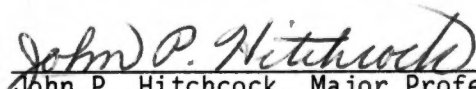


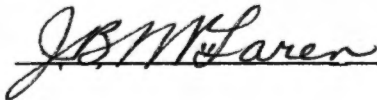
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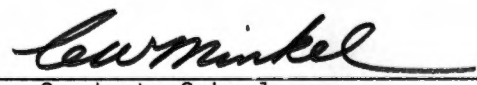
  
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IN VIVO AND IN VITRO AVAILABILITY OF LYSINE IN HEAT  
TREATED BLOOD MEAL FOR YOUNG POULTRY  
AND SWINE

A Thesis  
Presented for the  
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Tina Maynard Shipley

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## ABSTRACT

Three experiments were conducted utilizing 498 Hubbard x White Mountain 8 day-old chicks, 162 in experiments 1 and 3 and 165 in experiment 2. One other experiment was conducted utilizing 42 cross-bred pigs allotted by weight within litter replicate. The objectives of these experiments were to evaluate the effect of method of incorporation of the test protein, blood meal, on growth response to supplemental lysine; to determine the effect of heat treatment on lysine bioavailability from two sources, either purified spray-dried whole beef blood (BBM) or a commercially obtained vat dried beef-pork blood meal (BPBM) using the slope-ratio technique; and finally to test the validity of using the chick as a biological model for young pigs. Chicks were allotted by weight to one of 9, 11, or 9 dietary treatments in experiments 1-3. Diets contained 45% of the chicks or pigs requirement for lysine (0.43% and 0.36%, respectively), served as control diets. In experiment 1, experimental diets were supplemented with 0.10% or 0.20% lysine as L-lysine-HCl or from spray-dried whole beef blood that had undergone no further processing plus the addition of excess amino acids to simulate the amino acid pattern of the blood meal. The experimental diets in experiment 2 were supplemented at graded levels with L-lysine-HCl or at graded isonitrogenous levels with spray-dried whole beef blood that had been treated in one of four ways: (1) untreated blood meal was included in the diet with no further processing; (2) blood

meal A was mixed with 5% dextrose and 40% water, then dried at 60°C for 28 hours; (3) blood meal B was mixed with 5% dextrose and 20% water, then dried at 100°C for 14 hours; (4) blood meal C was mixed with 10% dextrose and 20% water and then autoclaved at 13.78 bars and 122°C for 20 minutes. Diets in experiment 3 were supplemented with graded levels of L-lysine-HCl or a mixture of commercial beef-pork blood meal that had received the same treatments as blood meals B and C above, with an untreated sample used as a control. In experiment 4, diets were supplemented with L-lysine-HCl, untreated beef-pork blood meal and beef-pork blood meal C. Body weight and feed consumption were recorded for all four experiments. Blood plasma samples and aliquots of diets in experiment 4 were collected for total lysine analysis.

Results from experiment 1 indicated that crystalline lysine always supported more growth, and graded additions of 0.10% and 0.20% lysine from either source regardless of amino acid pattern resulted in linear responses in gain, feed intake, and gain feed ratio. The percent bioavailable lysine from BBM was  $11.3 \pm 1.08\%$  for young poultry.

Results from experiment 2 indicated linear increases in gain, feed intake, and gain feed ratio with graded levels of supplementation. There were differences in these parameters among treatments of blood meal, indicating that the treatment of blood meal significantly reduced the percent bioavailable lysine in blood meal B ( $P < .05$ ) and C ( $P < .001$ ). The percent bioavailable lysine of BBM, BBM-A, BBM-B and BBM-C were  $9.08 \pm 0.65$ ,  $8.16 \pm 0.68$ ,  $7.49 \pm 0.07$  and  $3.88 \pm 0.87\%$ , respectively.

Performance data from experiment 3 exhibited the same trends in gain, feed intake, and gain feed ratio as in experiment 2. Heat treatment had a significant effect on the blood meal samples, reducing the percent bioavailable lysine in BPBM-B and BPBM-C ( $P < .001$ ). However, BPBM-B was not significantly different from BPBM-C ( $P > .4$ ). The percent bioavailable lysine contained in BPBM, BPBM-B and BPBM-C were  $8.57 \pm 0.25$ ,  $5.98 \pm 0.33$  and  $5.66 \pm 0.39\%$ , respectively.

Pigs from experiment 4 exhibited linear increases in gain and gain feed ratios. Feed intake of pigs fed beef-pork blood meal C was lower than for all other test groups, indicating lysine bioavailability was significantly reduced under reducing conditions at elevated temperatures ( $P < .001$ ). The lysine bioavailability of untreated beef-pork blood meal and beef-pork blood meal C was  $9.89 \pm 0.73\%$  and  $0.50 \pm 0.55\%$ . Data from this experiment suggested that the chick may not be a good biological model for the pig when low quality proteins are utilized as the test protein. The plasma lysine values for each treatment were not significantly different.

Multi-enzyme, pepsin digestibility and a rapid dye-binding procedure were performed. The blood meal samples were also analyzed for mineral composition and total lysine. These factors were used as independent variables and regressed on the dependent variable, bioavailable lysine, to obtain prediction equations. The best model with a  $R^2$  value of 0.99 for BBM contained pepsin digestibility and multi-enzyme values; the best single variable model for BPBM included pepsin digestibility values, which also had a  $R^2$  value of 0.99.

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## CHAPTER I

### INTRODUCTION

The nutritive value or protein quality of a food protein depends not only on the concentration of amino acids, but also the physiological availability of the amino acids. General requirements, which vary from animal to animal, are based upon adequate amounts of certain essential amino acids with a reasonable balance among all. However, bioavailability varies with the protein source, interaction with other dietary components, and processing treatment. Limitations and costs of protein feeds make the hunt for new protein sources and the evaluation of existing sources urgent.

Cereal grains are quite often limiting in one or more amino acid, most notably lysine. As a result, lysine is usually first, second, or third limiting in most swine and poultry rations. However, animal by-products used as protein supplements tend to be of a higher overall protein quality and should be considered as dietary protein supplements in ration formulation. These sources are generally subjected to various types of processing which may result in substantial decreases in amino acid bioavailabilities. Heating of proteins has been shown to reduce availability of amino acids (Miller et al., 1965; Osner and Johnson, 1968), in particular, lysine (Narayana Rao and McLaughlan, 1967), with the severity of heat damage dependent upon both the duration and temperature of heating, and upon the presence of other compounds in the heated material.

Lysine is the only essential amino acid that has a free amino group (epsilon) when in condensed form within the peptide chain. Work with casein-glucose model systems has indicated that these free amino groups react with the aldehydic groups of reducing sugars even under relatively dry conditions to form compounds that have no nutritional value, but that could be hydrolyzed by strong acids to free lysine (Elred and Rodney, 1946). Such behavior is explained by the Maillard or browning reaction (Maillard, 1912). Carpenter and his associates (1957) were the first to relate the structure of lysine to its nutritional bioavailability and definitely demonstrated by chemical means that in certain circumstances not all of the lysine present in a protein is biologically available to an animal, and total lysine content is not a reliable guide to the true quality of a protein in relation to lysine.

Rapid, in vitro methods of predicting bioavailable lysine have been attempted in the food industry but to date no such assay has been accepted as satisfactory for animal feeds. Methods of predicting bioavailable lysine must be developed to enable feed manufacturers to market protein supplements of known minimum bioavailable lysine content.

The high cost of all protein feedstuffs and the relatively high requirement of lysine for chicks and pigs suggest that a feed source high in lysine should be favorably considered in formulation of rations. Blood meal might be such a product.

Therefore, with these considerations, this study's objectives were to determine the effect of heat treatment on blood meal samples obtained from two sources. Also, experimentation utilizing chicks was employed to determine if the chick could serve as a biological model for pigs; to determine the effect of excess amino acid pattern on growth response to supplemental lysine and to determine if in vitro methods of assessing protein quality or combinations of in vitro methods can be used to accurately assess bioavailable lysine concentrations in blood meal.

## CHAPTER II

### LITERATURE REVIEW

#### I. FACTORS AFFECTING LYSINE AVAILABILITY

Considerable attention has been devoted to the nutritional value of proteins and to the amino acid profile which has been used in ration formulation. Furthermore, interest in the availability of each of these amino acids and variation among samples has increased, as well as the effect of processing or treatment on protein quality.

Lysine bioavailability is varied among feedstuffs; but more importantly, it is also quite variable among samples of the same feed ingredient. Ranges of estimates of a particular amino acid can be quite large, even as much as 30% for lysine in soybean meal (Baker, 1978).

Netke and Scott (1970) reported that the lysine in soybean meal was 78% available; however, in a later report, Robel and Frobish (1977) reported a range of 87-90% available lysine. Batterham et al. (1979) and Major and Batterham (1981) have reported values of 89-95% and 84-89%, respectively. However, both Ousterhout et al. (1959) and Cheeke and Myer (1975) have reported values in excess of 100%.

Discrepancies in availability values for lysine are not limited to cereal grains or proteins of plant origin; indeed, ranges for the animal by-product protein supplements may be even greater.

In meat and bone meal, Major and Batterham (1981) reported estimates of available lysine ranging from 49-64% of the total lysine content. In earlier work, Batterham et al. (1979) found a higher estimate of 64-70%. However, Ousterhout et al. (1959) reported that over 100% of the lysine present in meat and bone samples was theoretically available.

These differences among samples are probably not only due to different varieties, strains, species, or geographic origins of the sample; processing procedures may have had a profound impact on the availability of lysine. Processing feeds for the livestock industry has increased in recent years and many of these processes utilize heat as a factor (Costa et al., 1977).

Subjection of proteins to heat has been demonstrated (Miller et al., 1965; Ford and Salter, 1966; Narayana Rao and McLaughlan, 1967) to substantially reduce the availability of amino acids, particularly lysine. The severity of damage to the protein quality is dependent upon both the duration and temperature of the heat treatment, as well as upon the presence of other compounds in the heated material.

Lysine is extremely heat labile and readily undergoes the Maillard or browning reaction (Costa et al., 1977). Therefore, compounds of importance in feedstuffs are the reducing sugars. In the Maillard reaction (Maillard, 1912) an intermediate Schiff's base is formed between an amino group and a carbonyl group of the sugar (Hodge, 1953), thus effectively rendering the lysine unavailable to the animal. Carpenter and associates (1957) and

Lea et al. (1958) were the first to relate the structure of lysine to its nutritional availability and definitely demonstrated that under certain circumstances not all of the lysine in a protein is nutritionally available or recoverable to an animal.

Blood meal made from blood collected from pigs, cattle, and poultry at slaughter is a high protein, lysine rich supplement; however, it has found limited use in ration formulation (Parsons, 1975) since it is low in isoleucine and is susceptible to the Maillard reaction during processing where significant losses of total and available lysine may be incurred. Conventional methods of processing utilize high temperatures for extended periods of time and include processes commonly referred to as "vat dried" and "drum dried." These procedures may reduce levels of available lysine to zero (Waibel et al., 1974). But recently new methods of processing that utilize lower temperatures such as ring process or spray-drying may significantly improve lysine availability estimates (Parsons, 1975; Kratzer and Green, 1957).

Hamm and Searcy (1976) conducted a series of experiments on the effect of heat treatment on lysine availability from poultry blood meal. Raw blood was utilized as a control, and contained 7.0% available lysine on a dry weight basis. Aliquots from the control sample were subjected to various conditions at temperatures ranging from 23°C to 193°C for two different time periods, 30 and 60 minutes. As temperature and time of exposure increased, available lysine decreased. Temperatures of 183°C and above, regardless of duration

of treatment, resulted in essentially no available lysine. When the batch processing of blood meal was simulated (105°C for 170 minutes with pressure of 500 mm Hg), the drop in lysine availability was 0.9% from the control.

