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AMINO ACIDS AND INGESTED PROTEIN IN  
SWINE.**

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RELATIONSHIP OF BLOOD PLASMA FREE  
AMINO ACIDS AND INGESTED PROTEIN IN SWINE

by

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## INTRODUCTION

About a century ago, protein was recognized as an indispensable dietary component. For many years thereafter nitrogen was thought to be the essential constituent of proteins, but by 1900 it had become apparent that proteins differed little in nitrogen content, yet greatly in nutritional value. Although we have progressed from nitrogen nutrition through protein nutrition to amino acid nutrition, our growing basic knowledge has not altered the fact that protein is the dietary source of nutritionally indispensable amino acids.

The concept which has emerged from recent nutrition research is that the proportion of amino acids in the dietary protein determines the efficiency of utilization of the protein (Flodin, 1957; Harper and Kumta, 1959). In addition, the amino acids should be released and absorbed at such a rate to present the correct combination of amino acids to the anabolic centers of the tissues.

This amino acid balance principle is not entirely new as Osborne and Mendel (1915) attributed the high nutritive value of lactalbumin for promoting growth to the "more perfect balance in the proportions of the essential amino acid groups which it contains". Later, Almquist (1954) studied in more detail the importance of amino acid balance. The essence of the amino acid balance theory is that the more closely the amino acids in the protein resemble the amino acid requirements of the animal, the greater is the protein efficiency obtained.

Many differences in protein quality have been expressed quantitatively by concepts such as biological value, protein efficiency, and chemical

score. These functions are correlated with the amino acid composition of the protein (Block and Mitchell, 1946). Almquist (1954) has proposed that the free amino acids in blood plasma be used as a sensitive index to protein quality. Blood amino acid levels were found by Howell (1906) to be affected by the ingestion of a meal. Recently, Frame (1958) has presented the first detailed study relating the amino acid composition of the dietary protein to plasma amino acid levels. Longnecker and Hause (1959) have indicated that the free plasma amino acid concentration reflects the amino acid composition of the ration, if it was postulated that the amino acids were removed from the plasma in proportion to their requirements. Puchal (1962) has presented data which indicate that the plasma amino acid levels of baby pigs reflect the amino acid composition of the ingested protein.

One of the main objectives of protein nutritionists has been to devise a single and rapid method of predicting the efficiency of utilization of proteins. Recently, Longnecker and Hause (1961) have reported a shortened plasma amino acid ratio technique to evaluate the amino acid adequacy in a food protein especially applicable to humans.

This study was conducted in an attempt to establish the interrelationships between plasma amino acid changes and supplementation of various limiting essential amino acids to soybean protein in the early weaned pig. In addition, information was sought concerning the degree of proteolysis of soybean protein and milk protein occurring in the digestive tract.

## REVIEW OF LITERATURE

## Dietary Protein and Blood Plasma Free Amino Acid Interrelationships

In 1912, Van Slyke and Meyer presented evidence of the presence of amino acid nitrogen in the nonprotein fraction of blood plasma. That amino acids circulating in the blood stream are rapidly removed by the tissues was indicated by Van Slyke and Meyer (1913) who further proved that intravenously administered amino acids were removed from the circulation as well as those from protein digestion, and that when the diet was the source of the amino acids entering the blood stream, the liver assumes particular importance in their disposal.

As early as 1916, Bang established a correlation between the disappearance of amino acids from the digestive tract and the increase in blood plasma free amino acid nitrogen which followed the ingestion of amino acids. Seth and Luck (1925) observed that the response of blood plasma amino acid nitrogen in the rabbit and dog to amino acid ingestion was not the same when different amino acids were introduced in the digestive tract. The amino nitrogen content of the blood not only attained a very high value rapidly but the high level persisted for six hours after the ingestion. Shambaugh et al. (1931) fed tyrosine and phenylalanine to rabbits but found no rise in the amino acid nitrogen of the blood at any period. Feeding glycine has been reported to reflect an increased concentration of glycine in the plasma with a small increase in the level of alanine (Gutman and Alexander, 1947).

Many other amino acids were soon studied in order to determine the amino acid changes occurring after their administration. Leucine, isoleucine,

threonine, and valine were observed to increase rapidly in systemic plasma following ingestion. The remaining amino acids in the plasma were observed to vary in their response to a sudden large dosage of any one amino acid. When leucine increased in the plasma, the other amino acids decreased, except for cystine, histidine, lysine, and tryptophan (Hier, 1947). After administration of isoleucine, arginine decreased in the plasma, while phenylalanine, tyrosine, and valine decreased significantly, with the remaining amino acids showing no marked change. High levels of threonine and valine did not appear to affect the level of other amino acids in the plasma.

When methionine was ingested, its plasma level increased markedly and remained so for 24 hours, a phenomenon which was not characteristic of any other amino acid. A decreased plasma level was observed simultaneously for isoleucine, phenylalanine, tyrosine, and valine.

Administration of phenylalanine resulted in an increased concentration of this amino acid in the plasma concomitantly with tyrosine, no other amino acid being affected. After ingestion of tyrosine, the tyrosine plasma level increased but not that of phenylalanine.

Steele and LeBovit (1951) administered purified amino acids, one at a time and together to humans in a fasting state. After the ingestion of a large amount of leucine or histidine, the plasma concentration of the ingested amino acids reached very high levels. When intact proteins were fed to humans (Frame, 1958), the concentration of most amino acids in the plasma did not peak until two to six hours after the test meal was ingested.

There is some evidence that in human subjects the amount of essential amino acids required for nitrogen balance is increased when nonessential nitrogen is added to diets containing minimal quantities of essential amino acids (Tuttle et al., 1959). Sauberlich (1961b) investigated the effect of adding 5 percent levels of nonessential as well as essential amino acids to low-protein diets and found varying degrees of growth depression in weanling rats. High values for plasma free amino acids were usually demonstrated when the amino acids were supplemented in excess to the diet.

Swendseid et al. (1962) reported that various nonessential nitrogen supplements (glutamic acid, glycine, diammonium citrate, and a mixture of alanine, aspartic acid, glutamic acid, glycine, and serine) were found to retard the growth rate of young rats if they were added to diets containing 8 percent casein and 0.3 percent methionine. Increases in the nonessential amino acids fed as supplements and their metabolic derivatives were found in the plasma and muscle tissue. In some instances, the essential amino acids appeared to decrease. This indicated that the decrease in the ratio of essential to nonessential nitrogen, which characterizes the supplemented diets, results in a decrease in the ratio of essential to nonessential amino acids in the plasma. Similar data have been observed in humans receiving low protein diets for periods of 30 days or longer which resulted in a decreased plasma essential to nonessential amino acid ratios (Swendseid et al., 1963a).



Further, Swendseid et al. (1963b) concluded that reduced essential to nonessential amino acid ratios are associated with suboptimal protein intakes in the growing rat. These decreased ratios occurred not only when the ratio of essential to nonessential amino acids in the diet was lowered by nonessential amino acid supplementation, but when the dietary ratio remained the same involving different dietary levels of casein. Glycine supplementation appeared to reduce essential amino acid plasma levels whereas glutamic acid supplementation did not have this effect.

These results indicate that the effect of the administration of any one amino acid on its blood plasma level may differ or vary depending upon whether the amino acid is given in the free form or fed as a constituent of a protein.

Attempts to evaluate the nutritional value of proteins by blood plasma free amino acids have shown discrepancies between this physiological criteria and the amino acid composition of the protein. Levels of plasma amino acids in the portal vein after feeding different protein meals have been studied in dogs (Dent and Schilling, 1949), rats (Denton and Elvehjem, 1954b; Wheeler and Morgan, 1958) and chickens (Charkey et al., 1953). The results of these studies have conflicted with one another. Many researchers seem to agree that the concentration of blood plasma free amino acids, after ingesting different proteins, corresponds roughly to the amino acid composition of the protein fed. However, the lack of a consistent correlation has been repeatedly demonstrated (Frame, 1958; Longnecker and Hause, 1959; Guggenheim et al., 1960; and Goldberg and Guggenheim, 1962).

Almquist (1954) summarized the literature on blood plasma amino acids of the chicken in relation to the amino acid composition of the diet, and concluded that in the chick there is a high degree of correlation between the free amino acid content of plasma and the amino acid composition in the dietary protein. Almquist further concluded that when the dietary supply is closely comparable to known requirements, there is little tendency for a relative accumulation of the amino acids in the blood. On the other hand, a surplus or deficiency of an amino acid in the diet is reflected to an exaggerated degree in the blood. This is supported by the work of Hill et al. (1961) who added zein to a ration already deficient in lysine for the chick and obtained a further decrease in the lysine level in the blood. Arginine, glycine, histidine, and tryptophan also decreased while isoleucine, leucine, threonine, and valine increased in concentration. Owings and Balloun (1961) found that addition of arginine to a casein diet for chicks increased the free arginine level in the plasma. Lysine, proline, glutamic acid, and serine increased in concentration whereas threonine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, and histidine decreased. These authors concluded that either excessive essential amino acids in casein were not absorbed to the extent that they were on the low arginine diet or that chicks were able to utilize these amino acids from the plasma more completely when arginine was added to the diet.

Working with the young pig, Puchal et al. (1962) found that plasma amino acid levels were related to the amino acid content of the dietary protein. Different plasma amino acid patterns were observed when five

different protein sources were fed (dried skim milk, soybean meal, fish meal, cottonseed meal, and meat meal). By comparing the plasma amino acid pattern of animals fed milk protein with that of the other proteins, an indication was obtained of the limiting essential amino acids which may be responsible for limiting growth rate in these poorer quality proteins.

McLaughlan et al. (1963) observed that levels of lysine, methionine, threonine, and tryptophan rose rapidly in peripheral blood after normal human subjects ingested a protein meal. The extent and duration of the increases were roughly proportional to the amount of protein eaten and the amino acid composition of the protein. On the other hand, working with the dog, Longnecker and Hause (1959) failed to observe a consistent correlation between the changes in the plasma amino acid concentrations and the amino acid composition of the ingested diet. They concluded, however, that a direct relationship between the postprandial plasma amino acid pattern and the amino acid composition of the ingested protein did exist. Guggenheim et al. (1960) and Goldberg and Guggenheim (1962) similarly reported that the extent and duration of a rise in concentration of lysine and methionine in portal plasma of rats during digestion were not in complete agreement with the amino acid composition of the protein source.

Recently, Peraino and Harper (1963) compared amino acid patterns in the portal plasma of rats after zein, casein, or their respective hydrolysates were fed. They observed that the amino acids of zein and about one-half of those of casein were absorbed into the portal blood more rapidly when

hydrolysates were fed. This indicates that digestion of the protein was for the most part a more limiting factor than amino acid absorption in determining the rate of entry of the dietary amino acids into the portal blood. The comparison of the free portal plasma amino acid concentrations after feeding either casein or zein does show that the amino acids in casein are more available than those of zein.

These results indicate that the amino acid composition of the ingested protein is only partially reflected in the blood plasma free amino acid levels of various species, however, it may be an indication of amino acid availability from the ingested protein and hence digestibility.

Level of dietary protein has been shown to influence blood plasma amino acid levels in chicks and swine. Olsen et al. (1959) noted that as the protein content of purified diets for chicks was increased the concentration of blood plasma amino acids exhibited a distinct rise. Ritchey and Richardson (1959) conducted a similar study with weanling pigs and observed that 12 of the amino acids studied increased in plasma concentration as dietary protein was increased from 0 to 15 to 30 percent. When going from the nonprotein diet to the 15 percent protein diet, a marked increase was observed in the concentration of glutamic acid, isoleucine, and tyrosine while aspartic acid, threonine, serine, methionine, leucine, ornithine, and arginine remained practically unchanged and lysine decreased. When the protein level was increased to 30 percent of the diet, the concentration of most of the amino acids increased.

It would appear that the protein level in the diet affects the concentration of free amino acids in the plasma, or plasma amino acids partially reflect dietary protein level.

#### Amino Acid Absorption

It is well known that proteins are hydrolyzed in the intestinal tract to amino acids which are absorbed through the intestinal wall and transported as such in the blood. It has been shown by Christensen (1949) that the majority of ingested protein is absorbed into the blood as free amino acids and not as peptides. In vitro studies have indicated that the rates of release of amino acids from dietary proteins may vary (Melnick et al., 1946; Denton and Elvehjem, 1953).

Previous studies of amino acid absorption by the small intestine have been limited to observations with single amino acids, or with mixtures of only a few amino acids. These studies have not clarified the interaction that results when mixtures of many amino acids are present in the small intestine. The interaction of amino acids occurring during their absorption has been emphasized by Nasset (1957). Indirect evidence of preferential absorption of individual amino acids from mixtures has been obtained from studies of amino acid patterns in portal plasma following the ingestion of a protein meal (Dent and Schilling, 1949; Guggenheim et al., 1960).

Present knowledge indicates that the L-isomers of amino acids are absorbed more rapidly than the corresponding D-forms (Gibson and Wiseman, 1951). The possible equivalence of D- to L-forms is a complicated procedure. The DL-forms have been used in many nutrition studies and the question that arises concerns the nutritive effectiveness of the D-isomers and the degree

of substitution for the corresponding L-isomer in supporting growth and maintenance. Greenstein and Winitz (1961) have prepared an excellent review concerning DL-amino acids and their interactions upon one another.

Differences have been observed, in vitro, in the rates of absorption of different amino acids from the intestine (Wiseman, 1953; Friedlander and Quastrel, 1955). When administered separately each amino acid is absorbed at a characteristic rate (Wiseman, 1955), but this rate may be altered by the presence of other amino acids, suggesting a competition for the transfer mechanism (Pinsky and Geiger, 1952; Hagihira et al., 1960). All L-amino acids are absorbed quite rapidly after digestion and only small amounts of amino acids can be found at any time in the intestinal contents (Hightower, 1962). Wiseman (1955) has reported that those amino acids which, when present alone, are transferred across the small intestine of the hamster at the lower rates (methionine and histidine) inhibits the transfer of those amino acids transported at higher rates (proline and glycine).

Agar and co-workers (1956), studying the inhibitory effect of several amino acids on the intestinal absorption of histidine, concluded that the amino acids with the largest nonpolar side chains (tryptophan, methionine, leucine, and isoleucine) inhibit strongly, whereas those amino acids with polar side chains (glutamic acid, aspartic acid, lysine, and arginine) inhibit weakly or not at all. Recent studies by Spencer and Samiy (1960, 1961) suggest or indicate that the rate of absorption of tryptophan and phenylalanine is markedly reduced when high levels of either amino acid are present in the intestinal lumen.

Delhumeau et al. (1962) showed that, in general, those amino acids reported by Wiseman (1955) and Agar et al. (1956) to have the highest

inhibitory effect (greatest affinity for the transfer mechanism) are those absorbed most rapidly, with those having a low inhibitory effect being absorbed slowly. A marked selectivity occurred in the absorption of different individual amino acids from mixtures of 18 L-amino acids into sacs of rat intestine in situ. The relative rank order, based on the percentage absorbed of the amino acids, however, was found to be significantly constant and independent of the molar ratios found in the mixtures. These ratios influenced principally the magnitude of the percentage of each amino acid absorbed. The absorption of the individual amino acids from a mixture with the amino acid ratios found in egg albumin hydrolysate was markedly higher as compared to when two or four micromoles of each amino acid were present in the intestine or a mixture simulating casein or zein hydrolysates.

The observations made in these studies are of particular importance in view of the necessity for the simultaneous presence of amino acids in liver and tissues in certain patterns required for optimum protein synthesis.

#### Portal Versus Systemic Blood Plasma Free Amino Acids

Since blood plasma free amino acids have been used as a criterion for determining the amino acid adequacy of a protein, the question arises as to whether systemic or portal plasma amino acids are more accurate in judging this relationship. Amino acids absorbed from the intestinal tract are carried by the portal vein to the liver, where selective retention and anabolic and catabolic reactions occur. Hence, the concentrations and

proportions of amino acids reaching the systemic circulation may be different as compared to concentrations in the portal vein during digestion and active absorption of amino acids from the small intestine.

Seth and Luck (1925) were among the first investigators to present evidence of the difference encountered between portal and peripheral blood plasma free amino acid concentrations. Different amino acids were fed to dogs and their comparative results showed that at any particular time the free amino acid concentration of portal blood was higher than the corresponding arterial values.

Dent and Schilling (1949) reported that portal blood of dogs after a test meal has a higher concentration of amino acids than systemic blood obtained from the jugular, although no significant differences in the ratios of one amino acid to another were found. These results are supported by Christensen (1949) who found that the total and free amino nitrogen of the blood was higher in the portal blood of dogs than in systemic blood at either one or two and a half hours after feeding a test meal.

On the other hand, Denton et al. (1953) cannulated the portal vein of dogs and observed appreciable differences both quantitatively and in amino acid ratios between the portal and radial blood plasma free amino acids. This was interpreted as being due to amino acid removal from the plasma by tissues as well as to a dilution effect.

Further reports by Denton and Elvehjem (1954a, 1954b) showed that concentrations of essential amino acids in the portal vein increased



rapidly after feeding beef or casein to dogs and that the increases in individual amino acids were proportional in degree to the amounts supplied by the proteins. These increases were reflected in the radial vein, though to a lesser extent. After feeding zein, an initial drop occurred in the total free amino acid level suggesting synthesis and secretion of digestive enzymes from the plasma amino acids. A gradual increase in the plasma amino acid concentrations occurred as digestion proceeded. Lysine levels remained depressed in the portal and radial veins five to six hours after the meal.

Recently, Puchal (1962) reported that no appreciable differences were observed in the amino acid pattern of portal or anterior vena cava plasma in the ratios of one amino acid to another or in the quantitative presence of these amino acids in young pigs. However, only values for ornithine, lysine, histidine, arginine, and tyrosine-phenylalanine were reported.

Perraino and Harper (1963) compared portal and systemic (heart puncture) plasma amino acids when rats were force-fed either zein, casein or their respective hydrolysates. They found that portal plasma amino acid concentrations increased with time and the concentration of systemic plasma amino acids reflected approximately the same pattern observed in the portal plasma, though to a lesser extent.

Most of the data indicates that, after ingesting a protein meal, the portal plasma free amino acids rise concomitantly and that systemic plasma amino acids reflect the concentrations observed in portal plasma, but to a lesser extent.

### Proteolysis of Proteins in vivo

In the early part of the century, Abderhalden et al. (1907) established the presence of several amino acids in the combined chymes of large groups of animals after feeding a protein meal. Almost 50 years had elapsed before analytical procedures had been sufficiently developed to enable feasible quantitative amino acid analysis.

Few quantitative determinations of free amino acids occurring in the small intestine during digestion of a protein meal have been performed. Nasset et al. (1955) were the first investigators to determine to any extent the amino acids in the intestinal contents of dogs. This work suggested that the qualitative free amino acid composition of intestinal contents is not greatly altered by the type of protein meal fed. They concluded that hydrolytic enzymes, other secreted proteins and sloughed mucosa provide sufficient endogenous amino acids to the ingested mixture to obscure the amino acid composition of the ingested protein.

Comparing the amino acid composition of two different proteins, egg albumin and zein, Nasset (1957) found that the total nitrogen recovered from the various sections of the small intestine remained constant regardless of the type of dietary protein in the ration. Amino acid analysis of the intestinal contents revealed that although the qualitative differences between the amino acid composition of the two proteins is still evident in the gastric contents, the distinguishing features have been lost in the jejunum. When a nonprotein meal was fed, the jejunum amino acid mixture could not be distinguished from those derived from protein meals.

More recently, Twombly and Meyer (1961) reported evidence of an increased amount of endogenous nitrogen secreted into the intestinal tract with increasing protein level. Nasset and Soon Ju (1961) recovered the nitrogen attributable to labeled casein from the jejunum of dogs and found it to be diluted more than four-fold with endogenous nitrogen. Similar studies in the rat showed exogenous nitrogen to be diluted up to seven-fold with endogenous nitrogen in the intestine, while the molar ratios of free amino acids were markedly different from those found in the ingested casein.

The type of dietary protein had a profound effect upon the quantity of nitrogen recovered from the small intestine (Rogers et al., 1960). When diets containing soybean protein, casein or gliadin were fed to rats, very little accumulation of nitrogen was observed in the small intestine over that found after feeding a nitrogen-free diet. More nitrogen accumulated in the small intestine of rats fed zein and gelatin than in the intestines of those fed casein.

Nasset (1962) found that the relative molar ratios of free amino acids in the intestinal lumen were not greatly different whether protein or nonprotein test meals were fed to enterostomized dogs. The relative molar ratios of amino acids in the intestinal contents after feeding lard or lean beef resemble each other much more closely than either one resembles the ratios obtained from amino acids present in lean beef itself. Ganapathy and Nasset (1962) concluded that molar ratios of amino acids in the plasma remain fairly constant even after ingesting a high quality protein. Plasma

amino acid molar ratios were shown to differ from the molar ratios of free amino acids present in intestinal contents.

The preponderance of data presented to date indicates that ingesta is mixed with relatively large quantities of endogenous protein in the small intestine and therefore ingested protein does not greatly alter the molar ratios of free amino acids in intestinal contents.

#### Effects of Fasting on Blood Plasma Free Amino Acids

Studies with chicks have shown that fasting usually results in an increase in the plasma lysine and threonine concentration, whereas the concentration of the other plasma amino acids usually remains constant or decreases (Charkey et al., 1953; Charkey et al., 1954; Gray et al., 1960). After fasting humans for 48 hours, a decreased concentration of lysine, threonine, methionine, arginine, and tryptophan occurred, while the plasma concentrations of leucine and valine increased (Charkey et al., 1955), indicating a species difference.

Hill and Olsen (1963a) reported that in chicks fed a diet containing 15 percent isolated soybean protein and then deprived of feed for periods of 12 to 48 hours, a marked elevation in the concentrations of lysine and threonine was observed. Concentrations of glutamine, isoleucine, leucine, and valine were also elevated, but to a lesser extent, whereas concentrations of arginine, histidine, phenylalanine, tryptophan, and tyrosine were depressed. After feeding chicks the nonprotein ration, rather than being completely deprived of feed, the concentrations of all amino acids investigated, including lysine and threonine, were below values obtained when the complete diet was fed.

Similar data have been reported by Sanahuja and Harper (1963) who observed that plasma concentrations of most of the indispensable amino acids decreased in protein depleted rats. After feeding the depleted rats a 6 percent fibrin ration, the indispensable amino acids decreased further in the plasma, apparently attributed to a high rate of protein synthesis. The depleted rats were also fed an imbalanced diet (less histidine) and the plasma concentration of all essential amino acids, except histidine, differed little from the basal protein depleted group. Histidine was present at a very low plasma level. When the nonprotein depleted rats were fed the imbalanced diet, histidine fell more rapidly in the plasma than in the protein depleted rats and a depression in food intake occurred more rapidly. The concentration of the other indispensable amino acids rose more rapidly in the plasma than in the protein depleted rats. The fall in plasma concentration of the most limiting amino acids in rats fed an amino acid imbalanced diet resembles that observed by Longnecker and Hause (1959) in dogs fed a meal lacking tryptophan.

Recent reports in the literature have suggested that fasting plasma amino acid levels may be used in determining amino acid requirements or protein efficiency. Chance et al. (1962) determined the fasting plasma amino acid levels in young swine and used these values in predicting the amino acid requirements of this species.

Longnecker and Hause (1959, 1961) used fasting plasma amino acid levels in calculating the plasma amino acid ratio used in determining protein adequacy in dogs. By this method, the amino acid(s) deficient in a protein could be easily calculated and in order of limitation.

Hill and Olsen (1963b) used a modification of the method proposed by Longnecker and Hause (1959). These authors calculated plasma amino acid ratios by relating the plasma amino acid concentration, corrected for the concentration obtained when a nonprotein diet was fed, to the chick's amino acid requirement. It was found that these ratios revealed the first and possibly the second limiting amino acids for soybean or casein diets.

Jarowski (1963) has recently obtained a patent relating to amino acid nutrition and more particularly to balanced foodstuffs and methods for improving dietary amino acid utilization. His claim is that the efficiency of amino acid utilization of a diet can be improved by determining the relative proportions of the essential amino acids in the blood plasma of the contemplated recipient of the diet upon fasting and then supplementing the diet so as to adjust its nutritionally available content of at least the first three limiting amino acids to conform with the blood plasma amino acid proportions.

In general, fasting lowers and increases some of the indispensable amino acids depending upon the species. Several investigators have employed different techniques in using the fasting levels of blood plasma free amino acids in determining either amino acid requirements or improving the efficiency of utilization of amino acids in a ration. Further investigation may lead to more refined techniques in determining the amino acid adequacy of a ration by using plasma amino acid concentrations.

## EXPERIMENTAL

### General Experimental Procedure

The data from the experiments which comprise this dissertation are on file in the Animal Science Department of the Iowa Agricultural and Home Economics Experiment Station under the title of Swine Nutrition Experiments 1136, 1143, 1153, 1157, 1175, 6302, and 6326.

The methods used in conducting these experiments are similar and these common features will be described to avoid unnecessary repetition in the discussion of each individual experiment.

All experimental animals were crossbred pigs of Landrace, Yorkshire, and Black Poland China breeding and were obtained from the swine nutrition farm breeding herd. Within 24 hours after birth, each pig was individually weighed, ear marked, needle teeth clipped, and received two milliliters of iron-dextran complex (50 mg. Fe/ml) intraperitoneally. The males were castrated at approximately five days of age.

Experimental pigs were weaned between 10 and 14 days of age. They were group fed for a pre-experimental period of two days under heat lamps, during which time they were introduced to eating dry rations. All pigs were randomly allotted from littermate outcome groups to blocks of individual feeding pens to which experimental treatments had previously been randomly assigned.

All experiments were conducted in the same baby pig nursery which is temperature controlled, air-conditioned, and equipped with germicidal (ultraviolet) lamps at different locations in the nursery. The concrete

floor under the individual metal pens was radiant heated by thermostatically controlled circulating water. The room temperature was maintained at  $72 \pm 2$  degrees Fahrenheit. The floor temperature was maintained at  $85 \pm 2$  degrees the first week, dropped five degrees per week and finally maintained at  $70 \pm 2$  degrees Fahrenheit for the duration of the experiment. Each individual pen was furnished with a self feeder, a continuous flow waterer, and the pens were cleaned daily.

All pigs were individually weighed at the initiation of each experiment and at weekly intervals thereafter. Feed consumption was also determined at weekly intervals. Blood samples were obtained in each experiment after two weeks from the date of initiation of the experiment, except for Experiments 1136 and 6302. Blood samples from the latter experiments were obtained at one week from the initiation date of the experiment.

The data collected from each experiment were statistically analyzed and missing data were calculated by methods described by Snedecor (1956). In all experiments the pen was considered the experimental unit. Any reference to statistical significance pertains to the probability level of 5 percent or less.

#### Analytical Methods

All blood specimens were obtained from the anterior vena cava, except where otherwise indicated. A syringe was used for obtaining blood and the dead space contained a 10 percent solution of sodium citrate (Hewitt, 1932) as an anticoagulant. The blood was placed into test tubes that contained



0.1 ml. of the citrate solution. The blood samples were centrifuged immediately at 2,500 r.p.m. for 10 minutes and the plasma removed. When plasma samples were pooled, an equal proportion of plasma was taken from each pig.

A 10 ml. aliquot of the plasma was promptly deproteinized with picric acid and the deproteinized plasma was prepared for column chromatography as described by Stein and Moore (1954). When the protein-free plasma could not be prepared on the same day collected, it was frozen and stored in polyethylene bottles at -17 degrees Centigrade until processed.

The deproteinized plasma was concentrated down to approximately 3 ml. under vacuum with a rotary evaporator at 55 degrees Centigrade. The concentrated solution was then diluted to 10 ml. with 0.02 N HCl, mixed homogeneously and divided into three aliquots of four, four and two ml., respectively. One of the four ml. aliquots was subsequently neutralized to pH 7.0 (Hydrion paper) by dropwise addition of N NaOH and allowed to stand at room temperature for four hours, conditions which are known to convert cysteine to cystine (Stein and Moore, 1954). The sample was then adjusted to pH 2.0 by dropwise addition of N HCl and stored at -17 degrees Centigrade until analyzed.

The concentration of amino acids in the deproteinized plasma was analyzed by ion exchange chromatography as described by Moore and Stein (1954), but employing the Technicon Autoanalyzer and using the 12 chambered varigrad instead of the 9 chambered varigrad (Peterson and Sober, 1959) for gradient elution of the amino acids according to modifications recommended

by Piez and Morris (1960). Integration constants were determined for each amino acid from a standard micromole amino acid solution and used to calculate the amino acid concentrations in blood samples and intestinal contents.

