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INFLUENCE OF VARIOUS FACTORS ON THE PROTEIN  
REQUIREMENTS OF THE CHICK

by

Gerald Alton Donovan

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Approved:

Signature was redacted for privacy.

In Charge of Major Work

Signature was redacted for privacy.

Head of Major Department

Signature was redacted for privacy.

Dean of Graduate College

Iowa State College

1955

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# TABLE OF CONTENTS

	Page
INTRODUCTION . . . . .	1
REVIEW OF LITERATURE . . . . .	3
The Protein Requirement of the Chick. . . . .	3
Factors Affecting the Protein Requirement . . . . .	6
Amino acid availability. . . . .	6
Amino acid imbalance . . . . .	9
Amino acid supplementation . . . . .	12
Energy level . . . . .	14
Type of carbohydrate . . . . .	16
Antibiotics. . . . .	17
Lipotropic Effect of Protein. . . . .	19
Semipurified diets. . . . .	23
EXPERIMENTAL PROCEDURE . . . . .	25
Stock Used. . . . .	25
Methods of Feeding and Management . . . . .	26
Records and Experimental Design . . . . .	26
Chemical and Microbiological Tests. . . . .	29
Microbiological assay procedure. . . . .	29
Preparation of sample. . . . .	32
Liver fat determination. . . . .	34
Protein determination. . . . .	34
EXPERIMENTAL RESULTS . . . . .	35
Experiment I. . . . .	35
Objective. . . . .	35
Method . . . . .	37
Results. . . . .	37
Experiments II, III and IV. . . . .	38
Objective. . . . .	38
Method . . . . .	39
Results. . . . .	39

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	Page
Experiment V. . . . .	43
Objective. . . . .	43
Method . . . . .	44
Results. . . . .	44
Experiment VI . . . . .	46
Objective. . . . .	46
Method . . . . .	46
Results. . . . .	47
Experiment VII. . . . .	56
Objective. . . . .	56
Method . . . . .	56
Results. . . . .	57
Experiment VIII . . . . .	64
Objective. . . . .	64
Method . . . . .	65
Results. . . . .	65
Experiment IX . . . . .	73
Objective. . . . .	73
Method . . . . .	73
Results. . . . .	74
Experiment X. . . . .	76
Objective. . . . .	76
Method . . . . .	76
Results. . . . .	78
DISCUSSION . . . . .	82
The Protein Requirements of the Chick . . . . .	82
Amino acid supplementation and availability . . . . .	87
Lipotropic effect of protein . . . . .	98
SUMMARY. . . . .	101

	Page
CONCLUSIONS. . . . .	104
LITERATURE CITED . . . . .	106
ACKNOWLEDGMENT . . . . .	116
APPENDICES . . . . .	117

## INTRODUCTION

Protein, normally one of the most expensive ingredients used in the formulation of a poultry ration, is concentrated to the greatest extent in the diets of the young chick. This is true because protein is a primary constituent of many structural and protective tissues, such as ligaments, feathers, nails and skin, as well as the organs and muscles. Proteins also reduce morbidity and mortality as substances within the body that fight disease, for example, antibodies are themselves proteins and their ease of formation is dependent on the plane of protein nutrition.

The protein supplied as such to the chick is, in reality, a conveyor of amino acids. The chick reassembles these into orderly patterns to produce the various protein constituents of the body. Thus, for best growth and well-being it is pertinent that the proper amino acids in the proper concentration should be available at the synthesizing centers of the chick. Optimum results are dependent upon the rate of release of the amino acids during digestion and their rate of absorption into the blood. Assimilation may be slow or rapid so the young chick must be supplied with a source of protein containing a balanced supply of readily available essential amino acids.

The present day nutritionist is confronted with several

problems when attempting to supply amino acids to the chick. What level of protein will best fit the chick's need? What ratios of essential amino acids are optimum? Are all the amino acids in a particular protein readily available? What factors affect the protein requirements of the chick? Reports of research pertaining to these matters are quite varied and inconsistent.

Much of the present day research in chick nutrition is being conducted with semi-purified diets. An isolated soybean protein called "Drackett Assay Protein C-1" is commonly used as the amino acid source in these diets. No information is available, to the knowledge of this author, as to the protein quality of this product other than a complete protein analysis.

The following investigations were conducted to determine more accurately the protein requirements of the chick during the most critical nutritional period of its life, to determine the availability of the essential amino acids in the diet and to investigate the influence of several factors on the chick's protein requirement.

## REVIEW OF LITERATURE

## The Protein Requirement of the Chick

The common judgment of practical feed men in the early part of the twentieth century seemed to indicate that a relatively low protein ration was desirable early in the chick growing period. It was reported by Clark (1924) that the average protein analysis of 32 commercial chick starting rations was 16.7 percent, while 53 samples of chick growing mash contained 18.9 percent protein. According to Mussehl (1926) the plane of protein intake for the 1924 Wisconsin Chick Ration was 16.95 percent. This author also reported that growing chicks could tolerate a ration containing a 20 percent protein level during the first nine weeks.

A few years later Funk (1932) using a "free-choice" method of feeding found that Single Comb White Leghorns preferred a ration containing 17.3 to 19.2 percent protein during the first eight weeks of life. The feeds used in this experiment were corn meal, wheat bran, wheat shorts, dried buttermilk, dried skim milk, meat scrap, alfalfa leaf meal, bone meal and salt.