Kratzer and Green (1957) studied lysine availability as measured by chick growth assay. They reported that vat dried blood contained 6-8% available lysine. However, spray-dried soluble blood meal contained 10-12%. Parsons (1975) utilized a pig growth assay and determined that ring processed blood had a percent bioavailability of approximately 70, or values of available lysine of 6.5 to 7.0%. In a subsequent study to determine the lysine availability from flash ring dried blood meal, Parsons (1977) reported values of 6.7 to 7.0% bioavailable lysine.

## II. GENERAL CONSIDERATIONS FOR IN VITRO ASSAYS

In recent years, concern has been expressed by researchers from academic, food industries, and food regulatory agencies on the need for rapid assays that will allow for the close monitoring of protein nutritional quality in foods and feedstuffs during processing. Anderson (1978) stated that the food processing industry is in dire need of a rapid assay that could replace the slow Protein Efficiency Ratio (PER) bioassay. Methods of predicting protein nutritional quality should correlate well with data obtained from the PER bioassay. Any rapid assay, in addition to being fast and accurate, must be applicable to a wide range of feedstuffs

(Bodwell, 1977), including, but not limited to, processed cereal grain products, meat and milk products and animal by-products.

A major difficulty in developing a rapid chemical or enzymatic method(s) for measuring true protein nutritional quality are inherent physical and chemical differences in classes of food or feedstuffs. A methodology that works well in one class (i.e., cereal products) may not be applicable or suitable for another class (i.e., animal products). Among the limiting factors causing an assay to predict more accurately in one class than another (Satterlee et al., 1981) are:

1. Limited data base--only a single class or related classes of foods were used in the development of the assay.
2. Certain physical/chemical characteristics may prevent the accurate estimation of nutritional quality in certain classes of foods. For example, foods that contain proteolytic enzyme inhibitors, intact cellulosic cell walls, or oxidized sulfur amino acids may not be evaluated accurately. These factors plus an assortment of others will affect eventual amino acid availability and subsequent estimates of protein nutritional quality by rapid assays that are not sensitive to the presence of such factors.

Three basic parameters that dictate the nutritional quality of a protein are: (1) the quantities of the essential amino acids that are present in the protein; (2) the digestibility of the amino

acids that have been released from the protein through the digestive process; and (3) the bioavailability of the essential amino acids released during protein digestion. If a rapid assay can either directly or indirectly measure these three basic parameters, and is also constructed around a wide variety of foods, then the limiting factors of a rapid in vitro assay can be minimized.

Digestibility, whether estimated by in vivo or in vitro assays, is at best only a general indicator of possible effects of heat damage, overprocessing, etc. on amino acid bioavailability or of a possible inherent lack of availability. In part, in vitro enzymatic assays may greatly over estimate the availability of a specific amino acid (i.e., lysine) based on growth data (Ford and Salter, 1966). Data from Schuster and Bodwell (1979), Alsmeyer et al. (1974), and Lee et al. (1978) have demonstrated that the inclusion of digestibility in indices to predict protein nutritional quality made no apparent contribution to the results. Thus, it may be concluded that digestibility of proteins need not be incorporated if the developed assay is for a single class of food. But it must be noted that this would be a limited assay, and would have limited value in widespread quality control and regulatory applications.

The total amino acid composition of a protein is generally determined after an acid hydrolysis and does not necessarily reflect the amount of individual amino acid that is in the bioavailable state. Although the amino acid profile can be important in pure proteins, it has less value as a measurement for the nutritional

value of protein, particularly if the food is rich in lysine and has been processed or stored in the presence of reducing sugars, since the browning reaction or Maillard reaction may occur (Peterson and Warthesen, 1979). There is a growing awareness that "unavailable peptides" and premelanoidins representing the soluble early products of the Maillard reaction may be antinutritive and distort the normal processes of protein breakdown and absorption in the small intestine (Shorrocks and Ford, 1978). Thus, the nutritionally effective amino acid composition of damaged proteins may well be different from that indicated by the total amino acid analysis. Therefore, it would be imperative that an in vitro methodology be capable of measuring available lysine.

### III. IN VITRO ASSAYS FOR DETERMINING PROTEIN DIGESTIBILITY

Originally intended for use in estimating protein quality, Akeson and Stahmann (1964) are credited for developing one of the earliest assays for estimating protein digestibility, utilizing the enzymes pepsin and pancreatin. Saunders et al. (1973) used the pepsin pancreatin assay of Akeson and Stahmann (1964) on several alfalfa proteins and found a correlation of 0.88 between the in vitro digestibility data and digestibility data obtained from rat bioassays. Buchanan (1969) found that a papain-trypsin digestion of leaf proteins also yielded in vitro protein digestibilities that were highly correlated with their respective in vivo values. Trypsin (Maga et al., 1973) has been successfully used to estimate the

apparent digestibility of selected food proteins. Ford and Salter (1966) utilized a protease preparation to predict protein digestibility for a variety of food ingredients. Gauthier et al. (1982) presented a method based on the enzymatic hydrolysis of proteins with simultaneous dialysis of the digested products that they adapted from the work of Mauron et al. (1955) and Steinhart and Kirchgessner (1973) utilizing pepsin and pancreatin. Although they presented no correlations with in vivo data, the method appears to be a very promising method of evaluating the nutritional implications of technological treatments being carried out in processing, since it was able to detect the effect of heat on proteins.

Hsu et al. (1977) described a 10 minute multi-enzyme assay that could successfully predict ( $r = 0.90$ ) rat apparent protein digestibility. The assay utilized the pancreatic enzymes trypsin and chymotrypsin, and the intestinal enzyme, amino-peptidase. The Hsu et al. (1977) assay was found to have an excellent relationship to in vivo digestion in the rat, as noted by the in vitro method's sensitivity to: (1) trypsin inhibitors; (2) the effects of processing on food proteins; and (3) its insensitivity to fats and inherent buffering salts in the test proteins. However, over two-thirds of the samples in the work of Hsu et al. (1977) were of plant origin and the remainder of dairy origin. Therefore, the assay of Hsu et al. (1977) are limited to those classes of food. Satterlee et al. (1979) modified the above assay by increasing the test protein base to include meat and egg based foods and by adding additional enzyme

protease from Streptomyces griseus. Their method has been used to accurately establish apparent protein digestibility on a vast range of foods.

#### IV. IN VITRO MEASUREMENTS OF BIOAVAILABLE (REACTIVE) LYSINE

##### Chemical Methods

Chemical methods are all dependent on the principle that it is the reaction of the  $\epsilon$ -amino lysine groups in protein with carbonyl groups and other compounds that reduces the availability of the lysine in processed feeds to a greater extent than that of other essential amino acids. Carpenter and his associates (1957) were the first to relate the structure of lysine to its nutritional availability and definitively demonstrated by chemical means that the  $\epsilon$ -amino group of lysine could be blocked, thus rendering the lysine nutritionally unavailable. Therefore, chemical methods of assay take advantage of the reactivity of the  $\epsilon$ -amino group by a "tagging" compound to react with the test proteins at the  $\epsilon$ -group that have not already reacted with other components. The amount of "tagged lysine" may then be determined, thus yielding an estimate of bioavailable lysine.

Fluoro-dinitrobenzene. Sanger (1945) presented a classical use of a chemical reagent to determine the identity of free amino groups in a peptide chain with 1-fluoro-2,4-dinitrobenzene (FDNB). This reagent forms dinitrophenyl derivatives at each primary amino

group of lysine (i.e., the terminal  $\alpha$ -amino groups and the  $\epsilon$ -amino groups). Using this procedure, the structure of insulin was elucidated. Lea and Hannan (1950) then demonstrated that FDNB was unable to "tag" lysine units in casein that had been allowed to react with glucose.

Carpenter and Ellinger (1955) found that the FDNB procedure, using feedstuffs in a crude form, provided better results with higher correlations with biological data than any previous method. In 1960, Carpenter further simplified the methodology by using a chloroformate blank as opposed to chromatography. Booth (1971) further modified Carpenter's (1960) procedure, and it has been used extensively in many laboratories.

A major problem with the procedure is that with high levels of polysaccharides in a sample, there can be up to a 30% loss of dinitrophenyl lysine derivatives during acid hydrolysis. This is apparently due to the sugars released by hydrolysis having a reductive, thus decolorizing effect. Because of this problem Rao et al. (1963) suggested a different approach. Assuming that the total lysine value of a damaged protein accounts for both reactive or unblocked lysine and partial recovery of lysine from  $\epsilon$ -amino group blocked lysine, they conducted two total lysine analyses; the first used the test material prepared by acid hydrolysis for total lysine analysis, while the second used the test material pre-tested with FDNB. The difference between the two values would be the value of the reactive lysine.

The assumption holds well with proteins that are severely heated, either with or without carbohydrates, but is not as applicable for protein that has reacted with reducing sugars under mild conditions, as demonstrated by Hurrell and Carpenter (1974). Under the latter condition, the major Maillard compound formed would be a desoxyketosyl derivative. Finot and Mauron (1972) demonstrated that after FDNB treatment there were several reaction products that broke down in complex ways, yielding neither lysine nor dinitrobenzene lysine derivatives which has been confirmed by Hurrell and Carpenter (1980). Thus, this procedure lacks some degree of sensitivity, and although it has been adapted by the AOAC (1975), it must be used with discretion.

Trinitrobenzene Sulphonic Acid. As an alternative to FDNB, Kakade and Liener (1969) introduced the reagent trinitrobenzene sulphonic acid (TNBS). It is not only water soluble, but when TNBS is utilized, the protein does not need to be totally hydrolyzed; only soluble peptides are required. These peptides give the same color intensity per "tagged" lysine unit as a trinitrobenzene-lysine derivative. However, trinitrobenzene-lysine derivatives are more easily reduced than dinitrobenzene derivatives during acid-digestion in the presence of carbohydrates (Posati et al., 1972). Observations by Hurrell and Carpenter (1974) led to a second problem; TNBS will still react at the  $\epsilon$ -amino group in an early Maillard derivative of lysine, and the trinitrobenzene-lysine derivative remains after

acid hydrolysis. Therefore, the use of TNBS may not be applicable in all situations.

### Dye-Binding Methods

Dye-binding capacity (DBC) has been used as a rapid indicator of amino acid composition for many years (Ashworth, 1966; Udy, 1971). It depends on the attraction between negatively charged azo dyes and the basic amino groups in the protein.

With increased interest in finding and developing high lysine cereal grains, it was discovered that a high ratio of DBC-crude protein in a sample could be used as a screening process to select samples for more accurate analysis, although in principle DBC was only an indicator of the sum of histidine, arginine and lysine (Bhatty and Wu, 1975). Other workers reported that the same ratio could serve as an indicator of protein quality in fish meals (Moran et al., 1963; Carpenter and Opstuedt, 1976). However, the procedure measured all the basic groups and any early Maillard compounds between  $\epsilon$ -amino groups and reducing sugars which were still basic enough to attract the azo dyes (Lea and Hannan, 1950).

In order to measure lysine only, researchers pursued the idea of conducting two DBC determination on a single sample; the first DBC was determined by the standard DBC procedure and the second with the  $\epsilon$ -amino group of lysine blocked such that the difference between the two was lysine. Several compounds were tested as blocking agents in the early work. Sandler and Warren (1974) tried

ethyl chloroformate, and Jones and Lakin (1976) used TNBS, but neither compound was a suitable blocking agent.

Based on earlier experimentation (Bjarnason and Carpenter, 1969; Varnish and Carpenter, 1975), Hurrell and coworkers (1979) believed that propionic anhydride served as a reagent for a simple method of blocking  $\epsilon$ -amino groups by acylation. However, DBC procedures are not applicable where early Maillard damage has occurred. The first reading, or quantitation of histidine, lysine, and arginine, can be measured and is a true reflection, but Maillard damaged proteins do not react with propionic anhydride, and thus, remain charged and are measured in the second reading.

## V. IN VIVO ASSAYS

The slope ratio assay is a multiple protein level methodology. Several levels of the test material are fed, but only points falling on the linear portion of the curve are used in calculating the slope of the response line. The slope of the response line of the test protein is expressed as a percentage of the slope of a reference standard protein. However, this assay has several inherent faults as well as advantages.