Of the two unidentified peaks in human plasma mentioned by Stein and Moore (1954) and Frame (1958) the one between urea and aspartic acid was seen frequently while the post-leucine peak was not observed in any of the blood samples obtained from the vana cava. However, an unidentified peak was found to emerge before isoleucine in all blood and intestinal samples. An unidentified peak was observed to emerge after cystine in blood and intestinal samples. A standard solution of  $\alpha$ -amino-butyric acid was added to a sample and found to emerge identically with this unknown ninhydrin positive substance. This amino acid has been revealed in plasma by paper chromatography (Dent, 1947, 1948). Two small unidentified positive substances emerged after histidine in blood and intestinal samples. These substances were probably 1 or 3-methyl-histidine or perhaps carnosine or anserine, however, this postulation was not confirmed by analytical methods. Frequent overlappings of ornithine and ammonia resulted; however, in general, separation of all the amino acids was excellent. In the intestinal samples, a peak was frequently observed after serine and five different peaks were observed between phenylalanine and ammonia. These unknown peaks emerged quite close to ammonia indicating that these ninhydrin positive substances

were basic in nature. A peak emerged identical with that of citrulline in the plasma, however, it is doubtful that this unknown substance is citrulline (Ganapathy and Nasset, 1962).

Tryptophan was not observed in the eluates and in view of the rate of destruction reported for this amino acid (Stein and Moore, 1954) by this analytical procedure plus the low plasma concentration observed in most species (0.60 to 0.88 mg. per 100 ml.), it is not surprising that the small amount remaining was not detectable. The sensitivity for this analytical procedure is 0.01 micromoles with a precision of approximately 10 percent (Piez and Morris, 1960). Two of the 4 ml. aliquots of the same sample were prepared as previously described to check the quantitative error on duplicate samples. Quantitative recovery did not vary appreciably between samples and were within the sensitivity range as described by Piez and Morris (1960).

Tryptophan was determined by the method of Hess and Udenfriend (1959) with modifications as follows: The proteins were precipitated with 8 percent trichloroacetic acid and the benzene extraction was not performed. Fluorescence was measured by a G. K. Turner model 110 photofluorometer at 440 millimicrons. Tryptophan was analyzed in plasma samples of Experiments 1159 and 6302 only.

#### Experiment 1143 - Amino acid supplementation to young pig rations

Objective This experiment was designed to study the effects of methionine, threonine, valine, and lysine supplementation of soybean protein on growth rate and feed efficiency of young growing pigs.

Previous data reported by Puchal (1962) have shown that threonine, lysine, valine, and arginine exhibited a low blood plasma concentration when methionine was supplemented to soybean protein as compared to the unsupplemented soybean protein. Threonine and lysine have been estimated to be the second and third limiting amino acids of soybean protein for young pigs based upon the amino acid composition of soybean protein (Hays, 1957) and the pig's requirement for these amino acids. Puchal (1962) demonstrated that methionine, threonine, and valine supplementation to diets containing soybean protein improved the growth performance of young pigs as compared to pigs fed the basal soybean protein ration.

To provide a more accurate evaluation of Puchal's observations, it was decided to further investigate the effects of supplementing these amino acids to soybean protein for young pigs.

Experimental procedure      Thirty-five pigs averaging 17.0 days of age and 11.0 pounds of body weight were used in this experiment. The composition of the basal ration, shown in Table 1, contained soybean meal as the only source of protein and was formulated to provide 18.5 percent protein. Lactose and potassium chloride were added to the ration to equalize the amounts provided by a comparable dried skim milk ration, to reduce to a minimum any carbohydrate or mineral effect on amino acid absorption. The levels of DL-methionine, DL-threonine, L-lysine·HCl, and DL-valine were respectively, 0.19, 0.32, 0.34, and 0.38 percent. The five treatments were made isonitrogenous by the addition of ammonium citrate (dibasic).

Results and discussion      Total gains and feed required per pound of gain are summarized in Table 5 and graphically presented in Figure 1.

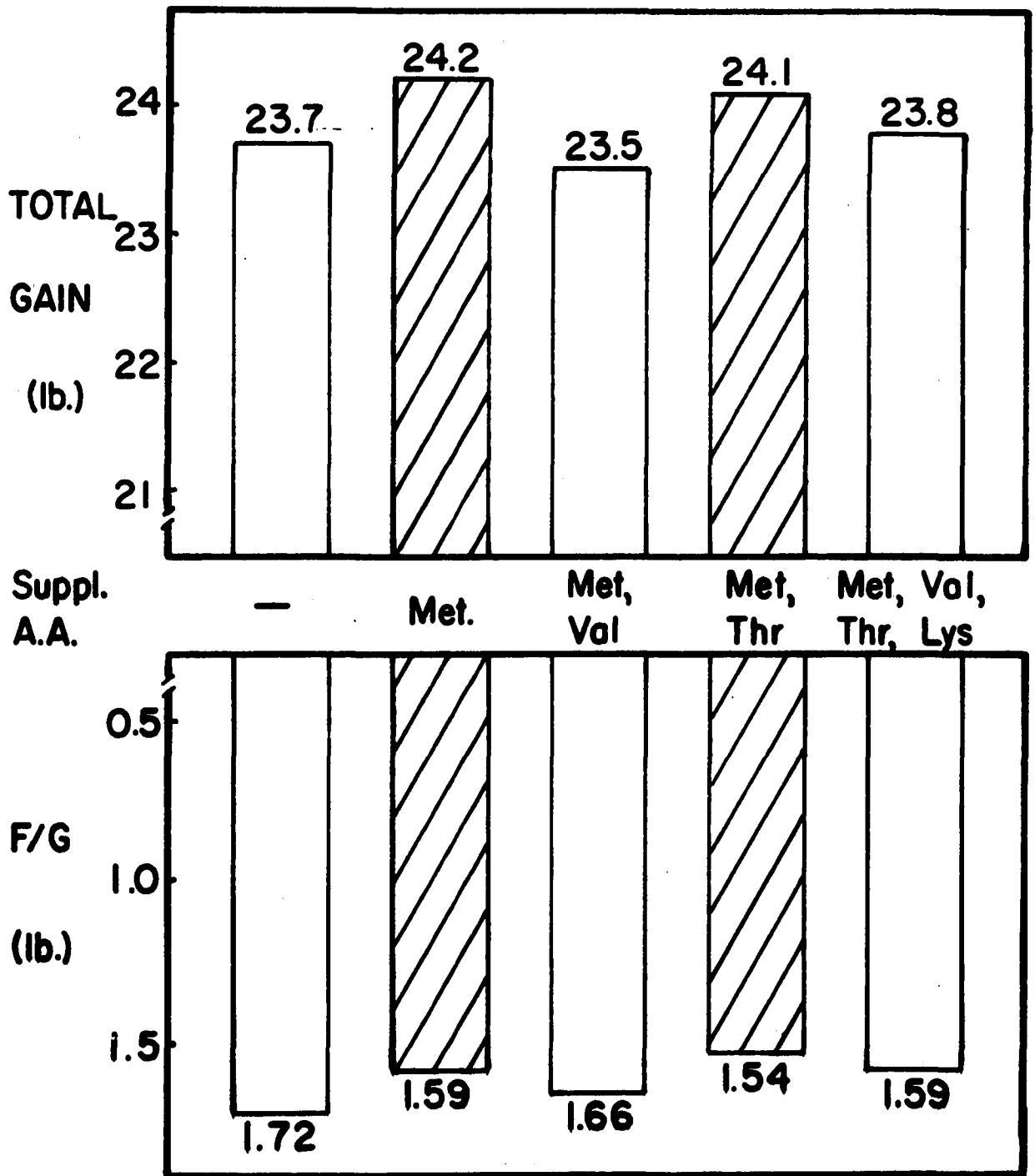
A surprisingly poor performance was observed for the pigs fed the rations supplemented with amino acids as compared to the pigs fed the basal ration. Previous published data have shown that methionine addition to soybean protein has repeatedly improved body gain and feed efficiency (Acker et al., 1959; Hays et al., 1959). When methionine and valine were added to the basal ration, pig gains were depressed slightly as compared to pigs fed the basal ration. The ration supplemented with methionine and threonine improved pig gains only slightly as compared to the basal ration. Essentially no difference was observed between the gains of pigs fed the basal ration and those fed the ration containing added methionine, threonine, valine, and lysine.

The rations supplemented with methionine or methionine and threonine were more efficiently utilized as compared to the basal ration. Essentially no difference in feed efficiency was observed when pigs were fed the ration supplemented with methionine and valine as compared to those fed the basal ration. Plasma amino acid concentrations were not determined in this experiment.

#### Experiment 1153 - Supplementation of young pig rations with amino acids

Objective      This experiment was designed to further investigate the effects of methionine, threonine, valine, and lysine supplementation to soybean protein on growth rate, feed efficiency, and blood plasma free amino acid concentrations of young pigs. The data in Experiment 1143 did not

Figure 1. Experiment 1143 - Effects of amino acid supplementation on growth rate and feed efficiency of young pigs



demonstrate any marked improvement in growth performance of young pigs fed soybean protein supplemented with methionine. Previous data reported by Puchal (1962) showed that threonine, lysine, arginine, and valine exhibited a low blood plasma concentration when the methionine supplemented soybean protein was fed to pigs as compared to those fed the unsupplemented protein. Reports by Hill et al. (1961) have indicated that correcting the low blood plasma level of a deficient amino acid in a given protein results in a marked decrease in the concentration of the next limiting amino acid(s) which supposedly are handicapping protein synthesis. The above considerations provided the basis for this experiment.

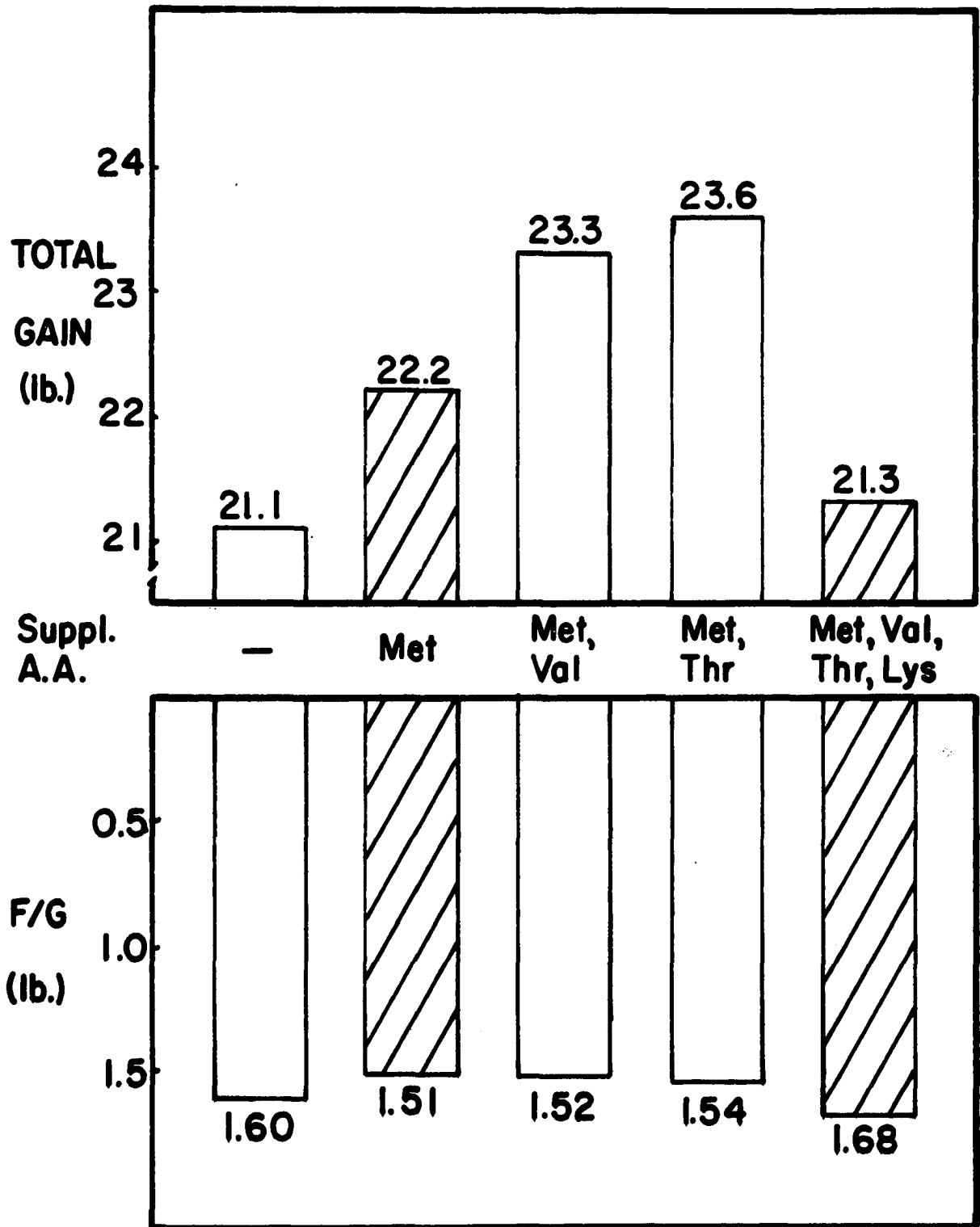
Experimental procedure      Thirty-five individually fed pigs averaging 18.5 days of age and 9.8 pounds of body weight were used in this experiment. The basal ration and levels of supplemental amino acids were identical to that of Experiment 1143. Blood samples were pooled across replications from pigs fed each ration treatment.

Results and discussion      A summary of total gains and feed required per pound of gain is presented in Table 6 and graphically illustrated in Figure 2. The experimental plan and analysis of variance are shown in Table 7.

Rations supplemented with methionine, methionine plus valine, or methionine plus threonine improved the growth rate of young pigs as compared to those fed the basal ration. Essentially no difference in growth rate was observed when pigs were fed the basal ration as compared to those fed the basal ration with added methionine, valine, threonine,



**Figure 2. Experiment 1153 - Effects of amino acid supplementation on growth rate and feed efficiency of young pigs**



and lysine. The above differences were not statistically significant. It would appear that lysine addition to soybean protein may be responsible for the growth depression as compared to those pigs fed the other amino acid supplemented diets, as a similar response was noted in Experiment 1143 and by Puchal (1962).

There were no statistically significant differences observed in feed conversion by pigs fed the diets used in this experiment. The basal soybean protein ration and the basal ration supplemented with the combination of amino acids were less efficiently utilized as compared to the other three amino acid supplemented rations.

The blood plasma free amino acid concentrations are summarized in Table 8. Supplementation of each amino acid to the diet resulted in a corresponding increase in concentration of that amino acid in the plasma. The ration supplemented with methionine resulted in a threefold increase in the plasma methionine concentration as compared to the methionine plasma level of pigs fed the basal ration. Except for aspartic acid, threonine, and glutamic acid, the other amino acids decreased in plasma concentration when pigs were fed the ration with added methionine as compared to those fed the basal ration. The reduction was most pronounced for lysine (from 4.40 to 2.04 mg./100 ml.) indicating that lysine was being more efficiently utilized by the tissues or that the absorption of lysine from the small intestine was altered in some manner. These data are not in complete agreement with data reported by Puchal (1962) who found that in some instances the amino acids were higher in plasma concentration, except for

glycine, lysine, histidine, and arginine which decreased when methionine was added to the basal ration. Methionine hydroxy analogue (Puchal, 1962) was used instead of methionine which may account for these discrepancies. Histidine plasma concentration did not show the marked decrease as reported by Puchal (1962), however, a 27 percent decrease in plasma concentration was observed confirming Puchal's (1962) data that under certain conditions histidine may be deficient in soybean protein for young pigs. The ration supplemented with methionine increased the plasma threonine concentration indicating that threonine would not be a limiting amino acid hindering growth rate which is in partial agreement with Puchal (1962).

The rations supplemented with methionine and valine resulted in an increase in plasma concentration of all the essential amino acids as compared to the plasma amino acid level of pigs fed the ration supplemented with methionine. Most of the nonessential amino acids decreased in plasma concentration, however, serine and proline increased in concentration.

Methionine and threonine supplementation to the basal ration resulted in an increased concentration in the plasma of almost all the amino acids, except citrulline, as compared to the plasma amino acid levels of pigs fed the ration supplemented with methionine. In this experiment, the greatest total gain was obtained from the diet supplemented with methionine and threonine with a corresponding high total level of plasma circulating amino acids also resulting as compared to the total plasma amino acid levels of pigs fed the other amino acid supplemented rations. The plasma

concentration of methionine increased almost fourfold as compared to the plasma methionine level of pigs fed the basal ration.

The ration supplemented with methionine, valine, threonine, and lysine resulted in an increased plasma concentration of these amino acids as compared to the level of these amino acids in the plasma of pigs fed the basal ration. Most of the amino acids were higher in plasma concentration, except methionine and citrulline, as compared to the plasma amino acid level of pigs fed the ration supplemented with methionine. Supplementation with lysine increased the plasma lysine concentration comparable to that observed when pigs were fed the basal ration. The total plasma amino acid concentration for pigs fed this ration is greater than that observed for the other three amino acid supplemented rations, however, no further improvement in growth rate of young pigs was observed when these four amino acids were supplemented to soybean protein. When any three of these amino acids were supplemented to soybean protein, excluding lysine, all the basic amino acids decreased in plasma concentration as compared to the plasma amino acid levels observed when pigs were fed the basal ration.

It would appear from the growth data of young pigs in this experiment that valine and threonine are partially unavailable from soybean protein, hence hindering protein synthesis and preventing maximum growth performance of young pigs fed diets supplemented with methionine. Puchal et al. (1962) reported that the amino acid composition of the protein reflects the amino acid concentration in the plasma. From the plasma data, lysine would be

suspected to be the next limiting amino acid after the methionine deficiency has been corrected, however, when lysine has been supplemented with methionine to soybean protein no significant improvement in growth rate of young pigs has been observed (Puchal, 1962; Berry et al., 1962), but an increase in plasma lysine has been observed. In this experiment some improvement in growth rate of young pigs was observed when methionine, valine, and threonine were added to the diet, but further addition of lysine actually depressed growth performance.

Experiment 1159 - Supplementation of soybean protein diets for young pigs with methionine, valine, threonine, isoleucine, and lysine

Objective This experiment was conducted to further study the effects of methionine, threonine, valine, lysine, and isoleucine supplementation to soybean protein on growth rate, feed efficiency, and blood plasma free amino acid concentrations of young pigs. Information was needed on the growth performance of pigs fed a ration supplemented with the combination of methionine, threonine, and valine. The data from Experiment 1153 indicated a growth response of young pigs when threonine or valine in combination with methionine was added to soybean protein. This growth response, plus the observed low plasma levels of valine, lysine, and isoleucine, when methionine was supplemented to soybean protein, provided the basis for this experiment.

Experimental procedure Thirty-six pigs averaging 10.0 pounds and 16.5 days of age were randomly allotted to a randomized block experimental design and individually fed to test the six ration treatments. The composition

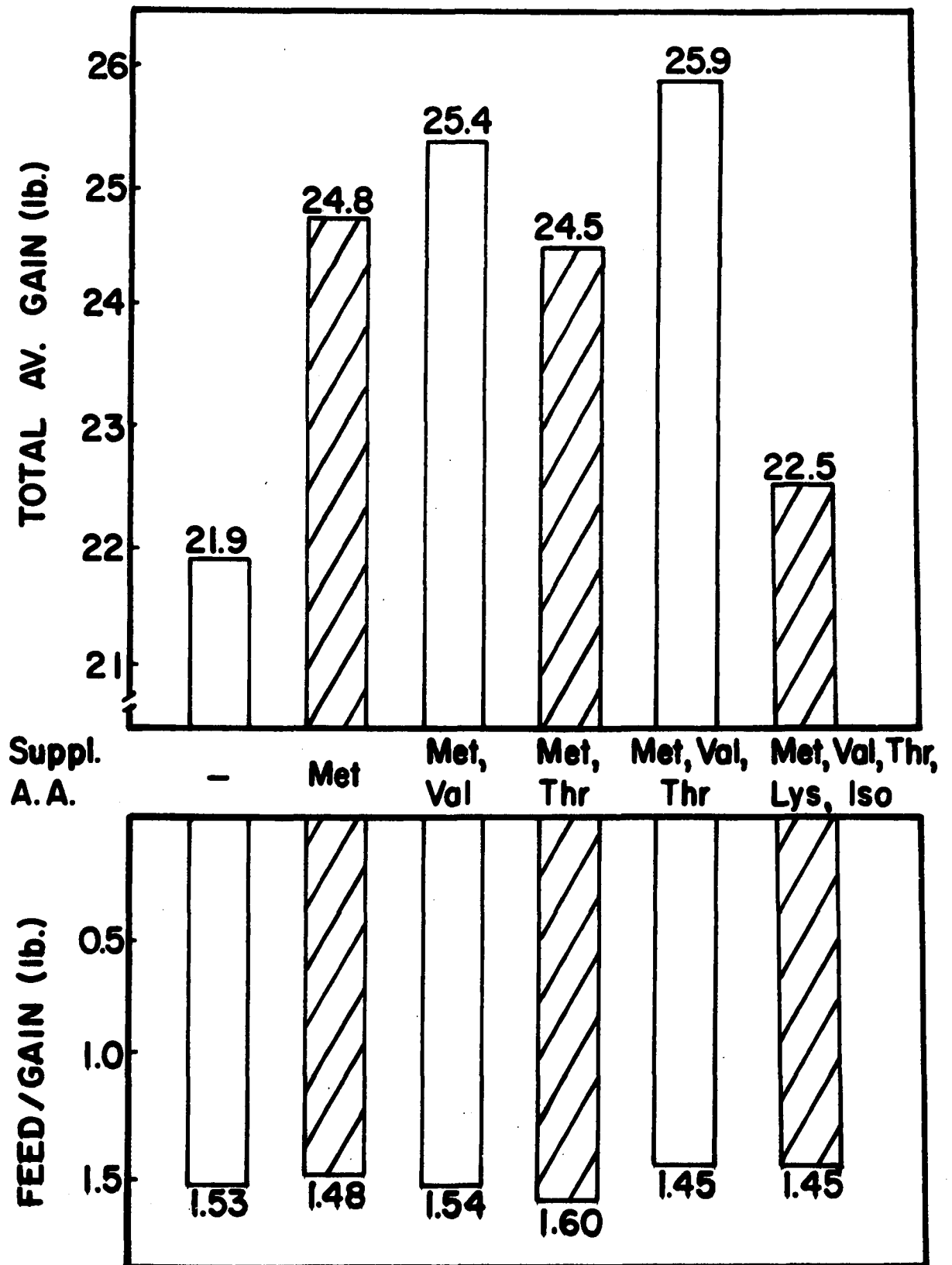
of the basal ration is shown in Table 1. Soybean meal was the only protein source and lactose was included to minimize any carbohydrate effect on amino acid absorption. Potassium chloride was added to all diets in accordance with the level found in dried skim milk. The amino acid levels used in this experiment were the same as reported in Experiment 1143 except that lysine was reduced from 0.34 to 0.15 percent. It was observed in the two previous experiments that lysine supplementation seemed to depress the growth rate of young pigs, and perhaps this could be attributed to the relatively high supplemental lysine level. The original lysine level (0.34 percent) was selected as the amount needed to increase the plasma lysine level to that observed in a diet containing 20 percent dried skim milk protein. In Experiment 1153, the concentration of isoleucine was observed to decrease in the plasma of pigs fed the ration supplemented with methionine as compared to pigs fed the basal ration. Isoleucine was added to soybean protein at the level of 0.23 percent. Blood samples were pooled across replications of pigs fed each treatment.

Results and discussion      The summary of total gains and feed efficiency is presented in Table 9 and graphically illustrated in Figure 3. The experimental plan and analysis of variance are shown in Table 10.

A significant improvement in growth rate of young pigs resulted with the supplementation of amino acids to soybean protein, except when lysine was added, as compared to pigs fed the basal ration. The greatest

**Figure 3. Experiment 1159 - Effects of amino acid supplementation on gains and feed efficiency of young pigs**





improvement in growth rate was observed as a result of supplementing methionine, threonine, and valine to soybean protein. Feed efficiency also improved when these three amino acids were added to the diet. The addition of methionine to the basal ration improved growth rate and reduced feed required per pound of gain.

Soybean protein supplemented with methionine and valine improved the growth performance of young pigs as compared to pigs fed the basal ration or the basal ration supplemented with methionine. The ration supplemented with methionine and valine resulted in no significant difference in feed utilization as compared to pigs fed the basal ration. The further addition of threonine to the methionine-supplemented ration improved body weight gain although feed efficiency was not improved.

On the other hand, the addition of valine, threonine, lysine, and leucine to the methionine-supplemented diet failed to improve growth performance, however, feed efficiency was improved. Here again the addition of lysine appeared to depress growth as in the previous trials.

The blood plasma free amino acid concentrations are summarized in Table 11. The addition of methionine to soybean protein resulted in a fourfold increase in the blood plasma methionine level when compared to the plasma level of pigs fed the basal ration. The other amino acids decreased in plasma concentration, except glutamic acid and glycine. The plasma concentration of threonine decreased by 32 percent which is opposite to data reported in Experiment 1153 where the plasma concentration of threonine increased when methionine was added to the ration.

Lysine was markedly reduced in the plasma (5.10 to 2.49 mg./100 ml.) and histidine and arginine also decreased confirming similar data reported by Puchal (1962) and data from Experiment 1153.

The supplementation of soybean protein with methionine and valine resulted in an increased concentration of these two amino acids in the plasma as compared to the plasma level of pigs fed the basal ration. Threonine, methionine, phenylalanine, lysine, histidine, and arginine were lower in concentration in the plasma as compared to that of pigs fed the ration supplemented with methionine. Lysine showed a further decrease in plasma concentration.

The addition of methionine and threonine to soybean protein resulted in an increased plasma concentration of these two amino acids as compared to the plasma level of pigs fed the basal ration. Except for tyrosine, the other amino acids increased in plasma concentration as compared to the plasma level of pigs fed the methionine-supplemented ration.

Soybean protein supplemented with methionine, threonine, and valine resulted in an increased plasma concentration of all the amino acids, except alanine, as compared to the plasma level of pigs fed the ration supplemented with methionine alone.

The addition of methionine, valine, threonine, lysine, and isoleucine to the basal ration resulted in an increased plasma concentration of these amino acids as compared to the plasma amino acid levels when the basal ration was fed. Lysine was unique in that the 0.15 percent added level to the diet increased the plasma lysine concentration as compared to the

plasma lysine levels observed with the other amino acid supplemented rations, but did not increase the plasma lysine concentration above that observed when the basal ration was fed. The addition of methionine, valine, threonine, lysine, and isoleucine to the basal ration resulted in an increased plasma concentration of almost all the amino acids, except glycine, as compared to the plasma level of pigs fed the methionine-supplemented ration.

In this experiment, each of the basic amino acids was observed to decrease in plasma concentration when methionine, valine, threonine, and isoleucine were added to the basal ration as compared to the plasma level of pigs fed the basal ration. This confirms the data observed in Experiment 1153.

It would appear that the addition of any one amino acid to a protein source has definite effects upon the concentrations of other amino acids in the blood plasma, and the incorporation of the amino acids into proteins by the tissues. The study of the interrelationships of one amino acid (including levels) affecting another or several amino acids may lead to the appropriate amino acid combination to add to a deficient protein and hence improve the nutritive value of this protein.

#### Experiment 1175 - Amino acid supplementation to soybean protein for young pigs

Objective      The study of blood plasma free amino acids in Experiments 1153 and 1159 indicated that the plasma proline level decreased whenever the amino acids used in these experiments were added to soybean protein. It has

been reported by Fischer and Abderhalden (1902) that peptides involving proline are resistant to enzyme cleavage and it is suggested that a similar phenomena may partially be responsible for the inefficient utilization of soybean protein at an early age. Previous experiments have indicated that several essential amino acids decreased in the plasma when methionine was added to the ration, and so a combination of all the essential amino acids was included as a treatment. This experiment was designed to study the effects of proline supplementation plus a combination of essential amino acids (methionine, threonine, valine, lysine, leucine, isoleucine, tryptophan, phenylalanine, and histidine) upon growth rate, feed efficiency, and blood plasma free amino acid levels of young pigs. It appeared from the data in Experiments 1153 and 1159 that the plasma level of methionine was low and perhaps methionine was still the limiting amino acid hindering growth rate, hence, the supplemental level of methionine was increased from 0.19 to 0.35 percent. Similarly, threonine and valine markedly increased in the plasma when pigs were fed a ration with added threonine and valine to soybean protein. Perhaps the level of these two amino acids was too high and was preventing maximum growth performance from occurring. Hence, the supplemental levels of these two amino acids were reduced.

