitum* water consumption.

The room temperature was initially maintained at 85 degrees Fahrenheit and reduced five degrees each week to a temperature of 68 degrees which was maintained for the remainder of the five week experimental period. Weight gains and feed consumption data were collected initially, at the initiation and termination of each collection period and on termination of the experiment.

Rations. The composition and calculated analysis of the soybean oil meal and dried skimmilk basal diets are presented in Tables 6 and 13. The six ration treatments consisted of four soybean oil meal type diets and two dried skimmilk type diets. The four soya diets were the (1) basal, (2) basal plus 0.05 percent supplemental DL-methionine, (3) basal plus 0.5 percent supplemental L-arginine and (4) basal plus 0.05 percent DL-methionine plus 0.5 percent L-arginine. The two milk diets were the (1) basal and (2) basal plus 0.84 percent supplemental L-arginine. This level of arginine raised the calculated arginine content of the milk diet to that of the soybean oil meal basal diet.

The 20 percent level of protein for these rations was provided by low heat, spray dried skimmilk (34 percent protein) or solvent processed soybean oil meal (50 percent protein) except that contributed by the dried beet pulp which amounted to less than 1 percent of the total protein fed. Chromium oxide marker was added to all rations at the rate of 1 percent as an index substance for other studies being

Table 6. Experiment 798 - Composition of soybean oil meal and dried skimmilk basal rations

Ingredient	Proportion SBOM ration	Proportion DSM ration
Cane sugar	15.00	15.00
Lactose	34.48	17.84
Solvent soybean oil meal (50% protein)	39.68	
Dried skimmilk (34% protein)		58.36
Stabilized lard	2.50	2.50
Dried beet pulp	2.00	2.00
Vitamin-antibiotic premix[a]	2.00	2.00
Calcium carbonate	0.04	
Dicalcium phosphate	2.65	0.65
Iodized salt	0.50	0.50
Trace mineral mix (35-C-41)[b]	0.15	0.15
Chromium oxide	1.00	1.00
Total	100.00	100.00

[a]Composition given in Table 16.

[b]Composition given in Table 14.

carried out simultaneously on the same animals. All animals were fed the dried skimmilk basal ration for the first four days of the experiment, after which time they were given their respective test diet.

Collection of excreta. Total urine and fecal collections were made for two periods of five days (120 hours) duration beginning with the eighth day and again with the 28th day on experiment. The urine was allowed to flow into jars containing toluene and a 10 percent aliquot was taken daily and stored under toluene at 34 degrees Fahrenheit until the end of the five day period at which time nitrogen determinations were made. The collection pan and walls of the pen were rinsed once daily with cool water and a 10 percent aliquot of the rinse water added to the urine containers.

A double screened flooring arrangement was used to separate the major portion of the fecal excreta from the urine excreta, however it is well realized that considerable contamination was unavoidable. The fecal excreta was collected daily and frozen immediately upon collection. Care was taken to recover as much of the fecal material as possible although there was definitely an appreciable quantity that could not be collected. A fraction of the portion remaining was therefore rinsed into the urine bottles and is ultimately reflected as digestible matter. After the total five day collection, the fecal material was pooled for each pig and the total quantity dried at 100 degrees Centigrade for 48 hours. Ration samples were taken and dried at 100 degrees Centigrade for 24 hours. After drying, the fecal and ration samples were passed through a micro-mill, thoroughly mixed and sub-samples taken for determinations. Standard macro-Kjeldahl procedures were used for the nitrogen determinations.

Analysis of data. The weight gain, feed/gain, and digestibility data were treated statistically according to the analysis of variance and co-variance plans presented in Tables 25, 26 and 27 in the Appendix. All statements concerning statistical significance are at a probability level of 5 percent or less.

Results and discussion

Growth data. As can be seen from the summary of weight gains and feed required per pound of gain presented in Figure 4 and Table 24, the animals receiving the milk diets gained an average of 24.8 pounds on 1.58 pounds of feed per pound of gain as compared to an average gain of 14.4 pounds on 2.12 pounds of feed per pound of gain for the pigs receiving the soya diets. These differences in rate of gain and feed conversion were statistically significant.

Two pigs receiving the soya diets died and two others were near death at time of removal from experiment. The animals were processed through the Iowa State College Veterinary Diagnostic Laboratory. Post-mortem examinations did not reveal any causes of death that could be associated with the ration treatments. The missing observations for these

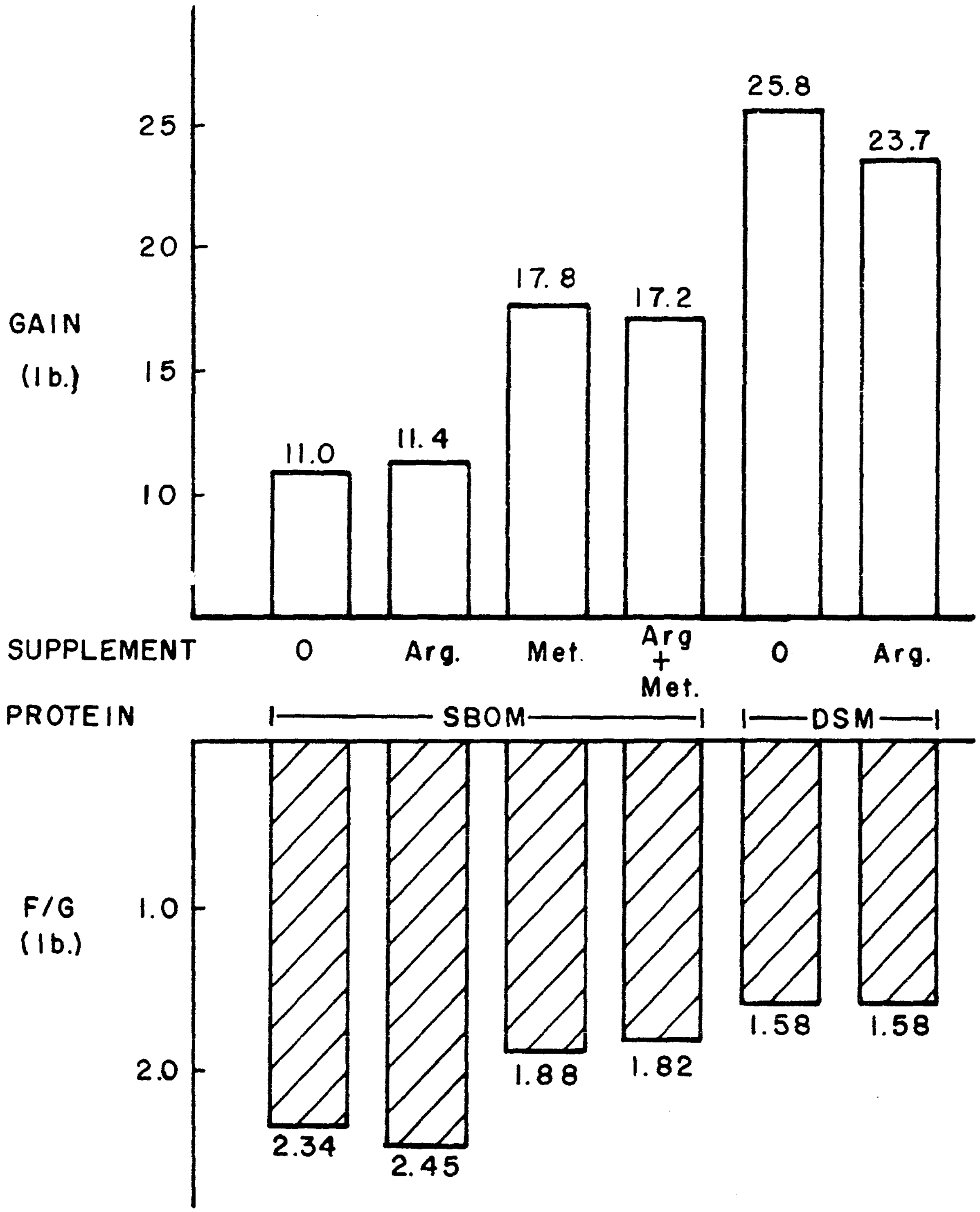


Figure 4. Experiment 798 - Effects of supplemental DL-methionine and L-arginine to soybean oil meal protein diets on gains and feed required per pound of gain

four pigs were calculated and treated accordingly in the statistical analysis. A mild outbreak of scours was observed in the pigs receiving the soya diets during the second week on experiment; however the intensity or persistency of scouring could not be associated with the performance of the pigs.

Supplementation of the diets with arginine had little or no effect on gains or feed conversion. For those pigs receiving milk diets, rate of gain was slightly lower on the average for those receiving the supplemental arginine than for those not receiving arginine; however feed conversion was similar for the two groups. Also if there was a detrimental effect from excess arginine, the pigs receiving the soya diet plus supplemental arginine should have been more severely affected. To the contrary, the pigs receiving soya diets plus arginine gained equally as rapid and essentially as efficient as those receiving no supplemental arginine.

Supplemental DL-methionine improved one to six week gains from 11.2 pounds to 17.5 pounds and decreased the pounds of feed required per pound of gain from 2.40 to 1.85. Analysis of the data from the four soybean oil meal diets showed that these differences between the methionine supplemented and non-supplemented diets were statistically significant.

The data from only the four soybean oil meal diets were used to test the effects of methionine as similar milk diets supplemented with methionine were not fed and also because of the apparent heterogeneity of variances of pigs fed the two types of rations. Bartletts' test for homogeneity of variance (Snedecor, 1956, p. 285) demonstrated that this apparent lack of homogeneity was highly significant, therefore the data obtained with soybean oil meal diets only were used to test the effects of methionine supplementation.

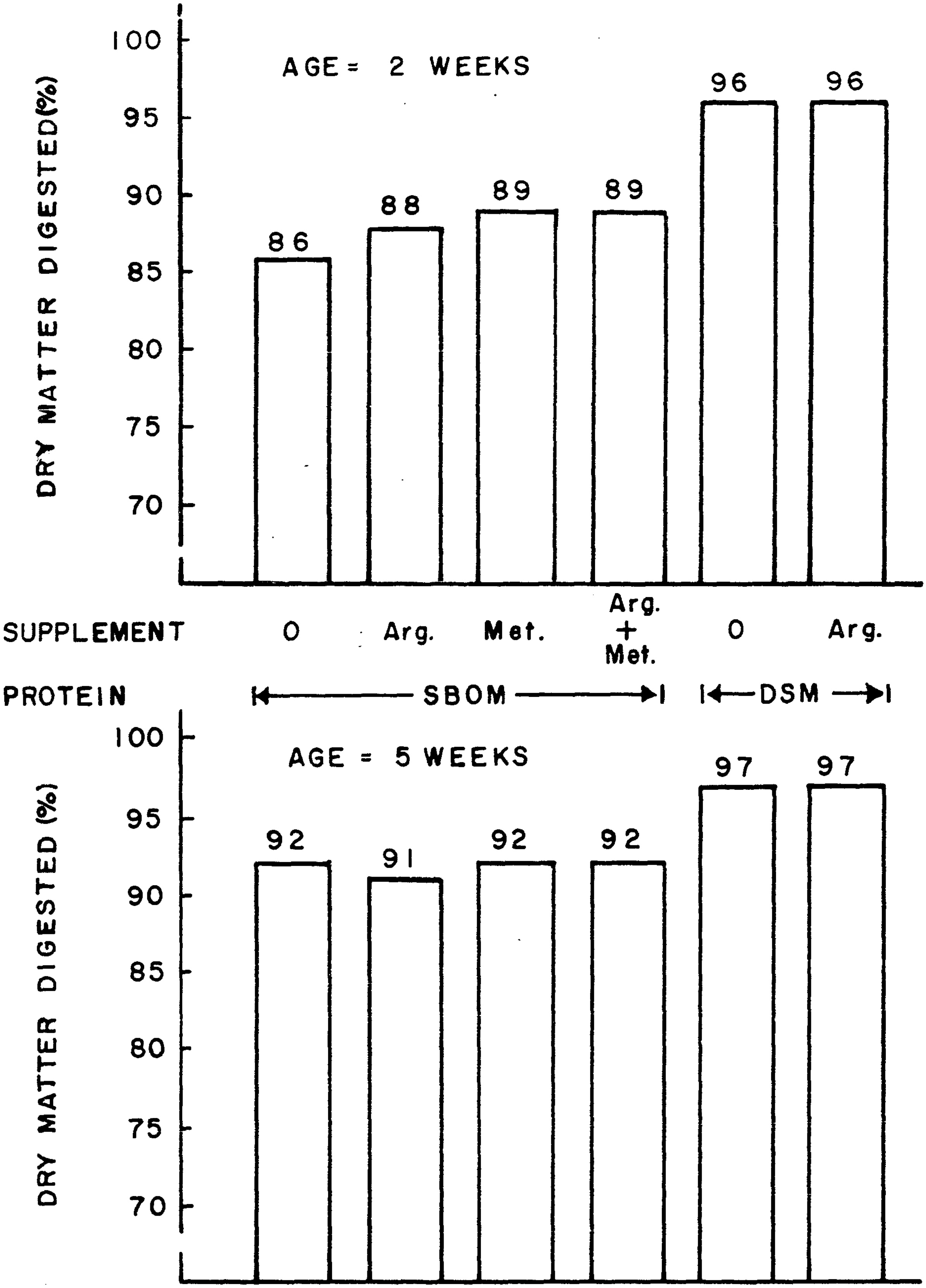
Balance data. A summary of the two and five week dry matter digestibility data is presented in Figure 5.

At two weeks of age the pigs digested 88 percent of the dry matter of the soya diets as compared to 96 percent of the dry matter of the milk diets. At five weeks of age the digestibility of the dry matter of the soya diets had increased to 92 percent, whereas the digestibility of the milk rations showed little change with a digestibility coefficient of 97 percent.

The same pattern of digestibility existed with respect to protein, Figures 6 and 9. This would be expected as lactose and sucrose, two readily available carbohydrates, were used as the sources of energy in the rations. The digestibility coefficients for soya protein were 77 percent and 82 percent for the two and five week age periods, respectively. The digestibility of milk protein changed very little with age (95 percent versus 96 percent for the two and five week age periods, respectively).