McLaughlan (1972) reported that the slope ratio assay gave invalid results for lysine deficient proteins, since they may seriously skew the slope when an inclusion of a non-protein group is included in the calculations. Nekte and Scott (1970) suggested that the meaningfulness of reported estimates of amino acid

availability is cloudy, especially because it has been shown that the growth of animals fed the diets used to establish the reference curve as well as those fed the test diets is influenced by nutritional factors other than the amino acid under study. The slope-ratio technique has also been criticized in that the standard and sample response curves do not always pass through the origin and may not be linearly true (Baker, 1978). However, the controversy in regard to linearity probably becomes academic, since linearity is at least approached (Ousterhout et al., 1959).

Other criticisms of growth assays in general are: (1) the possibility of overestimation of bioavailability (Soares and Kifer, 1971), and (2) short-term assays may even underestimate bioavailability (Robel and Frobish, 1977). Hill et al. (1966) reported that four-day chick growth assays, using the slope ratio technique produced a 10% lower estimate of lysine availability from soybean meal than did data from a five-day assay. In experiments by Robel and Frobish (1977), four-, six-, and eight-day assay data were used to determine relative availabilities for specific amino acids. The values obtained from the eight-day assay were the highest obtained estimates. They concluded that length of assay is associated with availability estimates.

However, Carpenter (1973) concluded that growth assays remain the only direct method of determining the nutritional significance of values obtained by other procedures, and several researchers since have successfully employed growth assays and slope-ratio

techniques in estimating availabilities of nutrients. Batterham et al. (1979; 1981) assayed for lysine using the slope-ratio technique with pigs and rats in various protein sources, and determined the availability estimates by chemical techniques. They reported little agreement between chemical values and the biological values. Major and Batterham (1981) repeated another series of experiments with chicks using a vegetable and animal protein source. The chemical techniques employed were the Silcock available-lysine test and the fluorodinitrobenzene procedure. There was a positive relationship between the in vivo and in vitro results and the amino acid availability estimates were higher for the chick than for the pig.

## CHAPTER III

### MATERIALS AND METHODS

#### I. EXPERIMENTAL ANIMALS AND TREATMENTS

A series of four experiments was conducted. The first three experiments utilized a total of 489 broiler chicks of the Hubbard x White Mountain strain. The last experiment utilized 42 crossbred pigs. All chicks were housed in wire bottom, thermostatically-controlled heated starter batteries kept at 28-30°C and a continuous light schedule was maintained. The chicks were feather-sexed upon hatching and only the males were allotted to experimental treatment. During the first seven days posthatching, the chicks were fed a corn-soybean meal starter diet, providing all nutrients at or above the National Research Council (NRC) (1977) requirements.

Following an overnight fast, the chicks were weighed to the nearest gram, wing banded, and randomly allotted to treatment such that the average initial weights and weight ranges or distributions were similar. Experiments 1 and 3 continued from 8 to 15 days posthatching while experiment 2 was conducted from 7 to 14 days posthatching. Water and feed were provided ad libitum. Mortality was recorded daily and feed consumption data were corrected for death losses.

The first experiment was conducted utilizing 162 8-day-old chicks with an average initial weight of 124 grams fed a purified

crystalline amino acid diet. The composition of the basal diet (diet 1) is depicted in Table 1. The basal amino acid mixture was based on the modified reference standard (MRS) of Sasse and Baker (1973) but was further modified such that the lysine content was 0.43% of the diet, or 45% of the chicks requirement as recommended by the NRC (1977). Furthermore, the amino acid pattern of the purified spray-dried whole beef blood meal (BBM) was accounted for by calculation. The diet contained approximately 4000 Kcal ME/Kg.

A second crystalline amino acid mix formulated to simulate the amino acid pattern of BBM at a dietary concentration providing increments of 0.1% lysine is shown in Table 2. Therefore, either the amino acid mix, amino acid mix plus lysine, amino acid mix plus BBM, or BBM alone were added to the lysine-deficient purified diet to add either 0.1% or 0.2% additional lysine. These additions were done at the expense of cornstarch.

The assay consisted of nine treatments replicated three times with six chicks per replication (Table 3). Diets 1-3 were used to construct the lysine standard curve. Diets 4 and 5 were isolysine to diets 2 and 3 and the amino acid pattern of these diets, with the exception of lysine, was equal to the modified reference standard. Diets 6 and 7 were also isolysine to diets 2 and 3, respectively, but had excesses of the other amino acids. Diet 8 was isolysine to diets 2, 4, and 6 but had half the excess amino acid pattern of diet 6. Diet 9 was included to evaluate the chick response to BBM.

TABLE 1. Percentage Composition of Basal Diet (Experiment 1)<sup>a</sup>

| Ingredient                | Percent | Amino Acid Mixture               | Percent |
|---------------------------|---------|----------------------------------|---------|
| Cornstarch                | To 100  | L-arginine-HCl                   | 1.046   |
| Amino Acid Mixture        | 17.22   | L-lysine-HCl                     | 0.500   |
| Corn Oil                  | 10.00   | L-histidine-HCl·H <sub>2</sub> O | 0.290   |
| α-Cell                    | 3.00    | L-phenylalanine                  | 0.352   |
| Glista Salts <sup>b</sup> | 5.37    | L-tyrosine                       | 0.394   |
| NaHCO <sub>3</sub>        | 1.50    | DL-methionine                    | 0.333   |
| Vitamins <sup>c</sup>     | 0.20    | L-cystine                        | 0.331   |
| Choline Chloride          | 0.20    | L-tryptophan                     | 0.120   |
|                           |         | L-threonine                      | 0.561   |
|                           |         | L-valine                         | 0.496   |
|                           |         | L-isoleucine                     | 0.580   |
|                           |         | L-leucine                        | 0.733   |
|                           |         | L-proline                        | 0.313   |
|                           |         | Glycine                          | 0.510   |
|                           |         | L-glutamic acid                  | 10.660  |

<sup>a</sup>Baker et al., 1979.

<sup>b</sup>As percentage of diet: CaCO<sub>3</sub>, .3; Ca(PO<sub>4</sub>)<sub>2</sub>, 2.8; K<sub>2</sub>HPO<sub>4</sub>, .9; NaCl, .88; MgSO<sub>4</sub>·7H<sub>2</sub>O, .35; MnSO<sub>4</sub>·H<sub>2</sub>O, .065; ferric citrate, .05; ZnCO<sub>3</sub>, .01; CuSO<sub>4</sub>·5H<sub>2</sub>O, .002; H<sub>3</sub>BO<sub>3</sub>, .0009; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, .0009; KI, .004; CoSO<sub>4</sub>·7H<sub>2</sub>O, .0001; Na<sub>2</sub>SeO<sub>3</sub>, .0062, total 5.37%.

<sup>c</sup>Provided per kilogram of diet: thiamin-HCl, 100 mg; riboflavin, 16 mg; niacin, 100 mg; D-calcium pantothenate, 20 mg; pyridoxine-HCl, 6 mg; biotin, 0.6 mg; cyanocobalamin, 20 µg; folate, 4 mg; menadione, 5 mg; vitamin A, 10,000 IU; cholecalciferol, 600 ICU; DL-α-tocopheryl-acetate, 20 mg.

TABLE 2. Percentage Composition of the Amino Acid Mix to Simulate 1.13% Dietary BBM (Experiment 1)<sup>a,b</sup>

| Amino Acid                       | Percent |
|----------------------------------|---------|
| L-arginine·HCl                   | 3.968   |
| L-histidine·HCl·H <sub>2</sub> O | 6.105   |
| L-phenylalanine                  | 5.647   |
| L-tyrosine                       | 2.137   |
| DL-methionine                    | 0.649   |
| L-cystine                        | 0.725   |
| L-tryptophan                     | 1.145   |
| L-threonine                      | 3.396   |
| L-valine                         | 7.402   |
| L-isoleucine                     | 0.763   |
| L-leucine                        | 10.187  |
| L-proline                        | 3.319   |
| Glycine                          | 3.433   |
| L-glutamic acid                  | 51.126  |

<sup>a</sup>Purified spray-dried whole beef blood meal obtained from United States Biochemical Corporation.

<sup>b</sup>Calculated values based on data from The Amino Acid Contents of Feeding Stuffs (Degussa, 1982).

TABLE 3. Experimental Treatments and Diet Description (Experiment 1)

| Treatment | Diet Description                            |
|-----------|---------------------------------------------|
| 1         | Basal + 2.621% amino acid mix               |
| 2         | As 1 + 0.100% lysine <sup>a</sup>           |
| 3         | As 1 + 0.200% lysine                        |
| 4         | As 1 + 1.130% BBM <sup>b</sup>              |
| 5         | As 1 + 2.260% BBM                           |
| 6         | As 2 + 1.3105% amino acid mix               |
| 7         | As 3 + 2.621% amino acid mix                |
| 8         | Basal + 1.138% BBM + 1.3105% amino acid mix |
| 9         | Basal + 2.260% BBM                          |

<sup>a</sup>Lysine provided as L-lysine-HCl.

<sup>b</sup>BBM obtained from United States Biochemical Corporation and calculated to contain 0.100% lysine when included at a dietary level of 1.130%.

Experiment 2 was conducted utilizing 165 8-day-old chicks with an average initial weight of 125 grams. The composition of the purified crystalline basal diet (diet 1) is presented in Table 4. In this assay, BBM that was included in the experimental diets was treated in one of four ways in 3 Kg allotments: (1) untreated BBM; no further processing was done; (2) BBM was mixed with 5% dextrose and 40% H<sub>2</sub>O, then dried at 60°C for 28 hours (BBM-A); (3) a mixture of 5% dextrose, 20% H<sub>2</sub>O and BBM was dried at 100°C for 14 hours (BBM-B); and (4) BBM was combined with 10% dextrose and 20% H<sub>2</sub>O, then autoclaved for 20 minutes at 13.78 bars and 122°C (BBM-C). Each experimental diet was fed to triplicate groups of five chicks per replicate. The diets differed only in lysine content and source of lysine. Equivalent amounts of cornstarch were subtracted from the basal diet as lysine, BBM, BBM-A, BBM-B, or BBM-C were added. Diets 2 and 3 had 0.100% and 0.200% supplemental lysine added as L-lysine·HCl respectively. Diets 4 and 5, 6 and 7, 8 and 9, 10 and 11 were formulated with BBM, BBM-A, BBM-B, and BBM-C, respectively, to add the equivalent of 0.100% or 0.200% lysine. To insure that these equivalents were included, percent nitrogen was determined by the Kjeldahl procedure (AOAC, 1975). Using BBM as the standard, the treated BBM samples were included in the diets at the same nitrogen level (Table 4).

In experiment 3, 142 8-day-old chicks averaging 106 grams were utilized. The composition of the basal diet (diet 1) is depicted in Table 5. Triplicate groups of six chicks per replicate were fed

TABLE 4. Percent Nitrogen of BBM and BPBM Samples and Lysine Equivalency Values

| Treatment      | Percentage Nitrogen |       | .100% Lysine Equivalency Percent |      |
|----------------|---------------------|-------|----------------------------------|------|
|                | BBM                 | BPBM  | BBM                              | BPBM |
| Untreated      | 14.44               | 14.80 | 1.13                             | 1.10 |
| A (60°C)       | 13.88               | -     | 1.18                             | -    |
| B (100°C)      | 14.30               | 14.30 | 1.14                             | 1.14 |
| C (autoclaved) | 12.60               | 13.70 | 1.29                             | 1.19 |

TABLE 5. Percentage Composition of Basal Diets (Experiments 2 and 3)<sup>a,b</sup>

| Ingredient                | Percentage<br>Experiment |        | Amino<br>Acid Mixture            | Percent |
|---------------------------|--------------------------|--------|----------------------------------|---------|
|                           | 2                        | 3      |                                  |         |
| Cornstarch                | To 100                   | To 100 | L-arginine·HCl                   | 1.15    |
| Amino Acids               | 19.84                    | 19.84  | L-lysine·HCl                     | 0.80    |
| Corn Oil                  | 10.00                    | 10.00  | L-histidine HCl·H <sub>2</sub> O | 0.45    |
| α-Cell                    | 3.00                     | 3.00   | L-phenylalanine                  | 0.50    |
| Glista Salts <sup>c</sup> | 5.37                     | 5.37   | L-tyrosine                       | 0.45    |
| NaHCO <sub>3</sub>        | 1.50                     | 1.50   | DL-methionine                    | 0.35    |
| Vitamins <sup>d</sup>     | 0.20                     | 0.20   | L-cystine                        | 0.35    |
| Choline Chloride          | 0.20                     | 0.20   | L-tryptophan                     | 0.15    |
|                           |                          |        | L-threonine                      | 0.65    |
|                           |                          |        | L-valine                         | 0.69    |
|                           |                          |        | L-isoleucine                     | 0.60    |
|                           |                          |        | L-leucine                        | 1.00    |
|                           |                          |        | L-proline                        | 0.40    |
|                           |                          |        | Glycine                          | 0.60    |
|                           |                          |        | L-glutamic acid                  | 12.00   |

<sup>a</sup>Baker et al., 1979.