Experimental procedure      Thirty-six pigs averaging 10.9 pounds body weight and 16.6 days of age were used in this experiment. The composition of the basal ration is shown in Table 1. Lactose and potassium chloride were added to equalize the amounts provided by a comparable dried

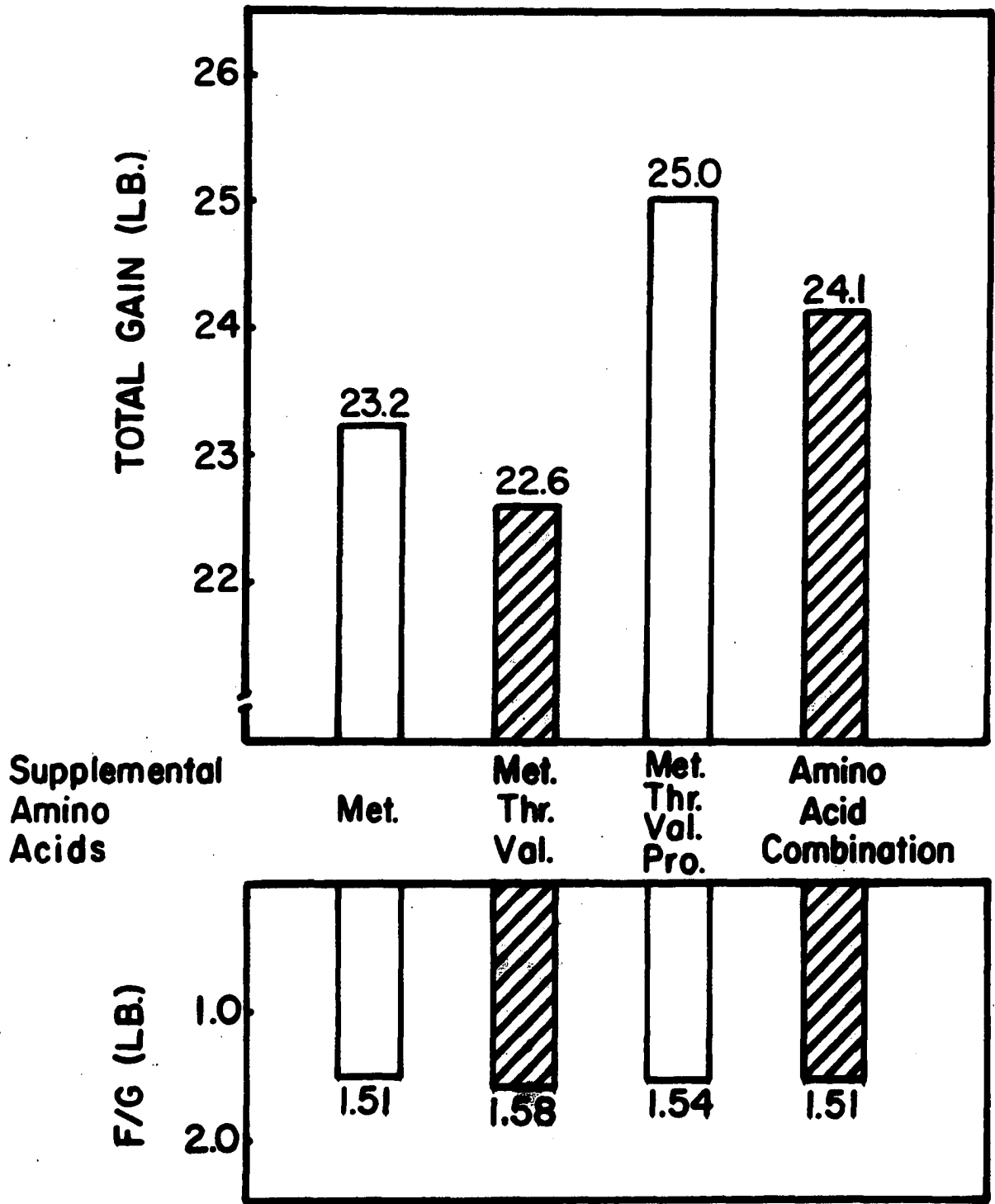
skim milk ration for reasons previously stated. All rations were made isonitrogenous with ammonium citrate (dibasic). The percent of amino acids added to the experimental rations was DL-methionine, 0.35; DL-threonine, 0.24; DL-valine, 0.22; L-lysine·HCl, 0.10; DL-tryptophan, 0.06; L-histidine·HCl, 0.10; DL-phenylalanine, 0.10; L-isoleucine with D-alloisoleucine, 0.23; DL-leucine, 0.32; and L-proline, 0.10. Blood samples were pooled across replications of pigs fed each treatment.

Results and discussion. Total gains and feed efficiency are summarized in Table 12 and graphically presented in Figure 4. The experimental plan and analysis of variance are shown in Table 13.

An improvement in growth rate of young pigs was obtained with the addition of methionine to soybean protein as compared to pigs fed the ration supplemented with methionine, threonine, and valine. Previous data have shown some growth response of young pigs when the ration was supplemented with valine or threonine in combination with methionine, whereas in this experiment superior growth performance was observed when methionine alone was added to soybean protein. Levels of threonine and valine were reduced in this experiment and perhaps the higher levels of these two amino acids used in previous experiments are more representative of the requirement of the young pig at this early age.

The ration with added methionine, threonine, valine, and proline resulted in superior growth performance as compared to pigs fed the other three amino acid supplemented rations. Further supplementation of the

**Figure 4. Experiment 1175 - Effects of supplementation of soybean protein diets with an amino acid combination on gains and feed efficiency of young pigs**





essential amino acids reduced the growth improvement that had been obtained with methionine, threonine, valine, and proline supplementation.

There were no significant differences in feed efficiency which could be attributed to any of the ration treatments.

The blood plasma free amino acid concentrations are summarized in Table 14. The addition of methionine, valine, and threonine to soybean protein resulted in an increased plasma concentration of all the amino acids as compared to the plasma aminogram of pigs fed the methionine-supplemented ration. Threonine and valine increased more than twofold in the plasma whereas little difference was observed between methionine plasma levels of pigs fed these two rations.

The addition of proline, methionine, valine, and threonine to soybean protein resulted in an increased plasma level of all the amino acids compared to the plasma aminogram obtained when pigs were fed the methionine-supplemented ration. The highest plasma level of proline and greater body weight gain were observed when pigs were fed this ration. It is possible that proline may be partially essential for maximum growth of young pigs. Little difference was observed between the plasma amino acid levels when pigs were fed this ration as compared to the plasma amino acid levels of pigs fed the ration supplemented with the amino acid combination.

Again, it appears that the addition of one or several amino acids to soybean protein has definite effects upon the levels of amino acids observed in the plasma and corresponding influences upon growth performance. Since most of the amino acids used in these studies were of the DL-

configuration, it is conceivable that the increase in concentration of amino acids in the plasma when one or more amino acids was added to soybean protein is mainly the D-configuration, the L-form being more efficiently utilized in protein synthesis. It is well known that the D-form of an amino acid per se is not used in the synthesis of protein within the animal body, however, the D-forms are found in nature. Furthermore, the DL-form(s) would be expected to influence the apparent requirement for any given amino acid (Fell et al., 1959).

Further studies of the plasma amino acid changes occurring when amino acids are added to a protein may reveal the correct amino acid proportions to supplement soybean protein with the maximum growth performance of young pigs.

#### Experiment 1136 - Protein digestion studies with baby pigs

Objective This experiment was conducted to investigate the degree of proteolysis occurring from the ingested protein in the intestinal lumen and amino acid absorption into the portal blood system. Portal blood plasma amino acid levels have been repeatedly reported as higher in concentration than in systemic plasma as a result of only limited absorption of amino acids into the mucosa before the blood reaches the liver where the amino acids are subject to selection (Seth and Luck, 1925; Dent and Schilling, 1949; Christensen, 1949). The ratios of one amino acid to another have been reported by some researchers to be no different in the portal versus systemic blood (Dent and Schilling, 1949; Denton and Elvehjem, 1954b; Puchal, 1962), and to vary both quantitatively and qualitatively by others (Denton et al., 1953).

Earlier work (Nasset et al., 1955) demonstrated that the qualitative free amino acid composition of intestinal contents is not greatly altered by the type of meal fed. The mixture present during digestion appears to contain amino acids derived from food proteins, autodigestion of hydrolytic enzymes, and other endogenous proteins (Nasset, 1957). Ingesta are mixed with relatively large quantities of endogenous protein in the small intestine, and therefore ingested protein does not greatly influence the molar ratios of free amino acids in the intestinal contents (Nasset and Ju, 1961).

This experiment was designed to study the release of amino acids from the ingested protein in the different segments of the intestinal tract and to compare the aminograms between portal and anterior vena cava blood plasma.

Experimental procedure Fourteen 10.0-day old pigs averaging 9.9 pounds body weight were used in this experiment. Two protein sources, dried skim milk and soybean meal, were individually fed to the pigs for one week prior to the collection of the samples. The composition of the two rations is shown in Table 15.

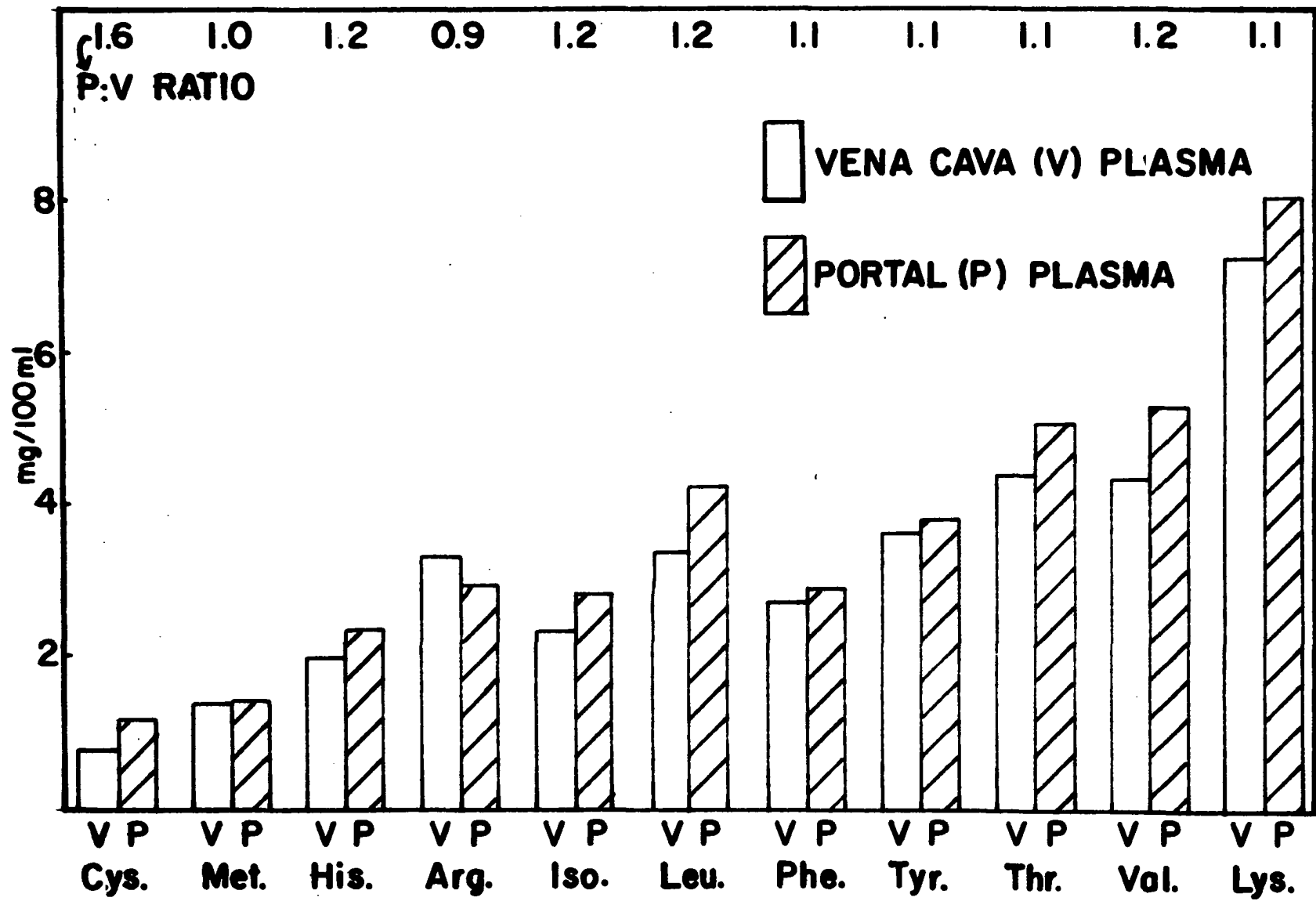
All pigs were fasted for 18 hours and then given the protein test meal. Two animals from each diet were sacrificed at one, two and three hours after the ingestion of the protein test meal. Two pigs, one fed the dried skim milk protein ration and the other fed the soybean protein ration, were fed a nonprotein test meal and sacrificed at three hours after ingesting this ration. The composition of the nonprotein meal diet is shown in Table 15.

Blood samples from the anterior vena cava were obtained after inducing general anesthesia with sodium pentobarbital. Immediately after obtaining the caval blood, the abdominal cavity was opened, the vena porta exposed and the corresponding portal blood sample obtained. Ligatures were then placed at the pylorus, first 60 centimeters of the small intestine, representing the duodenum (St. Clair, 1958), and at the mid section of the remaining small intestine and ileocecal region. The intestinal segments were removed and the contents collected quantitatively. The contents were then diluted to a known volume with distilled water, mixed thoroughly, and centrifuged at 2,500 r.p.m. for five minutes to remove food particles. Ten ml. of the supernatant were immediately deproteinized and prepared for column chromatography as previously described.

Results and discussion      Total gains and the amount of the test protein are shown in Table 16. The dried skim milk protein improved the gains of young pigs to a greater extent than those fed soybean protein. After fasting, the pigs ingested a greater quantity of the dried skim milk test meal as compared to those fed the soybean test meal.

No qualitative differences were observed in the plasma free amino acids between portal and vena cava samples. Portal plasma amino acids were almost invariably higher quantitatively as compared to vena cava plasma (Figure 5). Most of the portal/vena cava plasma amino acid ratios varied from 0.9 to 1.2, however the cystine ratio was 1.6, considerably higher than expected compared to the other essential amino acid ratios. Arginine was the only amino acid which had a ratio less than 1.0.

Figure 5. Experiment 1136 - Plasma amino acid concentrations of portal and vena cava blood (combined data of pigs fed dried skim milk and soybean protein)



These data confirm previous work reported by Dent and Schilling (1949) and Denton and Elvehjem (1953b) who observed that amino acid ratios did not vary appreciably in portal versus systemic plasma.

The portal and vena cava plasma amino acids were quantitatively different when dried skim milk protein or soybean protein was fed (Figure 6) with a preponderance of plasma amino acids occurring when dried skim milk protein was fed. The plasma amino acids in the vena cava blood reflected approximately the same pattern observed in portal blood, when either milk protein or soybean protein was fed (Figure 7), arginine being discrepant in this respect for the latter protein.

After feeding the dried skim milk protein, the total essential plasma amino acids increased sharply from one to two hours (46 percent) and then leveled off or declined at three hours (Figure 8). The total plasma essential amino acids decreased from one to two hours (32 percent) after feeding and then increased in concentration at three hours when soybean protein was fed (Figure 8). In Figures 9 and 10, the concentration of the designated amino acids in both the portal and vena cava plasma at each hour interval after feeding either dried skim milk or soybean protein is indicated by the points on the curves, respectively. The concentration of each essential amino acid increased quantitatively in the portal plasma from one to two hours after feeding and then decreased or leveled off at three hours when dried skim milk protein was fed. Some of the amino acids in the vena cava plasma corresponded to the amino acid pattern observed in the portal plasma whereas the other amino acids varied

Figure 6. Experiment 1136 - Plasma amino acid concentrations from pigs fed soybean or dried skim milk protein (combined data of 1, 2, and 3 hours after feeding)



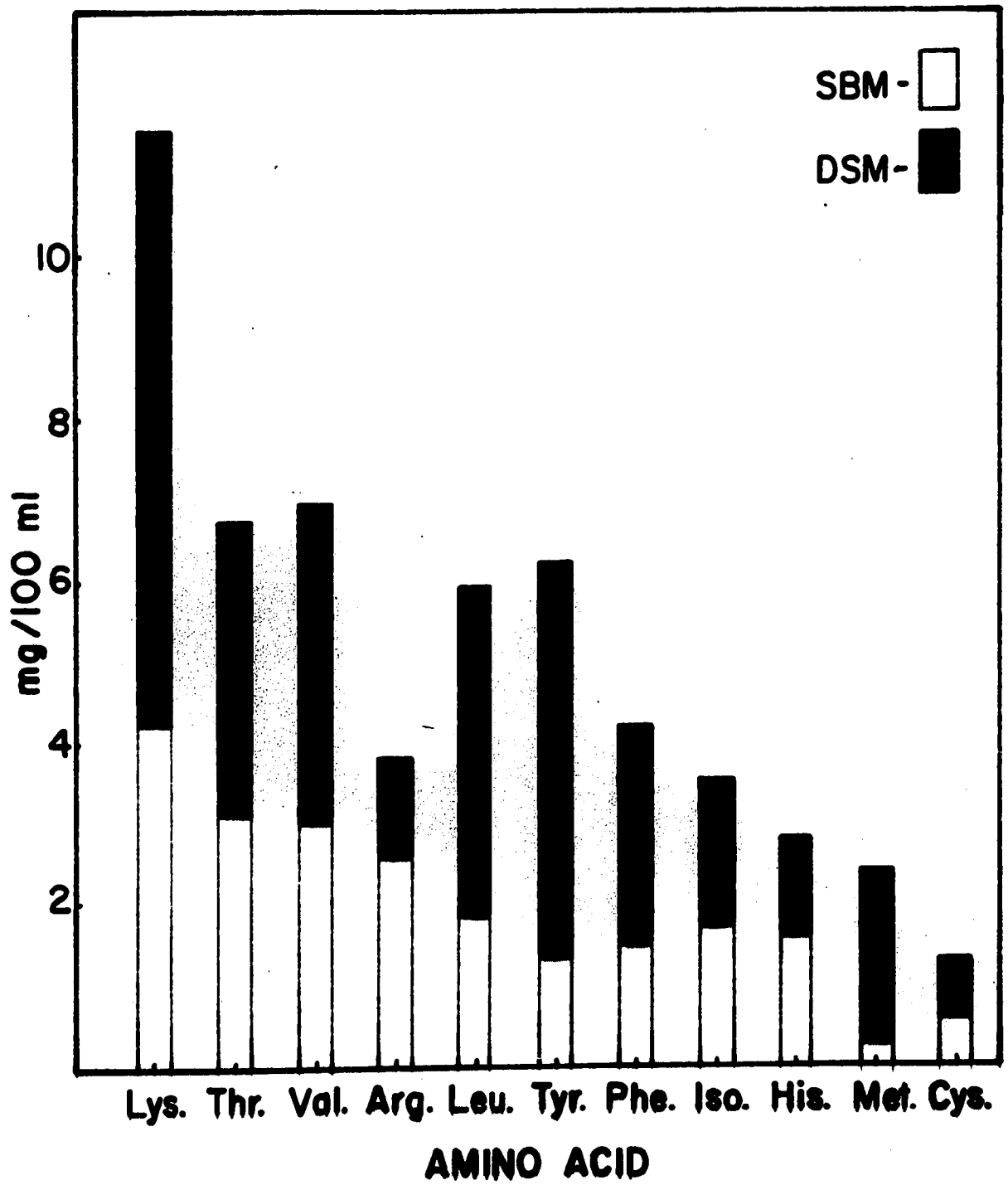


Figure 7. Experiment 1136 - Portal and vena cava plasma amino acid comparisons from pigs fed soybean protein, dried skim milk protein, or a nonprotein meal

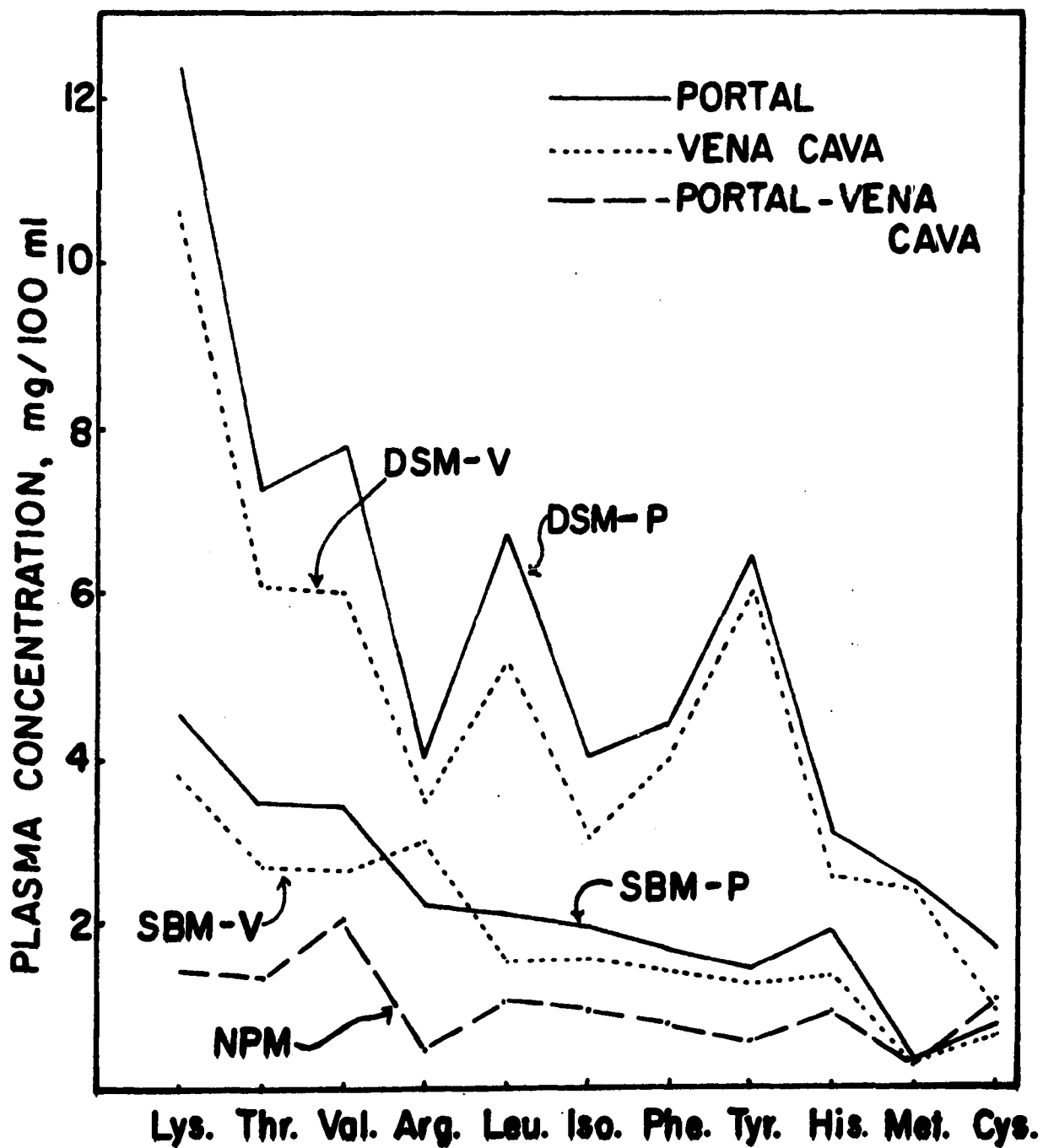
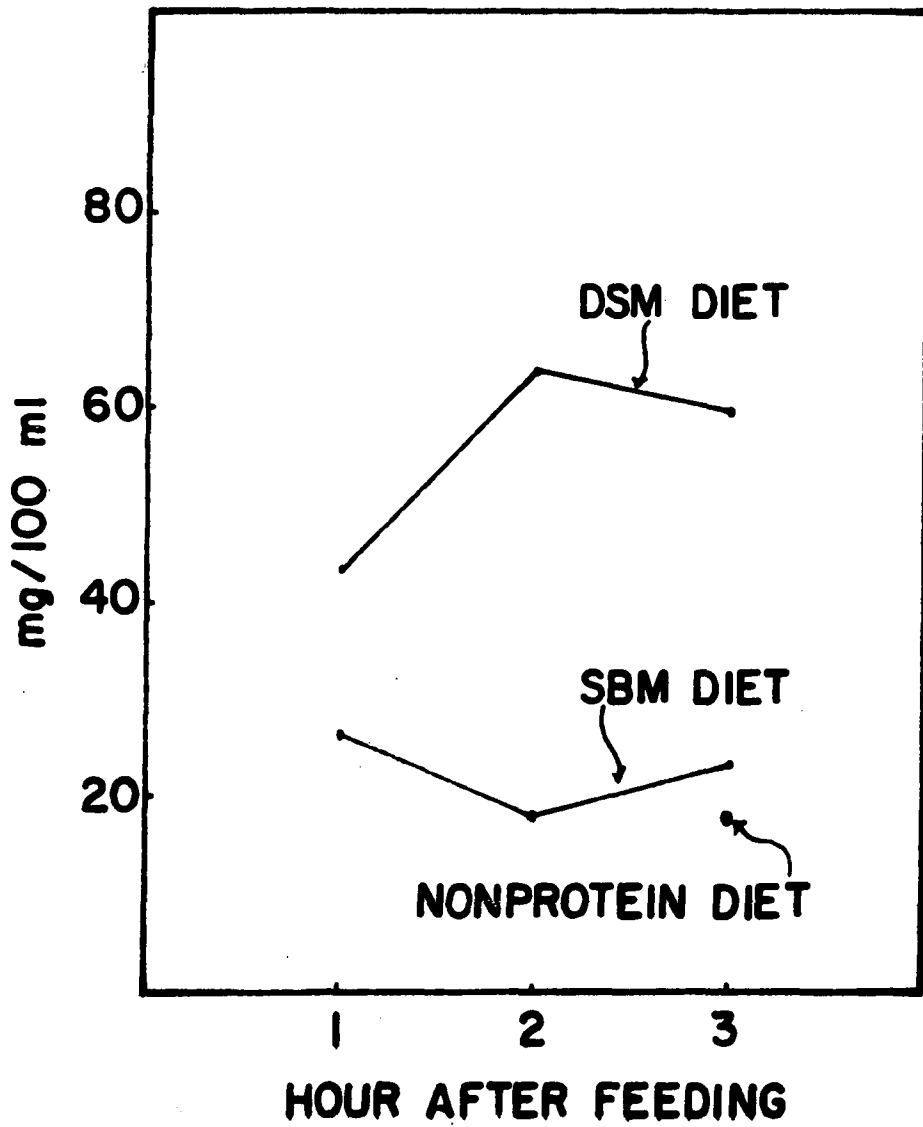


Figure 8. Experiment 1136 - The total concentration of free plasma essential amino acids, 1, 2, and 3 hours after ingesting soybean protein, dried skim milk protein, or a nonprotein diet



**Figure 9. Experiment 1136 - Concentrations of free amino acids in portal and systemic plasma at 1, 2, and 3 hour intervals after feeding dried skim milk protein**