Heuser (1941) has adequately reviewed the early literature with respect to protein in poultry nutrition. He concluded most workers were agreed that the early growth rate

of chicks was increased as the protein level was increased to about 20 percent of the diet. It should be pointed out that during this period the diets used were of low energy and B complex vitamin supplementation was not practiced to the extent it is in present day rations. Some investigators, however, such as Milne (1932) and McConachie, Graham and Branion (1935), found that higher protein levels were more desirable from the standpoint of health and well-being of the chicks.

Since the advent of high-energy poultry rations, vitamin supplementation, especially vitamin B<sub>12</sub>, the discovery of the growth-promoting properties of antibiotics and the great progress in the breeding of better quality chicks the improvement in chick growth (Table 1) and feed efficiency has been phenomenal. As a consequence it seemed

Table 1. Twenty-five years of progress

	Rations			
	1930	1938	1946	1954
Average 8-week weights, lbs. <sup>1</sup>				
Males	1.62	1.89	2.17	2.81
Females	1.36	1.68	1.98	2.16
Both sexes	1.49	1.79	2.07	2.49

<sup>1</sup>-20 N.H. male x B.P.R. female crossbred chicks used per group (Combs and Romoser, 1955).

reasonable to expect that nutrient requirements, expressed as a percentage of the ration, would be increased as was shown by Thayer (1953).

The advantage of protein levels higher than 20 or 21 percent in eliciting favorable growth and well-being in the young chick is supported by the data of Almquist and Asmundson (1944), Heller and Penquite (1945), Lloyd, Reed and Fritz (1949) and Anderson, Cunningham and Slinger (1952).

Conversely, Hill and Dansky (1950) suggested that no improvement in the growth rate of crossbred chicks to seven weeks of age was obtained by increasing the protein level above 20 percent in a diet of relatively high productive energy content. They stated that with adequate fish meal in the diet, all protein levels (20, 25 and 30 percent) promoted equal growth; with inadequate fish meal, high protein levels depressed growth. This suggests that possibly an improper balance of amino acids is more deleterious in high protein level feeds.

Singsen (1949) compared two protein levels, 20 and 28 percent, to determine the effect of protein level on the growth of crossbred chicks. His 20 percent diet contained about 960 kilocalories of productive energy per pound of ration while his 28 percent diet contained approximately 845. Thus, a variable dietary energy content confounded his results. He concluded that growth was similar at both

protein levels but that feed efficiency during the first three weeks of life was improved by the higher protein level.

Indications that 21 percent protein in the diet was optimum for early chick growth were shown by Swift et al. (1931), Matterson et al. (1952), Biely, March and Smith (1952), Scott, Goffi and Glista (1952) and others.

Saxena et al. (1953) reported the use of an 18 and 20 percent protein diet yielded equivalent growth and feed efficiency results. This concept of a lowered protein requirement for young chicks was upheld by the data of Machlin et al. (1952a). They demonstrated that at four and six weeks of age maximal or near-maximal weight was obtained at the 19 percent protein level when aureomycin was included in the ration. Without the antibiotic these authors found that greater chick weight was attained with 21 or more percent dietary protein.

#### Factors Affecting the Protein Requirement

##### Amino acid availability

In 1929 Ingvaldsen showed that excessive drying temperatures lowered the biological value and digestibility of fish proteins. Undoubtedly an inactivation of amino

acids, as has been specifically shown by Greaves, Morgan and Loveen (1938) to occur with the lysine in casein due to heating, contributed to this lowered biological value.

Block and Mitchell (1946) stated that most proteins showed a decrease in nutritive value when subjected to heat, especially under dehydrating conditions. That this is true of casein and meat digests was shown by Esh and Basu (1951). They suggested that the amino acid pattern might be altered due to an interaction of the fragmented molecules of the protein during heating, making them unavailable to the animal system.

A more thorough investigation of this concept was undertaken by Evans and Butts (1948) and Evans, Butts and Bandemer (1951). Using in vitro digestion techniques and microbiological assay procedures, they found a reduction in the availability of lysine, methionine, histidine, threonine, glycine, aspartic acid and glutamic acid of autoclaved soybean oil meal. These same authors (Evans and Butts, 1951) reported that their studies indicated three types of amino acid inactivation had occurred during autoclaving: (1) protein-bound threonine, glycine and glutamic acid reacted with sucrose or glucose to form linkages resistant to enzymatic but not to acid digestion; (2) protein-bound aspartic and glutamic acids reacted with some other constituent of the protein to form linkages resistant to

enzymatic digestion in vitro but hydrolyzed by acid digestion; (3) protein-bound arginine, lysine, histidine and tryptophan reacted with glucose or sucrose to form linkages resistant to enzymatic and acid hydrolysis.

The presence of enzyme-resistant linkages in making certain amino acids rather low in availability to rats was suggested by Geiger, Courtney and Geiger (1952). Their data indicated three amino acids, valine, proline and threonine, to be involved in such linkages.

Balloun, Johnson and Arnold (1953) reported that there was a significant decrease in the lysine content of soybean oil meals due to overheating, but the methionine content was unaffected. An explanation for the thermal susceptibility of lysine is suggested from the results of experiments conducted by Fox, Warner and Hurst (1955). These authors found that in soybean oil meal a large portion of the lysine is in the terminal position of the peptide chain and unprotected by enclosure within a folded protein molecule. Their work also indicated that neither methionine, leucine, phenylalanine or valine was terminal in soybean oil meal.