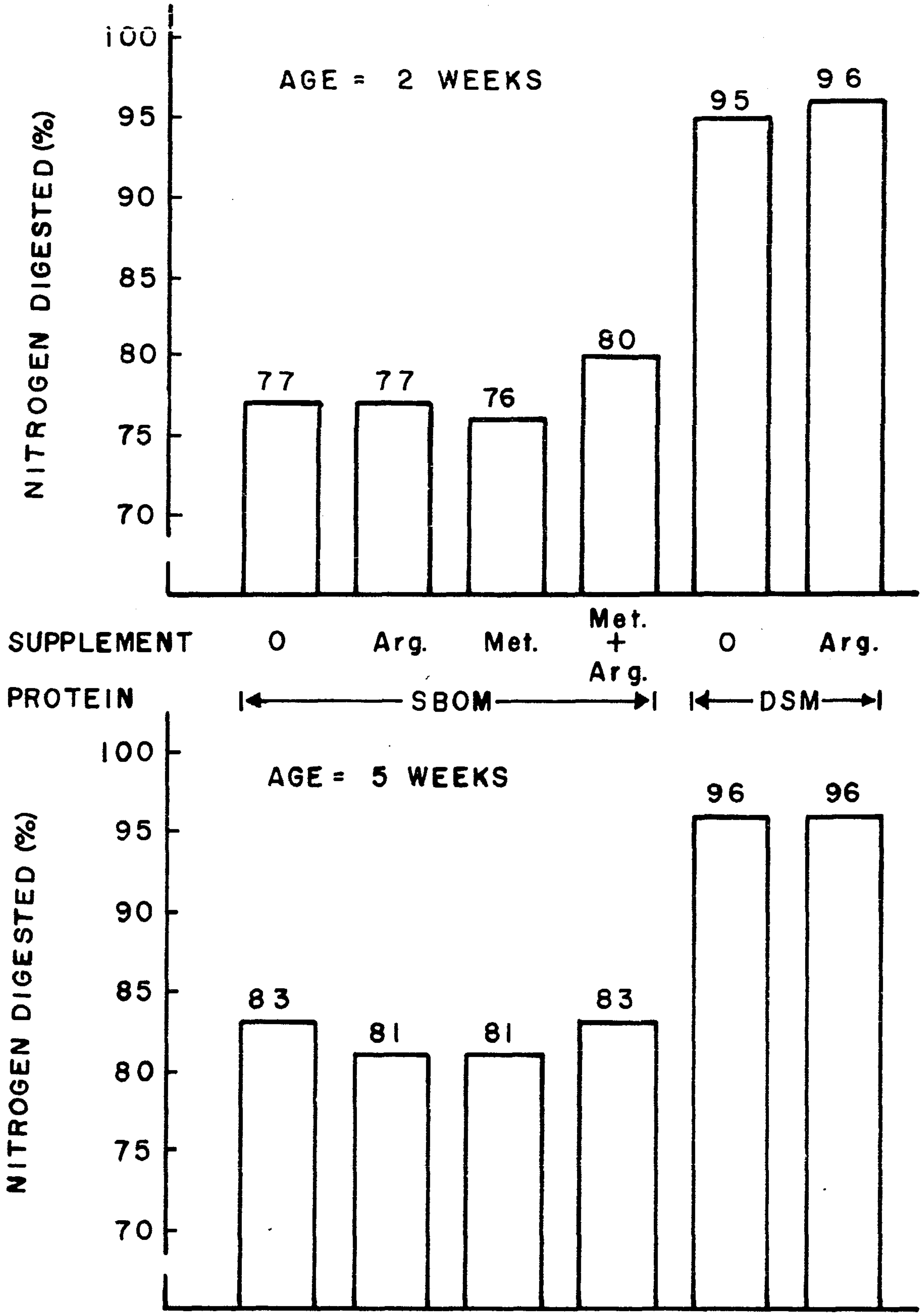
The effect of age on digestibility of both dry matter

Figure 5. Experiment 798 - Comparison of apparent dry matter digestibilities of soybean oil meal and milk protein diets at two and five weeks of age



100
95
90
85
80
75
70
DRY MATTER DIGESTED(%)
AGE = 2 WEEKS
86
88
89
89
96
96
SUPPLEMENT
0
Arg.
Met.
Arg. + Met.
0
Arg.
PROTEIN
SBOM
DSM
100
95
90
85
80
75
70
DRY MATTER DIGESTED (%)
AGE = 5 WEEKS
92
91
92
92
97
97

Figure 6. Experiment 798 - Comparison of apparent digestibilities of soybean oil meal and milk protein at two and five weeks of age



AGE = 2 WEEKS
NITROGEN DIGESTED (%)
100
95
90
85
80
75
70
77
77
76
80
95
96
SUPPLEMENT
0
Arg.
Met.
Met. + Arg.
0
Arg.
PROTEIN
SBOM
DSM
AGE = 5 WEEKS
NITROGEN DIGESTED (%)
100
95
90
85
80
75
70
83
81
81
83
96
96

and protein was statistically significant. The increased digestibility of the soya rations was largely responsible for these effects as indicated by the significant age x treatment interaction on dry matter digestibility; however this interaction was not significant for the protein data.

Averages of the two and five week protein digestibilities were used in the co-variance analysis of gain on digestibility and the adjusted gains arrived at by this analysis are presented in Table 28. A correlation coefficient of 0.492 was observed for gains and digestibility with a regression coefficient of 0.506 pounds of gain per percentage unit change in protein digestibility. This adjustment for differences in digestibility resulted in comparable gains for the pigs fed milk diets and soybean oil meal diets supplemented with methionine. Even with the adjustment, the performance on soybean oil meal rations without added methionine failed to approach that on the milk diet. Also, there was evidence that arginine had a depressing effect on gains. An adjustment for differences in digestibility of the arginine supplemented and unsupplemented rations probably would not be valid as the addition of 0.5 percent arginine to the soybean oil meal diets and 0.84 percent arginine to the milk rations should increase the apparent protein digestibility proportionally. However, this was not evident from the observed data.

The percentage nitrogen retention (Figures 7 and 9) was considerably greater for the milk diets at two weeks of age than for the soya diets (76 percent versus 51 percent). However, at five weeks of age the nitrogen retention had decreased to 59 percent for the milk diets whereas the retention on the soya diets had remained at 51 percent. This apparent age x source of protein interaction was significant and was probably a result of the improved digestibility of soya protein with age coupled with the 20 percent level of milk protein being more than adequate for the pig at five weeks of age.

Supplementation of the soya diets with arginine and methionine appeared to increase nitrogen retention at two weeks of age and to decrease nitrogen retention at five weeks of age. Analysis of the two week data showed these differences to be statistically non-significant. These differences did contribute to the significant age and treatment interaction in the combined analysis. The major portion of this interaction was accounted for by the age x source of protein fraction, but the age x arginine interaction was also significant.

The apparent biological values for these rations were no doubt influenced markedly by the over feeding of protein at five weeks of age, especially to those pigs receiving the milk diets. However, a review of these data (Figures 8 and 9)

Figure 7. Experiment 798 - Comparison of apparent nitrogen retention of soybean oil meal and milk protein diets at two and five weeks of age

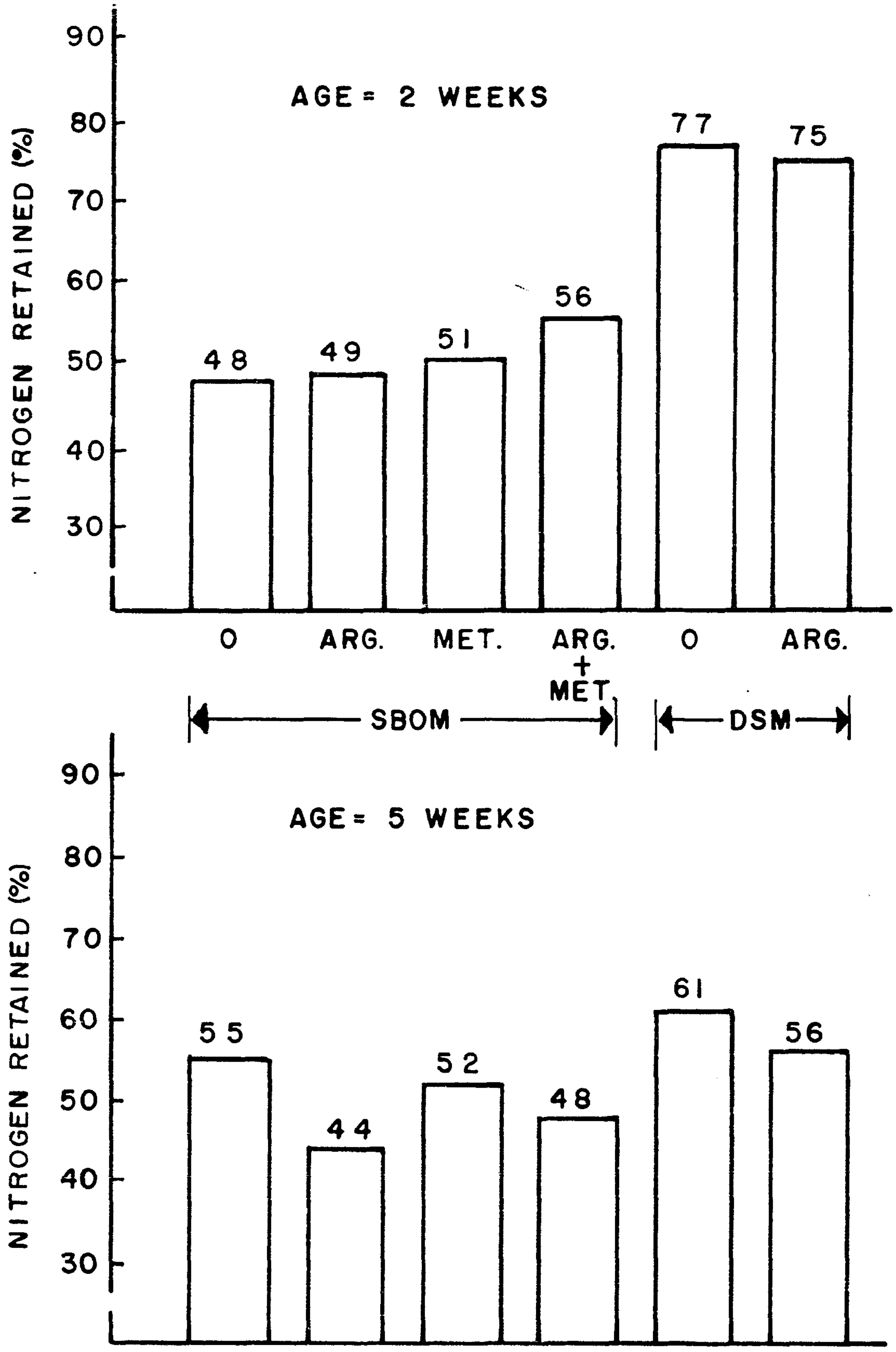
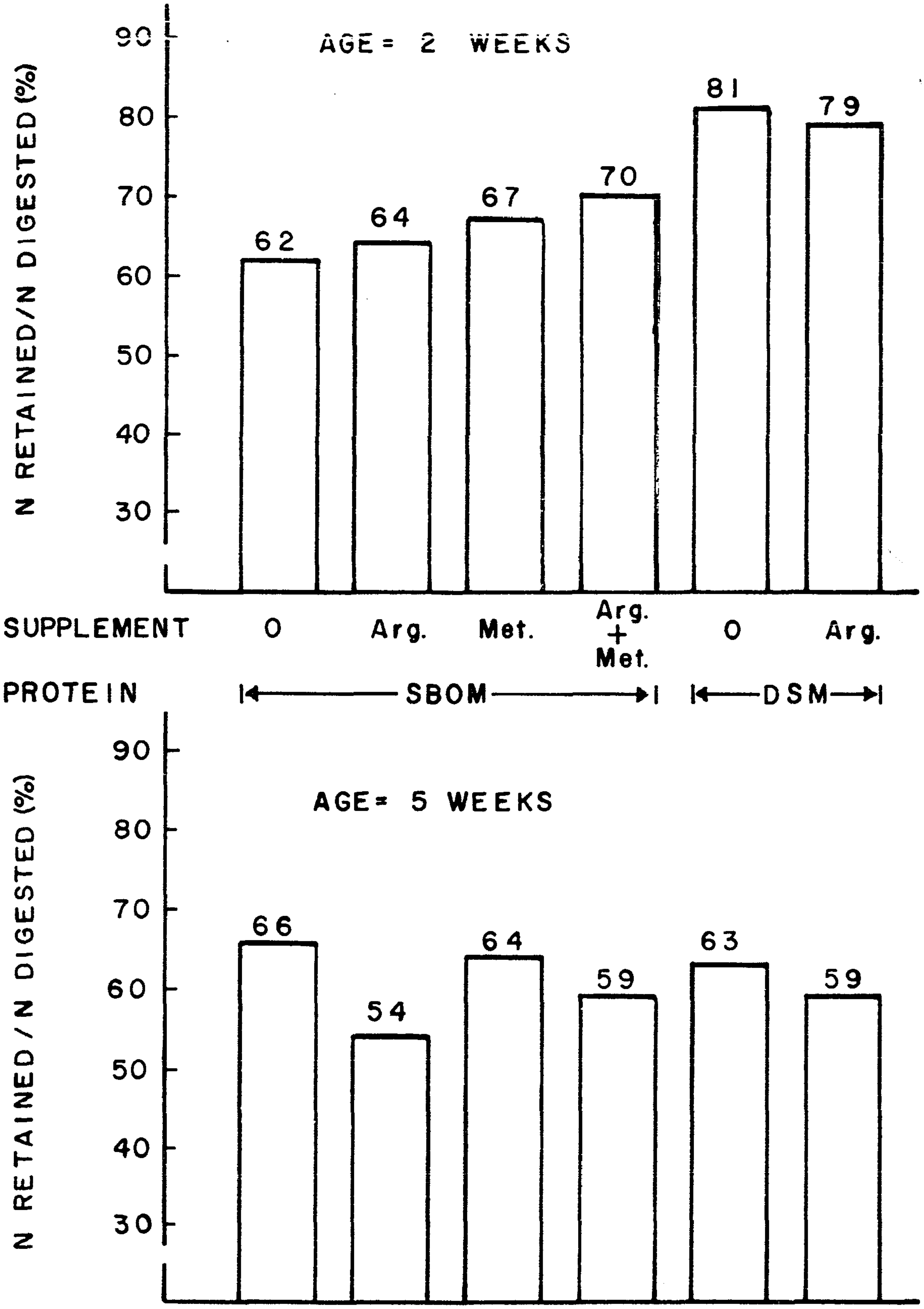
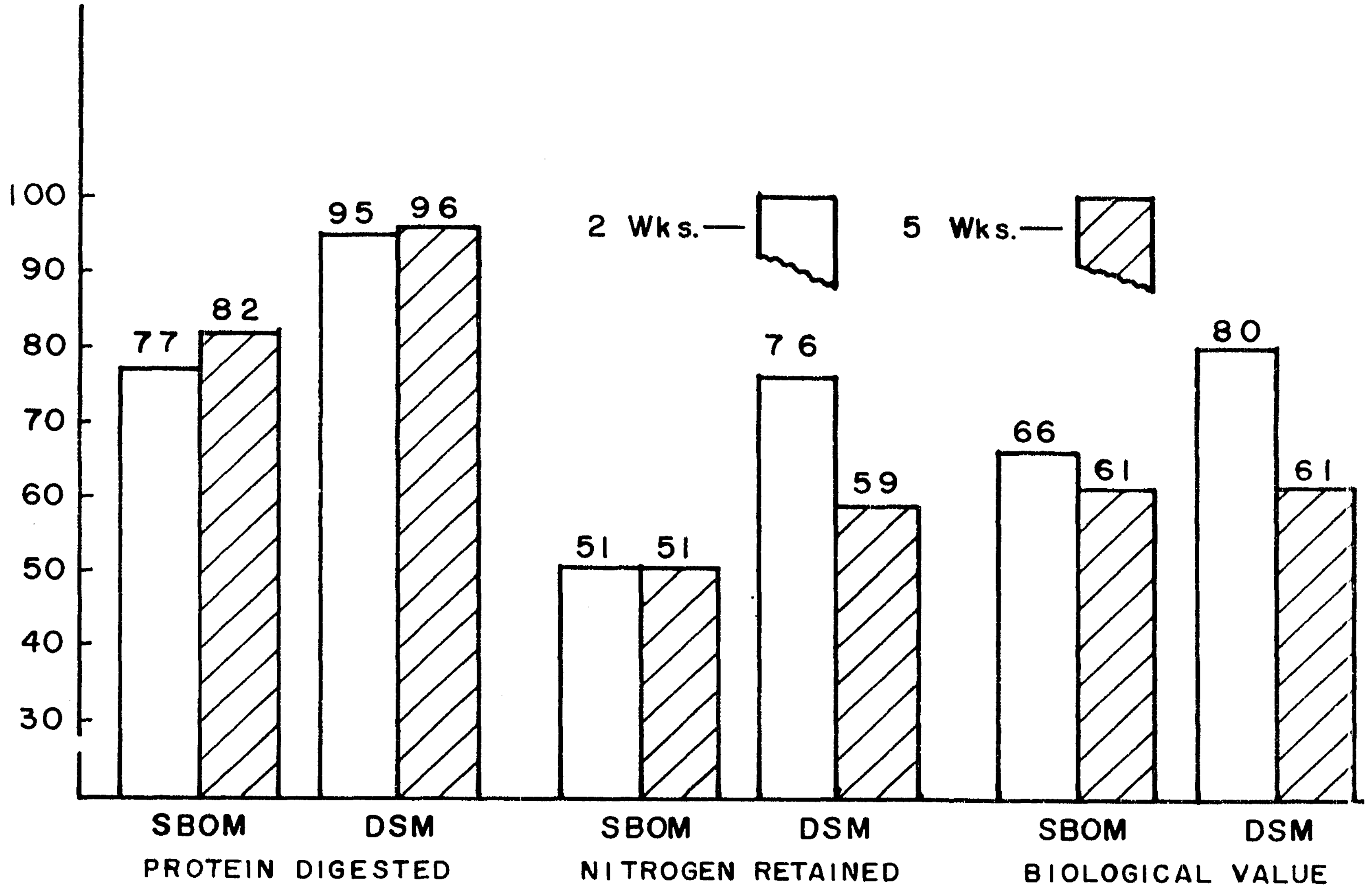