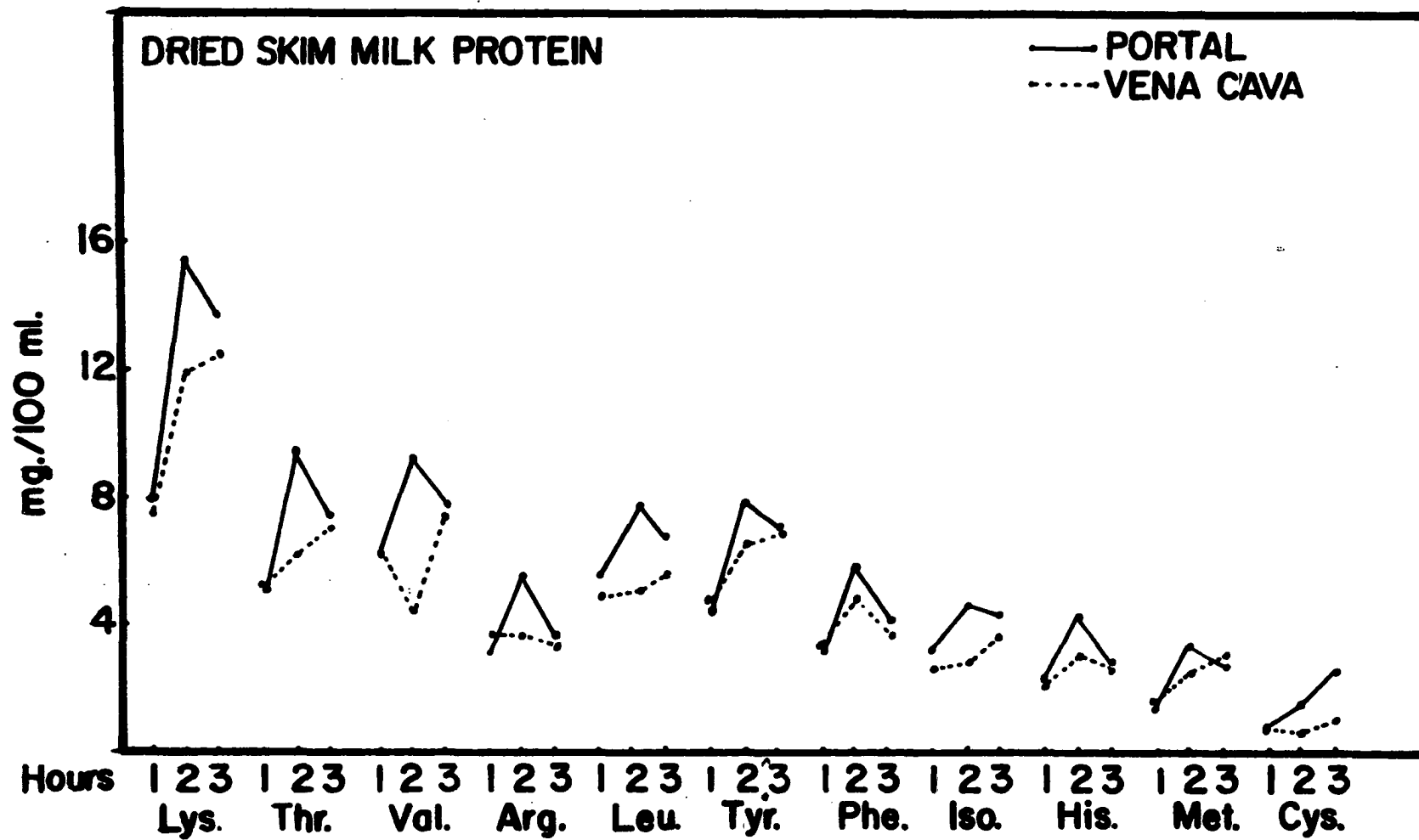
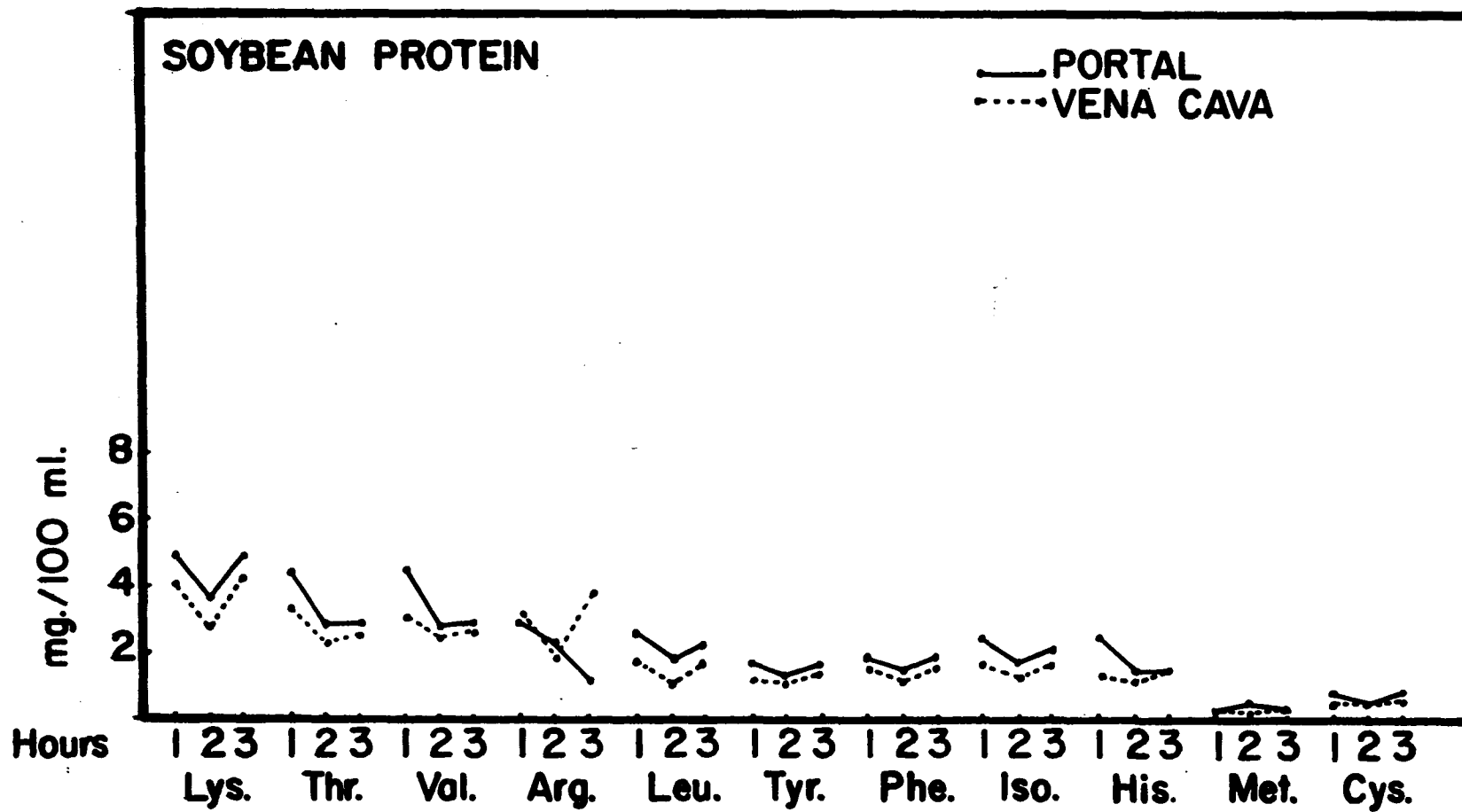


Figure 10. Experiment 1136 - Concentrations of free amino acids in portal and systemic plasma at 1, 2, and 3 hour intervals after feeding soybean protein.





in concentration with time. After feeding soybean protein, the concentration of the essential portal plasma amino acids was reflected in the vena cava plasma. Arginine was the only amino acid that deviated in this respect and a significant decrease in concentration occurred at three hours in the portal plasma.

One hour after feeding      The plasma and intestinal amino acid concentrations occurring one hour after ingesting either dried skim milk protein or soybean protein are summarized in Table 19. All amino acid values presented are the average of two observations.

The concentration of the amino acids, except threonine, citrilline, methionine, tyrosine, phenylalanine, and arginine increased in the portal plasma as compared to the vena cava plasma when pigs were fed the dried skim milk protein. Aspartic acid, glutamic acid, proline, glycine, and histidine increased linearly in concentration from the duodenum to the ileum. Threonine, serine, citrulline, alanine, cystine, valine, and isoleucine were higher in concentration in the jejunum as compared to the duodenum or ileum. Cysteic acid was found in the intestinal tract and the highest level appeared in the jejunum. Methionine, leucine, tyrosine, phenylalanine, and arginine progressively declined in concentration from the duodenum to the ileum indicating that these amino acids were released and absorbed at a faster rate in the upper parts of the digestive tract. Lysine was highest in concentration in the duodenum and lowest in concentration in the jejunum.

Except for methionine and arginine, the amino acids were higher in concentration in the portal plasma as compared with the vena cava plasma when pigs were fed soybean protein. Glutamic acid, glycine, and histidine were the only amino acids which were higher in the portal plasma of pigs fed soybean protein as compared to those fed dried skim milk protein. The other nonessential and essential amino acids were higher in concentration in either vena cava or portal plasma of pigs fed dried skim milk protein as compared to that of pigs fed soybean protein.

Except for cystine, the concentration of the amino acids increased linearly from the duodenum to the ileum indicating partial breakdown of soybean protein in the upper segments of the small intestine. The concentration of cystine was highest in the jejunum and lowest in the ileum.

The amino acids, except cystine, were higher in concentration in either the duodenum or jejunum of pigs fed dried skim milk protein as compared to those fed soybean protein. This was true for the ileum also except that lower levels of tyrosine, phenylalanine, and arginine were present when pigs were fed dried skim milk protein as compared to those fed soybean protein.

Two hours after feeding      The plasma and intestinal amino acid concentrations occurring two hours after ingesting either dried skim milk protein or soybean protein are summarized in Table 20.

All the plasma amino acids were higher in concentration in the portal vein as compared with the amino acid levels in the vena cava plasma of

pigs fed either dried skim milk protein or soybean protein, except cystine for the latter protein. The total concentration of amino acids was considerably higher for either portal or vena cava plasma when pigs were fed dried skim milk protein as compared to those fed soybean protein.

Methionine, leucine, cysteic acid, phenylalanine, and lysine decreased linearly in concentration from the duodenum to the ileum indicating rapid release and absorption of these amino acids from dried skim milk protein in the upper segments of the small intestine. Aspartic acid, threonine, serine, glutamic acid, proline, alanine, cystine, valine, isoleucine, and histidine increased in concentration from the duodenum to the jejunum and then declined in concentration in the ileum. Glycine increased linearly in concentration from the duodenum to the ileum.

Most of the intestinal amino acids, except aspartic acid and serine, increased in concentration from the duodenum to the jejunum and then decreased in concentration in the ileum when soybean protein was fed. When pigs were fed dried skim milk protein, the total intestinal amino acid concentration was considerably greater as compared to the total intestinal amino acid concentration of pigs fed soybean protein.

Three hours after feeding      The plasma and intestinal amino acid concentrations occurring three hours after ingesting either dried skim milk protein or soybean protein are summarized in Table 21.

Most of the amino acids were higher in concentration in the portal plasma than in vena cava plasma when pigs were fed dried skim milk protein. Except for glycine, alanine, histidine, and arginine, the other portal plasma amino acids were higher in concentration than in

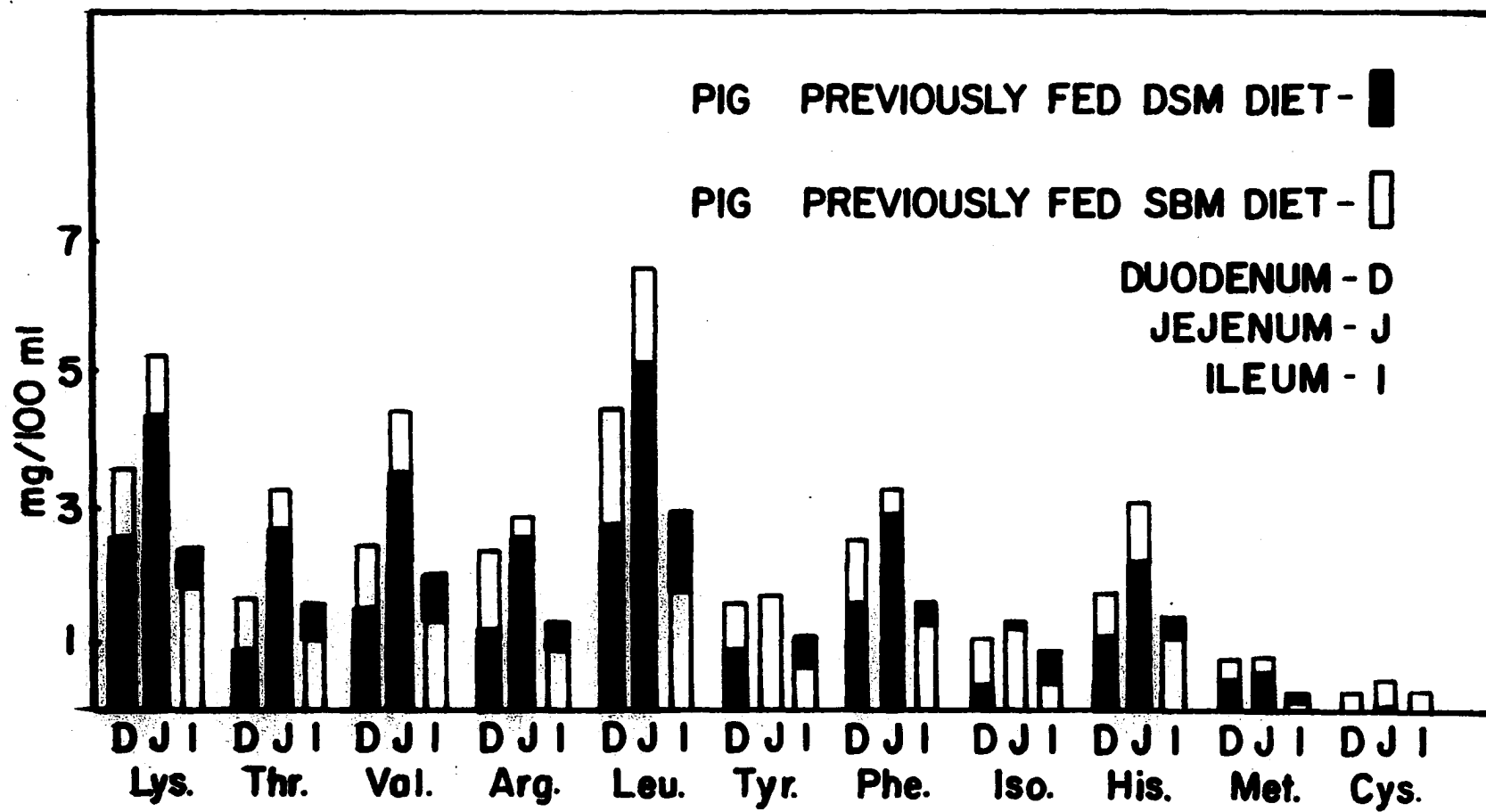
vena cava plasma when pigs were fed soybean protein. The total plasma amino acids (portal and vena cava) were higher quantitatively, when pigs were fed dried skim milk protein as compared to those fed soybean protein.

Most of the amino acids were lower quantitatively in the ileum as compared with the duodenum indicating a high rate of proteolysis and amino acid absorption occurring in the duodenum and jejunum when milk protein was fed. Except for tyrosine, the other amino acids increased in concentration from the duodenum to the jejunum and then declined in concentration in the ileum when pigs were fed milk protein. Aspartic acid, threonine, cystine, leucine, lysine, histidine, and arginine increased in concentration from the duodenum to the jejunum and then decreased quantitatively in the ileum when soybean protein was fed. Serine, glutamic acid, proline, alanine, valine, methionine, isoleucine, tyrosine, and phenylalanine increased linearly in concentration from the duodenum to the ileum. The highest concentration of amino acids appeared in the lower segments of the digestive tract indicating that the degree of proteolysis was not extensive for soybean protein.

Nonprotein meal (three hours after feeding)      The plasma and intestinal amino acid concentrations occurring three hours after ingesting the nonprotein meal ration are summarized in Table 22. The concentrations of the free amino acids in the different segments of the small intestine are shown graphically in Figure 11.

When the pig was fed the dried skim milk protein and switched to the nonprotein diet, except for methionine, the plasma amino acids were higher

Figure 11. Experiment 1136 - Intestinal free amino acid concentrations of pigs at three hours after ingesting a nonprotein diet



in concentration in the portal blood as compared to vena cava blood. Essentially no difference was observed between methionine levels in portal or vena cava plasma.

The portal blood sample was unavoidably lost and no amino acid comparisons can be made with vena cava blood when the pig was fed the soybean protein and switched to the nonprotein diet. In the vena cava plasma of the pigs fed the nonprotein diet, almost all of the amino acids were higher in concentration, except citrulline, glycine, alanine, and methionine in the pig previously fed soybean protein as compared to the pig previously fed dried skim milk protein.

The amino acids were highest in concentration in the jejunum as compared to amino acid levels observed in the duodenum or ileum of pigs previously fed either soybean protein or dried skim milk protein and changed to a nonprotein diet. Except for glycine and cystine, the concentration of amino acids was higher in the ileum of the pig fed milk protein followed by the nonprotein diet as compared to the pig fed soybean protein followed by the nonprotein diet. The level of all amino acids was higher in concentration in the duodenum of the pig fed soybean protein and changed to the nonprotein diet as compared to the pig fed dried skim milk protein and changed to the nonprotein diet.

The amino acid levels in the vena cava plasma or portal plasma and the intestinal segments were much lower in concentration of pigs fed the nonprotein diet as compared to those fed either milk protein or soybean and sacrificed at three hours after ingesting the protein meals. This was



especially true of the free amino acids in the different segments of the intestinal tract as a tremendous difference in concentration of all the amino acids was observed between pigs fed the protein rations and those fed the nonprotein meal rations.

Figure 12 presents a graphic summary of the essential amino acids occurring in each segment of the small intestine when either protein source was fed. When pigs were fed dried skim milk protein, except for cystine, the essential amino acids, except for cystine, were higher in concentration in the duodenum or jejunum with preponderance being in the jejunum. After feeding pigs soybean protein, the highest concentration of most of the essential amino acids occurred in the jejunum, the ileum being second in this respect. In almost every instance, the essential free amino acids were greater in quantity in each segment of the small intestine when dried skim milk protein was fed as compared to when soybean protein was fed.

In Figure 13 is presented a summary of the essential intestinal free amino acids occurring with time when either protein source was fed. The amino acids were higher in concentration at either two or three hours when dried skim milk protein was fed and at three hours when soybean protein was fed. It would appear that proteolysis is rapid for dried skim milk protein and due to the observed decreased concentration of free amino acids in the ileum, rapid absorption must also occur as shown by the high amino acid levels in the plasma. On the other hand, proteolysis does not occur rapidly when soybean protein is fed as is indicated by the low concentration

Figure 12. Experiment 1136 - Free amino acid concentrations in the different segments of the small intestine of pigs fed soybean or dried skim milk protein

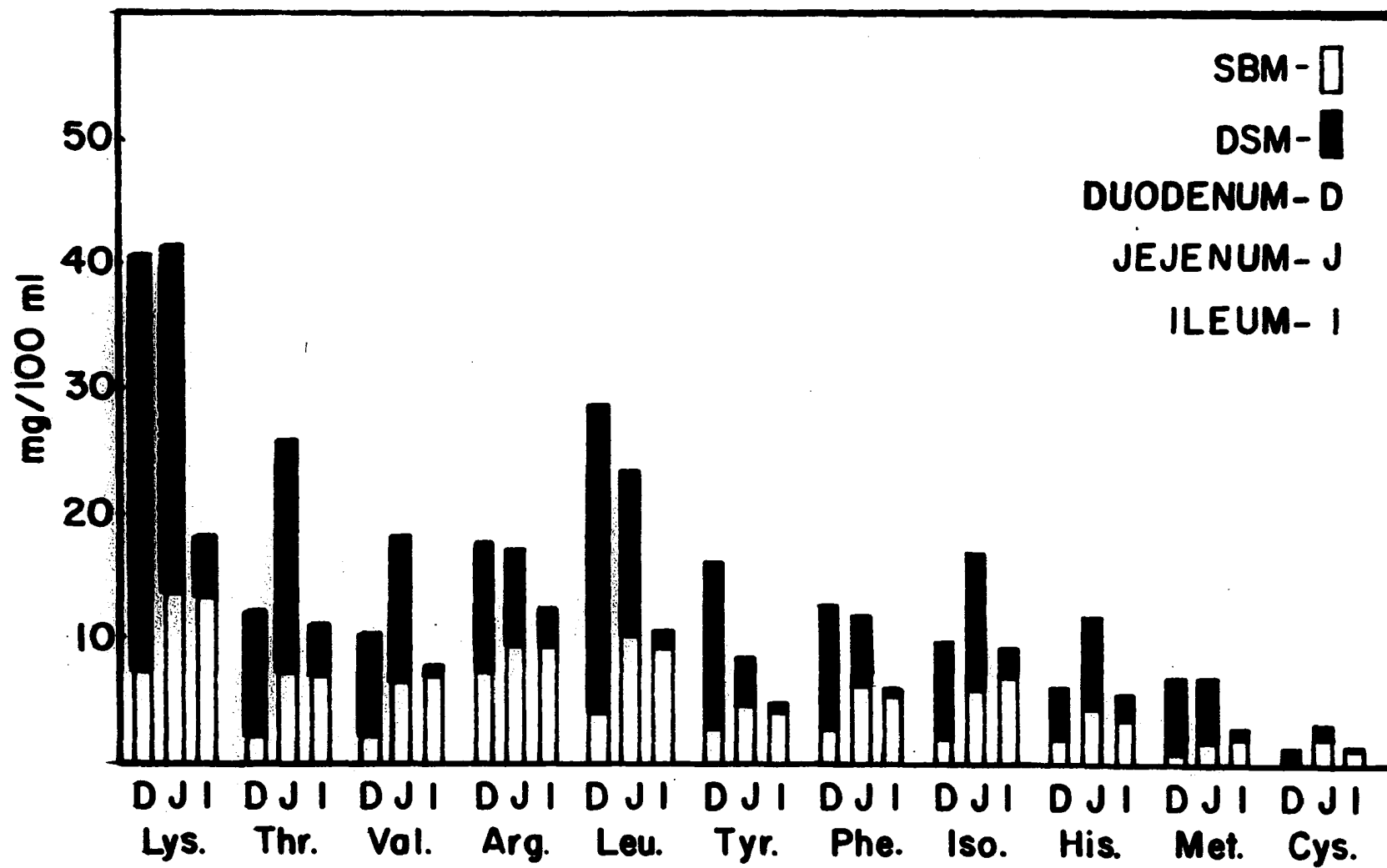
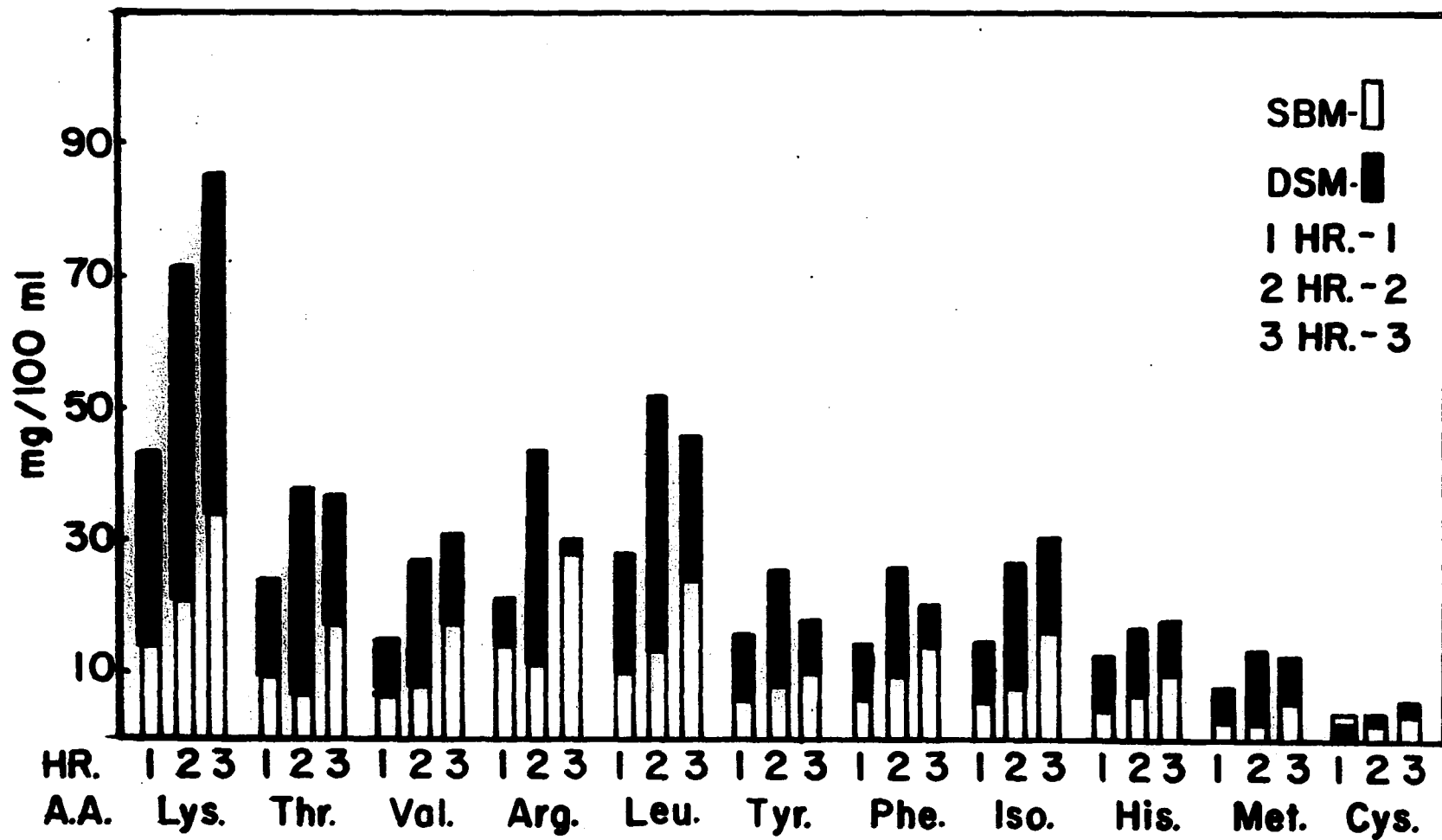


Figure 13. Experiment 1136 - Intestinal free amino acid concentrations of pigs at 1, 2, or 3 hours after ingesting soybean or dried skim milk protein



of free amino acids in each segment of the small intestine and the low levels of amino acids in the plasma reflect a slow rate of absorption.

Total nitrogen (Table 16) analyzed from the different intestinal sections was found to be higher than could be accounted for by nitrogen in the diet alone. This indicates the presence of endogenous nitrogen, in agreement with the reports by Nasset et al., (1955), Nasset and Soon Ju (1961) and Puchal (1962).

From this experiment it would appear that amino acids in the vena cava blood of the pig are representative of portal plasma amino acids since no appreciable differences were observed in the ratios of one amino acid to another. Gross differences were observed in intestinal free amino acid concentrations when dried skim milk protein was ingested as compared to soybean protein. The intestinal free amino acids were extremely low in concentration when a nonprotein meal was fed as compared to the concentration of intestinal free amino acids when either milk protein or soybean protein was fed.

#### Experiment 6302 - Amino acid absorption curve

Objective This experiment was designed to study the concentration changes occurring in plasma amino acids with time in the anterior vena cava of baby pigs fed either dried skim milk protein or soybean protein allowing the rations ad libitum or controlled feeding. Frame (1958) has reported amino acid changes in systemic blood of humans fed a high protein meal. Most amino acids increased in concentration after the meal, but the

concentration changes did not parallel the relative amino acid composition of the ingested food.

Since previous amino acid data, Puchal (1962), has largely been determined from pigs allowed rations ad libitum and at no particular time after feeding, it was decided to determine amino acid changes occurring in the plasma of baby pigs at specified time intervals after ingesting a protein meal.

Experimental procedure      Twenty pigs averaging 9.8 pounds and 10.7 days of age were used in this experiment.

The rations in this experiment were identical to those used in Experiment 1136, Table 15.

The pigs were fed either a ration containing soybean meal or dried skim milk for seven days prior to obtaining the blood specimens. Blood samples were drawn at 2-hour intervals for an 8-hour period on either the ad libitum or controlled feeding regimen. Hematocrits were determined individually at the specified time intervals and some plasma volume dilution occurred (10 to 15 percent). No correction was made for the dilution error since for any particular blood specimen, the percentage change in concentrations for each amino acid should be similar.

The pigs allowed feed ad libitum were not fasted and were allowed the rations during the entire 8-hour period in which blood samples were obtained. Animals were observed to be eating the rations at different times during the 8-hour period.

All control fed animals were fasted for a period of 18 hours prior to obtaining blood samples. Immediately after obtaining the 0-hour blood

samples, the rations were placed before the animals for two hours, then removed, and these animals were not allowed access to any food until after the final samples were obtained.

Similar pigs previously fed the two protein sources were fed a nonprotein diet for 36 hours and blood samples collected the following eight hours.

An acid hydrolysis was performed on soybean protein and dried skim milk protein and the amino acid composition of the two proteins was determined by column chromatography.