It is thus apparent that the availability of the amino acids varies from one feed stuff to another and amino acid content is not sufficient basis for determining the nutritional value of a feed stuff.

### Amino acid imbalance

Either an excessive or deficient supply of any one of the essential amino acids in relation to the supply of the other amino acids has been shown to be detrimental to the growth of poultry, swine and rats. Excessive methionine and glycine seemed to have a greater inhibitory effect on chick growth than excessive isoleucine, tyrosine, cystine or histidine in a niacin-low diet according to Anderson (1950).

The toxic effects of excessive methionine in the ration of rats has been shown by Hardin and Hove (1951) to be partially overcome by the addition of molecular equivalent quantities of glycine and arginine. They theorized that since glycine and arginine are precursors of creatine the detoxification mechanism was accomplished by the using up of excess methyl groups from methionine to form creatine.

A relationship between the folic acid and vitamin B<sub>12</sub> content of a diet, and glycine toxicity has been demonstrated in the chick by Machlin et al. (1952b). They showed a decrease in the toxicity symptoms brought about by excess glycine in the diet by the administration of vitamin B<sub>12</sub>. Naber et al. (1951) suggested that as the level of folic acid was increased in the diet of young chickens the growth-depressing effect of excess glycine was diminished.

Henderson et al. (1947) observed a growth depression

in rats receiving a nine percent casein-sucrose, niacin-free diet upon supplementation with two percent glycine, two percent acid-hydrolyzed casein or the crystalline amino acids in amounts contained in two percent acid-hydrolyzed casein. The growth-depressing effect of glycine was overcome by substituting dextrin for sucrose in the diet. This work was confirmed and extended by Hanks et al. (1948). They tested each of the essential amino acids separately and found only DL-threonine and DL-phenylalanine to be inhibitory. They attributed the oral DL-phenylalanine effect solely to the D-configuration. Adding niacin, tryptophan or starch to the diet overcame the growth-depressing effect. In fact, when the diet contained niacin and tryptophan, excess DL-threonine markedly improved growth over the basal ration. These workers also injected 15.6 and 10.4 milligrams of DL-threonine and DL-phenylalanine, respectively. When injected intraperitoneally into rats only the former caused a growth depression.

Ebisuzaki, Williams and Elvehjem (1952) fed a similar casein-sucrose, niacin-free diet to rats as well as a diet containing synthetic amino acids in amounts similar to those in casein. Added DL-threonine was growth depressing in the casein diet but not in the synthetic amino acid diet. The authors concluded that the excess dietary threonine decreased the availability of amino acids in casein by inhib-

iting digestive processes. The injected threonine, they postulated, may have been returned to the intestinal lumen via the bile.

An interesting observation concerning the tryptophan-niacin relationship was reported by Fisher, Scott and Johnson (1954). They showed that 0.20 percent L-tryptophan in the chick's diet completely replaced niacin.

Using a casein-starch diet, Russell, Taylor and Hogan (1952) added each of the essential amino acids to a basal diet at a level 200 percent above the suggested requirements of the rat. They found growth depression with only DL-lysine, DL-methionine and DL-valine. These authors suggested that excess amino acids per se are not toxic to animals, but that the degree of toxicity is relative to a particular amino acid and to the nutritive make-up of the diet.

The adverse effects of an under-supply of any of the essential amino acids, particularly in growth reduction, is rather easily demonstrated according to Ewing (1951).

In discussing amino acid imbalance in a ration it is pertinent that one consider the effects of such imbalances upon the rate of absorption of amino acids from the intestinal lumen. Pinsky and Geiger (1952) presented results indicating that the absorption of proper concentrations of essential amino acids was in many cases hindered by the presence of excessive amounts of other amino acids. They

concluded that an imbalance of amino acids might result from the differential absorption of amino acids from the gastrointestinal tract. This theory is supported by the data of Kamin and Handler (1951, 1952). They showed that an excess of one amino acid almost invariably inhibited the intestinal absorption of another. They reported that the infusion of one amino acid markedly increased the excretion of other amino acids, indicating an inhibited renal tubular reabsorption.

#### Amino acid supplementation

Osborne and Mendel (1914) were among the first investigators to attempt the improvement of the protein quality of an animal ration by single amino acid supplementation. They found that the protein, gliadin of wheat, was inadequate for growth but the addition of lysine to such a diet resulted in growth at a "normal" rate.

Since that time a voluminous amount of data has been published with respect to synthetic amino acid supplementation to poultry, swine, rat and human diets. Hill (1953) has adequately reviewed the literature with respect to methionine and lysine supplementation of poultry rations. He concluded that an improvement in growth and/or feed efficiency resulted from the use of supplemental methionine

in chicken diets only when a deficiency of methionine for these functions existed. This concept was supported by Donovan et al. (1955) with turkeys.

In contrast to Hill's concept, Saxena and McGinnis (1952) stated that improved feed efficiency may have been induced in their experiments by some special property of free methionine, not associated with the need for protein formation. In support of this concept, Rosenberg, Waddell and Baldini (1955) observed significant growth improvement when they added methionine to diets which contained adequate amounts of methionine in the natural form.