AGE = 2 WEEKS
NITROGEN RETAINED (%)
90
80
70
60
50
40
30
48
49
51
56
77
75
O
ARG.
MET.
ARG. + MET.
O
ARG.
SBOM
DSM
AGE = 5 WEEKS
NITROGEN RETAINED (%)
90
80
70
60
50
40
30
55
44
52
48
61
56

Figure 8. Experiment 798 - Comparison of apparent biological values of soybean oil meal and milk protein diets at two and five weeks of age



AGE = 2 WEEKS
N RETAINED/N DIGESTED (%)
90
80
70
60
50
40
30
62
64
67
70
81
79
SUPPLEMENT
0
Arg.
Met.
Arg. + Met.
0
Arg.
PROTEIN
SBOM
DSM
AGE = 5 WEEKS
N RETAINED/N DIGESTED (%)
90
80
70
60
50
40
30
66
54
64
59
63
59

Figure 9. Experiment 798 - Comparison of the utilization of soybean oil meal and milk protein at two and five weeks of age


100
90
80
70
60
50
40
30
2 Wks.
5 Wks.
77
82
95
96
51
51
76
59
66
61
80
61
SBOM
DSM
PROTEIN DIGESTED
SBOM
DSM
NITROGEN RETAINED
SBOM
DSM
BIOLOGICAL VALUE

indicate further that the low digestibility of soya protein is the primary factor responsible for the poorer performance of pigs fed soya protein diets as compared to pigs fed milk protein diets. The apparent biological value of the milk protein was considerably greater than the soya protein (80 as compared to 66) at two weeks of age; however the values for each of the proteins had decreased to 61 at five weeks of age. This age x source of protein interaction was statistically significant.

The effects of supplemental arginine to the soya diets was to slightly improve the biological value at two weeks of age and to depress it at five weeks of age. The age x arginine interaction was statistically significant. Arginine decreased the biological value of milk protein at both two and five weeks of age. This coupled with its depressing effect on soya diets at five weeks of age was of such magnitude and consistency that the main negative effect of arginine proved to be statistically significant. This is consistent with the overall average depressing effect of arginine on gains and feed conversion; however in the latter cases the differences were not significant.

The main effects of methionine or the age x methionine interaction was not significant. However, methionine significantly improved gain and feed conversion and one would expect this to be reflected in the nitrogen utilization data. There was an apparent improvement in nitrogen retention and biological value at two weeks of age; however this was not evident at five weeks of age.

Evaluation of Depletion-Repletion Technique

Experiment 768 - Depletion-repletion versus conventional growth trials as methods for measuring the protein requirement of baby pigs

Objective. The depletion-repletion technique was used in three previous experiments; however the validity of the technique with growing pigs had not been adequately determined, therefore this experiment was conducted to compare the validity and sensitivity of the depletion-repletion technique with that of conventional growth trials.

Experimental

Animals. Twelve litters of six pigs each averaging 8.9 days of age and 6.9 pounds body weight were selected and randomly allotted by weight within litters to six ration treatments making use of a randomized block design in which each block was made up of a single litter. The animals were confined to individual metabolism cages; however the screen flooring was removed so that the pigs were on concrete floors with wood shavings provided as bedding. Six replications of the six treatments were conducted at each of two times due to the limitation of only 36 metabolism cages. The initial age, weight and environmental conditions were maintained as similar as possible for the two periods.

The pigs were housed in a controlled temperature, well ventilated baby pig nursery, the same as used for experiment 732 (Unit E). Room and floor temperatures were regulated in the manner described for experiment 732.

At the end of a one week preliminary feeding period, half of the pigs (three of each litter) were fed a protein-free ration for a one week depletion period and then fed the test rations for a two week repletion period. The other half received the test rations for the three week period. Feed and water were provided ad libitum and individual weight changes and feed consumption data were recorded weekly.

Rations. The two methods (depletion-repletion and conventional growth) were each used to evaluate three levels of protein (15, 20 and 25 percent). A mixture of equal parts solvent processed soybean oil meal (50 percent protein) and low heat spray-dried skimmilk (34 percent protein) was used as the major source of protein. The composition and calculated analysis of the 20 per cent protein ration are presented in Tables 7 and 13. The depletion ration used was identical to that used in experiment 732, Table 3. Peo (1956), using similar rations, found that 20 percent protein was adequate for the pig one to five weeks of age, thus these levels (15, 20 and 25 percent) were selected to approxi-

Table 7. Experiment 768 - Composition of the 20 percent protein ration

Ingredient	Proportion
Ground yellow corn (9.4% protein)	59.19
Cane sugar	10.00
Solvent soybean oil meal (49.9% protein)	9.06
Dried skimmilk (34% protein)	9.06
Dried whey (sweet) (15.8% protein)	2.50
Stabilized lard	2.50
Dried brewers' yeast (45% protein)	0.50
Dried beet pulp	2.00
Vitamin-antibiotic premix[a]	2.00
Calcium carbonate	0.27
Dicalcium phosphate	2.32
Iodized salt	0.50
Trace mineral mix (35-C-41)[b]	0.10
Total	100.00

[a]Composition given in Table 16.

[b]Composition given in Table 14.

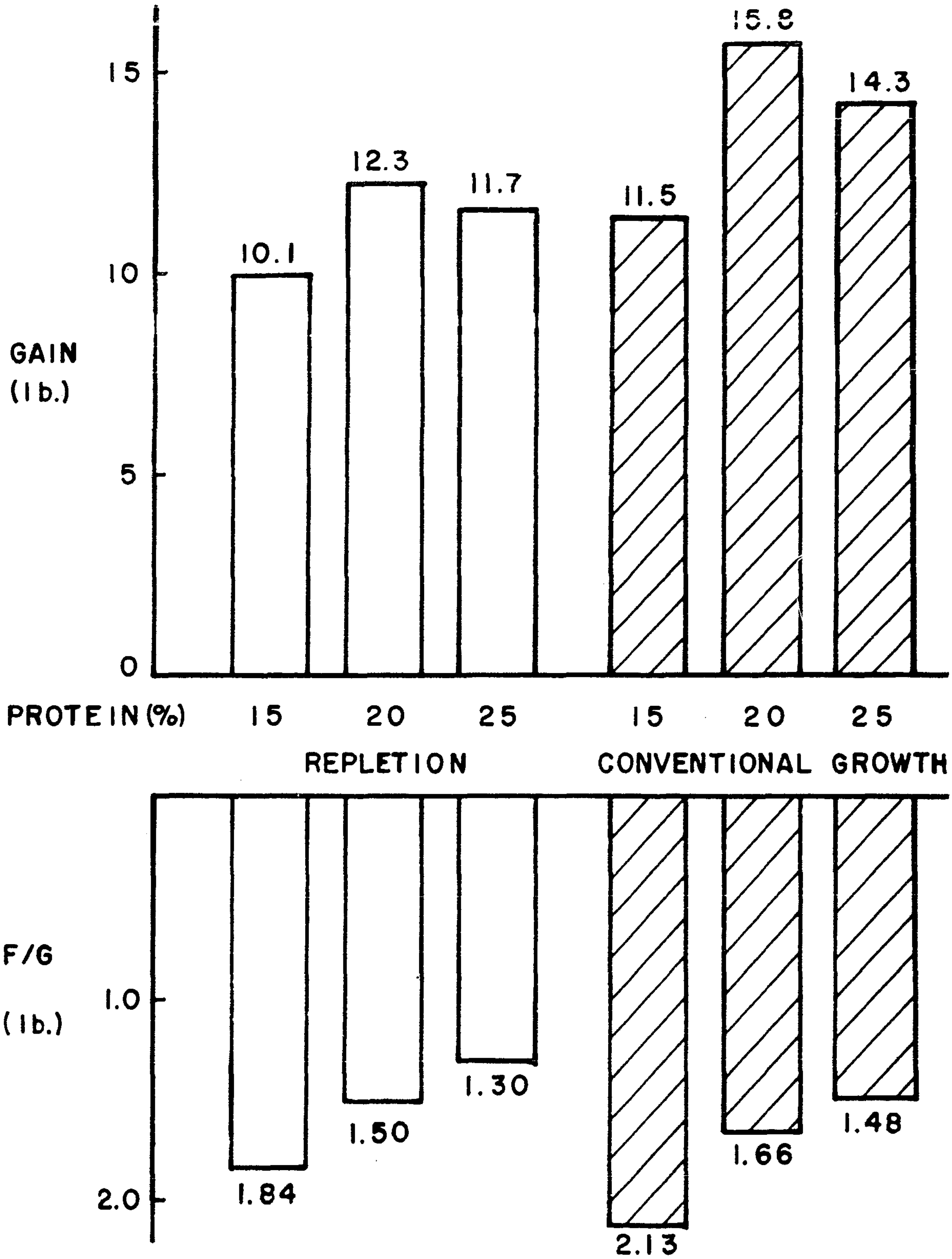
mate the optimum, and one level above and below the requirement.

Analysis of data. The total gain per pig during the repletion period or during the three week conventional growth period was used as the observation and the data were analyzed according to the analysis of variance plan presented in Table 29. Likewise, the pounds of feed required per pound of gain for the repletion period or conventional growth period were used in the statistical analysis and the observed mean squares are presented in the same table.

The data were treated statistically in two ways. The combined analysis of variance (pooled depletion-repletion and conventional growth data) was computed to test for interactions between method of measuring and protein levels fed. This was calculated as a measure of the validity of the results. Also the data from each method were analyzed individually to estimate the relative sensitivity of the two methods.

Results and discussion. Summaries of the gain and feed required per pound of gain data are presented in Figure 10. The response was similar in each method, that is, the 20 percent level of protein produced the greatest gains, with the higher level (25 percent) giving intermediate results and the low level (15 percent) being inadequate in either method. On the other hand, the feed required per pound of gain was least for the highest protein level fed and greatest for the lowest level of protein. The analysis of variance for the gain and feed/gain data showed the gain response to be a significant quadratic regression and the feed response to be

Figure 10. Experiment 768 - Effect of level of protein on gains and feed required per pound of gain as measured by depletion-repletion and conventional growth methods


15.8
14.3
12.3
11.7
11.5
10.1
15
10
5
0
GAIN
(lb.)
PROTEIN (%)
15
20
25
15
20
25
REPLETION
CONVENTIONAL GROWTH
F/G
(lb.)
1.0
2.0
1.84
1.50
1.30
2.13
1.66
1.48

a significant linear regression.

As can be seen in the combined analysis of variance (Table 29) and by inspecting the data, there did not appear to be an interaction of protein levels and method of measuring. The mean square term for interaction as measured by weight gains was only 1.4 times the mean square term for error, likewise the feed/gain data gave an interaction mean square/error mean square ratio of less than one. Obviously, neither of these approached statistical significance. This can be readily seen from the data and individual analysis of variance, as each method gave a quadratic regression of gains on protein levels with the maximum gains observed on the 20 percent protein ration, also each method resulted in a linear regression of feed/gain on protein levels with the maximum feed required per unit of gain being observed on the low (15 percent) protein ration.

The sensitivity of the two methods, as indicated by the relative coefficients of variation [(standard deviation/mean) x 100], did not differ greatly with respect to rate of gain, the observed coefficients of variation being 18.6 and 17.7 percent for the conventional growth and depletion-repletion methods, respectively. However, as methods in measuring feed required per pound of gain the conventional growth method resulted in a coefficient of variation of 12.6 as compared to 9.9 percent for the depletion-repletion method. This represented a 23 percent reduction in the coefficient of variation thus indicating an appreciable reduction in variability of response.

It should be pointed out that difficulties arose in making and interpreting direct comparisons of the two methods. The repletion data were collected over a two week period, whereas the conventional growth data were collected over a three week period. However, if one attempts to keep the length of growth periods equal, it is impossible to avoid an age difference because of the necessary depletion period. Since on an *ad libitum* feeding program there is less labor involved in a conventional growth test than in a depletion-repletion trial, there would be no advantage to using the depletion-repletion method unless the latter was more sensitive or required less time or materials. Therefore, it was decided to use the same total number of days for each method, that is, 28 days including the seven day preliminary feeding period.

The findings of this experiment indicate that the depletion-repletion technique gives valid results for the protein levels tested. The maximum level for most efficient feed utilization was not reached in this experiment, thus it is possible that had higher levels been used, one would have observed an interaction of method and protein levels on feed required per pound of gain.

A comparison of the sensitivity of the two methods demonstrated the repletion technique to be a more sensitive method for measuring feed required per pound of gain; however the two methods were quite similar in efficiency for measuring rate of gain.

Energy, Protein and Methionine Interrelationships

Experiment 715 - The effect of level of energy and level of protein on response to supplemental methionine

Objective. Previous work reported in the literature indicates that the energy content of the ration is an important factor in regulating feed intake. Therefore the nutrient concentration in the ration should be increased or decreased proportionally to assure optimum intake of a specific nutrient. This experiment was conducted to study the effect of energy content of the ration on the response to additional protein and supplemental DL-methionine.

Experimental

Animals. Ninety-six crossbred growing pigs averaging 27 pounds body weight and 48 days of age were selected and randomly assigned on the basis of initial weight within sex to the 16 ration treatments. The restriction was placed on allotment that no two litter mates receive the same ration treatment. The animals were maintained in individual wire floored metabolism crates throughout the experimental period with feed and water provided ad libitum. The animals were weighed, and feed consumption determined each two weeks. The experiment was terminated as each animal reached 75 pounds body weight plus or minus three pounds. Thus as the animals approached 75 pounds it was necessary to weigh them each week.

Due to a limitation of only 48 metabolism cages, it was necessary to conduct the experiment in two phases of three replications each. The first phase was initiated on April 2, 1956 and continued into May. The second phase was initiated May 21 and continued through the first two weeks in July. During each phase, the temperature of the building was kept above a minimum of 68 degrees Fahrenheit. However as warmer weather came the maximum temperature fluctuated with prevailing outside temperatures.

Rations. The 16 ration treatments were derived from a 2 x 2 x 4 factorial arrangement of two levels of energy, two levels of protein and four levels of supplemental DL-methionine. The composition of the rations and calculated analysis are presented in Tables 8 and 13, respectively.