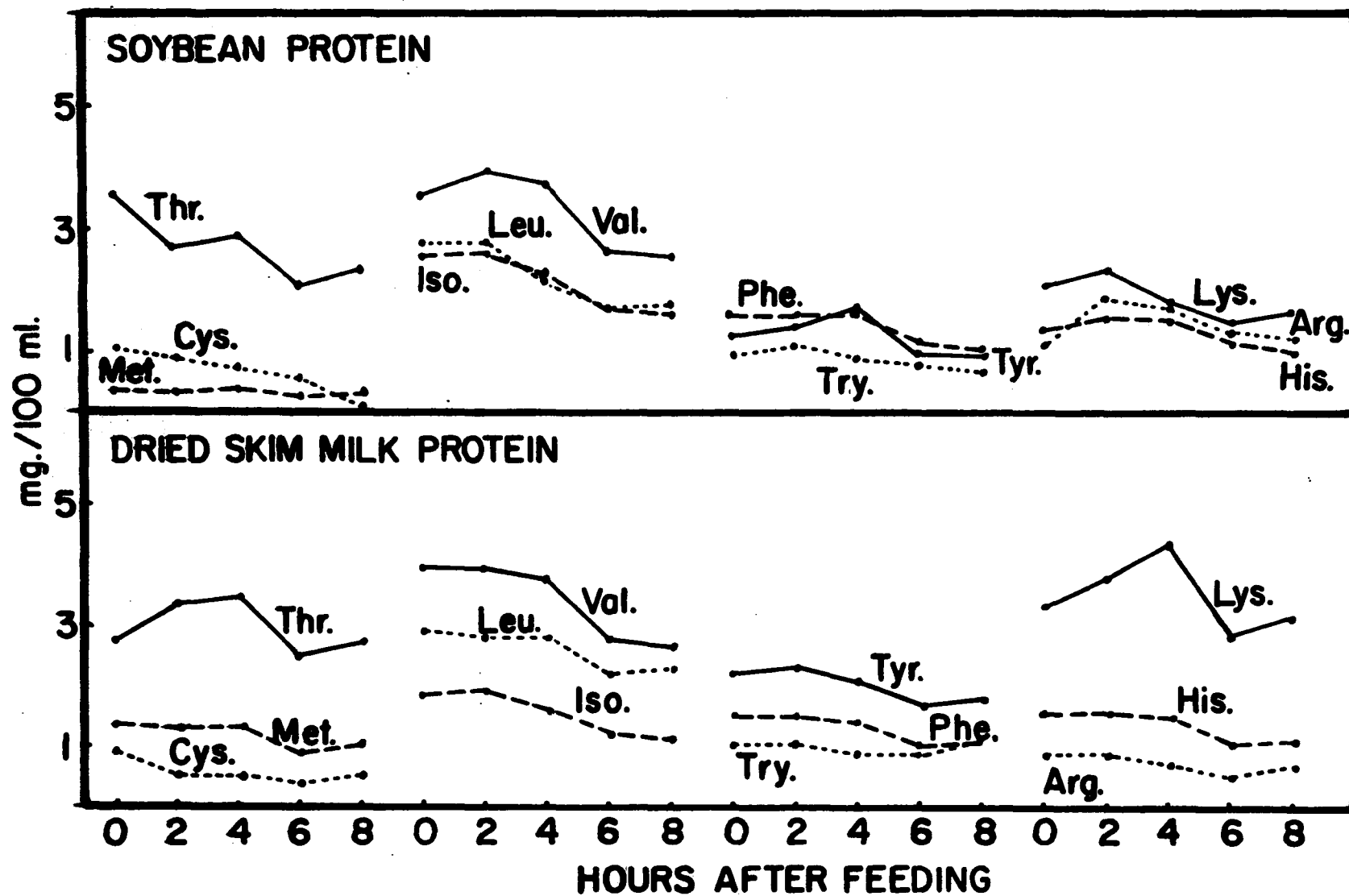
### Results and discussion

Ad libitum feeding      The plasma amino acids are summarized in Table 23 for the ad libitum fed pigs fed either dried skim milk protein or soybean protein. The concentrations of the essential amino acids are shown graphically in Figure 14. Most of the plasma amino acids appeared to peak at either two or four hours when pigs were fed dried skim milk protein. Glycine, cystine, valine, methionine, leucine, and arginine showed the highest concentration at 0 hours and then declined in concentration with time. Aspartic acid, proline, alanine, isoleucine, tyrosine, phenylalanine, tryptophan, lysine, and histidine peaked in the plasma at either two or four hours and then the concentration of these amino acids decreased at six and eight hours, except tryptophan which increased at eight hours.

Most of the plasma amino acids were higher in concentration at two hours as compared to the other time periods when pigs were fed the soybean



Figure 14. Experiment 6302 - Plasma free essential amino acid concentrations of pigs ad libitum fed a soybean meal or dried skim milk ration



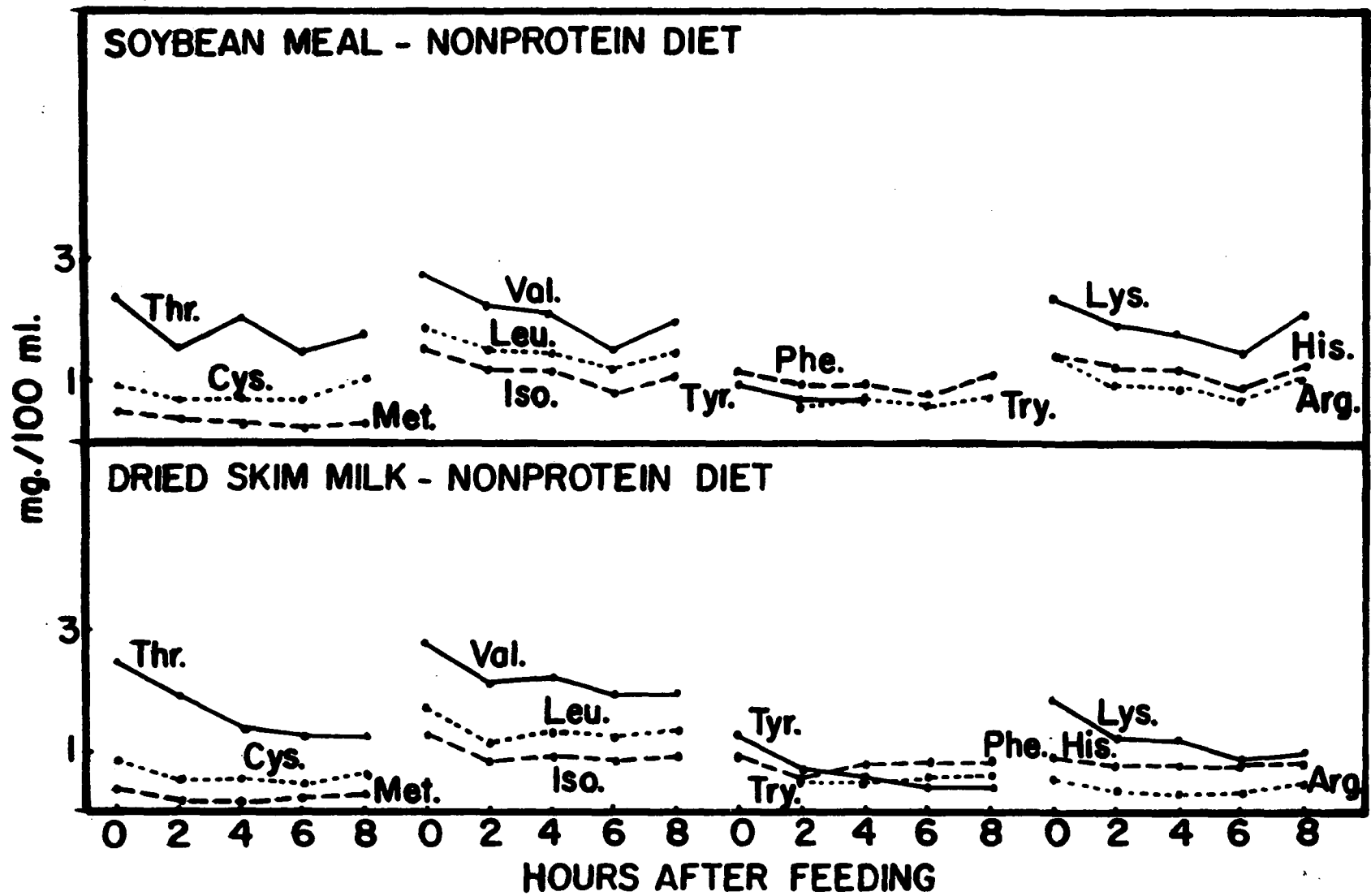
meal ration. Aspartic acid, threonine, serine, glycine, cystine, and leucine were highest at 0 hours and then progressively decreased in concentration with time. Glutamic acid, proline, alanine, valine, isoleucine, tyrosine, phenylalanine, tryptophan, lysine, histidine, and arginine had the highest concentration at two hours. The lowest concentration of all the amino acids appeared at the 6- and/or 8-hour intervals.

It appears for each protein that, in general, most of the amino acids increased in concentration at either two or four hours. The lowest concentration appeared at either six or eight hours. The total plasma amino acid level was considerably higher for pigs fed dried skim milk protein as compared to pigs fed soybean protein.

A summary of the plasma amino acids from pigs which had been previously fed either dried skim milk protein or soybean protein, ad libitum, and then switched to the nonprotein diet is shown in Table 24. The concentrations of the essential amino acids are shown graphically in Figure 15.

When the pigs were fed the soybean meal ration and then switched to the nonprotein diet, the plasma amino acids were lower in concentration as compared to those pigs fed the soybean protein. Essentially no difference was observed in the concentration of glycine, alanine, and methionine between the plasma levels of pigs previously fed either protein ration. Most of the essential amino acids were higher in concentrations at 0 hours as compared to the other time intervals, except tryptophan which peaked at four hours. No particular concentration pattern was noted

Figure 15. Experiment 6302 - Plasma free essential amino acid concentrations of pigs ad libitum fed a nonprotein diet following the feeding of a soybean meal or dried skim milk ration



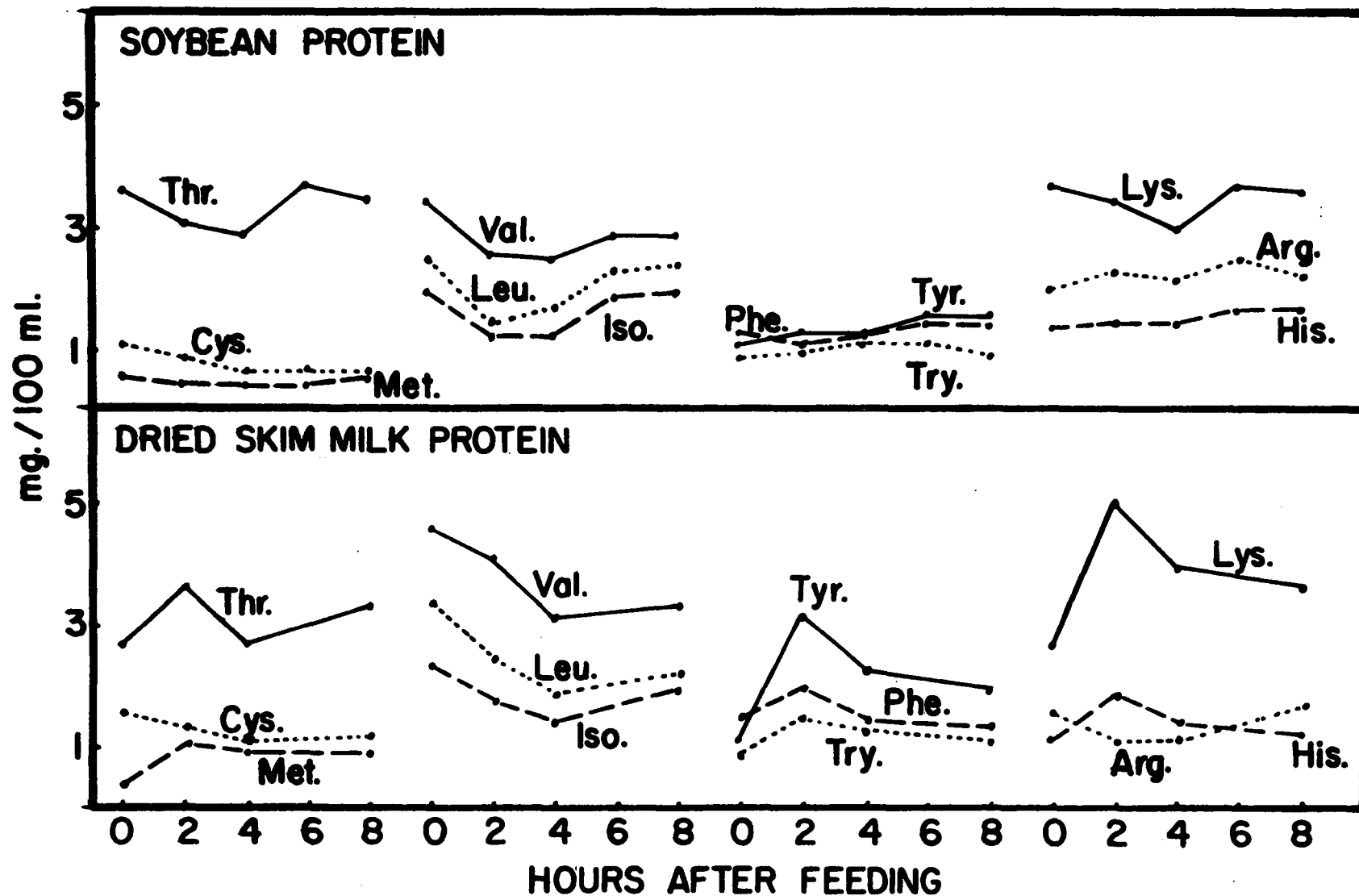
for the different amino acids as they varied in concentration at different time intervals. After feeding pigs the nonprotein meal ration (previously fed dried skim milk protein), most of the plasma amino acids, except aspartic acid, glycine, and alanine were lower in concentration as compared to the plasma amino acid levels of pigs fed dried skim milk protein. The highest concentration of the essential amino acids and most of the nonessential amino acids was observed at 0 hours as compared to the other time intervals. No definite trends were observed in the concentration of amino acids as they varied in concentration with time.

All of the essential plasma amino acids were lower in concentration when pigs were fed the nonprotein meal (previously fed dried skim milk protein) as compared to those fed the nonprotein meal (previously fed soybean protein), however, the reverse was true for most of the non-essential amino acids. In general when a nonprotein meal was fed to young pigs, it appeared that the essential and most of the nonessential amino acids decreased in plasma concentration regardless of whether the pigs had previously been fed milk or soybean protein.

Controlled feeding      A summary of the plasma amino acids is shown in Table 25 for pigs control fed dried skim milk protein or soybean protein. The concentrations of the essential amino acids are shown graphically in Figure 16.

Aspartic acid, threonine, proline, alanine, methionine, tyrosine, phenylalanine, tryptophan, lysine, and histidine were higher in concentration at two hours as compared to the other time intervals for pigs fed dried skim

**Figure 16. Experiment 6302 - Plasma free essential amino acid concentrations  
with controlled feeding of a soybean meal or dried skim milk ration**





milk protein. Glycine, valine, isoleucine, and leucine were highest in concentration at the 0 hour and then progressively decreased in concentration at the 2- and 4-hour intervals. A slight rise in concentration was observed at eight hours for these amino acids. Arginine was higher in concentration at eight hours as compared to the other time intervals. The plasma sample for the 6-hour interval was unavoidably lost and no amino acid data are reported. Threonine, proline, and alanine decreased in concentration at four hours as compared to two hours and then increased in concentration at eight hours. Cystine was higher in concentration at 0 hours, progressively decreased in concentration to four hours and then increased in concentration slightly with time.

Pigs fed the soybean meal ration had the highest concentration of cystine, valine, methionine, isoleucine, leucine, and lysine at 0 hours as compared to the other time periods. Cystine decreased linearly in concentration with time. Threonine, serine, glycine, valine, methionine, and lysine were lowest in concentration at four hours as compared to the other time periods. Proline increased linearly in concentration from 0 to six hours and decreased at eight hours. Methionine decreased linearly in concentration from 0 to four hours and then increased linearly to eight hours. Essentially no difference in concentration was observed between 0, six and eight hours with isoleucine. The 2- and 4-hour isoleucine levels were the lowest as compared to the other time intervals. Leucine decreased in concentration linearly to four hours. Histidine linearly increased in concentration from 0 to eight hours. Arginine was

lowest in concentration at 0 hours with the highest concentration observed at six hours. Tryptophan increased in concentration from 0 to four hours and decreased in concentration from six to eight hours.

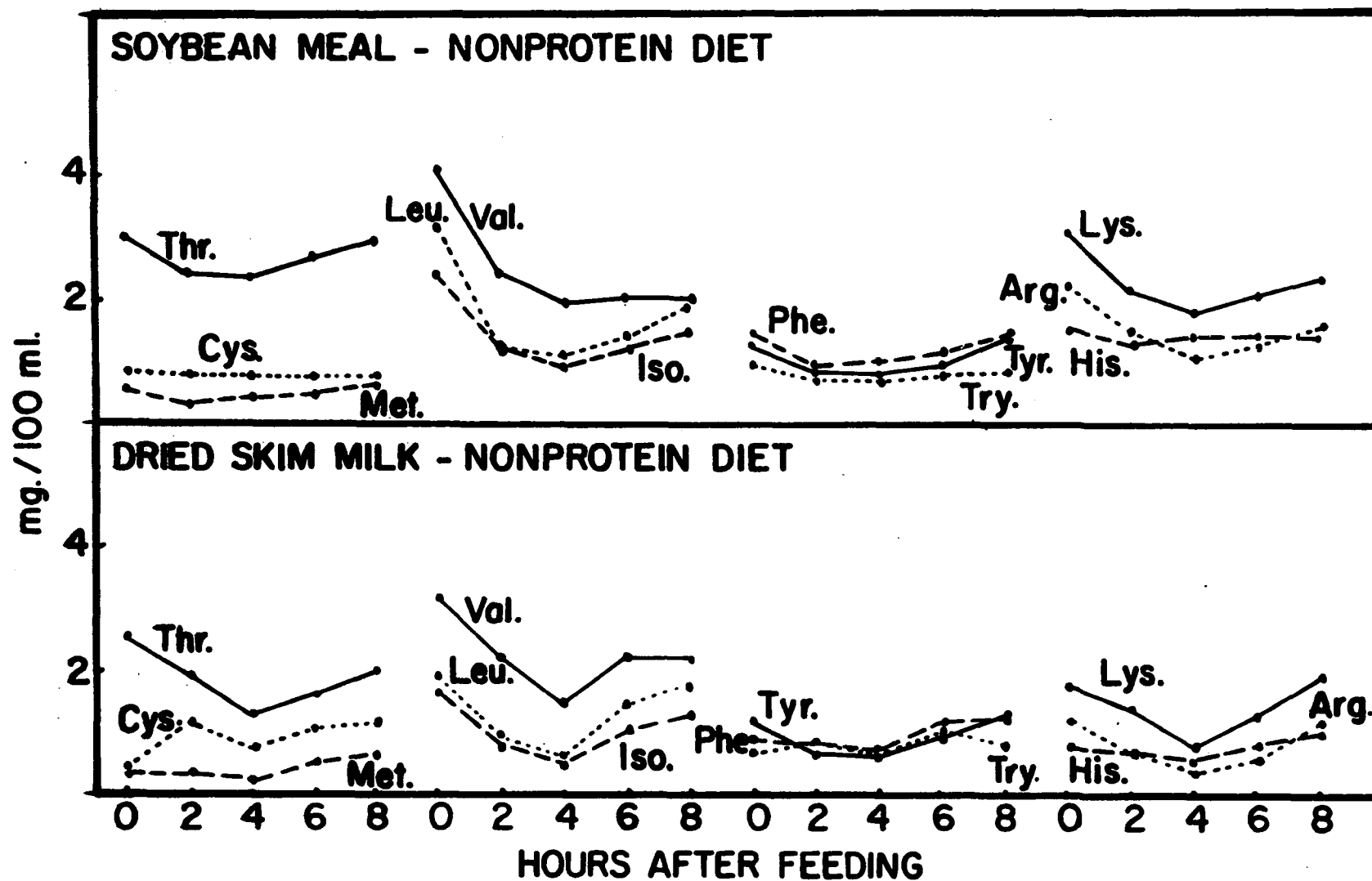
Again, the plasma amino acids were higher in concentration from pigs fed the dried skim milk protein as compared to the amino acid levels of pigs fed soybean protein and the concentrations of the plasma amino acids varied quantitatively at different times after ingesting a protein meal.

The plasma amino acids are summarized in Table 26 for the control fed pigs which were previously fed dried skim milk protein or soybean protein and changed to a nonprotein diet. The concentrations of the essential amino acids are shown graphically in Figure 17.

After feeding pigs a nonprotein meal (previously fed dried skim milk protein), the essential and most of the nonessential amino acids, except aspartic acid, glycine, and alanine, decreased in concentration in the plasma as compared to the amino acid levels of pigs fed dried skim milk protein. Except for cystine and tryptophan, the amino acids were lowest in concentration at four hours as compared to the other time intervals. Most of the essential amino acids were highest in concentration in the plasma at 0 hours except cystine, methionine, phenylalanine, lysine, and histidine. Most of the essential and nonessential amino acids linearly decreased in concentration from 0 to four hours and then linearly increased in concentration from four to eight hours.

When the pigs were fed the nonprotein meal (previously fed soybean protein), most of the amino acids decreased in the plasma, except aspartic

Figure 17. Experiment 6302 - Plasma essential amino acid concentrations of pigs control fed a nonprotein diet following the feeding of a soybean meal or dried skim milk ration



acid, methionine, and histidine, as compared to the plasma amino acid levels of pigs fed soybean protein. Threonine, serine, glutamic acid, valine, isoleucine, leucine, tyrosine, lysine, and arginine decreased linearly in plasma concentration from 0 to four hours and then increased linearly in concentration from four to eight hours. Glycine, methionine, and phenylalanine decreased in concentration from 0 to two hours and then increased in concentration from two to eight hours. The concentration of tryptophan was variable with time, the highest concentration appearing at 0 hours. The essential amino acids, except tryptophan, were higher in plasma concentration when pigs were fed the nonprotein meal (previously fed soybean protein) as compared to the plasma amino acid level of pigs fed the nonprotein meal (previously fed dried skim milk protein). The reverse was true for most of the nonessential amino acids.

Again, feeding a nonprotein meal to pigs previously fed either soybean protein or dried skim milk protein resulted in an overall decrease in the plasma amino acid concentrations. The concentration of the individual amino acids varied with time. The plasma essential amino acid concentrations were higher for pigs fed soybean protein and switched to a nonprotein meal as compared to the amino acid concentrations of pigs fed dried skim milk protein and switched to a nonprotein meal.

The essential blood plasma free amino acids generally declined in concentration with time when the two protein sources were allowed ad libitum or remained relatively unchanged in concentration when the proteins were control fed. This effect may possibly be attributed to the blood volume

dilution (10 to 15 percent) which should account for some of the decline in concentration especially at the 6- and 8-hour intervals.

In addition, these pigs were subjected to rather severe handling as blood samples were obtained from the vena cava five times in an 8-hour period. These samples were more difficult to collect at the latter time intervals as several animals developed large hematomas near the sternum. This severe handling probably caused some emotional stress to occur which would interfere with normal digestive processes and hence amino acid absorption into the blood stream.

Hydrolysis (acid)      Hydrolysis of dried skim milk protein and soybean protein used in this experiment was performed with 6 N hydrochloric acid at approximately 110° C for 24 and 48 hours, respectively. After hydrolysis, the samples were filtered and excess hydrochloric acid was removed by repeated concentration of the solution under reduced pressure. The samples were then prepared for column chromatography and the amino acid composition determined for the two proteins is summarized in Table 27. The amino acid values are expressed as percent of the protein fraction and as percent of soybean meal or dried skim milk. The 48-hour hydrolysis for dried skim milk protein increased all the amino acid levels except threonine and serine as compared to the amino acid levels obtained from the 24-hour hydrolysis. Threonine concentration remained unchanged whereas serine decreased in concentration with the 48-hour hydrolysis. The observed increased concentration of amino acids was slight for the 48-hour hydrolysis as compared to the 24-hour hydrolysis indicating that hydrolysis was almost complete at 24 hours.

The 48-hour hydrolysis for soybean protein significantly increased all the amino acid levels as compared to the 24-hour hydrolysis. It appears that hydrolysis was not complete at 48 hours. Another soybean protein sample was hydrolyzed for 72 hours and most of the amino acids were observed to increase slightly in concentration except serine and cystine. This confirmed the observation that the 48-hour hydrolysis for soybean protein was partially complete. This increased time of hydrolysis also indicates that soybean protein is more resistant to releasing its amino acids in the presence of a strong acid and at a relatively high temperature than is milk protein.

The amino acid composition of the two proteins is in agreement with values reported by Lyman et al. (1956), Block and Weiss (1956), and Hubbell (1963).

Experiment 6326 - Blood plasma free amino acid interrelationships of young pigs fed soybean and milk protein

Objective      This experiment was designed to study the effects of three protein levels within two protein sources on the free blood plasma amino acid levels in young pigs.

Various methods have been devised for determining amino acid availability in proteins, but none have been completely satisfactory. Among the methods used have been biological methods based on growth, nitrogen balance, chemical methods, and more recently blood plasma amino acid ratios (Longnecker and Hause, 1961). Some progress has been made in determining a shortened procedure for measuring amino acid availability (Longnecker and Hause, 1961), but it remains one of the

important problems in assessing food proteins and processed supplements. More rapid and accurate amino acid determinations are required.

It seemed reasonable to assume that the free plasma amino acids should increase in concentration with an increase in protein level. Once the free amino acids were determined between the protein levels, then by adding 5 percent increments of a protein to the basal protein the differences in the plasma amino acids should be the amino acids available from the 5 percent protein increment. Hence, a quick reliable method of measuring amino acid availability could be realized.

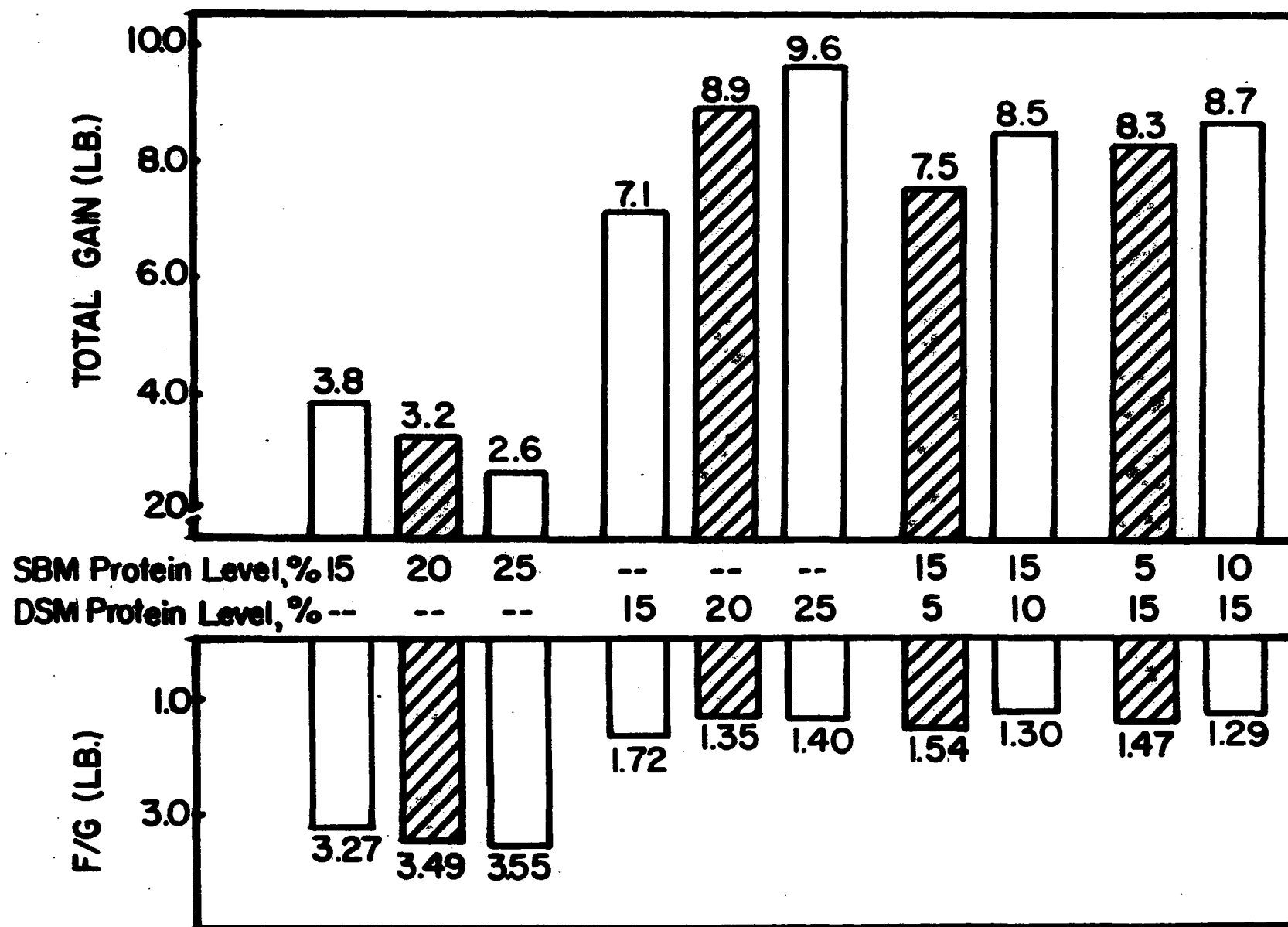
Experimental procedure      Thirty pigs averaging 9.6 pounds and 17.0 days of age were randomly allotted to ten treatments. The composition of the experimental rations is shown in Table 28. All animals were fasted for 18 hours prior to obtaining blood samples. The animals were allowed access to the rations for one hour and then the rations were removed. Blood samples were obtained at four hours after placing the rations before the animals. Blood samples were pooled from pigs on the same treatments. The experiment was terminated after two weeks.