Meat scraps have been consistently and extensively used in poultry rations as a source of protein. Kraybill and Wilder (1947) reported that some samples of this product may be deficient in methionine and tryptophan but lysine is usually plentiful. However, as has been pointed out previously, lysine is one of the more easily inactivated amino acids during heating (Evans and Butts, 1948). Thus March, Biely and Young (1950) found supplementation of a meat scraps diet with lysine gave improved growth. As the diet contained sufficient lysine to meet the chick's requirements the authors suggested the possibility that the meal was overheated during processing thus inactivating part of the lysine. Methionine was found to be the second limiting amino acid. Sure (1953a, 1953b) demonstrated that adding

amino acids improved wheat and corn protein for rat growth.

### Energy level

✓The work of Munro and Naismith (1953) has demonstrated that the energy content of the diet influences protein utilization. Using a rat diet which provided adequate amounts of protein they found that adding either carbohydrate or fat to a low-calorie, submaintenance diet a linear improvement in nitrogen balance was elicited. This was confirmed by Cox, Ellingson and Mueller (1953) who showed that the most efficient use of protein for growth occurred at the highest caloric intake.

✓Hill and Dansky (1950) found that the protein requirement of chicks for maximum growth appeared to be a relatively constant absolute quantity, but, as a percentage of the ration, it was related to the productive energy level through its influence on feed intake. This was shown to be true in chicks to a certain extent by Peterson, Grau and Peek (1954). They found that by adding cellulose to a low protein (16 percent) diet the metabolizable energy was lowered, thus the birds consumed more feed and in turn more total protein. This procedure resulted in a slight growth increase.

✓In later work Hill and Dansky (1954) found that at a

constant level of dietary protein the growth rate was unaffected, caloric efficiency was increased and feed efficiency was decreased by adjusting the dietary productive energy from 505 to 975 kilocalories per pound of diet or from 25.1 to 48.5 kilocalories per each percent of dietary protein. They further found that increasing the energy per each percent of dietary protein from 45.9 to 60.8 kilocalories had no influence upon the rate of chick growth.

✓ In contrast to this, Combs and Romoser (1955) presented data indicating that the growth rate of chicks was not affected until more than 45 kilocalories of productive energy per pound of feed were supplied for each percent crude protein. At this point there was also a decrease in the feed and caloric efficiency.

Baldini (1955) has reported evidence that the caloric content of the diet might be responsible for the varying response to methionine supplementation. He showed that on an 800 kilocalorie per pound ration birds responded by increased growth to methionine supplementation only until it reached 0.35 percent of the ration. On a 1000 kilocalorie diet, response to added methionine continued until the birds were on a diet containing 0.50 percent.

✓ Using a high-energy ration Heywang, Bird and Kemmerer (1953) reported that the optimum level of protein for growth of New Hampshire chicks during their first ten weeks is 24

or 25 percent. Similarly, Slinger et al. (1952) were able to show that a diet containing 885 kilocalories of productive energy per pound when fed to Barred Plymouth Rock male chicks gave better growth at the 23 percent protein level than at the 20 percent level. When the dietary energy content was increased in excess of 966 kilocalories a 26 percent level of protein supported even better growth than lower levels.

#### Type of carbohydrate

Various investigators have demonstrated that under certain experimental conditions either dextrin or cornstarch supported better growth of rats and chicks than did sucrose. The majority of the reports were studies of B vitamin deficiencies. It was shown that dextrin promoted greater intestinal synthesis of a number of the B vitamins than did sucrose, according to a review by Elvehjem (1948).

The presence of an unknown growth factor required by chicks fed sucrose rations, which was synthesized by intestinal microorganisms when dextrin was fed, or was present in dextrin, was offered by Monson, Dietrick and Elvehjem (1950) as an explanation for carbohydrate differences in eliciting chick growth responses in their experiments.

Harper et al. (1953) found that with each of several

protein sources (casein, egg albumin and wheat gluten) dextrin supported better rat growth than sucrose. Womack, Marshall and Parks (1953) improved a negative nitrogen balance of adult protein-depleted or undepleted rats fed rations containing low levels of amino acids by substituting dextrin for sucrose or by increasing the essential amino acid nitrogen intake on the sucrose diet. They concluded that the type of carbohydrate in the ration modified the rat's amino acid requirements.

Substituting dextrin for sucrose increased protein utilization by chicks on low levels of protein intake in the experiments of Monson et al. (1954). A further investigation of this subject was conducted by Dreisbach and Nasset (1954). They showed that in rats a much larger quantity of carbohydrate was available for absorption simultaneously with protein when starch rather than dextrin or glucose was the source of energy in the diet. They believed this to be due to the slower absorption of starch, possibly because of its necessary predigestion to glucose.

#### Antibiotics

Forbes (1954) found that the addition of antibiotics to the diet of rats increased nitrogen balance 7.5 percent, apparent digestibility of protein 2.2 percent, true

digestibility of protein 1.4 percent and decreased endogenous nitrogen excretion 16.8 percent. This apparent enhancement in utilization of protein has been demonstrated by Jensen (1953) who found that the feeding of antibiotics to swine from weaning to 200 pounds weight lowered the protein requirement from 16 percent to 14 percent.

The protein sparing effect of antibiotics in poultry is not quite so apparent. Scott, Goffi and Glista (1952) studied this problem using sub-optimal protein diets. They stated that since the improvement in protein efficiency when aureomycin was added to the diets was no better than the improvement in over all feed efficiency there was no evidence of a protein-sparing action. It was noted that the antibiotic supplementation did slightly improve chick growth on their sub-optimal diets.