The two energy levels were arrived at by adjusting the corn, soybean oil meal and stabilized yellow grease to a

Table 8. Experiment 715 - Composition of basal rations

Ingredients	970 Cal./lb. 12% protein	970 Cal./lb. 16% protein	1,120 Cal./lb. 12% protein	1,120 Cal./lb. 16% protein
Ground yellow corn[a]	88.50	74.94	77.72	64.17
Solvent soybean oil meal[b]	6.50	17.40	8.50	19.40
Stabilized yellow grease	0.00	2.75	8.75	11.50
Vitamin-antibiotic premix[c]	2.00	2.00	2.00	2.00
Dicalcium phosphate	1.25	1.05	1.35	1.15
Calcium carbonate	1.00	1.06	0.93	0.98
Iodized salt	0.50	0.50	0.50	0.50
Trace mineral mix[d]	0.10	0.10	0.10	0.10
Lysine - HCl (95%)	0.15	0.20	0.15	0.20
Total	100.00	100.00	100.00	100.00

[a]Analyzed 8.9% protein, 0.21% methionine.

[b]Analyzed 47.3% protein, 0.63% methionine.

[c]Solvent soybean oil meal carrier, for analysis see Table 15.

[d]Trace mineral mix 35-C-41, for analysis see Table 14.

calculated 970 and 1,120 productive Calories per pound. The energy values of the feed ingredients were taken from the report of Fraps (1946) and Titus (1955). These values are expressed as productive Calories per pound of feed for the chick, therefore are presented only for comparative purposes and not as actual values for the pig.

The protein levels within energy levels were arrived at by adjusting the corn, soybean oil meal and fat to give calculated levels of 12 and 16 percent crude protein. Ample amounts of corn and soybean oil meal were stored to complete the experiment and were analyzed for protein, methionine and lysine. Protein values were determined by standard Kjeldahl procedures, and the amino acid values were determined by microbiological methods. The corn assayed 8.9 percent protein, 0.21 percent methionine and 0.49 percent lysine; whereas the soybean oil meal assayed 47.3 percent protein, 0.63 percent methionine and 3.01 percent lysine. The lysine value for corn is considerably higher than average analysis, otherwise the values are comparable to values reported in the literature.

Each level of protein within each level of energy was supplemented with 0.0, 0.025, 0.05 and 0.10 percent DL-methionine to make up a total of 16 ration treatments. Since rations composed primarily of corn and soybean oil meal are also border line in lysine adequacy, all rations were supplemented with L-lysine at the rate of 1.0 percent of the calculated protein content of the diet. Thus the 12 and 16 percent protein rations were supplemented with 0.12 and 0.16 percent L-lysine, respectively.

The vitamin and mineral fortification of the rations was considered adequate according to standards set forth by the National Research Council (1953) plus a margin of safety for those nutrients which might be limiting in a ration of this type. The rations were mixed in quantities to assure an approximate two weeks supply, thus the feed was relatively fresh at all times.

Analysis of data. The gain and feed required per pound of gain data were analyzed according to the analysis of variance plan presented in Table 31 in the Appendix. Statements concerning statistical significance are at a probability level of 5 percent or less.

Results and discussion. A graphic summary of the gain and feed required per pound of gain data is presented in Figure 11. Also the data are presented in greater detail in Table 9, and Table 30 in the Appendix.

The level of energy in the ration had little or no effect on rate of gain when averaged across all protein and methionine levels. The rates of gain were 1.26 and 1.24 pounds per day for the high and low energy rations, respectively. On the low energy-low protein rations, the rate of gain was slightly improved over that on the high energy-low protein rations, however this was largely due to the low rate of gain of 1.04 pounds per day for the pigs receiving the ration containing 0.05 percent supplemental methionine.

Figure 11. Experiment 715 - Effects of varying levels of energy, protein and supplemental DL-methionine on rate of gain and feed required per pound of gain

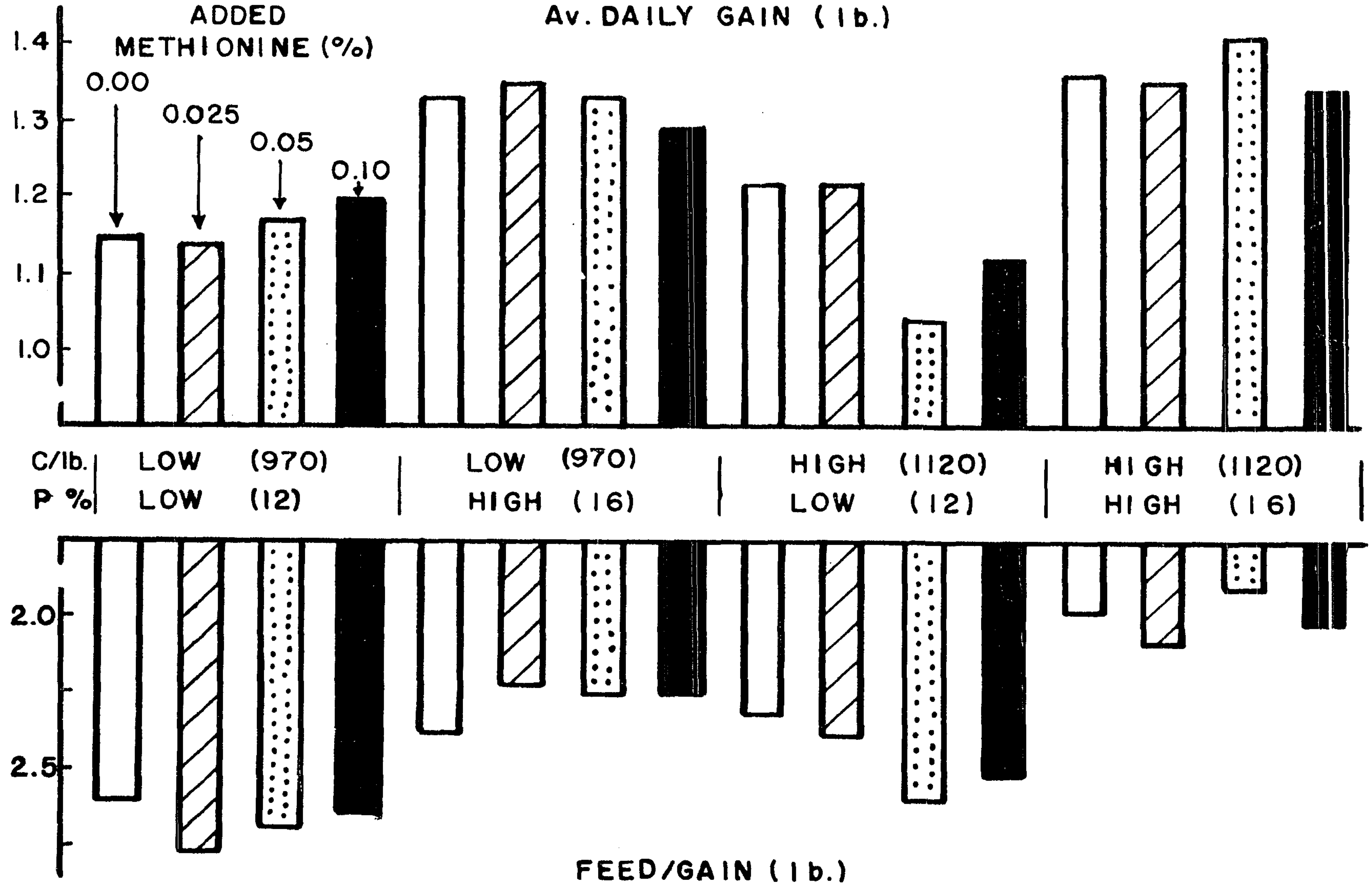

ADDED
METHIONINE (%)
0.00
0.025
0.05
0.10
Av. DAILY GAIN (lb.)
1.4
1.3
1.2
1.1
1.0
C/lb.
P %
LOW (970)
LOW (12)
LOW (970)
HIGH (16)
HIGH (1120)
LOW (12)
HIGH (1120)
HIGH (16)
2.0
2.5
FEED/GAIN (lb.)

Table 9. Experiment 715 - Summary of growth and feed data per pig

Energy level	Protein level	Added methionine	Treatment number	Daily gain	Daily feed	Feed/lb. gain
Cal./lb.	%	%			Pounds	
Low (970)	Low (12)	0.000	1	1.15	3.00	2.61
		0.025	2	1.14	3.16	2.77
		0.050	3	1.17	3.15	2.69
		0.100	4	1.20	3.17	2.64
	High (16)	0.000	5	1.33	3.17	2.38
		0.025	6	1.35	3.00	2.22
		0.050	7	1.33	3.01	2.26
		0.100	8	1.29	2.90	2.25
High (1,120)	Low (12)	0.000	9	1.22	2.83	2.32
		0.025	10	1.22	2.92	2.39
		0.050	11	1.04	2.70	2.60
		0.100	12	1.12	2.82	2.52
	High (16)	0.000	13	1.36	2.69	1.98
		0.025	14	1.35	2.81	2.08
		0.050	15	1.41	2.68	1.90
		0.100	16	1.34	2.71	2.02
		Main comparisons				
High energy				1.26	2.81	2.23[a]
Low energy				1.24	3.08	2.48
High protein				1.34[a]	2.87	2.14[a]
Low protein				1.16	2.99	2.58
	Levels of methionine					
		0.000		1.26	2.92	2.32
		0.025		1.26	2.97	2.36
		0.050		1.24	2.93	2.36
		0.100		1.24	2.93	2.36

[a]Differences significant at P = 0.05 or less.

This suggested interaction was not consistent throughout as the protein x energy component in the analysis of variance did not approach statistical significance.

The level of energy did have a marked effect on feed conversion. The feed required per pound of gain was decreased from 2.48 pounds to 2.23 pounds with the increased level of energy averaged across all levels of protein and methionine, with a slightly greater improvement on the higher protein rations than on the lower protein rations. Here again, this suggested interaction did not approach statistical significance although the main effect of energy was significant.

The level of protein exerted a marked effect on both rate of gain and feed conversion. The animals on the 16 percent protein rations gained at a significantly faster rate (1.34 versus 1.16) on significantly less feed per pound of gain (2.14 versus 2.58) than did those on 12 percent protein rations.

The average daily feed consumption data (Table 9) indicated that protein as well as energy content of the ration affects feed intake. Those animals receiving the low protein rations ate more feed per day than those on the high protein rations, although the total protein intake was greater on the high protein rations. Likewise, the animals receiving the low energy diets consumed more feed than did the animals receiving the high energy diets; however, the animals receiving the high energy diets consumed more total calories per day. The energy content of the ration did exert a greater effect on intake than did the protein content as the animals receiving the low energy diets consumed 95 percent of the total calories consumed by the animals on the high energy diets, whereas the animals receiving the low protein diets consumed only 89 percent of the total daily intake of protein consumed by the animals on the high protein diets.

Supplemental methionine had little or no consistent effect on rate of gain or feed conversion. When averaged acorss both protein and energy levels, the rates of gain and feed conversion were essentially identical for the four levels of methionine fed. The highest average rate of gain was observed for the animals receiving 0.05 percent supplemental methionine in the presence of the high energy and high level of protein; however neither of the other supplemental levels improved rate of gain on this energy-protein combination. Also, the most efficient feed conversion was observed on the same treatment. In the low energy-high protein rations, each of the three levels of supplementation appeared to be more efficient than did the ration containing no supplemental methionine. Thus, in the high protein rations (16 percent) there was an overall trend toward improvement in feed conversion; however, this was not the case for the low protein (12 percent) rations. Analysis of the first phase of the experiment alone (the first three replications) showed a significant protein x methionine interaction on feed conversion. When the two phases were combined this interaction approached but did not attain statistical significance.

The 12 percent protein rations were definitely inadequate, therefore a supplementation with the limiting amino acid should have improved both rate of gain and feed conversion. The data would suggest that methionine was not the limiting amino acid in the 12 percent rations. Calculation of the lysine content (Table 10) indicated that it may have been the limiting amino acid even though the diets were supplemented with 0.12 percent L-lysine. The work of Acker (1953) indicated that 0.10 percent L-lysine was the optimum supplemental level for a 12 percent protein corn-soya type ration and that there were other limitations in the supplemented ration, as it did not approach the performance of 14 percent protein rations.

Experiment 774 - The effect of level of energy on response to methionine supplementation

Objective. The results of experiment 715 clearly established that methionine supplementation did not improve a 12 percent protein ration; however the data did suggest that supplementation improved feed conversion on the 16 per-

Table 10. Summary of the calculated amino acid requirements and the quantities present in corn soybean oil meal type rations containing 12 and 16 percent protein[a]

Amino acid	12% protein		16% protein	
	Required	Present in ration	Required	Present in ration
	%	%	%	%
Arginine	0.22	0.65	0.29	0.92
Histidine	0.18	0.23	0.24	0.35
Isoleucine	0.42	0.47	0.56	0.85
Leucine	0.55	1.19	0.74	1.41
Lysine	0.60	0.49[b]	0.80	0.91[b]
Methionine & cystine	0.35	0.38	0.46	0.49
Phenylalanine	0.30	0.54	0.40	0.78
Threonine	0.43	0.68	0.58	0.82
Tryptophan	0.10	0.13	0.13	0.18
Valine	0.38	0.50	0.50	0.77

[a]Calculated for the 12 and 16 percent protein low energy (970 Cal./lb.) rations used in experiment 715.

[b]In addition to these quantities present from the natural ingredients the rations were supplemented with 0.12 and 0.16 percent L-lysine for the 12 and 16 percent protein rations, respectively.

cent protein ration and that the optimal level of supplemental methionine differed for the two energy levels fed. This experiment was conducted to further study the effect of level of energy on the response to methionine supplementation of a 16 percent protein ration.

Experimental

Animals. Twenty-four crossbred pigs averaging 36 pounds body weight and 53 days of age were randomly allotted by initial weight to three replications of a randomized block design involving eight ration treatments. The animals were maintained in wire floored metabolism crates with feed and water provided *ad libitum*. The animals were weighed initially, after two weeks on experiment and after five weeks on experiment, at which time the experiment was terminated. The experiment was conducted during the months of January and February, 1957, thus it was possible to maintain a uniform environmental temperature of 68 degrees Fahrenheit throughout the experiment.

Rations. The eight ration treatments consisted of a 2 x 4 factorial arrangement of two levels of energy and four levels of supplemental DL-methionine. The energy levels were slightly reduced from those in experiment 715 due to the difficulty of mixing and handling the high energy-high protein ration which contained 11.5 percent added fat. Thus, in this experiment, the two levels of energy were derived by the addition of 1.0 percent fat to the low energy rations and 9.0 percent to the high energy rations. Corn and soybean oil meal were adjusted accordingly to give a constant calculated 16.0 percent protein. This resulted in calculated productive energy values of 940 and 1,078 Calories per pound. Here again, these values are presented for comparative purposes only and not intended as absolute values for the pig. The rations were not supplemented with L-lysine as the rations were in experiment 715 since previous work at this station, Catron _et al_. (1953) and Jensen (1953), had not indicated a beneficial effect from lysine supplementation of 14 and 16 percent protein, corn-soya type ration, and also Meade and Teter (1956) reported that supplemental lysine failed to improve nitrogen balance of pigs fed 12, 14 and 16 percent protein diets of similar composition.

Supplemental DL-methionine was added at the rate of 0.0, 0.0125, 0.025 and 0.05 percent. In experiment 715, the ration containing low energy and 0.025 percent DL-methionine resulted in the most efficient gains and for the high energy ration, 0.05 percent resulted in the most efficient gains. Thus it was concluded that a level lower than 0.025 percent should be included as the level of energy was reduced in this experiment to 940 and 1,078 productive Calories per pound as compared to 970 and 1,120 Calories per pound in the previous experiment.