Results and discussion      Total gains and feed efficiency are summarized in Table 31 and graphically presented in Figure 18. The analysis of variance and experimental plan are shown in Table 33.

A linear decrease in total gain was observed when pigs were fed increasing levels of soybean protein whereas a linear increase in total gain was observed when increasing levels of dried skim milk protein were fed. When either soybean protein or dried skim milk protein was used as the basal ration and 5 percent protein increments were added, a linear



**Figure 18. Experiment 6326 - The effects of protein levels and protein sources upon total gains and feed efficiency**



increase in growth rate was observed with increasing protein levels. Rate of gain was significantly more rapid when pigs were fed dried skim milk protein as compared to those fed soybean protein.

Feed efficiency linearly decreased with increasing levels of soybean meal whereas feed efficiency increased with increasing levels of dried skim milk. Essentially no difference in feed utilization was observed between 20 percent and 25 percent dried skim milk protein levels. When either soybean meal or dried skim milk was used as the basal ration and 5 percent protein increments were added, a linear increase in feed efficiency was observed. Essentially no difference in feed efficiency was observed between protein levels when the two protein sources were combined. A highly significant increase in feed efficiency was observed when pigs were fed the dried skim milk ration as compared to those fed the soybean meal ration.

A summary of the free blood plasma amino acids is shown in Table 32. All the amino acids appeared to vary in concentration with increasing protein levels when the pigs were fed either soybean protein or dried skim milk protein. Previous plasma amino acid data have indicated that plasma amino acids are considerably higher in concentration when pigs were fed dried skim milk protein as compared to those fed soybean protein. The amino acid data from this experiment did not indicate this relationship. Methionine and tyrosine were the only two amino acids that were definitely higher in plasma concentration when pigs were fed milk protein as compared to those fed soybean protein.

When 5 percent protein increments of dried skim milk were added to the soybean basal ration, no definite trends in the concentration of the various amino acids were observed. Threonine was higher in concentration when 5 percent dried skim milk protein was added as compared to when 10 percent was added. The same trend was noted for serine, glycine, alanine, cystine, methionine, tyrosine, histidine, and arginine.

When 5 percent protein increments from soybean protein were added to the dried skim milk basal ration, no definite trends in the concentration of the amino acids were observed. Serine, glutamic acid, glycine, alanine, and cystine were lower in concentration when 5 percent soybean protein was added to the dried skim milk basal ration as compared to when 10 percent soybean protein was added. All the essential amino acids were higher in concentration when 10 percent soybean protein was added to the dried skim milk basal ration as compared to when the 5 percent soybean protein was added.

It would appear under the conditions of this experiment that the variability of free blood plasma amino acids is too great for plasma amino acid levels to serve as an accurate and quick method of determining amino acid availability by feeding increasing increments of soybean or milk protein.

## GENERAL DISCUSSION

Evidence presented by Puchal (1962) indicated that the blood plasma aminograms from pigs fed soybean protein did not resemble those fed dried skim milk protein and that slower growth occurred for the former group. Hence, appropriate amino acid supplementation of soybean protein should result in a blood plasma aminogram comparable to that of dried skim milk protein with a favorable growth response as well.

Supplementation of soybean protein with methionine in Experiments 1143, 1153, and 1159 improved the growth rate and feed efficiency of young pigs in agreement with published reports (Hays et al., 1959; Berry et al., 1962). In the latter two experiments, as expected, the plasma free amino acid level increased for the specific amino acid supplemented to soybean protein. The other amino acids decreased in plasma concentration, whereas threonine and glutamic acid increased in concentration in Experiment 1153. In Experiment 1159, the amino acids decreased in plasma concentration with the exception of glutamic acid and glycine.

It has been theorized that when the low plasma level of a deficient amino acid is corrected, protein synthesis will continue until the second limiting amino acid will limit further protein synthesis, which will occur with a simultaneous decrease in the plasma free concentration for this amino acid (Hill et al., 1961; Hill and Olsen, 1963a). The effect of amino acid supplementation on the presence of other amino acids in blood plasma presents a further complication in the evaluation and correction of amino acid deficiencies. Amino acids which appear to be equally

responsible for lack of growth under one set of conditions may not be the same amino acid(s) involved when the first limiting amino acid is corrected as has been observed in the increased plasma concentration of threonine in Experiment 1153 and a decrease in plasma concentration in Experiment 1159 when methionine was added to soybean protein.

In these two experiments, the amino acids which decreased in plasma concentration the greatest percentage were lysine, valine, leucine, and perhaps threonine, suggesting that these were the amino acids needed to further supplement soybean protein.

When valine or threonine was added to the methionine-supplemented soybean protein, a further small improvement in growth was obtained. This improvement in growth might be explained by studying the effects of valine and threonine supplementation on the amino acid changes in the plasma. Most of the plasma amino acids increased in concentration suggesting that their availability to the tissues had also increased as it has been frequently quoted in the literature that all the essential amino acids must be presented to the tissues almost simultaneously for their efficient utilization to occur (Cannon et al., 1947; Geiger, 1947; Geiger, 1948; Henderson and Harris, 1949).

The addition of lysine (Experiment 1153 and 1159) appeared to depress the growth response observed when either methionine, valine or threonine was added to soybean protein. The plasma amino acids increased in concentration when these four amino acids were added to the ration indicating increased availability, but further suggesting that blood plasma free amino acids alone cannot be used to detect the amino acid

deficiencies of soybean protein, when two or three of the most limiting amino acids have been added to the protein, due to the changes occurring in the absorption and utilization pattern of the blood plasma free amino acids.

It is possible that the decreased weight gains observed with lysine supplementation were caused by an amino acid imbalance. Most of the depression in weight gains attributed to amino acid imbalance have been demonstrated with diets grossly deficient in an essential amino acid (Fisher and Shapiro, 1961; Hill and Olsen, 1963a; (chicks); (rats), Kunita and Harper, 1960; Sauberlich, 1961a) or by adding excess quantities of an amino acid to the diet (Sauberlich, 1961b). The total dietary lysine level (including supplemented level) for Experiments 1153 and 1159 was 1.62 and 1.43 percent, respectively, with 0.54 and 0.35 percent excess lysine in the diets, respectively, as compared to the young pig's estimated lysine requirement (Becker, 1960). This depression in weight gains when lysine was added to soybean protein corresponds to the definition of "amino acid imbalance" stated by Harper (1958).

Adding 0.32 percent threonine and 0.38 percent valine to the methionine-supplemented soybean protein diet resulted in the most rapid growth rate (Experiment 1159). When the supplemental levels of threonine and valine were reduced to 0.24 and 0.22 percent, respectively, and the supplemental methionine level increased to 0.35 percent, growth was not improved to any significant extent (Experiment 1175). It would appear that the 0.32 and 0.38 supplemental levels of threonine and valine,

respectively, were perhaps more representative of the young pig's requirement at this early age and further suggests that these two amino acids were not readily available from soybean protein. Again, perhaps the supplemental level of methionine (0.35 percent) was too much and the optimum supplemental level is approximately 0.20 percent as shown by the pig gains observed in Experiment 1159.

The addition of 0.10 percent proline to the methionine, valine, and threonine supplemented ration resulted in the greatest improvement in pig gains as compared to pigs fed the other amino acid supplemented rations. It is doubtful that proline is an essential amino acid for the young pig as proline has been shown to be nonessential for the rat (St. Julian and Rose, 1932). Puchal (1962) suggested that proline may be partially required by the young pig due to the low levels of plasma proline obtained when methionine, valine and threonine were added to soybean protein. Similar decreases in concentration of plasma proline occurred in pigs fed the amino acid supplemented rations in Experiments 1153 and 1159.

Several investigators have shown that a diet containing only the essential amino acids supports a slow rate of growth (Kinsey and Grant, 1944; Wretlind, 1949) and that other sources of nitrogen (nonessential amino acids, Womack and Rose, 1949; other nonspecific sources of nitrogen, Lardy and Feldott, 1949; Rechicigl et al., 1947) are required for the attainment of normal or maximum growth rates. In this experiment, essential amino acid supplementation may have altered the proper balance of essential to nonessential amino acids, thereby causing a requirement



for a nonessential amino acid (Geiger, 1950) before maximum growth could occur. Sauberlich (1961a) has remarked that rapid early growth of rats required the presence of some of the nonessential amino acids. The highest level of plasma proline and greater body weight gains occurred when young pigs were fed the ration containing added methionine, threonine, valine, and proline suggesting a need for a nonessential amino acid.

The amino acid combination (which included all essential amino acids, except arginine) supplemented to soybean protein (Experiment 1175) resulted in no improvement in growth rate as compared to pigs fed the methionine, valine, threonine, and proline supplemented diet. A comparison between the plasma aminograms of pigs fed these two rations indicated little difference in amino acid concentrations.

Again, some degree of amino acid imbalance may be occurring which would alter the growth rate, however, the specific amino acid(s) causing the imbalance are not exactly known but lysine could be suspected.

The essential amino acids employed were racemic mixtures, except for L-lysine, L-histidine, and L-proline. It is well known that the D-form of an amino acid cannot be used per se within the animal body for protein synthesis. Analyses of the plasma demonstrated an increased concentration of the free amino acid when supplemented to soybean protein. A part of this increase may consist of D- essential amino acids circulating in the blood. The nonessential amino acids were observed to increase in concentration also and perhaps the nitrogen from the D- forms was used for the synthesis of the nonessential amino acids. Similar observations

have been reported to occur in chicks (Featherston et al., 1962).

Present knowledge indicates that the L- isomers of the amino acids are absorbed more rapidly than the corresponding D- form (Gibson and Wiseman, 1951; Edwards et al., 1963) and that some competition exists between absorption of D- and L- enantiomorphs of different amino acids from the intestinal lumen (Jervis and Smyth, 1959). Administration of racemic mixtures of essential amino acids to rats resulted in 62 percent of D-threonine, 51 percent of D-valine, and 65 percent of D-isoleucine excreted in the urine indicating poor utilization (Hedin and Schultze, 1961).

In the experiments reported herein, growth was not improved to any great extent when a combination (more than three essential amino acids) of amino acids was added to soybean protein suggesting that the D- isomers may have inhibited the efficiency of utilization of the L- isomers thereby preventing maximum growth from occurring or that these amino acids were not limiting.

Most of the experiments reported herein involved improving the amino acid adequacy of soybean protein by amino acid supplementation and utilizing the changes occurring in the plasma free amino acid pattern of the anterior vena cava as a means of determining which amino acid(s) would limit growth rate and verifying this by subsequent growth trials. It has been frequently reported that portal blood plasma free amino acids, although no different from a qualitative content than systemic plasma, are higher quantitatively due to products of protein digestion and amino acid absorption before they are removed from the

systemic and peripheral circulation by the various vital organs and muscles. Therefore, it has been suggested that only portal plasma amino acids should be used in amino acid nutrition studies, since the factors affecting the free amino acid composition of anterior vena cava plasma may invalidate the amino acid data obtained from this anatomical area of the circulation. In view of this, it was considered necessary to compare portal and anterior vena cava plasma free amino acids in order to evaluate any differences present.

Fourteen young pigs averaging 17.0 days of age were used in Experiment 1136 to obtain data on portal-anterior vena cava plasma free amino acid comparisons and on the degree of proteolysis occurring in the digestive tract. Two protein sources (dried skim milk and soybean meal) were individually fed and blood specimens from the vena porta and the anterior vena cava compared. Two pigs, each fed one of the protein sources, were changed to a nonprotein diet and similar comparisons were made.

No significant qualitative differences were detected in the amino acid composition of blood specimens obtained from either the anterior vena cava or the portal vein when either protein source was fed, but there were significant quantitative differences present. In almost every instance, the concentrations of all the amino acids were quantitatively greater in portal plasma as compared to anterior vena cava plasma regardless of protein source. What is more significant is that the portal-vena cava essential amino acid ratios did not vary appreciably from 1.0, except cystine, which indicates that the pattern of amino acids between the two

blood specimens is relatively constant even though significant quantitative differences were detected. These data substantiate the assumption that the amino acid composition of portal plasma is reflected in the anterior vena cava plasma and that the latter is capable of lending validity to the interpretations made in previous experiments of amino acid supplementation to improve the protein efficiency of soybean meal.

These data are also in agreement with published reports of portal-systemic plasma free amino acid comparisons (Denton and Elvehjem, 1954b; Peraino and Harper, 1963).

An overall comparison of the amino acid patterns in the portal plasma after the test proteins were fed shows that the amino acids of dried skim milk protein were absorbed into the portal blood more rapidly (indicating a high degree of digestibility) than those of soybean protein. This indicates that the digestibility or availability of amino acids from soybean protein was probably a more limiting factor than amino acid absorption in assessing the rate of entry of the dietary amino acids into the portal blood system. The relatively low concentrations of amino acids in the portal plasma at one, two, and three hours after feeding soybean protein reflects that the amino acids of soybean protein were not readily available for intestinal absorption into the portal system as compared to those of dried skim milk protein. This was partially expected due to the relatively low free amino acid levels present in the small intestine when soybean protein was fed.

The total concentration of circulating free plasma essential amino acids decreased significantly at two hours after ingesting soybean protein whereas the opposite effect occurred after ingesting milk protein. The former effect might be explained by the continuation of the fasting effect as amino acids have been observed to decrease in concentration with time in chicks upon fasting (Gray et al., 1960; Hill and Olsen, 1963b). However, two hours after ingesting a protein meal, the digestive processes are operating at a high intensity. The gastric, pancreatic, and intestinal secretory cells are eluting large volumes of digestive juices which contain enzymes, hormones, and other proteins (Ganapathy and Nasset, 1962). If these enzymes and proteins are being synthesized at a high rate, plasma amino acid levels might conceivably fall, especially when amino acid absorption is not occurring rapidly due to soybean protein's slow release rate of amino acids.

In this experiment, after feeding soybean protein or milk protein, the amino acids were not absorbed into the portal blood in proportion to their quantity in these proteins. This observation was especially true for soybean protein which can probably be explained by the poor availability of amino acids from this protein or that the rate of absorption of individual amino acids by the small intestine was affected by the presence of other amino acids (Pinsky and Geiger, 1952). Further, it could be that the molar ratios of the amino acids in the intestinal tract are responsible as Delhumeau et al. (1962) have presented data indicating that molar ratios influence the magnitude of the percentage of each amino acid absorbed. The frequent lack of correlation between

increases in amino acid concentrations in the portal plasma and the quantities of these amino acids ingested may be due in part to the effects of the amino acid mixture in the intestinal lumen upon the absorption processes. Goldberg and Guggenheim (1962) have reported that no close correlation exists between the amino acid composition of a given protein and the extent or duration of the rise in concentration of these amino acids in the intestine or plasma.

When considering the overall picture, the results indicate that the portal plasma amino acid pattern responds differently when these two proteins were ingested and that the mechanisms involved are complex and depend upon many factors.

The concentration of the individual circulating free amino acids was considerably lower when pigs were fed a nonprotein meal as compared to those fed either protein source. This decrease in levels of plasma amino acids is probably a continuation of the fasting effect.

After feeding the nonprotein meal, the intestinal free amino acids increased in concentration from the duodenum to the jejunum and declined in the ileum. This indicates the presence of endogenous proteins which were being autodigested and absorbed into the portal blood system as the portal free amino acids were higher in concentration as compared with the vena cava. No evidence is available to determine the quantity of endogenous protein present before feeding, but the average total nitrogen recovered from the small intestine was 0.14 gms. (Table 16), the equivalent of 0.9 gms. of protein, suggesting that the amount of endogenous proteins secreted

into and sloughed from the intestinal mucosa may be considerable, in agreement with published reports by Nasset et al. (1955); Nasset and Soon Ju (1961).

The degree of proteolysis which occurs in the different segments of the small intestine depends upon several factors: -- the amino acid composition of the protein, the rate at which the protein travels the length of the small intestine, the rate and degree of release of free amino acids from the protein during digestion, the effective secretion of gastric and intestinal enzymes and hormones, and the pH of the overall mixture.

Quantitative analysis of each segment of the small intestine showed gross differences in the concentrations of the free amino acids when dried skim milk protein was ingested as compared to soybean protein. This comparison suggests that the gastric and intestinal enzymes are rather effective in digesting dried skim milk protein as compared to that of soybean protein. Most of the intestinal amino acids were highest in concentration at either two or three hours after feeding, further indicating that the various enzymes were effectively promoting proteolysis of dried skim milk protein with increasing time. The rise in the portal plasma essential amino acid levels at two and three hours after feeding corresponds to the high levels of intestinal free amino acids present at these same time periods, further suggesting that rapid amino acid absorption occurs from the small intestine into the portal blood system. Most of the amino acids increased in concentration from the duodenum to the jejunum and

then declined in concentration in the ileum indicating that rapid amino acid absorption was occurring in the jejunum or in the upper parts of the ileum.

The relatively low level of free amino acids occurring in the different segments of the small intestine after ingesting soybean protein suggests that the gastric and intestinal enzymes were not effectively digesting this protein. Acute deficiencies of dietary essential amino acids in the rat have reduced pancreatic and intestinal enzyme activities which would impair the digestibility of ingested proteins (Lyman and Wilcox, 1963a; 1963b). Lack of methionine, valine, tryptophan, leucine, and isoleucine depressed pancreatic protease and lipase activities whereas lack of lysine and threonine depressed the enzyme activities to a lesser extent. It is well known that soybean protein is deficient in methionine and possibly lysine, threonine, and valine as compared to the young pig's requirement for these amino acids. It is conceivable that the poor digestion of soybean protein at an early age in the young pig is due to enzyme insufficiency (Lewis et al., 1955; 1957) through lack of dietary essential amino acids from soybean protein. The deficient missing amino acid(s) for maintaining the activity of the digestive enzymes in the pancreas would have to come from the tissues, which has been demonstrated by Charkey et al., (1954), rather than the diet thereby placing stress upon the animal and preventing maximum growth from occurring. The low digestibility of soybean protein may be in part due to its insolubility along with peptide bonds that are resistant to proteolytic attack. Chen



et al. (1962) have suggested that zein's low digestibility may be due to its insolubility and perhaps soybean protein would be similar in this respect.

The highest concentration of amino acids occurred in the jejunum and ileum with small differences between the two segments. No rise occurred in the portal plasma essential amino acid levels within the time intervals after feeding soybean protein, suggesting poor absorption of the free amino acids from the intestinal lumen into the portal blood system. The highest concentration of intestinal free amino acids occurred at three hours after feeding indicating that the rate of proteolysis of soybean protein is relatively slow and that a maximum rate had not yet been obtained.

The gross differences in the free plasma and intestinal amino acid concentrations between pigs fed dried skim milk protein and soybean protein appear to be much greater than what could be attributed to dietary differences. Earlier work (Nasset et al., 1955) suggested that the qualitative free amino acid mixture of the small intestine is not greatly altered by the type of meal fed. The ingested protein is mixed with large quantities of endogenous protein (autodigestion of hydrolytic enzymes and other proteins secreted into the small intestine and sloughed mucosa) which provide enough endogenous amino acids to obliterate the unique amino acid composition of the ingested food protein. It would appear from these studies that considerable endogenous amino acids are added to the mixture in the intestinal lumen and probably account for a major portion of the free amino acids present in the intestine. However, the fact remains that

there are gross differences in the total amount of free amino acids in the intestine and the plasma between these two protein sources suggesting that the amino acid pattern and physical or inherent characteristics of the ingested food protein have major influences upon the processes of protein digestion and amino acid absorption.

For purposes of comparing the plasma free amino acid changes with the amino acid composition of the ingested protein, most researchers (Frame, 1958; Longnecker and Hause, 1959; and Ganapathy and Nasset, 1962) have employed a period of fasting in order to exhaust the supply of free blood plasma amino acids contributed by any previously ingested food protein. Ganapathy and Nasset (1962) have stated that the body tissues tend to maintain a relatively uniform mixture of free amino acids in the plasma during periods of high quality protein intake. Puchal (1962) has theorized that the normal digestive physiology of the young pig allowed food ad libitum is accomplished through short feeding periods separated by short resting intervals of usually one or two hours duration. Further, it was believed that a young pig under normal feeding conditions would tend to have a uniform rate of incorporation of amino acids into the blood stream in view of the slow rate of food passage from the stomach to the small intestine and the different rate of release of the amino acids from the food proteins (Geiger et al., 1952) as well as the different rates of absorption of the amino acids (Wiseman, 1955; Friedlander and Quastrel, 1955).

Since a major proportion of the plasma amino acid data has been determined from pigs allowed food proteins ad libitum, the time had arrived

to evaluate the "dynamic" amino acid equilibrium maintained by pigs allowed food ad libitum and to compare this with animals fed a food protein following a period of fasting.

The essential and nonessential plasma free amino acids generally declined in concentration with time when the two protein sources were allowed ad libitum (Experiment 6302). Part of this effect can be attributed to the 10 to 15 percent plasma volume dilution especially at the six and eight hour intervals which should account for some of the decline in concentration. However, threonine and lysine showed definite increases in plasma concentration at two and four hours after feeding, when dried skim milk protein was ingested and valine and lysine responded similarly when soybean protein was fed. Even though some changes occurred in concentration of the plasma essential amino acids, these data suggest that the body tissues act homeostatically in maintaining a relatively constant level of circulating blood plasma free amino acids when food proteins are allowed ad libitum further lending validity to the plasma free amino acid interpretations by Puchal (1962) and to the amino acid supplemented experiments reported herein.

The essential and nonessential blood plasma free amino acids generally declined in concentration with time when the nonprotein diet was allowed ad libitum to pigs previously fed soybean or milk protein. Again, some decrease in the plasma amino acid concentration may be attributed to the plasma volume dilution but a larger amount is probably from the lack of food protein as these animals were ingesting a nonprotein

ration 36 hours prior to obtaining the initial blood specimen, therefore essentially in a state of fasting. Glycine and alanine were the only two amino acids that increased in plasma concentration as compared to the levels of these two amino acids when the two protein sources were fed. An increase in any amount in the concentration of these two amino acids during the ingestion of a nonprotein ration is indicative of arising from an endogenous source.

After fasting for 18 hours and then control feeding either soybean or milk protein, irregular changes appeared in the plasma amino acid levels especially for the latter protein source at two and four hours after feeding. These changes in plasma amino acid levels did not correspond with the relative amino acid composition of either ingested protein and varied with time. Similar results have been reported to occur in humans (Frame, 1958).

After control feeding the pigs milk protein, some of the essential plasma free amino acids increased in concentration at two and four hours indicative of the rapid rate of proteolysis and amino acid absorption occurring as observed in Experiment 1136. On the other hand, the essential plasma free amino acids did not begin to increase quantitatively until six and eight hours after feeding soybean protein, again indicative of the slow rate of proteolysis and ineffective amino acid absorption occurring as observed in Experiment 1136. The essential plasma free amino acids did not increase in concentration above the fasting level (0 hour) when soybean protein was control fed as compared to those fed

milk protein, further suggesting that soybean protein was not utilized efficiently. Part of the decrease in concentration of plasma free amino acids at two and four hours after ingesting either protein source may be due to the synthesis of enzymes and other proteins contained in the digestive juices even though some amino acid absorption may occur simultaneously due to protein digestion.

Following the controlled ingestion of the nonprotein diet, the majority of the essential and nonessential plasma free amino acids decreased in concentration when pigs had previously been fed milk or soybean protein. The fasting plasma amino acid levels (0 hour) appeared to be higher in pigs previously fed soybean protein as compared to those fed milk protein indicating that fasting plasma free amino acid levels are influenced by a previous feed regimen.

It has been theorized by Hill and Olsen (1963b) that a rapid breakdown of tissue protein during starvation results from a deficiency of available energy, therefore chicks that are not completely fasted and fed a nonprotein diet readily available in energy should not exhibit elevated levels of plasma free amino acids. The results of the 18-hour fast (Experiment 6302) and then feeding a readily available carbohydrate diet supports this hypothesis for young pigs also, as almost all the plasma free amino acids decreased in concentration at two and four hours after feeding and then began to slowly rise even though some plasma volume dilution occurred. From these data, it appears that the body tissues or body protein reserves contribute substantially to the plasma free amino acid levels during fasting or in a state of energy need.

Nasset (1962) has reported that free amino acids in intestinal contents were much higher when dogs ingested lean beef as compared to lard, however the relative amino acid molar ratios were not greatly different indicating that endogenous proteins contribute a major portion of the free amino acids present.

After employing an 18-hour fast and feeding a nonprotein diet, neither the free amino acids in the intestinal contents (Experiment 1136) nor in the plasma (Experiments 1136 and 6302) approached the amino acid values observed when an intact protein was ingested. It would appear that if the secretion of endogenous proteins is to the extent reported by Nasset (1962) then when a nonprotein diet is ingested the total amount of free amino acids should not be greatly different in the intestinal contents and some rise in amino acid concentrations should occur in the plasma due to autodigestion of the endogenous proteins. This did not occur (Experiments 1136 and 6302), therefore, intact protein per se must have major influences upon the protein digestion processes. Nasset (1962) and Nasset and Soon Ju (1961) have used mature dogs with jejunostomies under acute conditions whereas in the experiments reported herein young pigs were employed which also may account for some of the differences.

Protein nutritionists have long been searching for a rapid and accurate method of determining amino acid availability. Longnecker and Hause (1961) have developed a shortened procedure of measuring amino acid availability, however, time of analysis of free blood plasma amino

acids remains relatively long and the procedure of Longnecker and Hause (1961) has not been developed to a high degree of accuracy.

Experiment 6326 was designed to test a relatively quick method of determining amino acid availability by using three protein levels within a protein source and then combining two protein sources within the respective protein levels. The differences in amino acid concentrations between protein levels (Ritchey and Richardson, 1949) within protein sources should give a measure of the availability of the amino acids from the ingested protein. However, little differences in concentrations were observed in the blood plasma free amino acids between protein levels and within protein sources. The data in Experiments 1136 and 6302 indicated a much higher level of plasma free amino acids when dried skim milk protein was ingested as compared to soybean protein but the plasma amino acid data in this experiment did not suggest this relationship. Perhaps the reason no differences in plasma amino acid data were observed is that blood samples were obtained at four hours after feeding. Data from Experiment 6302 had not been summarized when this experiment was conducted and four hours after feeding was too long a time to observe the peak amino acid absorption time for milk protein and the amino acids from soybean protein were just beginning to be absorbed at this time as shown by the data in Experiment 6302. By using the method of Hill and Olsen (1963b) which is a modification of Longnecker and Hause's method (1959), the design of this experiment suggests a quick method for determining amino acid availability.

Feed efficiency and growth rate followed expected trends except when soybean protein was fed alone. When soybean protein levels were linearly increased, growth rate and feed efficiency linearly decreased which is unaccountable.



## SUMMARY

Eight experiments involving 206 young pigs were conducted to study the effects of amino acid supplementation on plasma amino acid patterns and growth rate, the degree of proteolysis of milk protein and soybean protein occurring in the small intestine with time, the absorption of amino acids into the blood stream after ingesting a protein meal, and the plasma free amino acid comparison between portal and anterior vena cava blood.

The plasma amino acid concentrations and the changes in those patterns that resulted from amino acid supplementation of the diet were used to predict amino acid deficiencies. Methionine deficiency of soybean protein was found to result in a markedly depressed concentration of this amino acid in the blood plasma. Methionine deficiency of soybean protein was shown to be corrected by "free" amino acid supplementation through faster growth rate and increased blood plasma methionine concentration. After correcting for the first limiting amino acid (methionine), other free plasma amino acids decreased in concentration. Threonine and valine declined to a greater extent than other amino acids, suggesting that these were the next limiting amino acids hindering maximum growth rate. This observation was confirmed by the improved growth performance when valine and threonine were added to soybean protein.

The addition of three essential amino acids (methionine, valine, and threonine) to soybean protein resulted in significant changes in the free blood plasma amino acid patterns, thus reflecting a change in intestinal absorption or utilization of these and other amino acids by the various tissues as compared to that of pigs fed unsupplemented soybean protein.

However, these changes in the plasma are so profound that the methodology does not appear to be sensitive enough to accurately point out the next limiting amino acid.