Anderson, Cunningham and Slinger (1952) suggested that penicillin enhances protein utilization in chicks. This concept is supported by the work of Machlin et al. (1952a). The data of Biely, March and Smith (1952) suggest that the increased growth and feed efficiency due to antibiotic supplementation in reality increased the protein requirement of the chick.

### Lipotropic Effect of Protein

In 1932 it was discovered by Best and Huntsman that an excessive accumulation of fat occurs in livers of rats suffering from a specific dietary deficiency - lack of adequate choline.

Channon and Wilkinson (1935) and Best and Huntsman (1935) suggested that the deposition of liver fat might be linked to protein metabolism. Later Beeston and Platt (1939) investigated alanine, arginine, histidine, hydroxyproline, leucine, lysine, proline, valine, aspartic acid, tyrosine and cystine for lipotropic activity. They added these amino acids to a diet low in casein and found that under certain dietary conditions tyrosine exerted a questionable activity and that cystine produced an antilipotropic effect.

Methionine was shown to possess lipotropic activity in a low-protein diet by Tucker and Eckstein (1937). This has been confirmed by other investigators, but Best and Ridout (1940) demonstrated that factors other than cystine and methionine were involved in the explanation of the lipotropic effect of dietary protein. This theory was extended by Beveridge, Lucas and O'Grady (1944). Their results indicated that the nature and level of protein intake markedly affected the liver fat of rats. These workers also suggested

that the adequacy of the essential amino acids in the diet was an important factor in this effect.

Incorporating singly each of the ten essential amino acids into a choline-free, purified diet, Eckstein (1952) found that only methionine exhibited a lipotropic effect. The nitrogen source in these experiments was a mixture of amino acids equal to that in a five percent casein diet. Yet, Griffith and Nawrocki (1948) showed that threonine markedly increased liver lipid deposition in weanling rats fed an eight percent casein diet supplemented with cystine and choline. To further confuse the situation Singal et al. (1953) presented data indicating a lipotropic effect due to threonine supplementation to a nine percent casein diet including added cystine and choline. This effect has been confirmed by several workers including Harper et al. (1954a) and Nino-Herrera, Harper and Elvehjem (1954). In the work reported by Harper et al. (1954c) the effectiveness of a constant amount of threonine in reducing the liver fat deposition in rats increased as the protein level of a casein diet was increased.

Winje et al. (1954) found that threonine was more effective in reducing liver fat in rats fed certain proteins than it was when other proteins were fed, even when the actual amounts of threonine in the diets were the same. This suggests a difference in the availability of threonine among

the different proteins or that other amino acids were involved. Rats fed a nine percent egg albumin diet demonstrated normal liver fat deposition when threonine, lysine and histidine were added to the diet but not when threonine or lysine alone were added. It would be well to note that, according to amino acid calculations, egg albumin as a sole source of protein meets the rat's minimum amino acid requirements for all the essential amino acids except lysine and histidine when the tryptophan content is equated to the rat's tryptophan requirement. The above workers suggest that possibly the threonine of albumin is not completely available to the rat.

Fatty infiltration of the liver is one aspect of the syndrome known as kwashiorkor, a malady occurring in children in the weaning and post-weaning ages in many tropical and subtropical parts of the world. This is of interest because diets prevalent in these areas are low in protein and what little protein is in the diet usually comes from one cereal, either corn or rice according to a report by the Nutrition Foundation (1955). Flodin (1953) reports rice and corn to be unbalanced with respect to their essential amino acid make-up. Threonine in rice and zein has also been shown to be somewhat unavailable biologically by

Pecora and Hundley (1951) and Geiger, Courtney and Geiger (1952) respectively.

Other compounds, such as glycine, serine, choline and betaine, are able to reduce the accumulation of liver fat as indicated by the work of Harper et al. (1954c). They demonstrated that the level of protein in the diet may influence this effect. Recently Lucas and Ridout (1955) confirmed this. They reported a larger percentage of fat in the livers of rats on methionine-supplemented, low-protein diets than in rats on diets higher in protein. They concluded that protein exerts a lipotropic effect which is mediated by some process not directly involving choline or its known precursors.

In summary it is noted that of the essential amino acids only methionine has a lipotropic effect when the diet is low in choline. Threonine and to some extent glycine and serine have a similar effect in choline-sufficient casein diets fed to rats. Also the amino acid balance in a protein as well as the level of protein in the diet appear to influence the fat content of the livers of rats. Lucas and Ridout (1955, p. 29) stated:

Protein contributes to protection of the liver in at least three ways: (1) by supplying methionine which, by providing methyl groups for formation of choline, prevents accumulation of excessive amounts of fat in the centrolobular regions, (2) by exerting a lipotropic effect which is independent of the methionine content

and which seems to be exerted specifically in the periportal regions, (3) by providing the building blocks (essential amino acids) necessary for the maintenance and repair of the liver cells.

### Semipurified Diets

In recent years improvements have been made in highly purified diets used in experimental studies until it is now possible to maintain an extremely rapid rate of growth of chicks without supplements of crude materials. Under these conditions amino acids in the diet must be readily available to the animal and special demands may have to be met rapidly to prevent a lag during early growth.

Maddy and Swift (1952) using the paired feeding technique, concluded that rats cannot utilize nitrogen and energy derived from free amino acids as efficiently as when the nitrogen is fed in the form of intact casein. Glista (1951) was able to obtain normal growth responses from chicks force-fed a diet containing only free amino acids as a source of nitrogen.