<sup>b</sup>Calculated to contain 0.36% lysine.

<sup>c</sup>As percentage of diet: CaCO<sub>3</sub>, .3; Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>, 2.8; K<sub>2</sub>HPO<sub>4</sub>, .9; NaCl, .88; MgSO<sub>4</sub>·7H<sub>2</sub>O, .35; MnSO<sub>4</sub>·H<sub>2</sub>O, .065; ferric citrate, .05; ZnCO<sub>3</sub>, .01; CuSO<sub>4</sub>·5H<sub>2</sub>O, .002; H<sub>3</sub>BO<sub>3</sub>, .0009; Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O, .0009; KI, .004; CoSO<sub>4</sub>·7H<sub>2</sub>O, .0001; Na<sub>2</sub>SeO<sub>3</sub>, .00002, total 5.37.

<sup>d</sup>Provided per kilogram of diet: thiamin·HCl, 100 mg; riboflavin, 16 mg; niacin, 100 mg; D-calcium pantothenate, 20 mg; pyridoxine·HCl, 6 mg; biotin, 0.6 mg; cyanocobalamin, 20 µg; folate, 4 mg; menadione, 5 mg; vitamin A, 10,000 IU; cholecalciferol, 600 ICU; DL-α-tocopheryl acetate, 20 mg.

the experimental diets. All diets were similar except for level of lysine and source of lysine. The sources were crystalline L-lysine-HCl or a mixture of commercially obtained bovine-porcine dried blood meal (BPBM) that was treated in the same manner as BBM, BBM-B and BBM-C in experiment 2. Diets 2 and 3 contained 0.100% and 0.200% supplemental lysine respectively added as the crystalline amino acid. Diets 4 and 5, 6 and 7, and 8 and 9 were supplemented with BPBM, BPBM-B, and BPBM-C at levels of 0.100% and 0.200%, respectively. To insure these levels of lysine were added, percent nitrogen was determined by the Kjeldahl procedure (AOAC, 1975). The untreated BPBM was used as the standard (Table 5). Additions of lysine and the test proteins were done at the expense of cornstarch. Each pair of diets in experiments 2 and 3 were used in establishing the response curves corresponding to the source of lysine in the experimental diets.

The 42 crossbred pigs in experiment 4 were allotted within litter blocks and by weight with an equal number of males and females utilized. The pigs were individually housed in expanded metal pens measuring 76 centimeters (cm) in length x 60 cm in width x 57 cm in height. Animals were hand fed twice daily, receiving a constant near ad libitum amount of their diets based on the consumption of the pig with the lowest intake. Pigs were hand watered after each feeding and water was also added to the diets to form a more palatable gruel feed. The pigs were 6 to 8 weeks of age and had treatment group averages of 10.5 to 11.8 Kg body weight at the initiation of the

experiment. Seven diets were fed over a three-week period after an adjustment period of three days and replicated six times with a single replication represented by a pig. They were fed a modified semi-purified diet (Baker et al., 1970; Robbins and Baker, 1978; Robbins and Baker, 1977) depicted in Table 6. The basal diet (diet 1) was formulated with both corn and soybean held at dietary levels to restrict lysine to 45% of the pigs NRC recommended intake (.36% lysine) and to further depress the cost of the amino acid mixture (Table 6) by accounting for the levels of the amino acids (calculated from published values, NRC, 1979) provided by corn and soybean meal added at dietary levels of 50% and 6.24%, respectively. Diets 2, 4, and 6 were supplemented with 0.200% lysine as lysine or the BPBM or BPBM-C utilized in experiment 3, respectively. Diets 3, 5, and 7 were supplemented at dietary levels of lysine, BPBM and BPBM-C, respectively to provide 0.400% additional lysine. In all cases, an equal weight of cornstarch was replaced in the diets by the substitutions. Diets 2 and 3, 4 and 5, and 6 and 7, respectively were used in calculating the response curves of lysine, BPBM and BPBM-C.

## II. DATES AND SAMPLE COLLECTION

Chicks utilized in experiments 1-3 were weighed at the end of the assay period. Feed consumption was determined. Pigs in experiment 4 were weighed on a weekly basis for three weeks and bled after an overnight fast via anterior vena cava puncture on day 16

TABLE 6. Percent Composition of Basal Diet (Experiment 4)<sup>a,b</sup>

| Ingredient                        | Percent | Amino Acid Mixture               | Percent |
|-----------------------------------|---------|----------------------------------|---------|
| Corn                              | 50.000  | L-Histidine·HCl·H <sub>2</sub> O | 0.050   |
| Cornstarch                        | 9.645   | L-isoleucine                     | 0.256   |
| Soybean Meal (48)                 | 6.240   | DL-methionine                    | 0.274   |
| Amino Acid Mixture                | 1.291   | L-threonine                      | 0.274   |
| Dextrose                          | 5.000   | L-tryptophan                     | 0.076   |
| Cellulose                         | 4.000   | L-valine                         | 0.248   |
| CaHPO <sub>4</sub>                | 2.600   | L-phenylalanine                  | 0.113   |
| NaHCO <sub>3</sub>                | 1.000   |                                  |         |
| CaCO <sub>3</sub>                 | 0.500   |                                  |         |
| NaCl                              | 0.500   |                                  |         |
| Vitamin Premix <sup>c</sup>       | 0.120   |                                  |         |
| Trace-Mineral Premix <sup>d</sup> | 0.080   |                                  |         |
| Antibiotic <sup>e</sup>           | 0.500   |                                  |         |
| Antioxidant <sup>f</sup>          | 0.005   |                                  |         |

<sup>a</sup>Robbins and Baker, 1978.

<sup>b</sup>Calculated to contain 0.360% lysine.

<sup>c</sup>Provided per 1,000 kg of diet: vitamin A, 10,000,000 IU; vitamin D, 600,000 IU; vitamin E, 11,000 IU; vitamin K, 5,000 mg; riboflavin, 16 g; nicotinic acid, 100 g; D-calcium pantothenate, 20 g; choline-chloride, 110 g; cyanocobalamin, 20 mg; sodium selenite, 100 mg; ascorbic acid, 250 mg.

<sup>d</sup>Contained (%) zinc, 20; iron, 10; manganese, 5.5; copper, 1.1 and iodine, .15.

<sup>e</sup>Tylan 10 (tylosin phosphate).

<sup>f</sup>Butylated Hydroxy Anisole or toluene.

of the assay. Feed pans were scraped following each feeding and refusal feed was dried and weighed in order to make a more accurate determination of feed consumption. Aliquots of each of the blood meals (BBM, BBM-A, BBM-B, BBM-C, BPBM, BPBM-B, BPBM-C) and of the experimental diets from experiment 4 were also obtained.

### III. PROCESSING AND ANALYSES OF SAMPLES

The whole blood samples collected from the pigs utilized in experiment 4 were centrifuged at 5000 rpm for 10 minutes. Plasma was then removed and prepared for amino acid analysis. Plasma preparation for amino acid analysis consisted of the addition of 50 mg of 5'-sulfo-salicylic acid to 2 milliliters of plasma. The samples were placed in ice for 10 minutes and centrifuged. The protein-free plasma (supernatant) was collected, filtered and diluted with lithium citrate buffer (pH = 2.2). Amino acid concentrations were determined using a 5 buffer-lithium citrate system with norleucine as an internal standard per Dionex (1980).

Blood meal samples and experimental diet samples were analyzed for total lysine content using a hydrolytic technique similar to that of Davies and Thomas (1973). A sample weight equivalent to 20 mg of protein (see p. 25) was placed in 5 ml Reacti-Vials (Pierce Chemical Co., Rockford, IL) with 2.5 ml of 6N hydrochloric acid. The vials were then maintained at 110°C for 16 hours, cooled, filtered through Whatman 42 Ashless filters, and washed with approximately 5 ml of glass distilled H<sub>2</sub>O. These solutions were then evaporated to dryness, resuspended in 2.4 ml of lithium citrate dilution buffer, filtered,

and further diluted. Norleucine was used as the internal standard (Dionex, 1980) and analyses were conducted utilizing a Dionex D-400 amino acid analyzer.

Blood meal ash samples were prepared for mineral analysis by AOAC (1975) procedures and analyzed spectrophotometrically for calcium, phosphorus, iron, zinc, sodium, magnesium, potassium, and copper.

#### IV. IN VITRO ANALYSES OF BLOOD MEAL SAMPLES

The in vitro techniques utilized in this study included a rapid dye-binding capacity procedure, multi-enzyme and a pepsin digestibility method.

The rapid dye-binding procedure of Hurrell et al. (1979) was used to assess the ability of lysine in various treated blood meal samples to bind acid orange. This procedure requires approximately two hours per sample run and their dye-binding procedure utilizing ordinary laboratory equipment was followed.

The multi-enzyme procedure (ME-1) described by Hsu et al. (1977) which measures the rate of carboxyl group release was utilized in this study. The multi-enzyme system consists of trypsin, chymotrypsin and peptidase. Aqueous protein suspensions of the test proteins were adjusted to pH 8.0 while stirring in a 37°C water bath. The multi-enzyme solution adjusted to pH 8.0 was added to the protein suspension and the decline in pH of the solution was recorded over a 10 minute period. The pH drop at 15 seconds and 10 minutes were related to the in vivo lysine bioavailability estimates. Since the procedure

is based on the change of pH over time, the presence of some remaining particulates in the aqueous protein solutions were disconcerting; it was possible that they would interfere with a true pH reading. Therefore, a second series of the test proteins were subjected to a modification of the multi-enzyme procedure (ME-2). Aqueous solutions of the proteins were stirred then centrifuged at 5000 rpm for 10 minutes. Fifty milliliters of the supernatant were then drawn and treated in the same manner as the aqueous protein solutions in ME-1.

The pepsin digestibility method utilized was a modification of the AOAC (1975) procedure number 7.037. This method is applicable to the animal proteins only and requires approximately two days. One gram samples of blood meal were defatted by soxhlet extraction for three hours. The residue was dried and quantitatively transferred to 200 ml polypropylene bottles. A 0.2% pepsin solution (150 ml) was added to each bottle which had been prewarmed to 42-45°C. Bottles were agitated in a reciprocating type water bath at 42-45°C for 16 hours with periodic bottle inversion to prevent solid particles from collecting on the sides or bottom of the bottle without contacting the pepsin solution. After incubation the bottles were removed and allowed to settle in a 45° angle settling rack for at least 15 minutes. Samples were filtered utilizing preweighed 50 ml coarse porosity fritted-glass crucibles containing 22 g dry, acid washed ashed sand as a filtering aid. Particles of residue were rinsed from the bottles with warm water and acetone if filtration rate became slow. Crucibles and residue were washed three times with warm water

and vacuum dried. Crucibles were removed, dried in an oven at 100°C for 24 hours, cooled and weighed. Percent indigestible residue for each sample was calculated.

## V. STATISTICAL ANALYSIS OF DATA

The data were analyzed by the General Linear Model (GLM) procedure of the Statistical Analysis System (SAS) (Barr et al., 1979). Orthogonal single-degree-of-freedom comparisons were made to test the significance of treatment of blood meals against the standard, crystalline lysine.