Quantitative differences were noted in plasma free amino acid levels when milk protein was ingested as compared to soybean protein. Two hours after feeding milk protein, the essential portal plasma free amino acids increased markedly, suggesting a rapid rate of proteolysis and amino acid absorption for milk protein as compared to soybean protein.

Gross differences in free amino acid concentrations occurred in each segment of the small intestine with time indicating a high and slow rate of proteolysis for milk protein and soybean protein, respectively, and further suggesting that the digestive enzymes were promoting the effective proteolysis of dried skim milk protein but were less effective in hydrolyzing soybean protein. The difference in total intestinal free amino acids between pigs fed these two protein sources was too great to have resulted entirely from the difference in composition of the ingested food protein suggesting a marked difference in degree of hydrolysis. After feeding a nonprotein diet, free amino acids were detected in each segment of the small intestine confirming the presence of endogenous proteins being autodigested. Again, the differences in intestinal free amino acid concentrations resulting from ingesting a food protein and a non protein diet were large.

Portal plasma free amino acid concentrations were invariably higher, quantitatively, than anterior vena cava plasma free amino acids regardless of protein source ingested. Since the portal-vena cava plasma amino acid

ratios did not vary significantly from 1.0, previous published reports were confirmed that anterior vena cava blood is appropriate for amino acid nutrition investigations.

After feeding soybean or milk protein ad libitum and following the pattern of the free plasma amino acids during an 8-hour period, the majority of the plasma free amino acids remained relatively constant further confirming the validity of previous reported plasma free amino acid data concerning amino acid supplementation studies. When a non-protein diet was allowed ad libitum, the plasma free amino acids were reduced in concentration probably due to the continuation of the fasting state.

After an 18-hour fast and control feeding the two protein sources, irregular changes appeared in the plasma free amino acid pattern during an 8-hour period. These changes did not correspond to the relative amino acid composition of the ingested protein and the relative concentrations of the different plasma free amino acids did not remain constant. However, some of the plasma free amino acids increased in concentration at two hours after ingesting milk protein indicative of effective proteolysis and amino acid absorption for this protein, whereas six hours were required before any increase in plasma free amino acid concentrations were observed when soybean protein was ingested.

When a nonprotein diet was control fed, the plasma free amino acid concentrations were depressed significantly at two and four hours suggesting that during fasting, energy is a major requirement resulting in rapid breakdown of body proteins.

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**APPENDIX**

Table 1. Experiments 1143, 1153, 1159, and 1175 - Composition of experimental diets

Ingredient	Experiment	
	1143 - 1153 - 1159	1175
Soybean meal	37.00	37.00
Lard, stabilized	2.00	2.00
Sucrose	14.00	14.00
Lactose	36.00	36.00
Wood flock	1.00	1.00
Corn starch	4.00	3.00
Amino acid carrier <sup>a</sup>	2.00	3.00
Vitamin premix <sup>b</sup>	1.00	1.00
Calcium carbonate	0.40	0.40
Dicalcium phosphate	1.80	1.80
Iodized salt	0.60	0.60
Trace mineral premix <sup>c</sup> (35-C-41)	<u>0.20</u>	<u>0.20</u>
Total <sup>d</sup>	100.00	100.00

<sup>a</sup>Amino acid composition given in text, KCl added at the level of 0.47 pounds and ammonium citrate substituted for sucrose.

<sup>b</sup>Composition given in Table 2.

<sup>c</sup>Composition given in Table 3.

<sup>d</sup>Calculated analysis is given in Table 4.



Table 2. Experiments 1143, 1153, 1159, and 1175 - Amounts of vitamins and antibiotic added per pound of complete diet

Source	Unit	Experiment
		1143 - 1153 - 1159 - 1175
Vitamin A	I.U./lb.	3000
Vitamin D <sub>2</sub>	I.U./lb.	500
Riboflavin	mg./lb.	5.0
Pantothenic acid	mg./lb.	8.0
Niacin	mg./lb.	26.5
Choline	mg./lb.	19.0
Vitamin B <sub>12</sub>	mcg./lb.	20.0
Vitamin E	mg./lb.	10.0
Menadione (Vitamin K)	mg./lb.	0.5
Thiamine	mg./lb.	3.0
Pyridoxine	mg./lb.	0.5
Folic acid	mcg./lb.	450
Biotin	mcg./lb.	3.0
Chlortetracycline	mg./lb.	50.0

Table 3. Composition of trace mineral mix (35-C-41)

Ingredient	Percent in premix element	Parts per million contributed to ration by 0.20%
Manganese sulphate (25.4% Mn)	5.68	113.6
Ferrous sulphate (20% Fe)	7.00	140.0
Copper sulphate (25% Cu)	0.48	9.5
Cobalt sulphate (33% Co)	0.17	3.3
Zinc sulphate (36% Zn)	8.10	162.0
Potassium sulphate (44.8% K)	0.75	15.0
Calcium carbonate (39% Ca)	5.28	10.6

Table 4. Experiments 1143, 1153, 1159, and 1175 - Calculated analysis of the complete diets

Constituent	Unit	Experiment
		1143 - 1153 - 1159 - 1175
Protein	%	18.52
Fat	%	2.18
Fiber	%	2.12
Calcium	%	0.70
Phosphorus	%	0.56
Vitamin:		
Vitamin A	I.U./lb.	3000
Vitamin D <sub>2</sub>	I.U./lb.	500
Riboflavin	mg./lb.	5.0
Pantothenic acid	mg./lb.	10.0
Niacin	mg./lb.	30.0
Choline	mg./lb.	500
Vitamin B <sub>12</sub>	mcg./lb.	20.0
Vitamin E	mg./lb.	10.0
Menadione (Vitamin K)	mg./lb.	0.5
Thiamine	mg./lb.	3.0
Pyridoxine	mg./lb.	2.0
Folic acid	mcg./lb.	549
Biotin	mcg./lb.	3.0
Chlortetracycline	mg./lb.	50.0

Table 5. Experiment 1143 - Summary of total gains and feed required per pound of gain

Replication	Treatment				
	SBM	Met	Met + Val	Met + Thr	Met + Val + Thr + Lys
<u>Total gains (lb.)</u>					
1	17.3	17.1	24.4	20.4	20.8
2	26.6	25.6	26.6	26.8	24.8
3	20.6	24.6	17.2	24.8	18.5
4	22.9	21.0	27.1	25.2	24.8
5	26.9	28.9	24.8	27.3	27.8
6	25.0	24.5	23.4	23.7	23.5
7	26.3	27.7	20.8	20.8	26.4
Average	23.7	24.2	23.5	24.1	23.8
<u>Feed/gain</u>					
1	1.91	1.82	1.53	1.49	1.49
2	1.71	1.56	1.74	1.44	1.58
3	1.81	1.72	1.98	1.58	1.68
4	1.74	1.71	1.47	1.66	1.62
5	1.61	1.38	1.63	1.50	1.56
6	1.73	1.48	1.63	1.57	1.54
7	1.52	1.48	1.52	1.56	1.66
Average	1.72	1.59	1.66	1.54	1.59

Table 6. Experiment 1153 - Summary of total gains and feed required per pound of gain

Replication	Treatment				
	SBM	SBM + Met	SBM + Met + Val	SBM + Met + Thr	SBM + Met + Val + Thr + Lys
<u>Total gains (lb.)</u>					
1	25.9	25.4	27.3	24.6	28.0
2	20.4	19.8	23.3	19.2	25.0
3	25.7	20.7	20.2	25.4	16.1
4	19.9	21.6	23.4	24.5	18.9
5	17.5	24.1	24.2	23.3	16.2
6	16.6	21.9	25.1	22.5	24.4
7	21.7	22.1	19.6	25.4	20.4
Average	21.1	22.2	23.3	23.6	21.3
<u>Feed/gain</u>					
1	1.25	1.52	1.64	1.64	1.78
2	1.59	1.55	1.58	1.50	1.54
3	1.67	1.35	1.41	1.56	2.14
4	1.78	1.52	1.46	1.60	1.63
5	1.73	1.52	1.41	1.51	1.55
6	1.58	1.49	1.50	1.46	1.58
7	1.60	1.59	1.64	1.51	1.53
Average	1.60	1.51	1.52	1.54	1.68

Table 7. Experiment 1153 - Analysis of variance of total gains and feed required per pound of gain

Source	d. f.	Mean squares	
		Total gains	Feed/gain
Totals	34	9.9264	0.0214
Replications	6	15.6352	0.0060
Treatments	4	8.8447	0.0351
SBM vs. SBM - Met, Met + Val, Met + Thr, Met + Val + Thr + Lys	1	12.4803	0.0083
SBM - Met vs. SBM - Met + Val, Met + Thr, Met + Val + Thr + Lys	1	1.2386	0.0275
SBM - Met + Val vs. SBM - Met + Thr, Met + Thr + Val + Lys	1	3.6021	0.0372
SBM - Met + Thr vs. SBM - Met + Val + Thr + Lys	1	18.0579	0.0672
Error	24	8.6795	0.0230

Table 8. Experiment 1153 - Summary of free blood plasma amino acid concentration<sup>a</sup>

Amino acid	Treatment				
	SBM	Met	Met + Val	Met + Thr	Met + Val + Thr + Lys
Taurine	1.33	3.00	3.68	5.86	2.24
Urea	21.11	18.39	23.58	27.12	16.51
Aspartic acid	0.40	0.53	0.50	0.61	0.53
Threonine	2.46	3.70	5.18	9.06	7.80
Serine	3.03	2.62	3.17	3.69	3.50
Glutamic acid	2.31	3.44	3.10	3.71	3.71
Citrulline	1.95	1.44	1.41	1.41	1.08
Proline	4.67	3.39	3.52	4.12	3.85
Glycine	8.42	7.88	7.46	8.70	8.75
Alanine	6.77	5.30	4.89	6.19	5.88
Cystine	0.85	0.83	1.05	1.08	1.15
Valine	5.24	3.94	7.73	5.22	9.60
Methionine	0.24	0.72	0.74	0.88	0.67
Isoleucine	3.65	2.86	3.11	3.72	3.26
Leucine	3.45	2.22	2.68	3.04	2.90
Tyrosine	2.83	2.07	2.28	2.31	2.24
Phenylalanine	1.66	1.27	1.54	1.69	1.58
Ornithine	3.27	2.67	2.60	3.13	3.12
Lysine	4.40	2.04	2.94	2.45	4.63
Histidine	1.84	1.35	1.65	1.76	1.72
Ammonia	1.08	0.87	0.98	1.10	1.13
Arginine	4.55	3.43	4.37	4.23	3.94

<sup>a</sup>Amino acid concentration expressed as mg./100 ml.

Table 9. Experiment 1159 - Summary of total gains and feed required per pound of gain

Replication	Treatment					
	SBM	Met	Met + Val	Met + Thr	Met + Val + Thr	Met + Thr + Val + Lys + Iso
<u>Total gain (lb.)</u>						
1	18.5	26.9	29.1	21.7	27.4	21.6
2	26.3	28.1	23.5	24.1	27.6	26.7
3	24.3	26.9	23.7	29.2	28.5	24.4
4	24.2	25.4	28.7	27.0	26.9	24.5
5	20.6	18.9	23.7	22.2 <sup>a</sup>	24.4	21.5
6	17.6	22.3	24.0	22.9	20.5	16.5
Average	21.9	24.8	25.4	24.5	25.9	22.5
<u>Feed/gain</u>						
1	1.58	1.37	1.35	1.59	1.44	1.44
2	1.47	1.47	1.93	1.41	1.36	1.48
3	1.49	1.39	1.41	1.70	1.41	1.47
4	1.56	1.60	1.56	1.67	1.53	1.49
5	1.57	1.50	1.54	1.63 <sup>a</sup>	1.51	1.48
6	1.52	1.52	1.48	1.58	1.43	1.35
Average	1.53	1.48	1.54	1.60	1.45	1.45

<sup>a</sup>Estimated value (Snedecor, 1956, page 310).



Table 10. Experiment 1159 - Analysis of variance of total gains and feed required per pound of gain

Source	d. f.	Mean squares	
		Total gains	Feed/gain
Totals	35	11.0699	0.0128
Replications	5	34.8578	0.0102
Treatments	5	15.3438*	0.0214
SBM vs. SBM - Met, Met + Val, Met + Thr, Met + Thr + Val, Met + Val + Thr + Lys + Iso	1	36.7205**	0.0041
SBM + Met vs. SBM - Met + Val, Met + Thr, Met + Val + Thr, Met + Val + Thr + Lys + Iso	1	0.1140	0.0059
SBM - Met + Val vs. SBM - Met + Thr, Met + Val + Thr, Met + Val + Thr + Lys + Iso	1	5.8368	0.0098
SBM - Met + Thr vs. SBM - Met + Thr + Val, Met + Thr + Val + Lys + Iso	1	0.3803	0.0870**
SBM - Met + Thr + Val vs. SBM - Met + Thr + Val + Lys + Iso	1	33.6675**	--
Error	25	5.4576	0.0116

\*Indicates significant difference (P = 0.05 or less).

\*\*Indicates significant difference (P = 0.01 or less).

Table 11. Experiment 1159 - Summary of free blood plasma amino acid concentration<sup>a</sup>

Amino acid	Treatment					
	SBM	Met	Met + Val	Met + Thr	Met + Val + Thr	Met + Val + Thr + Lys + Iso
Taurine	0.46	1.31	1.14	1.84	1.97	1.26
Urea	27.01	18.68	23.43	29.73	20.26	18.44
Aspartic acid	0.31	0.34	0.39	0.39	0.36	0.40
Threonine	6.53	4.42	3.74	9.69	8.66	8.60
Serine	5.01	2.87	2.54	3.66	3.68	2.51
Glutamic acid	2.35	2.56	3.03	2.96	3.18	4.33
Citrulline	2.34	1.14	1.63	1.62	1.29	1.31
Proline	5.92	3.82	3.86	4.55	4.77	4.27
Glycine	6.44	6.90	6.68	7.46	8.93	6.32
Alanine	9.42	6.64	6.92	6.65	6.28	8.96
Cystine	0.73	0.65	0.71	0.87	1.13	0.94
Valine	5.33	3.37	9.17	4.20	8.29	9.80
Methionine	0.28	1.00	0.93	1.22	1.59	1.38
Isoleucine	3.99	3.13	3.34	3.36	4.72	4.75
Leucine	3.47	2.30	2.39	2.41	3.82	2.65
Tyrosine	3.24	2.49	2.52	2.42	2.50	2.62
Phenylalanine	1.82	1.57	1.41	1.58	1.69	1.63
Ornithine	3.08	2.06	2.25	2.89	2.67	2.36
Lysine	5.10	2.49	1.51	2.79	2.51	3.00
Histidine	2.23	1.44	1.34	1.52	1.57	1.55
Arginine	5.55	3.73	3.70	5.10	4.73	4.14

<sup>a</sup>Amino acid concentrations expressed as mg./100 ml.

Table 12. Experiment 1175 - Summary of total gains and feed required per pound of gain

Replication	Treatment			
	SBM + Met	SBM + Met + Thr + Val	SBM + Met + Thr + Val + Pro	SBM + amino acid combination <sup>a</sup>
	<u>Total gains (lb.)</u>			
1	26.1	21.7	23.9	18.9
2	22.0	23.7	24.5	24.2
3	31.3	29.8	33.0	27.6
4	27.3	25.6	31.9	26.1
5	19.9	19.3	22.3	27.2
6	24.2	25.4	21.7	23.0
7	21.5	22.5	29.2	25.3
8	17.2	21.7	17.2	25.0
9	19.1	13.7	21.6	19.2
Average	23.2	22.6	25.0	24.1
	<u>Feed/gain</u>			
1	1.43	1.45	1.47	1.47
2	1.57	1.53	1.49	1.49
3	1.54	1.58	1.62	1.43
4	1.55	1.76	1.57	1.55
5	1.47	1.52	1.36	1.58
6	1.57	1.49	1.54	1.51
7	1.52	1.66	1.64	1.58
8	1.44	1.56	1.71	1.52
9	1.52	1.66	1.48	1.45
Average	1.51	1.58	1.54	1.51

<sup>a</sup>Amino acid combination included proline and all essential amino acids except arginine.

Table 13. Experiment 1175 - Analysis of variance of total gains and feed required per pound of gain

Source	d. f.	Mean squares	
		Total gain	Feed/gain
Totals	35	18.6740	0.0069
Replications	8	52.7769**	0.0098
Treatments	3	10.1574	0.0095
SBM - Met vs. SBM - Met + Thr + Val, Met + Thr + Val + Pro, amino acid combination	1	3.4848	0.0065
SBM - Met + Thr + Val vs. SBM - Met + Thr + Val + Pro, amino acid combination	1	22.6852	0.0171
SBM - Met + Thr + Val + Pro vs. SBM - amino acid combination	1	4.3022	0.0050
Error	24	8.3710	0.0056

\*\*Indicates significant difference ( $P = 0.01$  or less).

Table 14. Experiment 1175 - Summary of free blood plasma amino acids (mg./100 ml.)

Amino acid	SBM + Met	SBM + Met + Thr + Val	SBM + Met + Thr + Val + Pro	SBM + amino acid combination <sup>a</sup>
Taurine	1.58	1.76	3.01	1.61
Urea	31.94	25.86	34.73	28.63
Aspartic acid	0.26	0.38	0.35	0.42
Threonine	2.48	6.23	6.50	5.22
Serine	1.82	2.80	2.90	3.08
Glutamic acid	2.40	3.11	3.07	3.82
Citrulline	1.03	1.38	1.42	1.04
Proline	2.55	4.63	4.98	3.71
Glycine	4.98	6.83	6.75	8.97
Alanine	4.74	9.38	8.31	7.46
Cystine	0.72	1.28	1.36	1.15
Valine	2.96	7.17	6.98	4.04
Methionine	1.58	1.68	2.26	1.63
Isoleucine	2.18	3.29	3.29	3.00
Leucine	1.84	2.53	2.30	3.28
Tyrosine	1.42	1.74	2.11	1.72
Phenylalanine	1.15	1.34	1.48	1.70
Ornithine	1.82	2.30	2.34	2.12
Ammonia	0.88	0.92	1.18	0.94
Lysine	1.30	2.28	1.74	3.20
Histidine	1.04	1.34	1.38	1.62
Arginine	3.00	4.18	4.23	3.50

<sup>a</sup>Amino acid combination included proline and all essential amino acids except arginine.

Table 15. Experiments 1136 and 6302 - Composition of the experimental diets<sup>a</sup>

Ingredient	Experiments 1136 - 6302		
	Dried skim milk	Soybean meal	Nonprotein diet
Ground yellow corn	9.00	9.00	--
Dried skim milk	58.30	--	--
Soybean meal	--	39.65	--
Lactose	--	30.00	82.10
Sucrose	5.00	5.60	10.00
Wood flock	1.70	0.55	2.00
Stabilized lard	2.00	2.10	--
Corn starch	21.50	7.45	--
Potassium chloride	--	0.50	--
MgCl <sub>2</sub> ·6H <sub>2</sub> O	0.30	--	--
Vitamin premix <sup>b</sup>	2.00	2.00	2.00
Calcium carbonate	--	--	--
Dicalcium phosphate	--	1.70	3.20
Iodized salt	--	0.75	0.50
Trace mineral mix <sup>c</sup> (35-C-41)	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>
Total	100.00	100.00	100.00

<sup>a</sup>Calculated analysis given in Table 18.<sup>b</sup>Composition given in Table 17.<sup>c</sup>Composition given in Table 3.

Table 16. Experiment 1136 - Summary of total gain, feed consumption, dry matter and nitrogen recovered from the small intestine at different hours after a test meal

Pig number	Treatment	Postprandial time (hrs.)	Total gain (lbs.)	Test meal consumed (gms.)	Total dry matter of small intestine (gms.)	Total nitrogen recovered from small intestine (gms.)
1362S	DSM <sup>a</sup>	1	1.4	154	--b	--b
1360S	SBM <sup>c</sup>	1	0.0	121	--b	--b
1376B	SBM	1	-0.5	76	--b	--b
1370S	DSM	1	1.5	104	--b	--b
1364S	SBM	2	-0.1	142	13.86	0.62
1365B	DSM	2	1.9	216	6.03	0.39
1378B	SBM	2	-0.5	115	19.40	0.67
1375B	DSM	2	0.6	101	20.08	1.01
1361S	SBM	3	0.1	158	12.50	0.62
1366B	DSM	3	1.4	230	16.89	0.74
1377B	DSM	3	0.8	175	9.73	0.53
1379B	SBM	3	-0.2	107	13.99	0.56
1391S	DSM-NPD <sup>d</sup>	3	0.9	141	8.76	0.13
1399B	SBM-NPD	3	-1.6	64	8.66	0.15

<sup>a</sup>Dried skim milk.

<sup>b</sup>Values not calculated.

<sup>c</sup>Soybean meal.

<sup>d</sup>Nonprotein diet (pigs previously fed soybean or milk protein).

Table 17. Experiments 1136 and 6302 - Amounts of vitamins added per pound of complete diet

Source	Unit	Dried skim milk	Soybean meal	Nonprotein diet
Vitamin A	I.U./lb.	3000	3000	3000
Vitamin D <sub>2</sub>	I.U./lb.	500	500	500
Riboflavin	mg./lb.	0.0	3.0	5.0
Pantothenic acid	mg./lb.	1.1	8.0	10.0
Niacin	mg./lb.	26.2	25.5	30.0
Choline	mg./lb.	19.1	0.0	500
Vitamin B <sub>12</sub>	mcg./lb.	20.0	20.0	20.0
Vitamin E	mg./lb.	10.0	10.0	10.0
Menadione (Vitamin K)	mg./lb.	0.5	0.5	0.5
Thiamine	mg./lb.	3.0	3.0	3.0
Pyridoxine	mg./lb.	2.0	0.5	2.0
Folic acid	mcg./lb.	325	450	500
Biotin	mcg./lb.	3.0	3.0	3.0



Table 18. Experiments 1136 and 6302 - Calculated analysis of the completed diets

Constituent	Unit	Dried skim milk	Soybean meal	Nonprotein diet
Protein	%	20.72	20.70	0.00
Fat	%	2.67	3.19	0.00
Fiber	%	1.96	1.98	2.00
Calcium	%	0.70	0.70	0.70
Phosphorus	%	0.55	0.55	0.55
Vitamins:				
Vitamin A	I.U./lb.	3000	3000	3000
Vitamin D <sub>2</sub>	I.U./lb.	500	500	500
Riboflavin	mg./lb.	5.3	5.5	5.0
Pantothenic acid	mg./lb.	10.0	10.0	10.0
Niacin	mg./lb.	30.0	30.0	30.0
Choline	mg./lb.	500	535	500
Vitamin B <sub>12</sub>	mcg./lb.	20.0	20.0	20.0
Vitamin E	mg./lb.	10.0	10.0	10.0
Menadione (Vitamin K)	mg./lb.	0.5	0.5	0.5
Thiamine	mg./lb.	3.0	3.0	3.0
Pyridoxine	mg./lb.	2.0	2.4	2.0
Folic acid	mcg./lb.	500	500	500
Biotin	mcg./lb.	3.0	3.0	3.0

Table 19. Experiment 1136 - Summary of plasma and intestinal amino acids occurring one hour after ingesting the dried skim milk ration or soybean meal ration<sup>a</sup>

Protein source	Dried skim milk					Soybean meal				
	Vena cava	Portal	Duo-denum	Jejunum	Ileum	Vena cava	Portal	Duo-denum	Jejunum	Ileum
Cysteic acid	--b	--b	9.92	17.15	9.95	--b	--b	0.48	1.08	2.08
Taurine	1.50	2.13	--b	--b	--b	2.08	1.68	--b	--b	--b
Urea	24.41	30.34	--b	--b	--b	30.94	32.24	--b	--b	--b
Aspartic acid	0.18	0.56	3.25	10.56	15.72	0.38	1.95	1.84	2.28	8.83
Threonine	5.16	5.04	6.88	15.18	13.92	3.36	4.40	2.06	3.80	8.01
Serine	2.12	2.62	6.58	10.70	9.29	2.28	3.21	1.87	3.17	5.76
Glutamic acid	2.10	5.20	12.48	45.44	75.26	4.02	10.01	5.03	12.75	26.22
Citrulline	1.08	0.94	--b	--b	--b	1.29	1.61	--b	--b	--b
Proline	6.74	7.41	4.87	12.89	21.82	2.50	3.14	0.85	2.50	8.02
Glycine	5.22	5.37	3.71	8.32	13.83	4.45	6.19	1.96	4.60	9.02
Alanine	7.00	7.74	8.31	12.12	10.12	3.16	6.80	2.86	4.91	8.34
Cystine	0.84	0.97	0.47	1.56	1.50	0.52	0.86	2.00	2.20	1.44
Valine	6.24	6.34	6.28	8.60	7.73	3.08	4.54	1.11	2.55	5.85
Methionine	1.60	1.48	4.40	4.19	2.98	0.37	0.34	0.46	0.81	2.05
Isoleucine	2.62	3.07	4.73	8.84	8.40	1.68	2.39	1.04	1.99	5.22
Leucine	4.93	5.54	19.59	12.88	8.98	1.92	2.61	2.02	4.00	8.84
Tyrosine	4.77	4.58	13.29	6.64	3.32	1.23	1.62	1.52	1.95	4.68
Phenylalanine	3.38	3.33	10.45	6.32	4.23	1.58	1.96	1.38	2.36	5.08
Ornithine	1.76	2.09	--b	--b	--b	1.52	--b	--b	--b	--b
Ammonia	0.78	1.58	2.96	2.64	6.16	1.05	--b	2.10	3.00	6.76
Lysine	7.48	7.98	23.63	23.05	18.42	4.06	4.95	4.42	5.16	11.33
Histidine	2.10	2.28	3.02	7.50	7.84	1.44	2.54	0.90	1.78	3.48
Arginine	3.70	3.07	12.12	12.04	7.42	3.09	2.94	4.84	5.06	10.18

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Values not calculated or undetectable.

Table 20. Experiment 1136 - Summary of plasma and intestinal amino acids occurring two hours after ingesting the dried skim milk ration or soybean meal ration<sup>a</sup>

Protein source	Dried skim milk					Soybean meal				
	Vena cava	Portal	Duo-denun	Jej-urum	Ileum	Vena cava	Portal	Duo-denun	Jej-urum	Ileum
Cysteic acid	--b	--b	14.90	6.00	1.93	--b	--b	1.44	3.02	1.78
Taurine	2.14	3.38	--b	--b	--b	6.14	3.10	--b	--b	--b
Urea	25.52	24.26	--b	--b	--b	29.60	31.73	--b	--b	--b
Aspartic acid	0.57	1.36	8.59	15.65	6.34	0.24	1.06	1.26	0.40	0.35
Threonine	6.09	9.26	17.80	29.60	9.06	2.26	2.88	2.40	4.82	2.58
Serine	2.83	5.14	11.76	15.48	7.39	1.70	2.77	1.08	0.30	1.16
Glutamic acid	3.82	11.75	26.70	74.42	25.54	3.12	8.68	14.38	32.34	29.38
Citrulline	1.38	2.06	--b	--b	--b	1.04	1.00	--b	--b	--b
Proline	8.56	12.74	10.96	30.98	7.77	2.07	3.26	3.25	12.64	9.79
Glycine	4.74	6.56	5.10	21.46	25.49	3.96	5.56	8.80	29.58	15.05
Alanine	8.38	12.82	16.27	18.68	9.94	4.29	5.95	5.40	11.83	9.58
Cystine	0.77	1.59	1.66	2.44	1.37	0.48	0.45	0.90	1.34	0.82
Valine	4.42	9.22	12.62	20.84	6.80	2.16	2.60	2.38	6.06	2.63
Methionine	2.50	3.28	9.96	6.95	2.69	0.27	0.33	0.82	1.96	0.56
Isoleucine	2.86	4.64	14.34	18.57	6.86	1.33	1.57	2.20	6.08	2.63
Leucine	4.99	7.71	39.21	25.70	12.56	1.14	1.68	3.73	11.61	4.14
Tyrosine	6.50	7.79	21.79	8.88	7.30	1.04	1.25	2.85	6.58	2.25
Phenylalanine	4.81	5.75	16.56	14.27	9.23	1.09	1.40	2.36	8.34	3.04
Ornithine	2.48	8.03	--b	--b	--b	3.66	11.32	--b	--b	--b
Ammonia	1.15	1.32	5.06	10.48	7.64	0.82	1.08	6.48	13.56	12.03
Lysine	11.90	15.38	55.78	29.48	21.58	2.66	3.64	7.34	15.00	8.60
Histidine	3.01	4.21	8.82	11.42	4.59	1.18	1.52	2.22	5.08	2.08
Arginine	3.58	5.43	25.04	17.76	22.11	1.88	2.08	5.54	7.16	3.40

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Values not calculated or undetectable.