The availability of amino acids in casein, beef and zein was measured by Denton and Elvehjem (1953) using the rate of liberation of the amino acids by enzymes as the basis. They found that arginine was liberated very rapidly into a microbiologically available form from the three proteins during

pepsin digestion. Isoleucine and methionine were liberated much more slowly from casein and zein than from beef. Isoleucine, histidine and methionine appeared to be liberated from casein less readily than the other amino acids.

Geiger, Courtney and Geiger (1952) demonstrated an absence of proline and threonine in an enzymatic digest of zein, and considerable valine was excreted in the feces of zein-fed rats, suggesting that these amino acids are not available for absorption in sufficient quantities.

The failure to obtain as good early growth of chicks when arginine and glycine were supplied by gelatin as when supplied in the free form caused Monson et al. (1955) to suggest that the availability of these two amino acids from the protein was the limiting factor.

Hartsock and Johnson (1953) demonstrated the need for supplementing isolated soy protein with methionine when this was used as the sole source of dietary protein for rats. In chick diets Carver and Johnson (1954) used only methionine to supplement isolated soy protein. Doctor et al. (1954) used both supplemental methionine and glycine in similar diets for chicks. It thus appears that nutritionists are not agreed as to the completeness of purified proteins for use as the sole source of protein in a diet.

## EXPERIMENTAL PROCEDURE

### Stock Used

New Hampshire chicks from the Iowa State College Poultry Farm flock were used in all experiments. In Experiment I straight-run chicks were used, while in all other experiments the chicks were vent-sexed by commercial chick sexors. Except when otherwise indicated the chicks were ten days of age when the tests were started.

### Methods of Feeding and Management

The experiments were done in five-deck battery brooders equipped with wire floors and thermostatically controlled electric heating elements of the back-warming type. The temperature under the hovers was adjusted to the comfort of the chicks from an initial temperature of 110° F. The hovers in the battery used in most experiments were stationary at a height of four inches throughout the experimental period. In Experiment X the temperature under the hovers was maintained at 95° F. during the first week of brooding and then gradually reduced each week to a temperature of 80° F. at the end of the 30-day period. At the same time, the hovers were raised each week to compensate for

increased chick size.

All experiments were carried out in either of two battery rooms where the temperature was maintained at approximately 70° F. except during the summer months when the temperature was occasionally as high as 90° F.

The experimental rations and water were provided ad libitum. At the start of each experiment sufficient feed for the test period was mixed for each experimental lot and stored in metal cans with covers. The rations in Experiment VIII were refrigerated at 38° F. In all other experiments the rations were kept in the battery rooms.

#### Records and Experimental Design

All chicks were wing-banded and individually weighed at one day of age. In Experiment X, chicks were allotted at random to experimental pens at one day of age; in all other experiments, except Experiment I, the chicks were fed a basal diet for a ten-day preliminary period before being allotted to experimental pens. The preliminary period for Experiment I was 14 days. The basal diet fed during the preliminary period in Experiment VII is given in Table 2.

The preliminary period basal diet for all other experiments consisted of 54.4 percent glucose, 31.6 percent Drackett Assay Protein C-1, 4 percent soybean oil,

Table 2. Preliminary period basal diet for  
Experiment VII

Ingredients	Percent
Yellow corn, ground	38.3
Oats, ground	5.0
Wheat, flour middlings	5.0
Soybean oil meal	34.7
Meat scraps	5.0
Fish meal	2.5
Whey, dried	1.5
Alfalfa meal, dehydrated	5.0
Oyster shell, ground	1.0
Bone meal, steamed	1.0
Iodized salt	0.5
Penicillin - B <sub>12</sub> mix <sup>1</sup>	0.2
Crude protein <sup>2</sup>	26.0

The following vitamins were added per pound:

Choline chloride, mg.	213.0
Riboflavin, mg.	2.0
Niacin, mg.	10.0
Pantothenic acid, mg.	4.0
Vitamin A, I.U.	2500
Vitamin D <sub>3</sub> , I.C.U.	300
Vitamin B <sub>12</sub> , mcg.	3.0

<sup>1</sup>Vitamin B<sub>12</sub> and Antibiotic Feed Supplement Merck  
(3.0 mg. B<sub>12</sub> and 2.0 gm. penicillin).

<sup>2</sup>Calculated.

3 percent cellulose and 0.5 percent DL-methionine. Antibiotic, vitamins and minerals were supplied at the levels indicated in Appendices A and B. The calculated crude protein content was 26 percent.

At the completion of the ten-day preliminary period all chicks were again individually weighed and the gain in weight for each was calculated. Each chick was assigned to an "outcome" group according to weight gain during the preliminary period. An equal number from each outcome group was randomly allotted to each experimental pen. This procedure was utilized in an effort to reduce the variability in growth rates among pens or experimental units within a treatment. The experimental treatments were randomly assigned within blocks of pens or to all pens depending upon the experimental design used.

All chicks were weighed individually at the end of each experimental period. Mortality was recorded daily. Feed consumption data were corrected for a small wastage which was unavoidable.

Final weights, gains and percent average daily gains were calculated for several groups of birds treated alike in an effort to determine which criteria had the lowest coefficient of variation. From the results shown in Table 3 it appeared that it would be advantageous to report all growth results as percent average daily gains.

The analysis of variance of chick weight, feed efficiency, liver fat content and amino acid utilization data of all experiments which had replicated treatments was made according to the method reported by Snedecor (1946).