The soybean oil meal and corn used in this experiment analyzed 49.9 and 9.4 percent protein, respectively. The ration composition and calculated analysis are presented in Tables 11 and 13.

Table 11. Experiment 774 - Composition of low and high energy basal rations[a]

Ingredients	Proportion	
	Low energy	High energy
Ground yellow corn (9.4% protein)	78.1	68.2
Solvent soybean oil meal (49.9% protein)	15.4	17.3
Stabilized yellow grease	1.0	9.0
Vitamin-antibiotic premix[b]	2.0	2.0
Calcium carbonate	0.4	0.3
Dicalcium phosphate	2.5	2.6
Iodized salt	0.5	0.5
Trace mineral mix (35-C-41)[c]	0.1	0.1
Total	100.0	100.0

[a]The low and high energy rations calculated 940 and 1,078 productive Calories per pound, respectively.

[b]Composition given in Table 13.

[c]Composition given in Table 15.

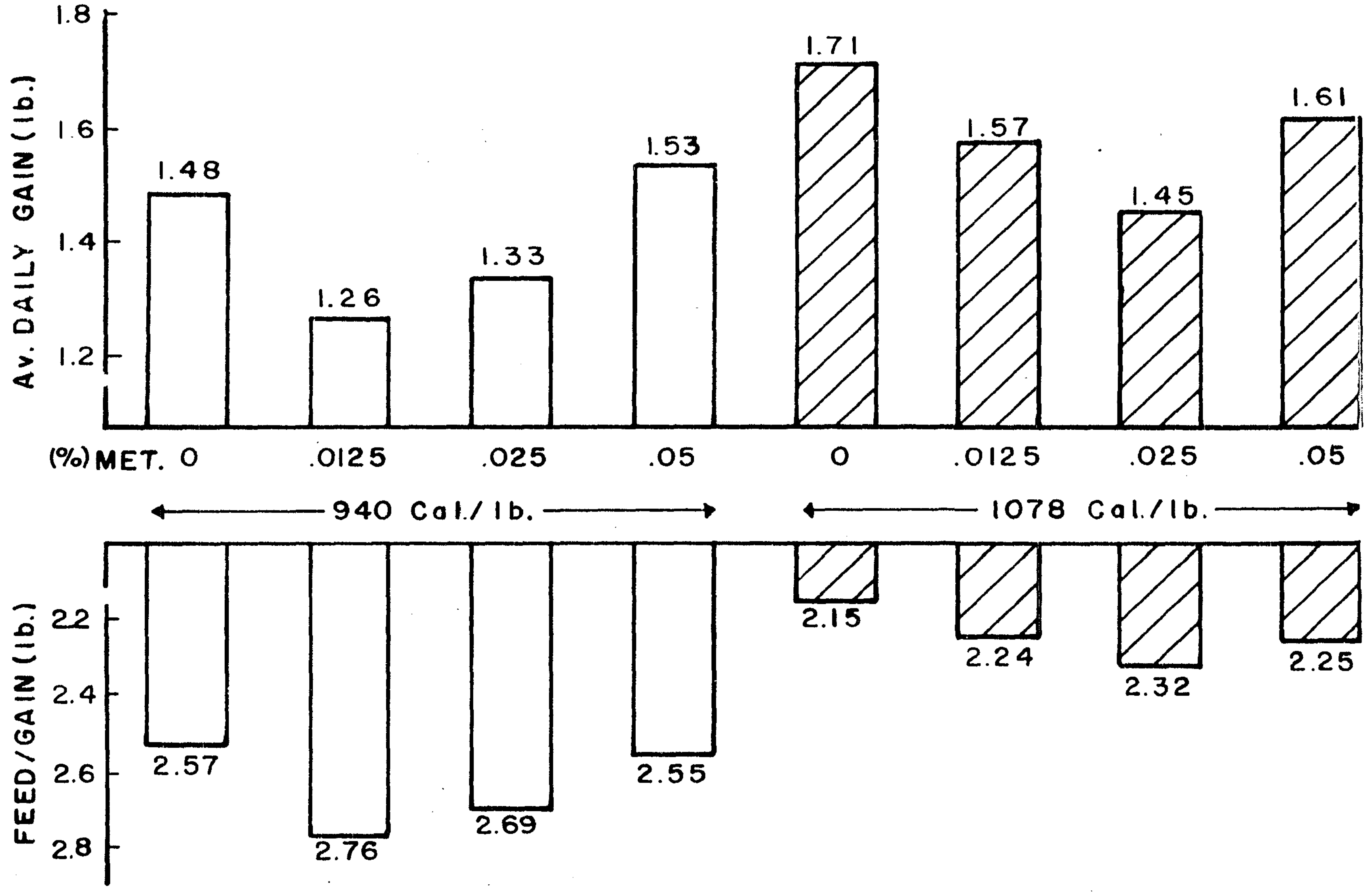
Analysis of data. The data for average daily gains and feed required per pound of gain were analyzed according to the analysis of variance plan presented in Table 33 in the Appendix. The table also contains the observed mean squares. Statements concerning statistical significance are at a probability level of 5 percent or less.

Results and discussion. Summaries of the average daily gains and feed required per pound of gain are presented in Figure 12 and Table 32.

An improved feed conversion was observed for the high energy rations as compared to the low energy rations similar to that observed in the previous experiment. Increasing the energy content from 940 to 1,078 Calories per pound of ration decreased the feed required per pound of gain from 2.63 to 2.24 pounds. This improvement in feed conversion was of such magnitude and consistency that it proved to be statistically significant. The improvement in gain with increased energy in the diet, even though it was 0.18 pounds per day, did not prove to be significant. This is in agreement with the results of experiment 715 in which the level of energy did not significantly affect rate of gain.

Supplementation with methionine had no significant effect on either rate of gain or feed conversion. The rations containing no supplemental methionine produced gains and feed conversion equal to or superior to those containing supple-

Figure 12. Experiment 774 - Effects of varying levels of energy and supplemental DL-methionine on rate of gain and feed required per pound of gain



Av. DAILY GAIN (lb.)
1.8
1.6
1.4
1.2
1.48
1.26
1.33
1.53
1.71
1.57
1.45
1.61
(%) MET. 0
.0125
.025
.05
0
.0125
.025
.05
940 Cal./lb.
1078 Cal./lb.
FEED/GAIN (lb.)
2.2
2.4
2.6
2.8
2.57
2.76
2.69
2.55
2.15
2.24
2.32
2.25

mental methionine. This was observed within energy levels and also when averaged across both levels of energy.

The variability was quite high in this experiment with the observed coefficients of variation being 15.5 and 7.9 percent for rate of gain and feed conversion, respectively. Thus, it was deemed advisable to repeat this study employing a split-plot design in which the energy levels would be the main-plot and the methionine levels the sub-plot treatments. By using such a design, litter mates could be controlled across sub-plot treatments which would be a definite advantage for measuring the effects of supplemental methionine and would also be comparable to the randomized block design for measuring the energy x methionine interactions.

Experiment 774a - The effect of level of energy on response to methionine supplementation

Objective. The results of the two earlier experiments demonstrated that increasing the level of energy in the ration improved feed conversion. The results of experiment 715 also suggested that supplemental methionine reduced the feed required per pound of gain; however the differences were not statistically significant. The data of experiment 774 were quite variable, thus it was decided to conduct a similar experiment using the same ration treatments but using a split-plot design in an attempt to control more of the variability and to more adequately test the effects of methionine supplementation.

Experimental

Animals. Twelve litter mate groups of four pigs each averaging 28 pounds body weight and 53 days of age were selected and allotted at random within litter to eight ration treatments in a split-plot design. The ration treatments were identical to those discussed in experiment 774. The two energy levels were considered as the main-plot treatments and the supplemental methionine levels were the four sub-plot treatments. Litter mates were controlled across sub-plot (methionine levels) treatments; thus any differences observed between energy levels (main-plots) are confounded with litter differences. Housing and management of pigs were similar to that described for experiment 774. The experiment was conducted in two phases of three replications each.

The second phase of the experiment continued into the month of June, thus it was not possible to maintain a constant environmental temperature of 68 degrees Fahrenheit as was done in the first phase of the experiment.

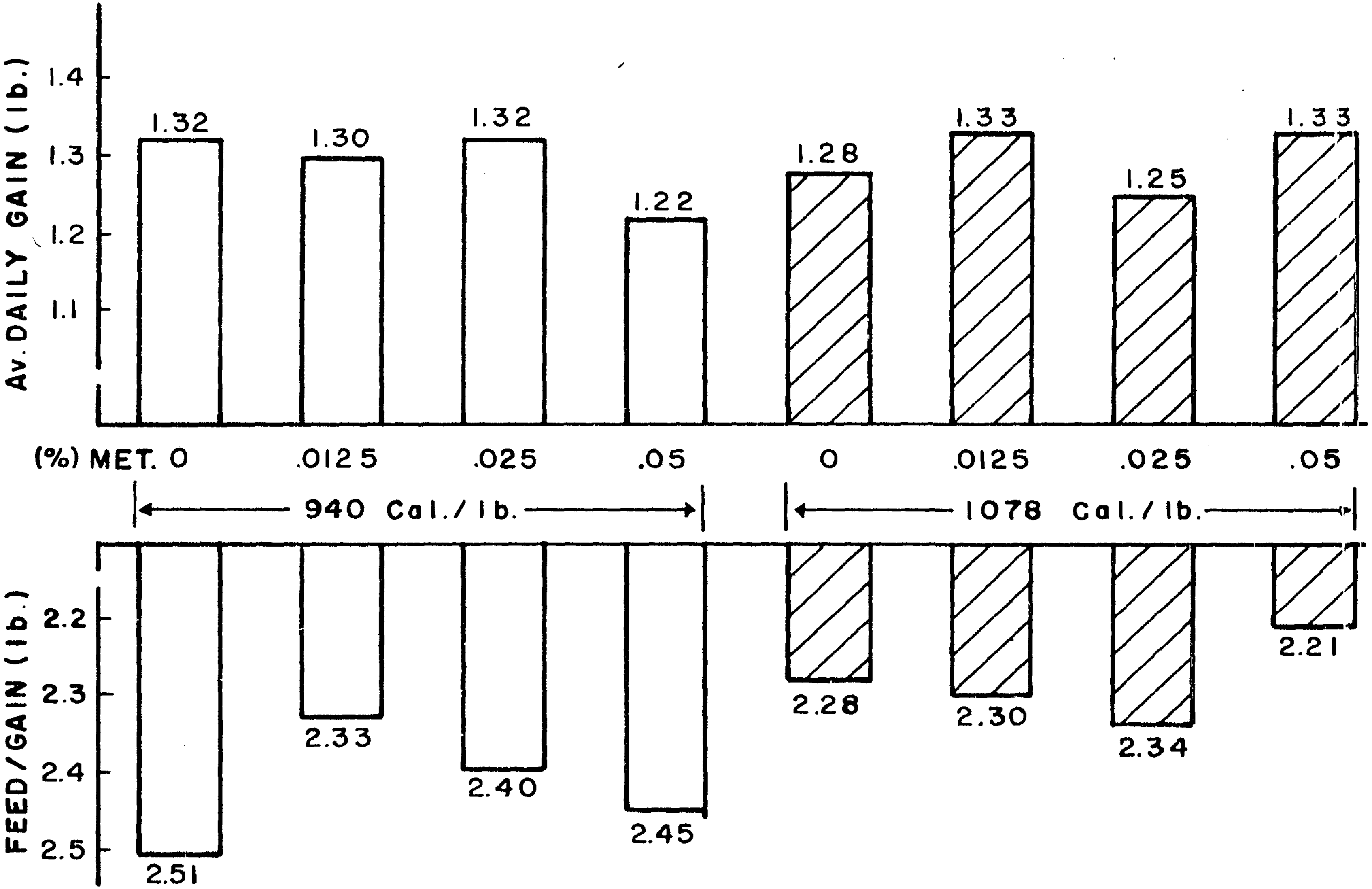
Analysis of data. The analysis of variance plan and observed mean squares for rate of gain and feed conversion data are presented in Table 34 in the Appendix. As in previously discussed experiments, statements concerning statistical significance are at a probability level of 5 percent or less.

Results and discussion. Summaries of gain and feed data are presented in Figure 13 and Table 35. The data includes a calculated value (Cochran and Cox, 1957, p. 302) for a pig that died. The pig was submitted to the diagnostic laboratory, however post mortem examination did not reveal the cause of death or any abnormalities that could be associated with ration treatment.

The data indicated that neither rate of gain nor feed required per pound of gain was significantly affected by supplemental methionine, which is in agreement with the results of the two previous experiments. Although here again there was an indication that supplemental methionine may have slightly improved feed conversion when averaged across both energy levels. One would expect a greater improvement on the high energy ration in view of the reports that the amino acid requirement is affected by level of energy in the diet. This was not observed as there was stronger evidence for beneficial effects in the presence of low energy.

The improvement in feed conversion on the high energy rations as compared to low energy rations (2.28 versus 2.42) approached significance. As mentioned earlier, the effect of energy was confounded with litter effect and thus this

Figure 13. Experiment 774a - Effects of varying levels of energy and supplemental DL-methionine on rate of gain and feed required per pound of gain


Av. DAILY GAIN (lb.)
1.4
1.3
1.2
1.1
1.32
1.30
1.32
1.22
1.28
1.33
1.25
1.33
(%) MET. 0
.0125
.025
.05
0
.0125
.025
.05
940 Cal./lb.
1078 Cal./lb.
FEED/GAIN (lb.)
2.2
2.3
2.4
2.5
2.51
2.33
2.40
2.45
2.28
2.30
2.34
2.21

experiment would not provide a sensitive test for the main effects of energy.

The improvement in efficiency of testing the effects of supplemental methionine by the use of a split-plot design appeared to be small in this instance as coefficients of variation of 8.4 and 12.4 percent were observed for the feed/gain and rate of gain data, respectively.

The results of this experiment and the two previous ones indicated that methionine supplementation slightly improved the efficiency of a 16 percent protein ration; however, this improvement would be of questionable practical significance.

GENERAL DISCUSSION

Levels of Protein for Baby Pig Rations

The results of experiment 768 comparing three levels (15, 20 and 25 percent) of a 50-50 misture of soybean oil meal (50 percent protein) and dried skimmilk (34 percent protein) protein demonstrated a quadratic regression of gains on protein levels with maximum two to five week gains being observed on the 20 percent protein ration. However, a linear regression of feed required per pound of gain on protein was observed with the 25 percent protein rations being the most efficient. The trends were the same whether measured by conventional growth trial or by the depletion-repletion technique.

These results are in agreement with the studies reported by Peo (1956) in which a similar protein mixture was used. Peo observed maximum gains on the 20 percent level of protein and also observed that 30 percent protein produced the most efficient gains; however a quadratic regression of feed/gain on protein levels indicated that the improvement in feed conversion was small as the protein level was increased above 25 percent. Becker et al. (1954) also observed that a level of approximately 22 percent protein was adequate for pigs one to four weeks of age.

Experiments 754 and 772, in which soybean oil meal was used as the primary source of protein for the pig two to six weeks of age demonstrated that 19 to 22 percent protein was adequate for maximum rate of gain. These data were also in agreement with experiment 768 in that 25 percent protein rations resulted in the most efficient feed conversion. These levels correspond well with the optimum level reported by Peo (1956) using dried skimmilk as the source of protein in the ration. This report also included a comparison of performance on levels of soya protein. He found no significant differences between levels ranging from 19 to 34 percent, although the data suggested that 28 to 31 percent protein resulted in the most efficient feed utilization.