Table 21. Experiment 1136 - Summary of plasma and intestinal amino acids occurring three hours after ingesting the dried skim milk ration or soybean meal ration<sup>a</sup>

Protein source	Dried skim milk					Soybean meal				
Sample	Vena cava	Portal	Duo-denum	Jejunum	Ileum	Vena cava	Portal	Duo-denum	Jejunum	Ileum
Cysteic acid	--b	--b	7.09	11.83	10.70	--b	--b	1.06	3.58	4.40
Taurine	1.71	1.32	--b	--b	--b	0.70	0.64	--b	--b	--b
Urea	35.41	31.77	--b	--b	--b	27.14	33.72	--b	--b	--b
Aspartic acid	0.32	0.92	6.40	30.76	14.02	0.43	0.52	1.68	9.02	2.08
Threonine	6.98	7.45	11.98	32.68	10.19	2.52	2.90	2.64	12.70	10.05
Serine	2.48	2.48	8.54	23.69	7.84	1.88	2.05	1.68	5.88	7.30
Glutamic acid	3.27	8.86	19.56	105.96	55.58	2.86	4.94	10.07	47.25	71.35
Citrulline	1.40	1.36	--b	--b	--b	1.00	1.47	--b	--b	--b
Proline	9.06	10.25	7.52	29.15	17.36	2.47	2.68	3.70	13.78	20.47
Glycine	3.86	4.38	3.86	16.74	15.37	4.77	4.58	17.42	10.45	15.29
Alanine	8.17	9.35	11.14	22.88	9.60	5.25	4.93	6.19	10.38	10.95
Cystine	1.05	2.63	0.84	5.76	1.36	0.82	0.93	2.07	2.53	1.46
Valine	7.35	7.72	12.21	24.95	8.82	2.68	2.88	3.20	10.66	11.60
Methionine	2.99	2.74	6.35	9.44	2.84	0.42	0.46	1.17	3.02	3.64
Isoleucine	3.63	4.33	9.68	23.19	12.33	1.73	1.98	2.64	9.00	12.26
Leucine	5.54	6.76	26.96	31.58	10.16	1.68	2.12	6.24	14.92	14.70
Tyrosine	6.76	6.94	12.80	10.04	3.70	1.47	1.61	4.27	4.84	5.42
Phenylalanine	3.70	4.07	10.72	14.64	4.58	1.56	1.74	4.36	7.98	8.15
Ornithine	4.46	3.95	--b	--b	--b	2.43	3.61	--b	--b	--b
Ammonia	1.18	1.22	3.72	6.72	7.39	1.01	1.00	7.28	7.70	12.96
Lysine	12.43	13.66	42.31	71.21	14.66	4.26	4.97	10.51	20.20	19.92
Histidine	2.63	2.74	6.18	16.70	4.02	1.51	1.50	2.78	6.24	4.91
Arginine	3.26	3.54	15.89	21.42	7.32	3.96	1.12	11.11	16.07	14.33

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Values not calculated or undetectable.

Table 22. Experiment 1136 - Summary of plasma and intestinal amino acids occurring 3 hours after ingesting a nonprotein diet<sup>a</sup>

Pre-experimental protein	Dried skim milk					Soybean meal				
	Vena cava	Portal	Duo-denum	Jejunum	Ileum	Vena cava	Portal <sup>b</sup>	Duo-denum	Jejunum	Ileum
Cysteic acid	--c	--c	--d	--d	--d	--c		--d	--d	--d
Taurine	--d	--d	--d	--d	--d	--d		--d	--d	--d
Urea	--d	--d	--d	--d	--d	--d		--d	--d	--d
Aspartic acid	0.20	0.81	0.43	1.81	1.50	0.46		1.05	2.48	0.89
Threonine	0.93	1.28	0.85	2.68	1.56	1.82		1.64	3.26	1.01
Serine	0.92	1.58	0.82	2.10	1.19	1.13		1.58	2.93	0.50
Glutamic acid	2.10	5.49	0.73	2.79	1.97	5.48		1.87	2.99	1.41
Citrulline	0.60	1.06	--c	--c	--c	0.56		--c	--c	--c
Proline	1.06	1.81	0.73	2.05	1.16	1.65		1.17	2.18	1.12
Glycine	2.90	4.17	0.76	2.02	1.79	2.18		1.46	3.59	2.19
Alanine	2.54	4.03	1.62	3.58	2.11	2.11		2.36	4.14	1.41
Cystine	0.70	0.84	--c	0.07	--c	1.67		0.29	0.46	0.27
Valine	1.69	2.12	1.50	3.51	2.00	2.32		2.43	4.41	1.30
Methionine	0.32	0.31	0.45	0.54	0.27	0.30		0.73	0.76	0.10
Isoleucine	0.62	0.93	0.36	1.30	0.88	1.37		1.04	1.20	0.38
Leucine	0.73	1.19	2.79	5.17	2.92	1.57		4.43	6.54	1.75
Tyrosine	0.52	0.65	0.89	1.69	1.06	0.57		1.57	1.70	0.61
Phenylalanine	0.61	0.92	1.59	2.91	1.59	0.84		2.51	3.28	1.24
Ornithine	--d	1.14	--c	--c	--c	0.97		--c	--c	--c
Ammonia	--d	1.22	0.70	0.97	1.65	1.03		0.77	1.35	0.85
Lysine	0.97	1.85	2.56	4.37	2.37	1.82		3.58	5.26	1.80
Histidine	0.69	1.08	1.06	2.21	1.37	1.05		1.72	3.06	1.13
Arginine	0.30	0.60	1.17	2.55	1.29	0.61		2.43	2.87	0.85

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Sample unavoidably lost.

<sup>c</sup>Undetectable.

<sup>d</sup>Values not calculated due to incomplete separation.

Table 23. Experiment 6302 - Plasma amino acid concentrations of pigs ad libitum fed either a dried skim milk ration or soybean meal ration<sup>a</sup>

Protein source	Dried skim milk					Soybean meal				
Hours after feeding	0	2	4	6	8	0	2	4	6	8
Aspartic acid	0.22	0.31	0.30	0.22	0.20	0.36	0.32	0.25	0.16	0.16
Threonine	2.77	3.32	3.39	2.50	2.71	3.52	2.70	2.85	2.03	2.26
Serine	2.05	2.14	2.12	1.68	1.65	2.36	2.26	1.99	1.57	1.59
Glutamic acid	3.28	3.42	3.29	2.94	2.17	2.44	2.89	2.83	1.95	2.01
Citrulline	0.69	0.74	0.66	0.51	0.48	0.97	0.92	0.84	0.66	0.62
Proline	3.46	3.83	3.66	3.02	2.96	2.37	2.50	2.05	1.83	1.96
Glycine	6.14	5.92	5.74	4.36	4.73	6.62	6.33	5.87	4.96	5.03
Alanine	4.43	4.83	5.55	4.41	4.21	4.04	4.14	3.62	3.28	3.59
Cystine	0.96	0.56	0.59	0.45	0.58	1.01	0.98	0.71	0.51	0.13
Valine	3.98	3.95	3.78	2.76	2.66	3.54	3.89	3.71	2.59	2.55
Methionine	1.47	1.35	1.36	0.96	1.06	0.40	0.36	0.40	0.28	0.28
Isoleucine	1.89	1.98	1.73	1.30	1.22	2.62	2.68	2.33	1.70	1.58
Leucine	2.93	2.87	2.81	2.22	2.29	2.74	2.71	2.23	1.73	1.73
Tyrosine	2.15	2.31	2.07	1.70	1.79	1.33	1.40	1.69	0.99	0.94
Phenylalanine	1.58	1.59	1.45	1.12	1.19	1.57	1.60	1.60	1.13	1.03
Ornithine	0.23	0.86	1.26	0.81	0.90	--b	--b	--b	--b	--b
Ammonia	1.16	1.40	1.44	1.17	1.20	--b	--b	--b	--b	--b
Lysine	3.32	3.80	4.34	2.94	3.16	2.06	2.29	1.82	1.49	1.58
Histidine	1.57	1.65	1.55	1.12	1.18	1.35	1.56	1.53	1.22	1.04
Arginine	0.91	0.91	0.84	0.55	0.73	1.20	1.84	1.68	1.34	1.19
Tryptophan	1.05	1.08	0.96	0.96	1.07	0.98	1.07	0.87	0.76	0.68

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Ornithine and ammonia did not separate chromatographically and values were not calculated.

Table 24. Experiment 6302 - Plasma amino acid concentrations of pigs ad libitum fed a nonprotein diet following the feeding of a soybean meal or dried skim milk ration<sup>a</sup>

Pre-experimental protein	Dried skim milk					Soybean meal				
Hours after feeding	0	2	4	6	8	0	2	4	6	8
Aspartic acid	0.38	0.35	0.33	0.27	0.38	0.33	0.24	0.42	0.36	0.26
Threonine	2.43	1.87	1.35	1.30	1.25	2.27	2.05	2.19	1.49	1.94
Serine	1.56	1.45	1.28	1.33	1.39	1.64	1.40	1.69	1.09	1.43
Glutamic acid	2.95	2.72	2.68	3.16	2.97	2.47	1.56	2.04	1.50	1.76
Citrulline	0.63	0.51	0.50	0.48	0.43	0.64	0.40	0.60	0.44	0.44
Proline	2.83	2.43	2.29	2.22	2.12	2.36	2.22	2.39	1.72	2.30
Glycine	7.90	7.88	7.12	6.70	7.04	6.38	6.49	5.98	4.35	6.48
Alanine	5.49	5.61	5.34	5.18	5.92	4.73	3.99	4.00	3.37	4.84
Cystine	0.85	0.55	0.57	0.48	0.57	0.89	0.72	0.68	0.71	1.01
Valine	2.77	2.14	2.20	1.96	1.97	2.74	2.24	2.07	1.54	1.98
Methionine	0.43	0.25	0.21	0.29	0.30	0.51	0.41	0.37	0.30	0.36
Isoleucine	1.27	0.88	0.93	0.87	0.91	1.52	1.22	1.21	0.84	1.14
Leucine	1.66	1.07	1.26	1.19	1.28	1.79	1.50	1.46	1.15	1.52
Tyrosine	1.22	0.72	0.63	0.48	0.49	0.96	0.67	0.67	0.58	0.73
Phenylalanine	0.95	9.67	0.79	0.81	0.80	1.13	0.93	0.97	0.67	1.01
Ornithine	--b	2.88	4.29	2.86	0.45	0.63	0.65	0.42	--b	--b
Ammonia	--b	0.09	0.09	0.71	0.90	0.96	0.69	0.73	--b	--b
Lysine	1.82	1.22	1.07	0.86	0.93	2.34	1.92	1.76	1.45	2.04
Histidine	0.95	0.78	0.77	0.84	0.85	1.39	1.24	1.21	0.87	1.22
Arginine	0.55	0.32	0.30	0.33	0.49	1.45	0.93	0.94	0.68	1.05
Tryptophan	--c	0.59	0.56	0.61	0.60	--c	0.62	0.70	0.67	0.69

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Ornithine and ammonia did not separate chromatographically and values were not calculated.

<sup>c</sup>Not analyzed.

Table 25. Experiment 6302 - Plasma amino acid concentrations of pigs control fed either a dried skim milk ration or soybean meal ration<sup>a</sup>

Protein source	Dried skim milk					Soybean meal				
Hours after feeding	0	2	4	6 <sup>b</sup>	8	0	2	4	6	8
Aspartic acid	0.25	0.26	0.22		0.16	0.18	0.24	0.18	0.28	0.21
Threonine	2.65	3.59	2.67		3.27	3.57	3.04	2.84	3.64	3.36
Serine	1.82	1.95	1.54		2.15	2.34	1.98	1.76	2.40	2.33
Glutamic acid	1.85	1.69	1.96		2.12	1.51	2.18	1.69	-- <sup>c</sup>	2.07
Citrulline	0.92	1.29	0.91		0.95	0.59	0.73	0.62	0.62	0.62
Proline	2.15	4.41	3.04		3.59	1.78	2.02	2.10	2.57	2.24
Glycine	6.50	4.70	3.56		5.36	7.16	6.26	5.93	6.98	7.22
Alanine	3.26	5.66	3.74		4.68	3.27	4.47	3.86	4.26	3.94
Cystine	1.52	1.29	1.06		1.12	1.06	0.86	0.63	0.60	0.52
Valine	4.54	4.09	3.08		3.26	3.34	2.49	2.36	2.84	2.79
Methionine	0.48	1.08	0.97		0.92	0.55	0.44	0.39	0.44	0.49
Isoleucine	2.35	1.69	1.41		1.87	1.87	1.19	1.23	1.83	1.86
Leucine	3.27	2.42	1.85		2.11	2.44	1.32	1.58	2.18	2.27
Tyrosine	1.14	3.13	2.21		1.90	1.02	1.17	1.20	1.48	1.45
Phenylalanine	1.36	1.98	1.48		1.31	1.16	1.11	1.25	1.45	1.35
Ornithine	0.81	0.85	0.81		1.16	0.96	1.03	0.95	0.96	1.09
Ammonia	0.86	0.86	0.82		1.15	0.62	0.85	0.91	1.25	0.80
Lysine	2.70	4.95	3.94		3.66	3.59	3.37	2.93	3.61	3.49
Histidine	1.07	1.78	1.36		1.19	1.31	1.40	1.42	1.55	1.63
Arginine	1.50	1.09	1.11		1.64	1.90	2.11	2.04	2.37	2.21
Tryptophan	0.88	1.47	1.25		1.03	0.84	0.99	1.10	1.05	0.86

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Samples unavoidably lost.

<sup>c</sup>Values lost during analysis.



Table 26. Experiment 6302 - Plasma amino acid concentrations of pigs control fed a nonprotein diet<sup>a</sup> prior to fasting and feeding the nonprotein meal, the pigs were fed diets containing either dried skim milk or soybean protein

Pre-experimental protein	Dried skim milk					Soybean meal				
Hours after feeding	0	2	4	6	8	0	2	4	6	8
Aspartic acid	0.41	0.34	0.21	0.28	0.21	0.31	0.32	0.29	0.31	0.30
Threonine	2.54	1.99	1.30	1.58	2.00	2.98	2.44	2.35	2.65	2.88
Serine	2.14	1.47	0.99	1.46	1.96	1.97	1.53	1.34	1.69	1.82
Glutamic acid	2.37	1.91	1.27	1.69	1.58	2.39	1.84	1.34	1.45	1.72
Citrulline	0.68	0.65	0.52	0.54	0.81	0.98	0.95	0.93	0.88	0.84
Proline	2.67	1.79	1.35	2.14	2.22	1.96	2.22	2.18	2.08	2.24
Glycine	9.18	6.14	4.40	7.58	7.92	6.68	5.06	5.38	5.84	6.78
Alanine	5.15	4.63	2.87	4.14	4.89	2.97	3.69	3.71	3.40	4.42
Cystine	0.44	1.13	0.74	1.01	1.18	0.84	0.77	0.78	0.79	0.76
Valine	3.22	2.21	1.47	2.20	2.23	4.02	2.38	1.93	2.00	1.99
Methionine	0.39	0.38	0.29	0.54	0.62	0.54	0.34	0.41	0.49	0.62
Isoleucine	1.69	0.81	0.59	1.05	1.30	2.46	1.20	0.98	1.20	1.52
Leucine	1.86	0.80	0.62	1.45	1.80	3.12	1.25	1.00	1.34	1.78
Tyrosine	1.17	0.72	0.65	0.98	1.29	1.25	0.87	0.83	0.90	1.30
Phenylalanine	0.95	0.83	0.71	1.16	1.33	1.39	0.88	0.98	1.07	1.38
Ornithine	--b	0.61	--b	0.59	0.68	1.26	0.60	0.58	--b	--b
Ammonia	--b	1.00	--b	1.14	0.99	0.84	1.31	0.74	--b	--b
Lysine	1.84	1.39	0.80	1.32	1.93	3.06	2.13	1.88	2.10	2.35
Histidine	0.81	0.76	0.62	0.85	1.03	1.55	1.32	1.44	1.40	1.39
Arginine	1.27	0.73	0.38	0.65	1.13	2.25	1.45	1.14	1.27	1.46
Tryptophan	0.76	0.85	0.79	1.04	0.98	0.94	0.72	0.78	0.75	0.80

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Ornithine and ammonia did not separate chromatographically and values were not calculated.

Table 27. Experiment 6302 - Amino acid composition of dried skim milk protein and soybean protein (acid hydrolysis)

Protein source	Dried skim milk						Soybean meal <sup>a</sup>				
Hours	24	48	24	48	-	24	48	72	24	48	72
	% of Protein		% of DSM			% of Protein			% of SBM		
Aspartic acid	6.60	6.63	2.18	2.19		6.79	10.44	11.25	3.39	5.22	5.63
Threonine	3.71	3.71	1.22	1.22		2.29	3.42	3.54	1.14	1.71	1.77
Serine	4.55	4.48	1.50	1.48		2.93	4.16	4.01	1.47	2.08	2.01
Glutamic acid	16.95	17.61	5.59	5.81		9.79	14.85	14.87	4.90	7.43	7.43
Proline	7.79	8.01	2.57	2.67		2.83	4.21	4.62	1.42	2.10	2.31
Glycine	1.71	1.83	0.57	0.60		2.49	3.88	4.36	1.25	1.94	2.18
Alanine	2.86	2.96	0.94	0.98		2.54	3.80	4.31	1.27	1.90	2.15
Cystine	0.50	0.69	0.16	0.23		0.85	1.15	1.05	0.43	0.58	0.52
Valine	5.47	5.87	1.81	1.94		2.77	4.46	5.09	1.39	2.23	2.55
Methionine	1.65	2.10	0.54	0.69		0.62	1.04	1.22	0.31	0.52	0.61
Isoleucine	4.67	5.11	1.54	1.69		2.79	4.46	4.79	1.39	2.23	2.39
Leucine	8.62	8.79	2.85	2.90		4.55	6.84	7.30	2.27	3.42	3.65
Tyrosine	4.38	4.50	1.45	1.48		2.20	3.30	3.34	1.10	1.65	1.67
Phenylalanine	4.25	4.38	1.40	1.44		2.97	4.50	4.71	1.48	2.25	2.36
Ammonia	1.92	2.17	0.63	0.72		1.25	1.90	2.32	0.63	0.95	1.16
Lysine	7.55	7.80	2.49	2.57		3.85	5.90	6.33	1.92	2.95	3.16
Histidine	2.74	2.94	0.90	0.96		1.72	2.65	2.74	0.86	1.33	1.37
Arginine	2.97	3.22	0.98	1.06		4.28	6.47	6.96	2.14	3.24	3.48

<sup>a</sup>72 hours were required for almost complete hydrolysis of soybean protein.

Table 28. Experiment 6326 - Composition of the experimental diets<sup>a</sup>

Ingredient	% Protein									
	15	20	25	15	20	25	20	25	20	25
Dried skim milk	45.45	60.60	75.75	--	--	--	15.51	30.30	45.45	45.45
Soybean meal	--	--	--	30.00	40.00	50.00	30.00	30.00	10.00	20.00
Lard, stabilized	2.00	1.95	1.85	2.10	2.05	2.00	2.00	1.95	1.95	1.90
Sucrose	20.00	20.00	17.75	20.00	16.45	6.90	17.75	12.45	20.00	15.50
Lactose	--	--	--	36.00	36.00	36.00	30.00	21.00	13.00	13.00
Woodflock	2.25	2.25	2.25	1.35	1.05	0.75	1.35	1.35	1.95	1.65
Corn starch	14.60	6.85	--	6.00	--	--	--	--	5.05	--
KCl	--	--	--	0.15	0.20	0.25	0.10	0.05	--	--
Vitamin premix <sup>b</sup>	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calcium carbonate	0.15	--	--	0.50	0.70	0.90	0.60	0.65	0.30	0.25
Dicalcium phosphate	0.25	--	--	1.70	1.35	1.00	0.85	0.05	--	--
Iodized salt	0.10	0.15	0.20	--	--	--	--	--	0.10	0.05
Trace mineral <sup>c</sup> mix (35-C-41)	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>	<u>0.20</u>
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

<sup>a</sup>Calculated analysis given in Table 30.

<sup>b</sup>Composition given in Table 29.

<sup>c</sup>Composition given in Table 3.

Table 29. Experiment 6326 - Amounts of vitamins added per pound of complete diet

Source of protein		Protein level (%)									
Soybean meal		15	20	25	--	--	--	15	15	5	10
Dried skim milk		--	--	--	15	20	25	5	10	15	15
Total		15	20	25	15	20	25	20	25	20	25
	<u>Unit</u>										
Vitamin A	I.U./lb.	3000	3000	3000	2918	2891	2864	2973	2945	2918	2918
Vitamin D <sub>2</sub>	I.U./lb.	500	500	500	414	385	356	471	442	414	414
Riboflavin	mg./lb.	5.0	5.0	4.5	1.0	0.0	0.0	3.3	2.0	0.8	0.7
Pantothenic acid	mg./lb.	8.5	7.6	7.0	3.2	1.0	0.0	6.0	3.66	2.6	2.0
Niacin	mg./lb.	27.2	26.2	25.2	27.7	27.0	26.2	26.4	25.6	26.8	25.8
Choline	mg./lb.	110	0.0	0.0	273	197	121	342	0.0	143	130
Vitamin B <sub>12</sub>	mcg./lb.	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin E	mg./lb.	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Menadione (Vitamin K)	mg./lb.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Thiamine	mg./lb.	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Pyridoxine	mg./lb.	1.0	0.5	0.0	2.0	2.0	2.0	1.0	0.6	2.0	1.5
Folic acid	mcg./lb.	419	392	365	364	318	273	374	328	337	310
Biotin	mcg./lb.	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Chlortetracycline	mg./lb.	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0

Table 30. Experiment 6326 - Calculated analysis of the completed diets

Source of protein		Protein level (%)									
Soybean meal		15	20	25	--	--	--	15	15	5	10
Dried skim milk		--	--	--	15	20	25	5	10	15	15
Units											
Protein	%	15.0	20.0	25.0	15.0	20.0	25.0	20.0	25.0	20.0	25.0
Fat	%	2.25	2.25	2.25	2.23	2.25	2.23	2.23	2.25	2.23	2.23
Fiber	%	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Calcium	%	0.70	0.71	0.71	0.70	0.76	0.95	0.71	0.71	0.71	0.71
Phosphorus	%	0.50	0.50	0.51	0.50	0.61	0.76	0.50	0.51	0.52	0.58
Vitamins:											
Vitamin A	I.U./lb.	3000	3000	3000	3000	3000	3000	3000	3000	3000	3000
Vitamin D <sub>2</sub>	I.U./lb.	500	500	500	500	500	500	500	500	500	500
Riboflavin	mg./lb.	5.4	5.5	5.1	5.1	5.4	6.8	5.0	5.1	5.0	5.0
Pantothenic acid	mg./lb.	10.3	10.0	10.0	10.0	10.1	11.4	10.1	10.0	10.0	10.0
Niacin	mg./lb.	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Choline	mg./lb.	500	520	650	500	500	500	500	542	500	500
Vitamin B <sub>12</sub>	mcg./lb.	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Vitamin E	mg./lb.	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Menadione											
(Vitamin K)	mg./lb.	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Thiamine	mg./lb.	3.3	3.4	3.5	3.0	3.0	3.0	3.3	3.3	3.1	3.0
Pyridoxine	mg./lb.	2.5	2.5	2.4	2.0	2.0	2.0	2.5	2.1	2.5	2.5
Folic acid	mcg./lb.	500	500	500	500	500	500	500	500	500	500
Biotin	mcg./lb.	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Chlortetracycline	mg./lb.	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0

Table 31. Experiment 6326 - Summary of total gain and feed required per pound of gain

Source of protein		Level of Protein (%)								
Soybean meal	15	20	25	--	--	--	15	15	5	10
Dried skim milk	--	--	--	15	20	25	5	10	15	15
Total	15	20	25	15	20	25	20	25	20	25
Replication		Total gain (lb.)								
1	5.5	2.7	2.0	5.9	10.8	10.4	5.5	6.7	7.2	8.3
2	3.2	2.2	1.9	7.0	7.2	9.2	9.6	9.4	8.7	10.9
3	2.7	4.6	3.9	8.4	8.6	9.2	7.5	9.5	9.1	7.0
Average	3.8	3.2	2.6	7.1	8.9	9.6 <sup>a</sup>	7.5	8.5	8.3	8.7 <sup>b</sup>
		Feed/gain								
1	2.35	3.44	2.98	1.82	1.30	1.38	1.68	1.32	1.49	1.25
2	3.46	4.23	5.16	1.86	1.50	1.58	1.46	1.35	1.64	1.30
3	4.00	2.80	2.51	1.47	1.25	1.25	1.47	1.24	1.28	1.32
Average	3.27	3.49	3.55	1.72	1.35	1.40	1.54	1.30	1.47	1.29

<sup>a</sup>Pigs fed milk protein gained significantly faster ( $P > 0.01$ ) and required significantly less feed/gain ( $P > 0.01$ ) as compared to pigs fed soybean protein.

<sup>b</sup>Pigs fed the milk-soybean protein combinations gained significantly faster ( $P > 0.01$ ) and required significantly less feed/gain ( $P > 0.01$ ) as compared to pigs fed soybean protein.

Table 32. Experiment 6326 - A summary of free blood plasma amino acids<sup>a</sup>

Source of protein	Protein levels (%)									
	15	20	25	--	--	--	15	15	5	10
Soybean meal	15	20	25	--	--	--	15	15	5	10
Dried skim milk	--	--	--	15	20	25	5	10	15	15
Total	15	20	25	15	20	25	20	25	20	25
Aspartic acid	0.31	0.67	0.35	0.28	0.22	0.27	0.27	0.27	0.32	0.28
Threonine	3.26	4.74	3.48	4.04	3.66	4.64	4.05	2.89	3.31	3.54
Serine	2.92	3.86	2.25	3.76	2.68	2.66	2.59	2.01	2.41	1.95
Glutamic acid	2.26	2.63	2.42	2.25	1.60	1.36	1.80	1.99	2.48	1.78
Citrulline	1.50	2.49	1.84	1.92	1.71	1.49	1.49	1.41	1.47	1.23
Proline	3.45	3.42	2.74	4.44	3.50	3.71	3.04	3.11	3.52	3.55
Glycine	5.80	6.24	4.35	4.80	3.37	2.48	4.20	3.62	4.12	2.64
Alanine	5.02	4.98	4.51	5.78	3.96	3.41	3.75	3.15	4.48	3.77
Cystine	1.05	1.66	1.06	0.87	1.04	1.02	1.09	0.83	0.74	0.48
Valine	3.01	3.86	3.50	4.04	3.67	4.30	3.28	4.05	4.36	4.48
Methionine	0.48	0.61	0.43	1.12	1.33	1.47	0.61	0.41	0.87	0.92
Isoleucine	2.37	2.48	2.21	2.14	1.91	1.86	2.02	2.14	2.16	2.55
Leucine	1.86	2.35	2.14	2.29	2.00	2.20	1.98	2.20	2.36	2.94
Tyrosine	1.96	1.78	1.71	3.27	3.19	3.11	2.61	2.54	2.96	3.39
Phenylalanine	1.59	1.88	1.61	1.85	1.75	1.98	1.81	1.91	1.89	2.08
Ornithine	2.21	2.91	-- <sup>b</sup>	1.61	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>	2.56
Ammonia	1.04	0.56	-- <sup>b</sup>	1.08	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>	-- <sup>b</sup>	0.95
Lysine	3.55	4.86	3.82	4.59	3.95	4.54	3.85	3.43	4.16	4.99
Histidine	1.50	1.50	1.34	1.34	0.93	1.04	1.32	1.17	1.15	1.30
Arginine	4.14	5.59	4.01	1.73	1.84	1.50	3.79	3.10	2.44	3.28

<sup>a</sup>Values expressed as mg./100 ml.

<sup>b</sup>Ornithine and ammonia did not separate chromatographically and values were not calculated.

Table 33. Experiment 6326 - Analysis of variance of total gains and feed required per pound of gain

Source	d. f.	Mean square	
		Total gain	Feed/gain
Totals	29	7.9048	1.1183
Replications	2	0.8364	0.7502
Treatments	9	20.5554**	2.8562**
Soybean and milk protein levels vs. protein levels of the two protein sources	1	42.4376**	8.1536**
Protein levels within each protein source	5	28.0142**	3.4837**
Levels of protein	2	0.7506	0.0088
Soybean protein vs. milk protein	1	128.0000**	17.0528**
Protein source x levels	2	5.2850	0.1741
Protein levels within combined protein sources	3	0.8300	0.0446
20% protein vs. 25% protein	1	1.4700	0.1261
15% soybean protein vs. 15% milk protein	1	0.7500	0.0052
Protein levels x protein sources combination	1	0.2700	0.0024
Error	18	2.3648	0.2902

\*\*Indicates significant difference ( $P = 0.01$  or less).