Table 3. Coefficients of variation for three criteria of chick growth

	Average percent daily gain <sup>1</sup>	Final weight	Gain
Group A <sup>2</sup>	6.07	8.56	8.80
Group B	12.86	14.21	20.38
Group C	8.54	13.12	14.72

<sup>1</sup>  $100(W_2 - W_1) / (W_2 - W_1)(1/2)(\text{No. days})$

<sup>2</sup> Twenty chicks per group.

The sequential range test used to test an array of means was described by Newman (1939), Keuls (1952) and Hartley (1955).

### Chemical and Microbiological Tests

#### Microbiological assay procedure

Four microorganisms were used for the determination of the amino acids involved in this work. Lactobacillus arabinosus 17-5 (ATCC No. 8014), Lactobacillus brevis (ATCC No. 8287) and Streptococcus faecalis R (ATCC No. 8043) were obtained from the American Type Culture Collection. Leuconostoc mesenteroides P-60 (ATCC No. 8042) was obtained from the Bacteriology Department of Iowa State

College. These cultures are maintained by the Chemistry Department of Iowa State College according to the technique described by De Fontaine (1952).

Prior to use in assays, the microorganisms were transferred to a complete medium containing no agar. The inoculum was then incubated for approximately 16 hours at 98.6° F. after which it was centrifuged. The residual cells were then washed free of the medium with a 0.9 percent sodium chloride solution and suspended in a similar solution.

The assay medium for methionine was that of Barton-Wright and Curtis (1948). Threonine was assayed with the synthetic Media III as suggested by Steel et al. (1949). The synthetic medium of Kuiken et al. (1943), as modified by De Fontaine (1952), was used for all other amino acid assays.

The procedure utilized involved adding two and one half milliliters of the appropriate assay medium to each of the tubes containing graded amounts of the particular amino acid to be assayed, graded amounts of a standard lysozyme sample and graded amounts of the sample. The volume of each solution was adjusted to five milliliters with distilled water. All solutions were then inoculated with the microorganism and incubated for 72 hours at 98.6° F.

The growth of the organism was measured by titration of the lactic acid produced in each tube with 0.05 N NaOH

or, in the case of isoleucine and leucine, 0.10 N NaOH to pH 6.8. This was determined by bromthymol blue indicator or by a Beckman pH meter. The concentration of the amino acid in the unknown was determined from a standard curve constructed from the titration values of the standard samples. The standard samples were run in quadruplicate at ten levels of concentration containing 0.0, 5.0, 10, 15, 20, 25, 30, 35, 40 and 50 micrograms of the amino acid per tube. The unknown and lysozyme samples were run at four appropriate levels and in quadruplicate. The lysozyme samples served to indicate the reliability of each assay.

The organisms used for assay of a particular amino acid are recorded in Table 4.

Table 4. Organisms used for assay of various amino acids

Amino acid	Microorganism
Arginine	<u>Streptococcus faecalis</u> R
Histidine	<u>Leuconostoc mesenteroides</u> P-60
Isoleucine	<u>Lactobacillus arabinosus</u> 17-5
Leucine	<u>Lactobacillus arabinosus</u> 17-5
Lysine	<u>Leuconostoc mesenteroides</u> P-60
Methionine	<u>Streptococcus faecalis</u> R
Phenylalanine	<u>Lactobacillus brevis</u>
Threonine	<u>Streptococcus faecalis</u> R
Valine	<u>Streptococcus faecalis</u> R

### Preparation of sample

The feed samples were ground in a Wiley mill until they would pass through a 40-mesh screen. Weighed portions of the sample were then hydrolyzed with enough 6 N constant-boiling hydrochloric acid to cover the sample. To facilitate hydrolysis the samples were autoclaved for 16 hours at 15 pounds pressure and 248° F. Excess acid was then removed by heating the samples over steam. The residue obtained was made up into solution and adjusted to pH 4.0. Humins are the least soluble at this pH according to a report by the U.S. Department of Agriculture (1954). The humin was filtered out and washed with pH 4.0 washing solution. The filtrate was neutralized to pH 6.8 as indicated by a Beckman pH meter and diluted to the desired volume. Appropriate amounts were taken for microbiological assay.

Chick excreta was collected over a 24-hour period during the eighth day of the experimental period. Forced air was used to remove most of the down before the excreta was put into glass jars for storage at -20° F.

The preparation of the chick excreta for assay involved vacuum drying the samples over calcium hypochlorite and sodium hydroxide pellets at 44° F. for seven days. The dried samples were then ground, hydrolyzed and prepared for assay using a procedure similar to that used for feed samples

with one exception. At pH 6.8, the excreta samples produced a precipitate which was filtered out and washed before the solution was brought to the desired volume.

The excreta analysis method used in these experiments for determining amino acid availability is considered a valid approach to the problem based upon the following assumptions: the percent of an amino acid in the excreta is relative to the total amount of excreta, the percent of the amino acid in the diet and the percent of the amino acid utilized by the chick. Thus the diets were formulated to differ only in the protein source. Since the carbohydrate, fat, vitamin, mineral and cellulose content of the diets were kept constant, it was assumed that the main variable contributing to the total excreta would be the chick's utilization of the different proteins.