One would suspect that higher levels of soya protein would be required than milk protein as milk protein is more efficiently digested (experiment 798); however, it appears that enzyme concentration (deficiency) is the limiting factor and that a lower rate of digestibility can not be offset by increasing the protein level (substrate concentration) in the ration. This difference could possibly be partially offset if isolated soya protein were used instead of solvent soybean oil meal since with the latter, increasing the protein level also decreases the available energy in the ration.

Evaluation of Depletion-Repletion Technique

The data of experiment 732 did not demonstrate a response to supplemental methionine in either the depletion-repletion group or the conventional growth group; however there was an indication that performance was less variable in the depletion-repletion group. This observation is in agreement with the low variability of response observed by Peo (1957a) under similar conditions.

Experiment 768 was designed to further test these observations using levels of protein of five percentage unit intervals to obtain obvious ration treatment differences. The responses to the varying levels of protein (15, 20 and 25 percent) were similar as measured by the two methods. A curvilinear response of gains on protein levels was observed with maximum gains occurring on the 20 percent level of protein. Whereas, a linear response of feed required per pound of gain on protein levels was observed with the 25 percent protein ration being the most efficient one.

The similarities of the responses to the protein levels in the depletion-repletion method and the conventional growth method are evidence that the depletion-repletion method does lead to valid conclusions concerning the protein adequacy of the diets. This observation is supported by the lack of a significant protein level x method of measuring interaction in either the gain or feed data.

An obvious difference in sensitivity between methods as measured by relative variation was not observed for the gain data. The variability was slightly greater in the conventional growth group as reflected by coefficients of variations of 18.6 percent and 17.7 percent for the conventional method and the depletion-repletion method, respectively. However, as methods in measuring feed required per pound of gain conventional growth resulted in a coefficient of variation of 12.6 as compared to 9.9 percent for depletion-repletion.

A portion of the data from experiments 754 and 772 can also be used to give a further indirect comparison of the two techniques by using the data from the 19, 22 and 25 percent protein rations for the two experiments. The data of these two experiments indicate that the two methods give differing pictures for gains on the three protein levels as 19 percent protein resulted in maximum gain in the conventional growth group whereas 25 percent protein resulted in maximum gains in the depleted group. The trends in feed required per pound of gain were similar for the two methods, with the 25 percent protein ration being the most efficient.

The data of these experiments indicate that the depletion-repletion technique may slightly exaggerate the protein requirement which could be expected as the depleted animal may be in a condition to utilize more protein than the animal of similar age in a repleted state. If this factor is taken into consideration, the technique should be applicable to young growing animals equally as well as for adult animals. Cannon (1954) points out that the utilization rate for a particular amino acid in protein repletion of an adult rat is two to five times the utilization rate for maintenance alone. Under the conditions of the experiments reported herein, the protein or amino acid requirement should be magnified considerably more in order to meet the requirements for maintenance, repletion of amino acid pools and also to meet the requirement demanded for rapid growth. This may partially account for the differences in results observed in experiment 768 as compared to 754 and 772. In experiment 768, a combination of milk and soya protein provided a diet quite well balanced in the essential amino acid make-up and therefore the animals in the depleted state met their requirements by a more efficient utilization of the protein present in the diet, resulting in little or no difference in the optimum level of protein as measured by the two methods. In the other experiments, soya protein and corn protein was not as efficient in repleting baby pigs, thus an increased level of protein appeared beneficial. If this is the case, one should expect a more dramatic response to supplementation with a limiting amino acid in a depletion-repletion study than in a conventional growth study. However, the depletion-repletion data in these studies did not indicate a consistent response to methionine supplementation of a corn-soybean oil meal diet, thus indicating that methionine was not the first limiting nutrient in the rations fed.

Difficulties arise in making and interpreting direct comparisons of the two methods; the repletion data were taken over a two week period whereas the conventional growth data were collected over a three or four week period. However, if one attampts to keep the length of growth periods equal, it is impossible to avoid an age difference because of the necessary depletion period.

There is more labor involved in a depletion-repletion study than in an _ad libitum_ feeding trial, thus the slight improvement in sensitivity by depletion-repletion could probably be compensated for by increasing the number of replications in a conventional growth experiment. In cases where the materials, animals or facilities are limiting it would be worthwhile to consider the use of the depletion-repletion technique.

Methionine Supplementation of Baby Pig Rations

Five baby pig (two to six weeks of age) experiments were conducted to study the influence of methionine supplementation of practical type rations on rate of gain and feed conversion. Solvent processed soybean oil meal (50 percent protein) was used as the major source of protein in the rations with sweet dried whey and ground yellow corn providing the remainder.

A comparison of the calculated amino acid content of these rations to the reported requirements indicated that methionine was the most limiting amino acid. Soya protein has been used as the major source of protein in studies determining the methionine requirement, thus adequately demonstrating that it is limiting in methionine. Whey protein is also quite limiting in its methionine content, leaving only the corn protein to offset a deficiency.

Experiments 732 and 746 were conducted to study the effects of various levels of supplemental methionine to a 20 percent protein ration on baby pig performance. The calculated sulfur amino acid content (methionine plus cystine) of the basal ration used in these experiments was 0.54 percent as compared to a requirement of 0.66 percent. The calculated values for the other essential amino acids were higher than their respective requirements.

The depletion-repletion technique was used to study the response to supplemental methionine. The results of the two experiments revealed no consistent trends in response to supplemental methionine in either the repletion gains or the feed requirements per pound of repletion gain. The lack of response to methionine would suggest that either the methionine was not the limiting nutrient, that the level of protein was not optimum for measuring such a response, or the animals failed to grow rapidly enough to measure a response.

There is considerable evidence that the requirement for a given amino acid is proportional to the protein content of the diet (Almquist, 1954); thus it was decided to study the effects of methionine supplementation of rations varying in protein levels from 16 to 31 percent. The ration ingredients used in these studies (experiments 754 and 772) were the same as those used in the two previous experiments. Only the corn and soybean oil meal content of the rations were adjusted to give the varying protein levels.

As the protein level of the rations was increased by increasing the soybean oil meal content of the ration, the ratio of methionine to protein decreased, which should result in greater responses to supplemental methionine up to the level of protein where the additional amino acid requirement is met by over feeding of protein. Some reports, Sewell et al. (1953b) and Crampton and Ness (1954), would indicate that the protein levels fed in these studies were not excessive.

Maximum gains and most efficient feed conversion in experiment 754 was observed on the 25 percent protein ration supplemented with 0.05 percent methionine; however there was no consistent improvement that could be associated with supplemental methionine as the level of protein was varied up or down from the 25 percent level. Supplemental methionine appeared to improve feed conversion on three of the five levels of protein fed; however the response was erratic and suggests that the observed differences were a result of random variation.

Experiment 772 was conducted to study the effects of supplemental methionine on the response to four levels of protein (16, 19, 22 and 25 percent). With the exception of the 22 percent level of protein, methionine appeared to slightly improve feed conversion, whereas gains were slightly increased on the supplemented rations with the exception of the 25 percent protein ration. The differences in either case were not statistically significant indicating again that supplemental methionine did not appreciably improve the performance under the conditions of these experiments.

Results of the previous four experiments indicated that factors other than the amino acid balance were responsible for the poor performance of soya protein diets. Experiment 798 was conducted to study the comparative performance of pigs on milk and soya protein diets and to also study the effects of supplemental methionine and arginine on the performance of baby pigs. Rate of gain, feed conversion and nitrogen balance at two and five weeks of age were used as the criteria of response.

The pigs fed the milk protein diets (dried skimmilk as the source of protein) gained at a significantly faster rate on significantly less feed per pound of gain than did the pigs on the soya protein diets (solvent soybean oil meal as the source of protein). This is in agreement with previous reports by Peo (1956) and Hudman (1956) in which they observed superior performance on diets containing dried skim-milk as the source of protein.

Arginine was chosen as a supplemental amino acid for this experiment because of its apparent abundance in soya protein. The thinking was that the imbalance of amino acids brought about by excessive quantities of arginine could be exerting a detrimental effect on the soya protein diets. Thus a further excess of 0.5 percent was added to the soya diets in the presence and absence of 0.05 percent supplemental DL-methionine and 0.84 percent L-arginine was added to a milk protein diet to bring its calculated arginine content to that of the soya protein basal diet.

Gains on the arginine supplemented milk protein diet were less than those observed for the non-supplemented diets; however the amounts of feed required per pound of gain were identical for the two diets. Also arginine exerted no obvious influence on the response to the soya protein diets, indicating that excess arginine in the soya rations is not seriously affecting performance.

Supplemental methionine significantly improved both rate of gain and feed conversion. This was expected as soybean oil meal was the only source of protein and previous work on the methionine requirement of the pig clearly establishes that soya protein is deficient in methionine.

The balance data demonstrated a significant difference in the digestibility of the milk and soya diets which was reflected in both protein and dry matter digestibilities. The pigs' ability to digest the soya diets increased with age (two versus five weeks of age); however the ability to digest the milk diets changed only slightly. This improvement in digestibility is in agreement with the postulation of Lewis et al. (1955) that the baby pig below five weeks of age has a proteolytic enzyme insufficiency, and is further substantiated by the digestibilities studies of Lloyd et al. (1957) using a practical type diet.

Although methionine appeared to improve nitrogen utilization at two weeks of age, the average effect was not significant. This observation is inconsistent with the gains and feed conversion data; however it is in agreement with the report by Curtin et al. (1952d). These workers, using a semi-purified soya protein diet, also observed slight improvements in gains, feed conversion and nitrogen retention but the differences were not statistically significant.

Adjusting the gains to equal protein digestibilities by covariance analysis resulted in similar gains for the pigs receiving methionine supplemented soya protein and milk protein diets. This indicates that the decreased ability to digest soya protein as compared to milk protein is largely responsible for the difference in baby pig performance on the two proteins. Also the improvement in digestibility with age explains why the performance of 25 to 35 pound pigs is quite satisfactory whereas the performance of baby pigs is very poor on all plant protein diets.

Energy, Protein and Methionine Interrelationships

Three experiments (715, 774 and 774a) were conducted to study the effects of caloric content of the ration on the growing pigs' response to increased protein and methionine levels in the ration.

The energy content of the ration significantly improved feed conversion in two of the three experiments and the data suggested an improvement in the third experiment. On the other hand, the energy level in the diet exerted no significant effect on rate of gain in any of the experiments.

In experiment 715, increasing the level of protein in the diets from 12 to 16 percent significantly improved both rate of gain and feed conversion. The degree of improvement was approximately the same for the two energy levels with a slightly greater improvement on the higher energy diets. This difference in response on the two energy levels (or energy x protein interaction) did not approach statistical significance.

Supplemental methionine failed to significantly improve gains or feed conversion on any combination of energy and protein levels; however there was a suggested improvement in feed conversion on the rations containing 16 percent protein with 0.025 percent added methionine appearing to be the optimum level for low energy rations and 0.05 percent appearing to be the optimum level for high energy rations. However, neither the main effects of methionine nor the energy x methionine interactions approached significance.

The failure of supplemental methionine to apparently improve the performance of pigs fed the 12 percent protein ration indicated that a nutrient(s) other than methionine was the more limiting as the performance on the 12 percent protein was definitely sub-optimal. The lysine content of the ration may have been limiting even though 0.12 percent supplemental lysine was added; however the data of Acker (1953) suggested 0.10 percent as the optimum supplemental level for a similar 12 percent protein ration.

Supplemental methionine added to corn-soybean oil meal type diets failed to improve rate of gain or feed conversion under the conditions of these experiments. These results are in agreement with the nitrogen balance studies reported by Meade (1956a and 1956b) in which the methionine failed to improve corn-soybean oil meal diets containing 12, 14 and 16 percent protein. Although these practical diets calculate to be low or border line in methionine adequacy, a consistent response was not observed.

The results of these experiments are not in agreement with reported chick data which has demonstrated that the calorie content of the ration has an effect on the response to increasing protein or amino acid levels. The rations used in these experiments were not nutritionally adapted to testing effects of energy on response to methionine as a consistent response to methionine was not observed; however the protein levels were such that any interactions of protein and energy should have been detectable.

Studies with the baby pig by Peo _et al_. (1954), comparing levels of protein and fat, also failed to demonstrate a significant interaction between protein and fat as measured by either gains or feed conversion. In this work constant levels of energy were not maintained for each level of protein, however the range of fat levels used (zero to 10 percent) was adequate to afford a thorough test of the effects of energy on the protein requirement.

Potter _et al_. (1956) concluded that the energy content of chick diets had a greater effect on performance than did the calorie/protein ratio. Their observations were that increasing the energy level of the ration improved both growth rate and feed conversion. The results of the experiments reported here would indicate that the energy level has comparatively less effect on rate of gain in the pig than in the chick, whereas the effect on feed conversion is similar for the two species.

Hill and Dansky (1950) concluded that the energy content of the ration indirectly affected the protein requirement of chicks through its effect on food intake. There may well be a species difference in that the data suggests that the energy content of the ration does not exert the controlling effect on the intake of feed by the pig as it does with the chick. The data reported by Rosenberg (1957) shows that chicks consumed essentially the same total calories on a diet containing 1,000 Calories per pound as a similar group did on a diet containing 1,200 Calories per pound, although this was not so evident in the work reported by Donaldson et al. (1956). In the three experiments reported herein, increased energy content of the diet did decrease the food intake per day; however the total caloric intake was greater for the high energy diets.

These observations would suggest that an interaction of protein and energy on pig performance could be demonstrated with extremely wide ranges of protein and energy, however these ranges would be beyond the limits of practicability.

SUMMARY AND CONCLUSIONS

Levels of Protein for Baby Pig Rations

Three experiments involving 288 pigs were conducted to determine the optimum level of protein for baby pigs. The feeding of rations containing three levels (15, 20 and 25 percent) of a 50-50 mixture of solvent soybean oil meal (50 percent protein) and low heat spray dried skimmilk (34 percent protein) as the source of protein resulted in a curvilinear response of gains on protein levels and a linear response of feed required per pound of gains on protein levels. Maximum gains were observed on the 20 percent protein rations, whereas most efficient feed conversion was obtained with the maximum level fed (25 percent).

In corn-soybean oil meal-whey type diets, 19 percent protein rations resulted in maximum rate of gain in conventional growth studies and 22 to 25 percent resulted in maximum repletion gains. In each case the response to levels of protein was curvilinear with levels above and below the stated optimums resulting in reduced rates of gain. The feed conversion data were similar for the two methods in that 25 percent protein rations produced the most efficient gains. The quadratic regression of feed required per pound of repletion gain was significant with the feed/gain decreasing as protein levels were increased to 25 percent, then leveling off with little change in efficiency of feed conversion up to 31 percent protein. In the conventional growth trials, feed conversion improved as the level of protein was increased up to the maximum (25 percent) level fed.

The optimum level of protein in the ration was approximately the same when the protein was provided by a mixture of soybean oil meal and milk protein as it was when corn, soybean oil meal and whey provided the protein. This indicates that over feeding of the plant proteins can not compensate for their lower digestibility as compared to milk protein.

Evaluation of Depletion-Repletion Technique

Direct comparisons of the depletion-repletion technique and conventional growth trials indicated that the depletion-repletion technique results in less variation within treatments. The responses to levels of protein in the ration were similar for the two methods indicating also that the results obtained with the depletion-repletion technique applied to baby pigs are quite valid. This observation was supported by the statistical analysis of the data in that significant ration treatment x method of measuring interactions were not observed.