The slope-ratio method (Netke and Scott, 1970; Sasse and Baker, 1973) was used to estimate lysine availability. The procedure relates the slope of the regression equation obtained from the GLM procedure in SAS describing the growth response from each of the treated blood meals with that describing the lysine standard response curve. Thus, when the slope of the blood meal curves was divided by the slope of the standard (crystalline lysine) curve, the unit of lysine per unit of blood meal was obtained, which yields the fraction of bioavailable lysine.

Statistical analysis was also conducted on data collected from the in vitro assays. A regression procedure was employed to determine the best (maximum  $R^2$ ) single or multiple variable model explaining variation in the dependent variable, bioavailable lysine.

A list of the independent variables considered and their corresponding acronyms is presented in Table 7. The dependent variable

TABLE 7. Independent Variables and Corresponding Acronyms

| Variable                   | Acronyms |
|----------------------------|----------|
| Crude Protein              | CP       |
| Multienzyme Technique 1    | ME-1     |
| Multienzyme Technique 2    | ME-2     |
| Pepsin Digestibility       | PEP      |
| Rapid Dye-Binding Capacity | DBC      |
| Calcium                    | Ca       |
| Magnesium                  | Mg       |
| Potassium                  | K        |
| Iron                       | Fe       |
| Zinc                       | Zn       |
| Sodium                     | Na       |
| Copper                     | Cu       |
| Plasma Lysine              | PL       |
| Hydrolyzate (Total) Lysine | HL       |

was regressed on the independent variable using an equation in the form:

$$\hat{Y} = b_0 + b_n X_n$$

where  $\hat{Y}$  = bioavailable lysine,  $X$  = in vitro component and  $n$  varying with the number of significant ( $P < .20$ ) main effects.

## CHAPTER IV

### RESULTS AND DISCUSSION

#### I. EXPERIMENT 1

The performance data for experiment 1 are presented in Table 8. Chicks fed the diets supplemented with graded levels of crystalline lysine, amino acid mix plus BBM or BBM responded with linear increases in weight gain, feed intake, and gain to feed ratio. Furthermore, at equivalent levels of supplemental lysine, crystalline L-lysine·HCl supported greater weight gains than those recorded for either the addition of BBM plus the amino acid mix or BBM. None of the difference appeared to be due to feed intake and lysine intake was similar between similar treatments regardless of source. The regression analysis presented in Table 9 was performed on source of lysine and amino acid pattern; however, the method of incorporation did not have a significant effect ( $B_1X_1$  vs  $B_2X_2$ ,  $P>0.10$ ;  $B_3X_3$  vs  $B_4X_4$ ,  $P>0.30$ ). Therefore, it was concluded that the method of incorporation, which accounts for the amino acid pattern of the test protein, did not cause any undetected growth stimulations or depressions, which could result in an over- or under-estimation of bioavailable lysine as Baker (1978) suggested is the case with soybean meal and by Ford (1962) and Osner and Johnson (1968). Thus, the curves corresponding to experimental diets 2 and 6, 3 and 7, 4 and 8, and 5 and 9 were combined to yield a simpler regression equation generated to determine

TABLE 8. Performance Data in Relation to Lysine Consumed (Experiment 1)<sup>a,b</sup>

| Treatment | Supplement to Basal Diet <sup>c</sup> | Gain (g) | Feed Intake (g) | Gain/Feed | Lysine Intake (mg) |
|-----------|---------------------------------------|----------|-----------------|-----------|--------------------|
| 1         | 2.62% amino acid mix                  | 49       | 125             | .395      | 537.5              |
| 2         | As1 + 0.10% lysine <sup>d</sup>       | 77       | 161             | .48       | 853.3              |
| 3         | As1 + 0.20% lysine                    | 104      | 178             | .59       | 1121.4             |
| 4         | As1 + 1.13% BBM                       | 72       | 149             | .48       | 789.7              |
| 5         | As1 + 2.26% BBM                       | 99       | 179             | .56       | 1127.7             |
| 6         | As2 + 1.3105% amino acid mix          | 72       | 146             | .49       | 773.8              |
| 7         | As3 + 2.621% amino acid mix           | 90       | 160             | .56       | 1008.0             |
| 8         | 1.13% BBM + 1.3105% amino acid mix    | 80       | 160             | .50       | 848.0              |
| 9         | 2.26% BBM                             | 104      | 185             | .57       | 1165.5             |

<sup>a</sup>Data represents means of six chicks per treatment.

<sup>b</sup>Pooled standard error of the mean (SEM) for gain, feed intake, and gain/feed were 3.79, 6.62, and 0.03, respectively.

<sup>c</sup>Calculated to contain 0.43% lysine.

<sup>d</sup>Lysine added as L-lysine-HCl.

TABLE 9. Regression Analysis of Performance Data (Experiment 1)<sup>a</sup>

| Supplement to Basal Diet                        | Regression Equation      | Coefficient of Determination ( $r^2$ ) | Standard Error of Coefficients |
|-------------------------------------------------|--------------------------|----------------------------------------|--------------------------------|
| Lysine <sup>b</sup><br>(MRS amino acid profile) | $Y = 52.42 + 117.986X_1$ | 0.917                                  | 10.62( $X_1$ )                 |
| Lysine<br>(BBM amino acid profile)              | + 97.93 $X_2$            |                                        | 11.75( $X_2$ )                 |
| BBM<br>(MRS amino acid profile)                 | + 12.93 $X_3$            |                                        | 1.13( $X_3$ )                  |
| BBM<br>(BBM amino acid profile)                 | + 11.72 $X_4$            |                                        | 1.17( $X_4$ )                  |

<sup>a</sup>td for  $X_1$  vs  $X_2 = 1.71$ ,  $P > 0.10$ ; td for  $X_3$  vs  $X_4 = 1.01$ ,  $P > 0.30$ .

<sup>b</sup>Lysine added as L-lysine-HCl.

the percent bioavailable lysine using the slope-ratio method (Netke and Scott, 1970; Sasse and Baker, 1973) in BBM (Table 10) which was  $11.30 \pm 1.08\%$ . This value is 4.00 to 4.60 percentage points higher than the estimates made by Parsons (1975, 1977, 1979) in assays using starter pigs. Kratzer and Green (1957) reported estimates of 6-8% bioavailable lysine in vat dried blood and 10-12% bioavailable lysine in spray dried soluble blood meal as measured by the chick growth assay. Hamm and Searcy (1976) presented data that suggests that raw poultry blood may contain 6.72-7.92% bioavailable lysine while a mildly processed sample may contain 6.34-7.04% bioavailable lysine, based on growth assays utilizing chicks.

The results of experiment 1 were the basis for the design of experiment 2. If an excess pattern of amino acids did not have a detrimental effect on the performance of the chicks in experiment 1, it was assumed that it would not need to be considered in experiment 2. Furthermore, the bioavailable value of lysine (11.3%) determined by the growth assay was considered to be a valid estimation, and addition of treated blood meal samples were based on this value.

## II. EXPERIMENT 2

Data concerning performance are depicted in Table 11. Linear increases in weight gain, feed intake, and gain feed ratio were observed with graded levels of crystalline lysine, BBM-A, BBM-B, or BBM-C dietary supplementation. As expected, there were also

TABLE 10. Composite Regression Analysis of Performance Data  
(Experiment 1)

| Supplement to<br>Basal Diet                                   | Regression<br>Equation  | Coefficient of<br>Determination<br>( $r^2$ ) | Standard<br>Error of<br>Coefficients |
|---------------------------------------------------------------|-------------------------|----------------------------------------------|--------------------------------------|
| Lysine<br>(MRS and BBM<br>amino acid<br>pattern) <sup>a</sup> | $Y = 52.07 + 109.96X_1$ | 0.917                                        | $7.89(X_1)$                          |
| BBM<br>(MRS and BBM<br>amino acid<br>pattern)                 | $+ 12.45X_2$            |                                              | $0.81(X_2)$                          |

<sup>a</sup>Lysine added as L-lysine-HCl.

TABLE 11. Performance Data in Relation to Lysine Consumed  
(Experiment 2)<sup>a,b</sup>

| Treatment | Supplement to<br>Basal Diet <sup>c</sup> | Gain<br>(g) | Feed<br>Intake<br>(g) | Gain/<br>Feed | Lysine<br>Intake<br>(mg) |
|-----------|------------------------------------------|-------------|-----------------------|---------------|--------------------------|
| 1         | 0.0% lysine                              | 54.3        | 143.5                 | 0.380         | 617.05                   |
| 2         | 0.10% lysine <sup>d</sup>                | 80.7        | 166.0                 | 0.487         | 879.80                   |
| 3         | 0.20% lysine                             | 120.8       | 217.5                 | 0.555         | 1370.25                  |
| 4         | 1.13% BBM                                | 79.9        | 180.5                 | 0.442         | 956.65                   |
| 5         | 2.26% BBM                                | 104.7       | 202.5                 | 0.516         | 1275.75                  |
| 6         | 1.18% BBM-A                              | 77.6        | 170.7                 | 0.457         | 904.71                   |
| 7         | 2.36% BBM-A                              | 96.0        | 191.9                 | 0.501         | 1208.97                  |
| 8         | 1.14% BBM-B                              | 68.3        | 152.9                 | 0.448         | 810.37                   |
| 9         | 2.28% BBM-B                              | 93.0        | 173.9                 | 0.532         | 1095.57                  |
| 10        | 1.29% BBM-C                              | 61.4        | 145.1                 | 0.423         | 769.03                   |
| 11        | 2.58% BBM-C                              | 72.0        | 158.9                 | 0.453         | 1001.07                  |

<sup>a</sup>Data represents means of five chicks per treatment.

<sup>b</sup>Pooled SEM for gain, feed intake, and gain feed ratio were 3.49, 8.21, and 0.011, respectively.

<sup>c</sup>Basal calculated to contain 0.43% lysine.

<sup>d</sup>Lysine added as L-lysine-HCl.

differences in these parameters among heat treatment of blood meal, with the greatest depressions seen in those chicks fed BBM-B or BBM-C. The subjection of proteins to various types of heat treatment, particularly for extensive periods of time, may result in reduced feed intake which is in agreement with several other studies (Miller et al., 1965; Ford and Salter, 1966; Narayana Rao and McLaughlan, 1967). Knipfel and coworkers (1975) have suggested that this may be due not only to poorer nutritive value which may be a factor in feed consumption, but also to reduced palatability. Due to the physical nature of blood meal, it is highly probable that palatability, which is difficult to objectively measure, had a role in reducing feed intake, thus lowering lysine intake. The regression analysis of the data presented in Table 12 included lysine consumption (Table 11) partitioned by source: crystalline lysine (L-lysine·HCl), BBM, BBM-A, BBM-B, or BBM-C. The treatment of blood meals significantly reduced the percent bioavailable lysine in BBM-B,  $7.49 \pm 0.87\%$ , ( $P < .05$ ) and BBM-C,  $3.88 \pm 0.87\%$ , ( $P < .001$ ) as compared to BBM which contained  $9.08 \pm 0.65\%$  bioavailable lysine. The sample treated at  $60^{\circ}\text{C}$  for 28 hours contained  $8.16 \pm 0.68\%$  and was not significantly different ( $P > .4$ ), indicating that the temperature and/or length of temperature subjection was not sufficient to reduced lysine bioavailability or protein quality. However, according to Osner and Johnson (1968) the treatment should have had a more significant effect. They have suggested, as a general rule, that very little protein damage will occur with temperatures less than  $100^{\circ}\text{C}$ , as was

TABLE 12. Regression Analysis of Performance Data (Experiment 2)

| Supplement to<br>Basal Diet | Regression<br>Equation  | Coefficient of<br>Determination<br>( $r^2$ ) | Standard<br>Error of<br>Coefficients |
|-----------------------------|-------------------------|----------------------------------------------|--------------------------------------|
| Lysine <sup>a</sup>         | $Y = 54.70 + 122.86X_1$ | 0.95                                         | $6.03(X_1)$                          |
| BBM                         | $+ 11.15X_2$            |                                              | $0.70(X_2)$                          |
| BBM-A                       | $+ 10.02X_3$            |                                              | $0.73(X_3)$                          |
| BBM-B                       | $+ 9.21X_4$             |                                              | $0.92(X_4)$                          |
| BBM-C                       | $+ 4.76X_5$             |                                              | $0.89(X_5)$                          |

<sup>a</sup>Lysine added as L-lysine·HCl.

the case here, but with a heating period no more than one hour, which was greatly exceeded in the treatment of BBM-A. In any case, the treatment should not be considered severe enough to have a significant effect on lysine bioavailability from blood meal and therefore was not considered in subsequent studies.