There is considerably more labor involved in a depletion-repletion experiment than in a conventional *ad libitum* feeding trial thus demanding a careful evaluation of the two methods. The increased sensitivity of the depletion-repletion technique, as demonstrated by its reduced within treatment variability, can no doubt be compensated for by increasing the number of replications in a conventional feeding trial, provided that other factors such as limitations in the number of experimental animals, available equipment and pen space or ration ingredients are not a problem. When experimental materials are limiting, it would be worth while to consider the use of the depletion-repletion technique.

Methionine Supplementation of Baby Pig Rations

Two experiments were conducted to study the effects of various supplemental levels of DL-methionine on the baby pig's response to corn-soybean oil meal-whey type rations containing 20 percent protein. Supplemental levels (0.0, 0.025, 0.05, 0.10 and 0.20 percent) of methionine exerted no significant effects on repletion gains or feed conversion, indicating that methionine was not the most limiting nutrient in the rations fed.

Further studies on the effects of 0.05 percent supplemental methionine added to similar rations containing varying levels of protein (16 to 31 percent) did not result in a consistent response that could be associated with supplemental methionine.

Nitrogen balance and growth studies were conducted to observe the relative value of soybean oil meal and dried skimmilk protein, to observe the effects of supplemental arginine on the utilization of the two proteins and to also observe the effects of supplemental methionine on utilization of soybean oil meal protein.

Pigs fed the milk rations gained at a faster rate on less feed per pound of gain than did pigs fed the soya diets. The supplemental arginine had no significant effect on either gains or feed conversion, even though the levels in the diets were three to four times the reported requirements. Supplemental DL-methionine significantly improved the rate of gain and efficiency of feed conversion in the pigs fed soya protein diets.

The digestibility of the dry matter and protein of the milk rations was high at two weeks of age (96 and 96 percent) and changed very little as the pigs increased in age to five weeks (97 and 96 percent). On the other hand the digestibility of the dry matter of the soya diets increased from 88 to 92 percent and the digestibility of protein increased from 77 to 82 percent as the pigs increased from two to five weeks of age. The percent of soya protein nitrogen retained remained constant for the two age groups, however the percentage of milk protein nitrogen retained decreased with increasing age of the pig. The biological value of the milk protein as compared to soya protein was considerably higher at two weeks of age (80 versus 66), however the biological value of the two proteins were similar at five weeks of age. The biological values are no doubt influenced by the apparent over feeding of protein at five weeks of age resulting in abnormally low values especially for the milk protein.

Supplemental arginine exerted its greatest influence on nitrogen retention at five weeks of age resulting in a significant depression in percent nitrogen retained. This was in agreement with the overall average effect on gains and feed conversion, however the latter effects were not significant. Supplemental methionine slightly improved nitrogen utilization of the soya diets at two weeks but did not at five weeks of age. This effect at two weeks of age, or the average effect at two and five weeks of age was not significant.

Adjusting the gains of the pigs on methionine supplemented soya protein diets with the use of covariance analysis on protein digestibility resulted in gains similar to that of the pigs fed milk protein diets.

Energy, Protein and Methionine Interrelationships

Three experiments were conducted using growing pigs 25 to 75 pounds body weight, to study the effect of the energy content of the ration on response to increased levels of protein and supplemental methionine in corn-soybean oil meal type diets. A consistent response to supplemental methionine was not observed on either 12 or 16 percent protein rations or in rations containing 940, 970, 1,078 or 1,120 productive Calories per pound; although there was some evidence that supplemental methionine slightly improved the efficiency of feed conversion of pigs fed 16 percent protein diets.

Increasing the level of protein from 12 to 16 percent significantly increased rate of gains and decreased the pounds of feed required per pound of gain independent of level of energy in the ration. Also, increasing the energy content of the ration from 970 to 1,120 Calories per pound significantly decreased the pounds of feed required per pound of gain. Statistical analysis of the data confirmed that these effects of protein and energy were independent.

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APPENDIX

Table 12. Composition and calculated analysis of pre-starter ration used in the preliminary feeding period of baby pig experiments

Composition

Ingredient		Proportion
Ground yellow corn		9.55
Cane sugar		10.00
Corn sugar		5.00
Sweet dried whey		2.50
Dried skimmilk		40.00
Solvent soybean oil meal (50% protein)		17.50
Condensed fish solubles		2.50
Stabilized lard		5.00
Corn steep water		1.00
Dried brewers' yeast		1.00
Dried beet pulp		2.00
Calcium carbonate		0.15
Dicalcium phosphate		0.60
Iodized salt		0.50
Trace mineral premix (35-C-41)[a]		0.20
Vitamin-antibiotic premix[b]		2.50
Total		100.00
Calculated analysis		
Protein	%	25.1
Fat	%	5.8
Fiber	%	1.2
Calcium	%	0.8
Phosphorus	%	0.7

[a]Composition given in Table 14.

[b]In addition to vitamins listed in calculated analysis the following were added at the indicated rates per pound of ration: folic acid, 0.5 mg.; thiamine, 5 mg.; pyridoxine, 1.5 mg.; PABA, 8.0 mg.; biotin, 20 mcg.; inositol, 250 mg.; ascorbic acid, 300 mg.; alpha tocopherol acetate, 10 mg.; menadione, 3.0 mg.

Table 12. (Continued)

Vitamin A	I.U./lb.	2,846
Vitamin D_2	I.U./lb.	600
Riboflavin	mg./lb.	6.7
Pantothenic acid	mg./lb.	12.7
Niacin	mg./lb.	27.6
Choline	mg./lb.	509
Vitamin B_{12}	mcg./lb.	20
Antibiotics[c]	mg./lb.	50

[c]Contained the following: chlortetracycline, 20 mg.; oxytetracycline, 20 mg.; penicillin, 5 mg.; bacitracin, 5 mg.

Table 13. Calculated analysis of basal rations

Item		Experiment 732	754	798	768	715	774	Protein-free[a]
Protein	%	20.0	19.0	20.0	20.0	12.0	16.0	0.0
Fat	%	4.1	4.2	2.7	4.4	3.4	4.1	2.5
Fiber	%	2.1	2.0	1.5	2.1	2.3	2.5	2.0
Calcium	%	0.8	0.8	0.8	0.8	0.7	0.8	0.8
Phosphorus	%	0.7	0.7	0.7	0.7	0.5	0.7	0.7
Vitamin A	I.U./lb.	5,000	3,031	3,000	3,443	2,885	2,281	5,000
Vitamin D_2	I.U./lb.	1,000	1,000	500	1,000	400	400	1,000
Riboflavin	mg./lb.	5.0	5.0	4.9	5.7	3.0	2.6	5.0
Pantothenic acid	mg./lb.	10.0	9.8	10.0	11.2	6.6	6.0	10.0
Niacin	mg./lb.	30.0	30.0	30.0	29.9	22.7	20.1	30.0
Choline	mg./lb.	560	527	516	556	479	400	450
Vitamin B_{12}	mcg./lb.	20	20	20	20	10	10	20
Antibiotic[b]	mg./lb.	50	50	50	50	10	10	50

[a]Depletion ration used in experiments 732, 746, 754 and 768.

[b]Chlortetracycline used in all experiments except 774, oxytetracycline used in experiment 774.

Table 14. Trace mineral premixes

	Element	% in premix	P.P.M. contributed to ration by: 0.10%	0.15%	2.0%
35-C-41	Iron	7.0	70.4	105.6	
	Copper	0.475	4.8	7.2	
	Cobalt	0.166	1.6	2.4	
	Zinc	8.10	81.6	122.4	
	Manganese	5.68	56.8	85.2	
	Calcium	5.28			
	Potassium	0.750	7.5	11.2	
35-D-10A	Iron	1.81			362.0
	Copper	0.038			7.6
	Cobalt	0.0155			3.1
	Zinc	0.250			50.0
	Manganese	0.507			101.5
	Potassium	19.90			3,980
	Iodine	0.0021			0.4
	Magnesium	2.65			530.0
	Calcium	7.35			

Table 15. Amounts of vitamins and antibiotics added per pound of complete ration

Ingredient	Protein[a] free	Experiment 732	754	715[b]	774[c]
Vitamin A, I.U.	5,000	4,678	2,678	2,000	1,500
Vitamin D_2, I.U.	1,000	1,000	1,000	400	400
Riboflavin, mg.	5.0	3.1	3.1	2.5	2.0
Calcium pantothenate, mg.	10.0	3.0	3.0	4.0	2.5
Niacin, mg.	30.0	22.7	22.7	14.0	12.0
Choline chloride, mg.	450			200	18
Vitamin B_{12}, mcg.	20	20	20	10	10
Alpha tocopherol acetate, mg.	10	10	10		
Ascorbic acid, mg.	300	100	100		
Menadione (vit. K), mg.	3.0	0.5	0.5		
Thiamine HCl, mg.	5.0	3.0	3.0		
Pyridoxine, mg.	1.5	1.5	1.5		
PABA, mg.	8.0	2.0	2.0		
Inositol, mg.	250	250	250		
Folic acid, mg.	0.5	0.5	0.5		
Biotin, mcg.	20	3.0	3.0		
Chlortetracycline, mg.[d]	50	50	50	5	10

[a]Used in experiments 732, 746, 754 and 768; lactose served as carrier for vitamins, in other premixes solvent soybean oil meal served as carrier.

[b]Amounts added to basal 12 percent protein ration (970 Cal./pound).

[c]Amounts added to basal ration (940 Cal./pound).

[d]Oxytetracycline used as antibiotic in experiment 774.

Table 16. Experiments 768 and 798 - Amounts of vitamins added per pound of complete ration

Ingredient	Experiment 768	Experiment 798 SBOM ration	Experiment 798 Milk ration
Vitamin A, I.U.[a]	3,000	3,000	3,000
Vitamin D_2, I.U.	1,000	500	500
Riboflavin, mg.	3.5	4.5	--
Ca Pantothenate, mg.	5.8	7.6	1.2
Niacin, mg.	22.0	26.1	26.9
Choline chloride, mg.	113	--	158
Vitamin B_{12}, mcg.	20	20	20
Alpha tocopherol acetate, mg.	10	10	10
Ascorbic acid, mg.	300	100	100
Menadione (vit. K), mg.	3.0	0.5	0.5
Thiamine HCl, mg.	5.0	3.0	3.0
Pyridoxine, mg.	1.5	1.5	1.5
PABA, mg.	8.0	2.0	2.0
Inositol, mg.	250	250	250
Folic acid, mg.	0.5	0.5	0.5
Biotin, mcg.	20	3.0	3.0
Chlortetracycline, mg.	50	50	50

[a]Lactose served as a carrier for the vitamins.

Table 17. Experiment 732 - Analysis of variance plan and observed mean squares for gains and feed required per pound of gain

Source of variation	d.f.	Conventional growth Gain	Conventional growth Feed/gain	Repletion Gain	Repletion Feed/gain
		Mean squares		Mean squares	
Replications	3	66.4556	0.1439	20.3150	0.2097
Treatments	3	17.4623	0.1591	3.5717	0.0485
Error	9[a]	10.9282	0.1509	6.1256	0.0754
Total	15[a]	24.2271	0.1512	8.4527	0.0969
Coefficients of variation (%)		24.4	22.2	19.0	17.9

[a]One degree of freedom subtracted from the conventional growth group for calculated value.

Table 18. Experiment 746 - Summary of repletion gains and feed required per pound of repletion gain

	Levels of added DL-methionine %				
	0.00	0.025	0.05	0.10	0.20
Replication	Repletion gain (lbs.)				
1	12.31	11.12	10.56	11.25	12.94
2	10.12	11.13	11.00	8.19	11.31
3	12.19	13.56	10.75	11.44	12.19
4	11.62	11.19	10.06	10.94	10.19
Average	11.56	11.75	10.59	10.46	11.66
Replication	Feed/repletion gain (lbs.)				
1	1.31	1.28	1.32	1.32	1.37
2	1.41	1.46	1.50	1.56	1.54
3	1.34	1.24	1.27	1.25	1.41
4	1.44	1.31	1.53	1.33	1.44
Average	1.38	1.32	1.41	1.36	1.44

Table 19. Experiment 746 - Analysis of variance plan and observed mean squares for average gains and feed required per pound of gain

Source of variation	d.f.	Mean squares Gain	Mean squares Feed/gain
Replications	3	2.9304	0.0393
Methionine levels	4	1.5627	0.0078
Linear regression	1	0.0001	0.0152
Quadratic regression	1	4.7516[a]	0.0018
Remainder	2	0.7496	0.0070
Error	12	0.8562	0.0034
Total	19	1.3325	0.0100

[a]Quadratic regression of gain on methionine levels significant (P = 0.05 or less).

Table 20. Experiment 754 - Summary of repletion gains and feed required per pound of gain

Level of protein (%)	19		22		25		28		31	
Methionine supple-mentation	-	+	-	+	-	+	-	+	-	+
Replication	Repletion gain (lbs.)									
1	8.25	7.00	9.44	7.58	8.38	9.88	9.75	6.62	8.44	10.38
2	8.38	7.94	12.00	11.00	10.38	12.69	8.94	9.55	9.06	9.12
3	7.44	8.25	10.31	9.08	9.44	10.25	8.75	11.75	10.12	6.88
Average[a]	8.02	7.73	10.58	9.22	9.40	10.94	9.15	9.31	9.21	8.79
	Feed/gain (lbs.)									
1	1.63	1.66	1.22	1.88	1.39	1.22	1.33	1.53	1.38	1.05
2	1.57	1.56	1.25	1.54	1.35	1.12	1.24	1.44	1.33	1.19
3	1.91	1.33	1.24	1.49	1.14	1.09	1.20	1.16	1.09	1.37
Average[a]	1.70	1.52	1.24	1.64	1.29	1.14	1.26	1.38	1.27	1.20

[a]Quadratic regression of gains and feed/gain on protein levels significant (P = 0.05 or less).

Table 21. Experiment 754 - Analysis of variance plan and observed mean squares for total gain and feed required per pound of gain

Source of variation	d.f.		Mean squares	
			Gain	Feed/gain
Replications	2		4.4494	0.0405
Treatments	9		2.8981	0.1099
Protein levels		4	4.8286	0.1588
Linear regression		1	1.4821	0.4541
Quadratic regression		1	13.9977[a]	0.1071[a]
Cubic regression		1	3.6704	0.0109
Quartic regression		1	0.1640	0.0631
Methionine supplementation		1	0.0410	0.0044
Methionine x protein level		4	1.6820	0.0873[b]
Error	18		1.6632	0.0200
Total	29		2.2386	0.0493

[a]Quadratic regression of gains and feed/gain on protein levels significant (P = 0.05 or less).

[b]Interaction of methionine supplementation and protein levels on feed/gain significant (P = 0.05 or less).

Table 22. Experiment 772 - Summary of gain and feed required per pound of gain

Level of protein	16		19		22		25	
Methionine supplementation[a]	-	+	-	+	-	+	-	+
Replication	4-week gain (lbs.)[b]							
1	18.38[c]	17.31	20.00	16.12	17.50	14.06	15.75	20.25
2	20.00	19.06	17.25	19.33	16.31	16.94	12.81	12.44
3	18.69	16.56	20.38	20.25	21.06	20.94	16.75	17.19
Average	19.02	17.64	19.21	18.57	18.29	17.31	15.10	16.63
Replication	Feed/gain (lbs.)[b]							
1	2.10[c]	2.15	1.82	1.84	1.79	1.70	1.82	1.67
2	2.04	2.13	1.99	1.76	1.82	1.80	1.70	1.79
3	2.19	1.95	1.93	1.92	1.76	1.89	1.92	1.75
Average	2.11	2.08	1.91	1.84	1.79	1.80	1.81	1.74

[a]DL-methionine added at the rate of 0.05%.