### III. EXPERIMENT 3

Performance data from experiment 3 are presented in Table 13. Chicks fed the diets supplemented with graded levels of crystalline lysine, untreated bovine-porcine blood meal (BPBM), BPBM treated at 100°C (BPBM-B), or autoclaved BPBM (BPBM-C) responded with linear increases in gain, feed intake, and gain feed ratio which was expected. As noted previously, this was a continued trend from the first two experiments, and the change in type of blood meal utilized (purified beef blood vs commercially obtained beef-pork blood) did not influence performance, although average gains and feed intakes were depressed when compared to the performance of chicks in experiment 2. This might have been due to two factors: (1) the average initial weight of the chicks in experiment 3 was lower (106 g vs 125 g), and (2) the chicks did not appear to be as healthy as those in the previous trial based on subjective evaluation. Only one death was recorded in experiment 2, whereas 11 deaths and one chick with a slipped tendon were recorded in experiment 3. The slope-ratio methodology based on the regressed data (Table 14) indicated percent bioavailabilities for BPBM, BPBM-B and BPBM-C as  $8.57 \pm 0.25\%$ ,  $5.98 \pm 0.33\%$ , and  $5.66 \pm 0.39\%$ ,

TABLE 13. Performance Data in Relation to Lysine Consumed  
(Experiment 3)<sup>a,b</sup>

| Treatment | Supplement to<br>Basal Diet <sup>c</sup> | Gain<br>(g)       | Feed<br>Intake<br>(g) | Gain/<br>Feed      | Lysine<br>Intake<br>(mg) |
|-----------|------------------------------------------|-------------------|-----------------------|--------------------|--------------------------|
| 1         | 0.00% lysine <sup>d</sup>                | 26.0              | 90.0                  | 0.289              | 387.0                    |
| 2         | 0.10% lysine                             | 25.3 <sup>e</sup> | 88.0 <sup>e</sup>     | 0.318 <sup>e</sup> | 466.4 <sup>e</sup>       |
| 3         | 0.20% lysine                             | 70.0              | 123.5                 | 0.567              | 778.1                    |
| 4         | 1.10% BPBM                               | 43.0              | 102.0                 | 0.420              | 540.6                    |
| 5         | 2.20% BPBM                               | 53.3              | 119.0                 | 0.449              | 749.7                    |
| 6         | 1.14% BPBM-B                             | 35.0              | 99.0                  | 0.352              | 524.7                    |
| 7         | 2.28% BPBM-B                             | 42.3              | 103.0                 | 0.412              | 648.9                    |
| 8         | 1.19% BPBM-C                             | 36.3              | 96.0                  | 0.374              | 508.8                    |
| 9         | 2.38% BPBM-C                             | 42.3              | 107.0                 | 0.395              | 674.1                    |

<sup>a</sup>Data represents means of six chicks per treatment.

<sup>b</sup>Pooled SEM for gain, feed intake and gain/feed were 8.43, 17.42, and .019, respectively.

<sup>c</sup>Calculated to contain 0.43% lysine.

<sup>d</sup>Lysine added as L-lysine·HCl.

<sup>e</sup>Data adjusted, due to death, for regression analysis.

TABLE 14. Regression Analysis of Performance Data (Experiment 3)

| Supplement to<br>Basal Diet | Regression<br>Equation  | Coefficient of<br>Determination<br>( $r^2$ ) | Standard<br>Error of<br>Coefficients |
|-----------------------------|-------------------------|----------------------------------------------|--------------------------------------|
| Lysine <sup>a</sup>         | $Y = 22.84 + 147.59X_1$ | 0.77                                         | $14.85(X_1)$                         |
| BPBM                        | + $12.66X_2$            |                                              | $1.74(X_2)$                          |
| BPBM-B                      | + $8.82X_3$             |                                              | $1.90(X_3)$                          |
| BPBM-C                      | + $8.36X_4$             |                                              | $1.75(X_4)$                          |

<sup>a</sup>Lysine added L-lysine·HCl.

respectively. These values are lower than their counterparts in experiment 2, but these may be due to the problems discussed previously. Parsons (1977) reported that there was no difference between flash ring dried beef blood and flash ring dried swine blood, both containing 6.7% bioavailable lysine, which he considered to be a conservative value. However, the BPBM (8.7%) was not significantly different from the BBM, and the lower value may have been due to a dilution factor. The commercially obtained BPBM contained fragments of bone and coal that could not be readily removed. Nonetheless, there were differences in BPBM among treatments; BPBM-B and BPBM-C were significantly different from BPBM ( $P < .001$ ), but bioavailable lysine from BPBM-C was not significantly different from BPBM-B ( $P > .4$ ). It is possible that the epsilon amino group of lysine in the commercially obtained blood meal, due to contamination and/or processing at the slaughter plant, may not have been as protected as that in the purified blood meal, such that a less severe treatment resulted in the same degree of destruction. Waibel and co-workers (1974) have reported that conventionally processed blood meal (for example, vat dried) may be lower in both total and available lysine than spray-dried or mildly treated blood meals. Therefore, it is quite possible that more severe treatments of blood meal samples obtained commercially may be needed before there are noticeable differences based on growth assays. On the basis of this, BPBM-B was dropped from the subsequent study.

## IV. EXPERIMENT 4

The performance data for experiment 4 are presented in Table 15. Linear increases in weight gain and gain feed ratio were observed with graded levels of crystalline lysine, BPBM, or BPBM-C dietary supplementation. However, feed intake of pigs fed graded levels of BPBM was not different, and was slightly higher than those pigs fed lysine as L-lysine-HCl. Pigs fed the two levels of BPBM-C also did not have feed intakes that were significantly different; however, their total feed intake was lower than that for pigs on treatments 2-5, with the most depression being noted with pigs fed 9.26% BPBM-C, who consumed less than any other group. The lower feed intake of the pigs on treatments 6 and 7 was most likely a palatability problem. Blood meal, especially a heat treated sample, has an unpleasant odor and undesirable color and texture. The results of the regression analysis are depicted in Table 16. The treatment of the blood meal allotments significantly reduced the percent bioavailable lysine in BPBM-C which had  $0.50 \pm 0.55\%$  ( $P < .001$ ) when compared to BPBM, which contained  $9.89 \pm 0.73\%$ . Interestingly, the BPBM value in experiment 4 was higher than that in experiment 3 (8.7%), but only slightly lower than the untreated purified spray-dried blood meal utilized in experiment 1 (11.03%), which is in agreement with Parsons (1977) when he found the two sources to be equivalent in lysine bioavailability. However, BPBM-C contained essentially no available lysine for the pig, although the chick assay resulted in an estimate of 5.6%. This

TABLE 15. Performance Data of Swine in Relation to Lysine Consumed (Experiment 4)<sup>a,b</sup>

| Treatment | Supplement to Basal Diet <sup>c</sup> | Gain (kg) | Feed Intake (kg) | Gain/Feed | Lysine Intake (g) |
|-----------|---------------------------------------|-----------|------------------|-----------|-------------------|
| 1         | 0.00% lysine <sup>d</sup>             | 5.15      | 10.68            | 0.49      | 38.45             |
| 2         | 0.20% lysine                          | 5.02      | 10.98            | 0.46      | 61.49             |
| 3         | 0.40% lysine                          | 6.07      | 11.71            | 0.53      | 88.99             |
| 4         | 2.67% BPBM                            | 4.95      | 12.04            | 0.41      | 67.42             |
| 5         | 5.34% BPBM                            | 6.42      | 11.80            | 0.54      | 89.68             |
| 6         | 4.63% BPBM-C                          | 3.83      | 10.95            | 0.35      | 61.32             |
| 7         | 9.26% BPBM-C                          | 4.60      | 10.66            | 0.43      | 81.02             |

<sup>a</sup>Data represents means of six pigs per treatment.

<sup>b</sup>Pooled SEM for gain, feed intake, and gain/feed were 0.20, 1.01, and .03, respectively.

<sup>c</sup>Calculated to contain 0.36% lysine.

<sup>d</sup>Lysine added as L-lysine·HCl.

TABLE 16. Regression Analysis of Performance Data (Experiment 4)

| Supplement to<br>Basal Diet | Regression<br>Equation | Coefficient of<br>Determination<br>( $r^2$ ) | Standard<br>Error of<br>Coefficients |
|-----------------------------|------------------------|----------------------------------------------|--------------------------------------|
| Lysine <sup>a</sup>         | $Y = 4.29 + 31.72X_1$  | .45                                          | $8(X_1)$                             |
| BPBM                        | + $3.14X_2$            | .                                            | $0.75(X_2)$                          |
| BPBM-C                      | + $0.16X_3$            |                                              | $0.47(X_3)$                          |

<sup>a</sup>Lysine added as L-lysine·HCl.

was probably a result of several factors: (1) reduced palatability; however, the lysine intake of these pigs was not significantly lower, and in fact was parallel to their counterparts fed crystalline lysine or BPBM; (2) depressed protein digestibility; and (3) an imbalance of the amino acids in the diet as a result of treatment. This suggests that the chick may not be valuable as a biological model for the pig when low quality proteins, as was BPBM-C, are the test proteins.

Results of the plasma lysine analyses are presented in Table 17, and were not significantly different among treatment. The plasma lysine levels tended to increase with increasing dietary levels of added lysine; however, there was a decrease in the plasma lysine levels of pigs fed 9.26% BPBM-C. Narayana Rao and McLaughlan (1967) reported that plasma lysine concentrations remained relatively stable unless rats were fed over an extended period of time a severely treated casein diet that had undergone the Maillard reaction. A similar trend was also noted when soy was utilized as the test protein.

## V. REGRESSION EQUATIONS FOR PREDICTING BIOAVAILABLE LYSINE

Equations for predicting bioavailable lysine in BBM and BPBM are presented in Tables 18 and 19, respectively. The best basic model was defined as that model with the highest  $R^2$ . An applied model was considered to be one that contained factors that were easily and quickly determined (total analysis time of 24 hours or less with a

TABLE 17. Plasma Lysine in Relation to Dietary Treatment  
(Experiment 4)<sup>a,b</sup>

| Treatment | Supplement to<br>Basal Diet <sup>c</sup> | Plasma Lysine<br>nmole/ml |
|-----------|------------------------------------------|---------------------------|
| 1         | 0.00% Lysine <sup>d</sup>                | 111.0                     |
| 2         | 0.20% Lysine                             | 80.7                      |
| 3         | 0.40% Lysine                             | 98.9                      |
| 4         | 2.67% BPBM                               | 84.9                      |
| 5         | 5.34% BPBM                               | 114.8                     |
| 6         | 4.63% BPBM-C                             | 102.7                     |
| 7         | 9.26% BPBM-C                             | 65.7                      |

<sup>a</sup>Data represents means of six pigs per treatment.

<sup>b</sup>There were no significant differences at the  $P < .05$  level.

<sup>c</sup>Calculated to contain 0.36% Lysine.

<sup>d</sup>Lysine added as L-lysine-HCl.

TABLE 18. Regression Coefficients for Models Predicting Bioavailable Lysine with In Vitro Components on Purified Beef Blood Meal (BBM)

| Model No. | Model                        | R <sup>2</sup> | C.V. <sup>a</sup> |
|-----------|------------------------------|----------------|-------------------|
| 1         | 19.93 - 0.06 PEP - 12.56 P   | 0.83           | 12.88             |
| 2         | 3.21 - 0.04 PEP + 4.47 ME-2  | 0.85           | 12.16             |
| 3         | 6.52 - 0.05 PEP + 0.19 HL    | 0.86           | 11.88             |
| 4         | 34.23 - 0.05 PEP - 12.75 Fe  | 0.93           | 7.93              |
| 5         | 29.92 - 0.05 PEP - 1.56 Na   | 0.95           | 6.52              |
| 6         | 34.02 - 0.08 PEP - 0.28 CP   | 0.96           | 6.25              |
| 7         | 14.73 - 0.07 PEP - 0.26 DBC  | 0.99           | 2.17              |
| 8         | 13.85 - 0.09 PEP - 3.84 ME-1 | 0.99           | 0.98              |

<sup>a</sup>Coefficient of variation.

TABLE 19. Coefficients for Models Predicting Bioavailable Lysine with In Vitro Components in Commercially-Obtained Beef-Pork Blood Meal (BPBM)

| Model No. | Model            | R <sup>2</sup> | C.V. <sup>a</sup> |
|-----------|------------------|----------------|-------------------|
| 1         | -34.15 + 17.44 P | 0.70           | 13.83             |
| 2         | -11.32 + 3.72 K  | 0.79           | 11.67             |
| 3         | 8.73 - 0.03 PEP  | 0.99           | 0.01              |

<sup>a</sup>Coefficient of variation.

minimal amount of equipment required). The best basic model was:

$$\hat{Y} = 13.85 - 0.09 \text{ PEP} - 3.84 \text{ ME-1}$$

and accounted for 99% of the variation in bioavailable lysine estimates ( $P < .02$ ). The best applied model was:

$$\hat{Y} = 34.02 - 0.08 \text{ PEP} - 0.28 \text{ CP}$$

with a  $R^2$  value of .96 ( $P < .02$ ).

The best apparent basic model, however, for explaining variation in bioavailable lysine in BPBM contained PEP and was not the best applied model. The best single variable from an applied standpoint that explained the most variation (79%) was potassium ( $P < .22$ ). It is interesting to note that unlike the models describing bioavailability of lysine in BBM, CP, or HL did not enter the single variable models for BPBM, as would be expected. This difference could not be accounted for in these previous studies. Therefore, more comprehensive studies need to be made to firmly establish the relationship between the components of the test protein and bioavailability of lysine.

## CHAPTER V

### SUMMARY

Three experiments were conducted utilizing 498 Hubbard x White Mountain 8 day-old chicks, 162 in experiments 1 and 3 and 165 in experiment 2. One other experiment was conducted utilizing 42 cross-bred pigs allotted by weight within litter replicate. The objectives of these experiments were to evaluate the effect of method of incorporation of the test protein, blood meal, on growth response to supplemental lysine; to determine the effect of heat treatment on lysine bioavailability from two sources, either purified spray-dried whole beef blood (BBM) or a commercially obtained vat dried beef-pork blood meal (BPBM) using the slope ratio technique; and finally to test the validity of using the chick as a biological model for young pigs. Chicks were allotted by weight to one of 8, 11, or 9 dietary treatments in experiments 1-3. Diets contained 45% of the chicks or pigs requirement for lysine (0.43% and 0.36%, respectively), served as control diets. In experiment 1, experimental diets were supplemented with 0.10% or 0.20% lysine as L-lysine-HCl or from spray-dried whole beef blood that had undergone no further processing plus the addition of excess amino acids to simulate the amino acid pattern of the blood meal. The experimental diets in experiment 2 were supplemented at graded levels with L-lysine-HCl or at graded isonitrogenous levels with spray-dried whole beef blood that had been treated in one of four ways: (1) untreated blood

meal was included in the diet with no further processing: (2) blood meal A was mixed with 5% dextrose and 40% water, then dried at 60°C for 28 hours; (3) blood meal B was mixed with 5% dextrose and 20% water, then dried at 100°C for 14 hours; (4) blood meal C was mixed with 10% dextrose and 20% water and then autoclaved at 13.78 bars and 122°C for 20 minutes. Diets in experiment 3 were supplemented with graded levels of L-lysine-HCl or a mixture of commercial beef-pork blood meal that had received the same treatments as blood meals B and C above, with an untreated sample used as a control. In experiment 4, diets were supplemented with L-lysine-HCl, untreated beef-pork blood meal and beef-pork blood meal C. Body weight and feed consumption were recorded for all four experiments. Blood plasma samples and aliquots of diets in experiment 4 were collected for total lysine analysis.

Results from experiment 1 indicated that crystalline lysine always supported more growth, and graded additions of 0.10% and 0.20% lysine from either source regardless of amino acid pattern resulted in linear responses in gain, feed intake, and gain feed ratio. The percent bioavailable lysine from BBM was  $11.3 \pm 1.08\%$  for young poultry.

Results from experiment 2 indicated linear increases in gain, feed intake, and gain feed ratio with graded levels of supplementation. There were differences in these parameters among treatments of blood meal, indicating that the treatment of blood meal significantly reduced the percent bioavailable lysine in blood meal B ( $P < .05$ ) and C ( $P < .001$ ). The percent bioavailable lysine of BBM, BBM-A, BBM-B and BBM-C were  $9.08 \pm 0.65$ ,  $8.16 \pm 0.68$ ,  $7.49 \pm 0.07$  and  $3.88 \pm 0.87\%$ , respectively.

Performance data from experiment 3 exhibited the same trends in gain, feed intake, and gain feed ratio as in experiment 2. Heat treatment had a significant effect on the blood meal samples, reducing the percent bioavailable lysine in BPBM-B and BPBM-C ( $P < .001$ ). However, BPBM-B was not significantly different from BPBM-C ( $P > .4$ ). The percent bioavailable lysine contained in BPBM, BPBM-B and BPBM-C were  $8.57 \pm 0.25$ ,  $5.98 \pm 0.33$  and  $5.66 \pm 0.39\%$ , respectively.

Pigs from experiment 4 exhibited linear increases in gain and gain feed ratios. Feed intake of pigs fed beef-pork blood meal C was lower than for all other test groups, indicating lysine bioavailability was significantly reduced under reducing conditions at elevated temperatures ( $P < .001$ ). The lysine bioavailability of untreated beef-pork blood meal and beef-pork blood meal C was  $9.89 \pm 0.73\%$  and  $0.50 \pm 0.55\%$ . Data from this experiment suggested that the chick may not be a good biological model for the pig when low quality proteins are utilized as the test protein. The plasma lysine values for each treatment were not significantly different.

Multi-enzyme (Hsu et al., 1977), pepsin digestibility (AOAC, 1975) and a rapid dye-binding procedure (Hurrell et al., 1979) were performed. The blood meal samples were also analyzed for mineral composition and total lysine. These factors were used as independent variables and regressed on the dependent variables, bioavailable lysine to obtain prediction equations. The best model with a  $R^2$  value of 0.99 for BBM contained PEP and ME-1; the best single variable model for BPBM included PEP, which also had a  $R^2$  value of 0.99.

LITERATURE CITED

## LITERATURE CITED

- Akeson, W. R. and M. A. Stahmann. 1964. A pepsin pancreatin digest index of protein quality evaluation. *J. Nutr.* 83:257.
- Alsmeyer, R. H., H. E. Cunningham and M. L. Happich. 1974. Equations predict PER from amino acid analysis. *Food Technol.* 28:34.
- Anderson, R. H. 1978. Protein quality testing: industry needs. *Food Technol.* 32:65.
- AOAC. 1975. Official Methods of Analysis. 12th ed. AOAC, Washington, D.C., 1970.
- Ashworth, U.S. 1966. Determination of protein in dairy products by dye binding. *J. Dairy Sci.* 49:133.
- Baker, D. H., D. E. Becker, A. H. Jensen and B. G. Harmon. 1970. Reproductive performance and progeny development in swine as influenced by protein restriction during various portion of gestation. *J. Anim. Sci.* 31:526.
- Baker, D. H. 1978. Nutrient bioavailability in feedstuffs: methodology for determining amino acid and B vitamin availability in cereal grains and soybean meal. Pp. 1-12, *Proc. Ga. Nutr. Conference.*
- Baker, D. H., K. R. Robbins and J. S. Buck. 1979. Modification of the level of histidine and sodium bicarbonate in the Illinois crystalline amino acid diet. *Poult. Sci.* 58:749.
- Barr, A. J., J. H. Goodnight, J. D. Said and G. T. Helwig. 1979. SAS Users Guide. SAS Institute Corporation, Raleigh, N.C.
- Batterham, E. S., R. D. Murison and C. E. Lewis. 1979. Availability of lysine in protein concentrates as determined by the slope-ratio assay with growing pigs and rats and by chemical techniques. *Brit. J. Nutr.* 41:383.
- Batterham, E. S., R. D. Murison and R. F. Lowe. 1981. Availability of lysine in vegetable protein concentrates as determined by the slope ratio assay with growing pigs and rats and by chemical techniques. *Brit. J. Nutr.* 45:401.
- Bhatty, R. S. and K. W. Wu. 1975. Lysine screening in barley with a modified Udy dye-binding method. *Can. J. Plant Sci.* 55:685.

- Bjarnason, J. and K. J. Carpenter. 1969. Mechanisms of heat damage in proteins. I. Models with acylated lysine units. Brit. J. Nutr. 23:859.
- Bodwell, C. E. 1977. Problems in the development and application of rapid methods of assessing protein quality. Food Technol. 31:73.
- Booth, V. H. 1971. Problems in the determination of FDNB-available lysine. J. Sci. Food Agric. 22:658.
- Buchanan, R. A. 1969. In vivo and in vitro methods of measuring nutritive value of leaf protein preparations. Brit. J. Nutr. 23:533.
- Carpenter, K. J. and G. M. Ellinger. 1955. Estimation of "available lysine" in protein concentrates. Biochem. J. 61:xi.
- Carpenter, K. J., G. M. Ellinger, M. I. Munro and E. J. Rolfe. 1957. Fish products as protein supplements to cereals. Brit. J. Nutr. 11:162.
- Carpenter, K. J. 1960. The estimation of the available lysine in animal-protein foods. Biochem. J. 77:604.
- Carpenter, K. J. 1973. Damage to lysine in food processing: its measurement and significance. Nutr. Abstr. Rev. 43:424.
- Carpenter, K. J. and J. Opstuedt. 1976. Application of chemical and biological assay procedures for lysine to fish meals. J. Agric. Fd. Chem. 24:389.
- Cheeke, D. R. and R. O. Myer. 1975. Protein digestibility and lysine availability in alfalfa meal and alfalfa protein concentrate. Nutr. Rep. Int. 12:337.
- Costa, D. M. A., A. H. Jensen, D. H. Baker and H. W. Norton. 1977. Lysine availability of roasted dried and high-moisture corns as determined by the chick growth assay. J. Ani. Sci. 46:457.
- Davies, M. G. and A. J. Thomas. 1973. An investigation of hydrolytic techniques for the amino acid analysis of foodstuffs. J. Sci. Food Agric. 24:1525.
- Degussa, A. G. 1982. The Amino Acid Contents of Feeding Stuffs. West Germany.