[b]Linear regression of gains and feed/gain on protein levels significant ($P = 0.05$ or less).

[c]All three replications on 16% protein conducted simultaneously with replication 3 of other treatments, thus they were not included in the statistical analysis of the data.

Table 23. Experiment 772 - Analysis of variance plan and observed mean square for gains and feed required per pound of gain

Source of variation	d.f.		Mean squares	
			Gain	Feed/gain
Replications	2		19.4982	0.0118
Ration treatments	5		6.7353	0.0104
Protein levels		2	14.0721	0.0176
Linear regression		1	0.7225	0.0310[a]
Quadratic regression		1	27.4216[b]	0.0042
Methionine supplementation		1	0.0047	0.0103
Protein level x methionine		2	2.7662	0.0033
Error	10		5.1698	0.0056
Total	17		7.3159	0.0774

[a]Linear regression of feed/gain on protein levels significant ($P = 0.05$ or less).

[b]Quadratic regression of gain on protein levels significant ($P = 0.05$ or less).

Table 24. Experiment 798 - Summary of total gains and feed required per pound of gain

Rep.	SBOM	SBOM + arg.	SBOM + meth.	SBOM arg. + meth.	DSM	DSM + arg.
			1-6 week gains (lbs.)			
1	17.4	13.3	25.4	18.5	28.5	26.2
2	6.4	8.8	14.6[a]	14.1[a]	21.6	22.5
3	10.2[a]	14.1	7.3	16.3[a]	26.3	27.3
4	8.3	10.5	22.3	20.9	24.8	20.0
5	10.1	3.2	22.9	14.0	25.6	21.9
6	13.9	18.3	14.2	19.7	28.3	24.1
Av.	11.0	11.4	17.8	17.2	25.8	23.7
			Feed/gain (lbs.)			
1	1.69	2.06	1.56	1.83	1.65	1.57
2	3.65	2.42	2.17[a]	2.11[a]	1.41	1.66
3	2.34[a]	1.93	2.62	1.82[a]	1.51	1.44
4	2.32	2.15	1.47	1.50	1.69	1.48
5	2.25	4.44	1.56	1.98	1.57	1.74
6	1.79	1.68	1.87	1.65	1.68	1.60
Av.	2.34	2.45	1.88	1.82	1.58	1.58

[a]Calculated values (Snedecor, 1956, p. 310).

Table 25. Experiment 798 - Analysis of variance plan and observed mean squares for gain and feed required per pound of gain

Source of variation	d.f.	Mean squares: Combined data Gain	Combined data Feed/gain	d.f.	DSM data Gain	DSM data Feed/gain	d.f.	SBOM data Gain	SBOM data Feed/gain
Replications	5	36.91	0.3794	5	10.75	0.0093	5	35.85	0.5870
Treatments	5	223.76[a]	0.8272	1	14.33	--	3	79.98	0.6141
SBOM vs. DSM	1	864.59[a]	2.2934[a]						
Arginine sup.	1	5.76	0.0017	1	14.33	--	1	0.07	0.0030
Methionine sup.	1	238.77[a]	1.7985[a]				1	238.77[a]	1.7985
Interactions	2	4.84	0.0211				1	1.09	0.0409
Error[b]	31	18.00	0.3335	5	3.28	0.0124	11	28.46	0.5311
Total	31	54.24	0.4205	11	7.68	0.0098	19	38.54	0.5589

[a]Differences significant (P = 0.05 or less).

[b]Bartlett's test (Snedecor, 1956, p. 285) for homogeneity revealed that the variances of DSM data and SBOM data are not equal.

Table 26. Experiment 798 - Analysis of variance plan and observed mean squares for apparent protein digestibility, dry matter digestibility, nitrogen retention and biological values

Source of variation	d.f.	Mean squares: Protein digestibility	Dry matter digestibility	Nitrogen retention	Apparent biological values
Replications (litter mates)	5	164.56[a]	16.52	80.96	32.35
Age (2 wks. vs. 5 wks.)	1	209.79[a]	106.82[a]	42.16	1,676.21[a]
Replications x age	5	11.03	0.58	43.94	51.88
Ration treatment	5	826.71	151.35	588.43	243.59
Milk vs. SBOM	1	4,084.28[a]	743.02[a]	2,578.95[a]	840.52[a]
Arginine vs. none	1	5.95	2.92	165.92	201.34[a]
Methionine vs. none	1	1.58	9.99	126.75	131.01
Interactions	2	20.87	0.42	35.28	22.54
Replications x treatment	25	37.84	6.17	51.02	34.00
Age x treatment	5	16.16	11.97	588.60[a]	246.02[a]
Replication x age x treatment	21	22.73	3.88	54.17	51.82
Total	67	100.38	18.58	133.80	96.47

[a]Differences significant (P = 0.05 or less).

Table 27. Experiment 798 - Analysis of covariance of average two and five week protein digestibilities on one to six week gains

Source of variation	d.f.	SSx^2	SSxy	SSy^2	d.f.	SSdy·x	M.S.
Total	31	2,847	1,647	1,681			
Replications	5	540	191	185			
Treatments	5	1,951	1,276	1,119			
Error	21	356	180	377	20	286	14.3
Treatment + error	26	2,307	1,456	1,496	25	577	
Adjusted treatments [a]					5	291	58.2

[a]Adjusted treatment "F" ratio = 58.2/14.3 = 4.1
Unadjusted treatment "F" ratio = 224/18.0 = 12.4
b = 0.506; r = 0.492

Table 28. Experiment 798 - Summary of observed one to six week gains and gains adjusted for differences in digestibility

Treatment	X digestibility	Y observed gains	$\hat{Y}$ adjusted gains[a]
	%	lbs.	lbs.
SBOM	80	11.0	13.5
SBOM + arg.	80	11.4	13.9
SBOM + meth.	79	17.8	20.8
SBOM + arg. + meth.	80	17.2	19.7
DSM	96	25.8	20.2
DSM + arg.	96	23.7	18.1

[a]$\hat{Y} = Y + b(\bar{x} - x) = Y + 0.506(85 - X)$

Table 29. Experiment 768 - Analysis of variance plan and observed mean squares for gains and feed required per pound of gain

Source of variation	d.f.	Combined analysis		d.f.	Separate analysis: Conventional growth		Separate analysis: Repletion	
		Gain	Feed/gain		Gain	Feed/gain	Gain	Feed/gain
		Mean squares			Mean squares		Mean squares	
Litters	11	19.9064[a]	0.0720	11	16.0833[a]	0.0790	7.4595	0.0302
Method	1	111.7513[a]	0.7771[a]					
Protein levels	2	66.0117[a]	2.2532[a]	2	57.6175[a]	1.3723[a]	15.2658[a]	0.9118[a]
Linear regression	1	56.3333[a]	4.3200[a]	1	46.2038[a]	2.5676[a]	14.5704	1.7876[a]
Quadratic regression	1	75.6900[a]	0.1863[a]	1	69.0312[a]	0.1770	15.9612	0.0360
Method x protein	2	6.8716	0.0310					
Error	55	4.8971	0.0365	22	6.3899	0.0490	4.0346	0.0236
Total	71	10.5046	0.1147	35	12.3637	0.1340	5.7528	0.0764
Coefficients of variation (%)					18.59	12.58	17.68	9.91

[a]Differences significant (P = 0.05 or less).

Table 30. Experiment 715 - Summary of average daily gains and feed required per pound of gain

	Added methionine %							
	0.00	0.025	0.05	0.10	0.00	0.025	0.05	0.10
Rep.	Low protein (12%)				High protein (16%)			
	Low energy (970 Cal./lb.)							
	Average daily gain (lbs.)							
1	1.21	1.26	1.37	1.41	1.38	1.53	1.59	1.50
2	1.29	1.10	1.23	1.31	1.37	1.40	1.13	1.29
3	1.29	1.10	1.21	1.16	1.28[a]	1.26	1.35	1.34
4	1.10	1.42	1.24	1.19	1.33	1.33	1.30	1.30
5	0.98	0.82	1.10	1.04	1.35	1.32	1.38	1.07
6	1.02	1.13	0.88	1.06	1.26	1.26	1.24	1.24
Av.	1.15	1.14	1.17	1.20	1.33	1.35	1.33	1.29
	Feed/gain (lbs.)							
1	2.49	2.98	2.61	2.50	2.35	2.18	2.06	2.08
2	2.37	3.09	2.90	2.57	2.54	2.12	2.35	2.42
3	2.53	2.37	2.15	2.57	2.40[a]	2.32	2.39	2.13
4	2.83	2.64	2.83	2.58	2.55	2.55	2.46	2.10
5	2.92	2.91	2.65	2.88	2.20	2.08	2.00	2.53
6	2.54	2.63	2.98	2.77	2.23	2.09	2.27	2.23
Av.	2.61	2.77	2.69	2.64	2.38	2.22	2.26	2.25
	High energy (1,120 Cal./lb.)							
	Average daily gain (lbs.)							
1	1.41	1.34	1.34	1.26	1.44	1.54	1.79	1.56
2	1.10	1.40	1.17	1.02	1.29	1.61	1.49	1.37
3	1.23	1.10	1.00	1.34	1.31	1.52	1.26	1.46
4	1.39	1.32	1.19	1.35	1.43	1.21	1.42	1.14
5	1.04	1.11	0.75	1.00	1.26	1.07	1.38	1.24
6	1.14	1.04	0.82	0.77	1.46	1.17	1.13	1.30
Av.	1.22	1.22	1.04	1.12	1.36	1.35	1.41	1.34

[a]Estimated value (Snedecor, 1956, p. 310).

Table 30. (Continued)

	Added methionine %							
	0.00	0.025	0.05	0.10	0.00	0.025	0.05	0.10
Rep.	Low protein (12%)				High protein (16%)			
	High energy (1,120 Cal./lb.)							
	Feed/gain (lbs.)							
1	2.24	2.28	2.50	2.48	1.78	1.78	1.80	1.86
2	2.38	2.29	2.67	2.51	2.12	2.01	1.92	2.06
3	2.19	2.33	2.63	2.40	2.13	1.96	1.98	1.98
4	2.24	2.47	2.32	2.38	2.02	2.53	1.79	2.25
5	2.56	2.41	2.71	2.31	2.04	2.06	1.80	1.96
6	2.29	2.55	2.80	3.02	1.81	2.15	2.12	2.04
Av.	2.32	2.39	2.60	2.52	1.98	2.08	1.90	2.02

Table 31. Experiment 715 - Analysis of variance plan and observed mean squares for average daily gain and feed required per pound of gain[a]

Source of variation	d.f.	Mean squares: Average daily gain	Mean squares: Feed/gain
Replications	5	0.2260	0.0796
Treatments	15	0.0702	0.4363
Methionine levels(M)	3	0.0054	0.0095
Protein levels(P)	1	0.8626[b]	4.4505[b]
Energy levels(E)	1	0.0060	1.5025[b]
M x P	3	0.0210	0.0808
M x E	3	0.0084	0.0317
P x E	1	0.0187	0.0189
M x P x E	3	0.0267	0.0688
Remainder	74[c]	0.0151	0.0352
Total	94[c]	0.0351	0.1015

[a]Individual pig considered the experimental unit, data analyzed on the per pig basis.

[b]Difference significant (P = 0.05 or less).

[c]One pig removed from experiment, missing value estimated (Snedecor, 1956, p. 310), one degree of freedom subtracted for calculated value.

Table 32. Experiment 774 - Summary of average daily gain and feed required per pound of gain

	Added DL-methionine (%)							
	Low energy				High energy			
	0.00	0.0125	0.025	0.05	0.00	0.0125	0.025	0.05
	Average daily gain (lbs.)							
Rep. 1	1.54	1.33	1.40	1.63	1.63	1.66	0.94	1.40
Rep. 2	1.50	1.33	0.99	1.57	1.86	1.63	1.76	1.74
Rep. 3	1.41	1.11	1.60	1.40	1.64	1.41	1.64	1.69
Av.	1.48	1.26	1.33	1.53	1.71	1.57	1.45	1.61
	Feed/gain[a] (lbs.)							
Rep. 1	2.68	2.71	2.69	2.46	2.02	2.16	2.70	2.26
Rep. 2	2.59	2.75	2.91	2.51	2.27	2.31	2.08	2.07
Rep. 3	2.31	2.83	2.47	2.67	2.15	2.25	2.18	2.42
Av.	2.53	2.76	2.69	2.55	2.15	2.24	2.32	2.25

[a]Improvement in feed efficiency of high energy vs. low energy significant at P = 0.05 or less.

Table 33. Experiment 774 - Analysis of variance plan and observed mean squares for average daily gains and feed required per pound of gain

Source of variation	d.f.		Mean squares: Average daily gain	Mean squares: Feed/gain
Replications	2		0.0227	0.0050
Ration treatments	7		0.0654	0.1553
Energy levels		1	0.1998	0.9243
Methionine levels		3	0.0690	0.0407
Linear regression		1	0.0006	0.0022
Quadratic regression		1	0.2028	0.1124
Cubic regression		1	0.0036	0.0075
Energy x methionine		3	0.0169	0.0135
Error	14		0.0534	0.0370
Total	23		0.0544	0.0702

Table 34. Experiment 774a - Analysis of variance plan and observed mean squares for average daily gains and feed required per pound of gain

Source of variation	d.f.	Mean squares: Average daily gain	Mean squares: Feed/gain
Replications	5	0.1184	0.2326
Energy levels	1	0.0005	0.2268
Error (a)	5	0.0159	0.0372
Methionine levels	3	0.0042	0.0161
Linear	1	0.0086	0.0128
Quadratic	1	0.0000	0.0039
Cubic	1	0.0041	0.0316
Methionine x energy	3	0.0190	0.0373
Error (b)	29[a]	0.0257	0.0385
Total	46[a]	0.0323	0.0620

[a]One degree of freedom subtracted for calculated value.

Table 35. Experiment 774a - Summary of average daily gains and feed required per pound of gain

	Added DL-methionine (%)							
	Low energy				High energy			
	0.00	0.0125	0.025	0.05	0.00	0.0125	0.025	0.05
	Average daily gain (lbs.)							
Rep. 1	1.23	1.37	1.63	1.17	1.37	1.66	1.37	1.26
Rep. 2	1.46	1.46	1.26	1.46	1.40	1.29	1.49	1.51
Rep. 3	1.49	1.46	1.46	1.40	1.34	1.35[a]	1.11	1.37
Rep. 4	1.29	1.26	1.44	1.14	1.40	1.43	0.94	1.54
Rep. 5	1.17	0.97	0.97	1.26	1.14	1.20	1.29	1.11
Rep. 6	1.29	1.31	1.14	0.89	1.06	1.03	1.29	1.20
Av.	1.32	1.30	1.32	1.22	1.28	1.33	1.25	1.33
	Feed/gain (lbs.)							
Rep. 1	3.21	2.39	2.47	2.70	2.61	2.27	2.49	2.60
Rep. 2	2.59	2.50	2.68	2.44	2.27	2.68	2.19	2.33
Rep. 3	2.63	2.30	2.28	2.38	2.63	2.51[a]	2.56	2.16
Rep. 4	2.19	2.28	2.32	2.38	2.08	2.23	2.77	2.05
Rep. 5	2.23	2.35	2.31	2.45	2.05	2.05	1.98	2.09
Rep. 6	2.22	2.15	2.32	2.34	2.05	2.08	2.04	2.04
Av.	2.51	2.33	2.40	2.45	2.28	2.30	2.34	2.21

[a]Estimated value (Cochran and Cox, 1957, p. 302).