- Dionex Corporation. 1980. D-400 Amino acid analyzer: operation and maintenance manual. Dionex Technical Bulletin No. 28304.
- Elred, N. R. and G. Rodney. 1946. The effect of proteolytic enzymes on raw and heated casein. *J. Biol. Chem.* 162:261.
- Finot, P. A. and J. Mauron. 1972. Blockage of lysine by the Maillard reaction N-(desoxy-1-D-fructosyl-1) and N-(desoxy-1-lactulosyl-1 lysine). *Helv. Chim. Acta.* 55:1153.
- Ford, J. E. 1962. A microbiological method for assessing the nutritional value of proteins. 2. The measurement of "available" methionine, leucine, isoleucine, arginine, histidine, tryptophan and valine. *Brit. J. Nutr.* 16:409.
- Ford, J. E. and D. N. Salter. 1966. Analysis of enzymically digested food proteins by sephadex-gel filtration. *Brit. J. Nutr.* 20:843.
- Gauthier, S. F., C. Vachon, J. D. Jones and L. Savore. 1982. Assessment of protein digestibility by in vitro enzymatic hydrolysis with simultaneous dialysis. *J. Nutr.* 112:1718.
- Hamm, D. and G. K. Searcy. 1976. Some factors which affect the availability of lysine in blood meals. *Poult. Sci.* 55:582.
- Hill, D. C., J. Singh and G. C. Ashton. 1966. A chick bioassay for lysine. *Poult. Sci.* 44:408.
- Hodge, J. E. 1953. Chemistry of browning reactions in model systems. *J. Agr. Food Chem.* 1:928.
- Hsu, H. W., D. L. Vavak, L. D. Satterlee and G. A. Miller. 1977. A multienzyme technique for estimating protein digestibility. *J. Food Sci.* 45(5):1269.
- Hurrell, R. F. and K. J. Carpenter. 1974. Mechanisms of heat damage in protein. 4. The reactive lysine content of heat-damaged material as measured in different ways. *Brit. J. Nutr.* 32:589.
- Hurrell, R. F., P. Lerman and K. J. Carpenter. 1979. Reactive lysine in foodstuffs as measured by a rapid dye-binding procedure. *J. Food Sci.* 44:1221.
- Hurrell, R. F. and K. J. Carpenter. 1980. The estimation of available lysine in foodstuffs after Maillard reactions. In "Maillard Reactions in Foods," Proceedings of Symposium, Sweden.

- Jones, G. P. and A. L. Lakin. 1976. Determination of lysine in barley by a modified dye-binding procedure. *Proc. Nutr. Soc.* 35:44A.
- Kakade, M. L. and I. E. Liener. 1969. Determination of available lysine in proteins. *Anal. Biochem.* 27:273.
- Knipfel, J. E., H. G. Botting and J. M. McLaughlan. 1975. From: *Protein Nutritional Quality of Foods and Feeds*. Mendel Friedman, Editor. Dekker, New York, N.Y. pp.235.
- Kratzer, F. H. and N. Green. 1957. The availability of lysine in blood meal for chicks and poultry. *Poult. Sci.* 36:562.
- Lea, C. H. and R. S. Hannan. 1950. Reaction between proteins and reducing sugars in the dry state. III. Nature of the protein groups reacting. *Biochim. Biophys. Acta.* 5:433.
- Lea, C. H., L. J. Parr and K. J. Carpenter. 1958. Chemical and nutritional changes in stored herring meal. *Brit. J. Nutr.* 12:297.
- Lee, Y. B., J. G. Elliott, D. A. Rickansrud and E. C. Hagberg. 1978. Predicting protein efficiency ratio by the chemical determination of connective tissue content of meat. *J. Food Sci.* 43:1359.
- Maga, J. H., K. Lorenz and O. Onayemi. 1973. Digestive acceptability of proteins as measured by the initial rate of in vitro proteolysis. *J. Food Sci.* 38:173.
- Maillard, L. C. 1912. Action of amino acids on sugars. Formation of melanoids in a methodical way. *Compt. Rend.* 154:66.
- Major, E. J. and E. S. Batterham. 1981. Availability of lysine in protein concentrates as determined by the slope-ratio assay with chicks and comparisons with rat, pig, and chemical assays. *Brit. J. Nutr.* 46:513.
- Mauron, J., F. Motter, E. Berjard, and R. H. Egli. 1955. The availability of lysine, methionine and tryptophan in condensed milk and milk powder in vitro digestion studies. *Arch. Biochem. Biophys.* 59:433.
- McLaughlan, J. M. 1972. Nutritional evaluation of proteins by biological methods. *Cereal Sci. Today*, 17:162.
- Miller, E. L., K. J. Carpenter and C. K. Milner. 1965. Availability of sulfur amino acids in protein foods. 3. Chemical and nutritional changes in heated cod muscles. *Brit. J. Nutr.* 19:547.

- Moran, E. T., L. S. Jensen and J. McGinnis. 1963. Dye binding by soybean and fish meal as an index of quality. *J. Nutr.* 79:239.
- Narayana Rao, M. and J. M. McLaughlan. 1967. Lysine and methionine availability in heat casein-glucose mixtures. *J.A.O.A.C.* 50(3):704.
- National Research Council. 1977. Nutrient requirements of domestic animals. 1. Nutrient requirements of poultry. 7th ed. National Research Council, National Academy of Science, Washington, D.C.
- National Research Council. 1979. Nutrient requirements of domestic animals. 2. Nutrient requirements of swine. 8th ed. National Research Council, National Academy of Science, Washington, D.C.
- Netke, S. P. and H. M. Scott. 1970. Estimates of the availability of amino acids in soybean oil meal as determined by chick growth assay: methodology as applied to lysine. *J. Nutr.* 100:281.
- Osner, R. C. and R. M. Johnson. 1968. Nutritional changes in proteins during heat processing. *J. Food Technol.* 3:81.
- Ousterhout, L. E., C. R. Grau and B. D. Lundholm. 1959. Biological availability of amino acids in fish meals and other protein sources. *J. Nutr.* 69:65.
- Parsons, M. J. 1975. Bioavailability of lysine in ring process blood meal for swine. Michigan State University, Swine Research Report.
- Parsons, M. J. 1977. Bioavailability of lysine in flash ring dried cattle blood (FRDCB) vs flash ring dried swine blood (FRDSB). Michigan State University, Swine Research Report.
- Parsons, M. J. 1979. Effects of incorporating 5% flash ring dried blood meal and 5% tallow into starter grower and finisher diets. Michigan State University, Swine Research Report.
- Peterson, W. R. and J. J. Warthesen. 1979. Total and available lysine determinations using high-pressure liquid chromatography. *J. Food Sci.* 44:994.
- Posati, L. P., V. H. Herbinger, E. D. DeVillbliss and M. J. Pallansch. 1972. Factors affecting the determination of available lysine in whey with 2,4,6-trinitrobenzene sulfonic acid. *J. Dairy Sci.* 55:1660.

- Rao, S. R., F. L. Carter and V. L. Frampton. 1963. Determination of available lysine in oilseed proteins. *Anal. Chem.* 35:1927.
- Robbins, K. R. and D. H. Baker. 1977. Phenylalanine requirement of the weanling pig and its relationship to tyrosine. *J. Anim. Sci.* 45:113.
- Robbins, K. R. and D. H. Baker. 1978. Notes to the editor. Development of a semisynthetic research diet for young swine. *Can. J. Anim. Sci.* 58:533.
- Robel, E. J. and L. T. Frobish. 1977. Evaluation of the chick bio-assay for estimating sulfur amino acid, lysine, and tryptophan availability in soybean meal. *Poult. Sci.* 56:1399.
- Sandler, L. and F. L. Warren. 1974. Effect of ethyl chloroformate in the dye binding capacity of protein. *Anal. Chem.* 46:1870.
- Sanger, F. 1945. The free amino groups of insulin. *Biochem. J.* 39:507.
- Sasse, C. E. and D. H. Baker. 1973. Modification of the Illinois reference standard amino acid mixture. *Poult. Sci.* 52:1970.
- Satterlee, L. D., J. G. Kendrick, D. K. Jewell and W. D. Brown. 1981. Estimating apparent protein digestibility from In vitro assays. In: Protein Quality in Humans: Assessment and In Vitro Estimation. AVI Publishing Company, Inc., Westport, CT., p. 316.
- Satterlee, L. D., H. F. Marshall and J. M. Tennyson. 1979. Measuring protein quality. *J.A.O.C.S.* 56:103.
- Saunders, R. M., M. A. Connor, A. N. Booth, E. M. Bickoff and G. O. Kohler. 1973. Measurement of digestibility of alfalfa protein concentrates by in vivo and in vitro methods. *J. Nutr.* 103:530.
- Schuster, E. M. and C. E. Bodwell. 1979. Relationship between protein nutritive value as estimated in young men and rats, essential amino acid composition and protein digestibility. *Federation Proc.* 38:313.
- Shorrocks, C. and J. E. Ford. 1978. Metabolism of heat damaged proteins in the rat: Inhibition of amino acid uptake by unavailable peptides isolated from enzymic digests of heat damaged cod fillet. *Brit. J. Nutr.* 40:185.

- Soares, J. H. and R. R. Kifer. 1971. Evaluation of protein quality based on residual amino acids of the ileal contents of chicks. Poult. Sci. 50:41.
- Steinhart, H. and R. Kirchgessner. 1973. In vitro verdauungsapparatur zur enzymatischen Hydrolyse von Protein. Arch. Tierernaehr. 23:449.
- Udy, D. C. 1971. Improved dye method for estimating protein. J.A.O.C.S. 48:29A.
- Varnish, S. A. and K. J. Carpenter. 1975. Mechanisms of heat damage in proteins. 5. The nutritional values of heat-damaged and propionylated proteins as sources of lysine, methionine, and tryptophan. Brit. J. Nutr. 34:325.
- Waibel, P. E., K. J. Carpenter and B. R. Behrends. 1974. Nutritional availability of lysine in blood meal. Federation Proc. 33:657.

APPENDIX

TABLE A1. Mineral Composition of BBM and BPBM<sup>a</sup>

| Supplement | PPM     |           |           |      |            |      |        |        |
|------------|---------|-----------|-----------|------|------------|------|--------|--------|
|            | Calcium | Magnesium | Potassium | Iron | Phosphorus | Zinc | Sodium | Copper |
| BBM        | 232     | 102       | 2079      | 1983 | 913        | 16   | 1338   | 5      |
| BBM-A      | 215     | 107       | 2257      | 2066 | 937        | 18   | 1409   | 7      |
| BBM-B      | 208     | 103       | 2142      | 2040 | 914        | 17   | 1397   | 6      |
| BBM-C      | 227     | 108       | 2209      | 2052 | 917        | 18   | 1427   | 7      |
| BPBM       | 1294    | 221       | 5338      | 2690 | 2443       | 21   | 1648   | 12     |
| BPBM-B     | 1637    | 202       | 4503      | 2371 | 2266       | 20   | 1439   | 11     |
| BPBM-C     | 1544    | 216       | 4712      | 2746 | 2321       | 20   | 1503   | 10     |

<sup>a</sup>All values corrected for dilution and expressed on a pure basis.

TABLE A2. In Vitro Quality Measures of BBM and BPBM<sup>a</sup>

| Supplement | CP, % | Pepsin <sup>b</sup><br>% | ME-1 <sup>c</sup> | ME-2 <sup>c</sup> | DBC <sup>d</sup> | HL, % |
|------------|-------|--------------------------|-------------------|-------------------|------------------|-------|
| BBM        | 90.26 | 0.12                     | 1.25              | 1.16              | 21.40            | 10.08 |
| BBM-A      | 91.32 | 0.29                     | 1.46              | 1.20              | 25.37            | 10.66 |
| BBM-B      | 94.09 | 4.67                     | 1.56              | 1.07              | 25.89            | 7.44  |
| BBM-C      | 87.5  | 74.16                    | 0.95              | 0.89              | 21.00            | 6.06  |
| BPBM       | 92.31 | 0.82                     | 0.74              | -                 | 19.10            | 7.52  |
| BPBM-B     | 93.85 | 81.13                    | 0.95              | -                 | 14.74            | 6.97  |
| BPBM-C     | 95.29 | 89.77                    | 1.32              | -                 | 9.89             | 5.41  |

<sup>a</sup>All values corrected for dilution and expressed on a pure basis.

<sup>b</sup>Percent indigestible residue.

<sup>c</sup>Decrease in pH over 10 minutes.

<sup>d</sup>Mmoles reactive lysine per 100 g protein.

## VITA

Tina Maynard Shipley was born on January 17, 1959 in Maryville, Tennessee. She is the daughter of Tony E. and Janice E. Maynard. She received her elementary and secondary education in Maryville, finishing her secondary education graduation requirements in Lyon, France and at The University of Tennessee, Knoxville.

In September 1977, she entered The University of Tennessee, Knoxville and was graduated with a Bachelor of Science degree in Agriculture in December 1980. She subsequently was admitted as a graduate student in Animal Science.

She was married to W. Dean Shipley, Jr. on December 13, 1980.