The data are reported as the percent amino acid in the diet divided into the percent amino acid in the excreta thus putting all of the amino acids within a diet and between diets on a comparable basis. Consequently any differences between these rations should be due to differences in the chick's utilization of the amino acids. It was necessary to assume that the enzymatic, bacterial, mucus and sloughed tissue contribution from the gastrointestinal tract, plus the endogenous amino acid contribution, would be about the same for all lots.

#### Liver fat determination

After the close of an experiment two males and two females from each pen were chosen at random for liver fat determinations. As soon as the birds were killed the livers were excised, wrapped in aluminum foil and stored at -20° F.

The pooled livers, from male and female chicks within a replicate, were washed of adhering blood and fatty tissue and blended in a Waring Blendor with a small amount (15 ml.) of distilled water. The resulting homogenous mixture was then poured into a petri dish and dried for 16 hours at 212° F. The dried residue was cooled in a desiccator and ground in a Wiley mill until the sample would go through a 40-mesh screen. Crude fat determinations were then made according to the method of the Association of Official Agricultural Chemists (1950).

#### Protein determination

The crude protein content of the feed samples was determined according to the method of the Association of Official Agricultural Chemists (1950).

## EXPERIMENTAL RESULTS

## Experiment I

Objective

The primary interests in this series of experiments were to reevaluate the protein requirements of the chick during the most critical phase of its life cycle, to investigate the influence of various factors upon the chick's protein requirements and to determine the availability of the essential amino acids in the diet.

The amino acid requirements of the chick, which in turn are reflected in the protein requirements, have been for the most part determined from standardized trials using ten to 14 day old chicks, with experimental periods lasting approximately ten days. The following experiments were similarly standardized and advantage was taken of the ten-day pretest period in an attempt to reduce some of the variability and mortality involved in chick experimentation.

The purpose of Experiment I was two fold. First, an examination of the essential amino acid content of the isolated soy protein used (Table 5) revealed it to be quite deficient in methionine, slightly submarginal in glycine and marginal in tryptophan with respect to the chick's

Table 5. Isolated soy protein as a source of essential amino acids for the chick

Amino acid	Amount in a 20% protein diet (%)	
	Recommended by NRC <sup>1</sup>	DAP <sup>2</sup> as protein source
Arginine	1.2	1.7
Glycine	1.0	0.8
Histidine	0.15	0.5
Isoleucine	0.6	1.3
Leucine	1.4	1.5
Lysine	0.9	1.4
Methionine)	0.45)	0.2)
Cystine )	0.35) 0.8	0.1) 0.3
Phenylalanine)	0.9 )	1.0)
Tyrosine )	0.7 ) 1.6	0.7) 1.7
Threonine	0.6	0.8
Tryptophan	0.2	0.2
Valine	0.8	1.1

<sup>1</sup>National Research Council (1954)

<sup>2</sup>Calculated from amino acid content of Drackett Assay Protein C-1 as determined by the Wisconsin Alumni Research Foundation.

requirements. Thus this experiment was conducted to determine the results on growth of supplementing a diet, which derives its entire amino acid source from isolated soy protein, with these three amino acids. Secondly, the experiment was designed to determine if supplementing an 18 percent protein diet with amino acids, which appear to be deficient in the diet, would result in as good growth response as that obtained with a 21 percent protein diet.

### Method

Ten straight-run New Hampshire chicks were started in each pen at 14 days of age. The basal diet consisted of glucose and soy protein<sup>1</sup> adjusted to give the desired protein levels, 4 percent soybean oil and 3 percent cellulose. Antibiotic, vitamins and minerals were supplied at the levels indicated in Appendices A and B.

### Results

As shown in Table 6, improved growth resulted when supplemental DL-methionine or a combination of DL-methionine, DL-tryptophan and glycine was added to a 21 percent protein diet. The addition of DL-methionine also appeared to be beneficial to the 18 percent protein diet. Supplementation with DL-methionine, DL-tryptophan and glycine gave no better growth response than the addition of DL-methionine alone. The 21 percent protein diet supplemented with DL-methionine produced more rapid chick growth than a similarly supplemented 18 percent protein diet.

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<sup>1</sup>Drackett Assay Protein C-1 (a commercially isolated soybean protein).

Table 6. Amino acid supplementation at two protein levels

Modification of diet %	Dietary protein %	Average percent <sup>1</sup> daily gain
Basal	21	9.15
0.56 DL-methionine	21	11.92
0.56 DL-methionine ) 0.156 glycine ) 0.044 DL-tryptophan )	21	11.43
Basal	18	9.27
0.56 DL-methionine	18	10.44

$$^1 100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$$

## Experiments II, III and IV

Objective

These experiments were conducted to determine as precisely as possible the protein requirements of the chick during its early life. A semi-purified diet, in which such variables as energy, vitamin and mineral contents could be kept practically constant, was employed. An experimental design utilizing a constant interval between protein levels was used to determine the curvilinear response to increments of added dietary protein.

### Method

In each experiment two pens of ten male chicks and two pens of ten female chicks were assigned to each of five different protein level rations. The basal diet consisted of glucose, the proper amount of soy protein, 4 percent soybean oil, 3 percent cellulose and 0.5 percent DL-methionine. Antibiotic, vitamins and minerals were supplied at the levels indicated in Appendices A and B. A split-plot experimental design was used to determine more precisely whether or not a difference exists between sexes with regard to growth and feed efficiency responses as influenced by the amount of dietary protein.

### Results

The protein levels and results of Experiments II, III and IV are given in Table 7. Analyses of the data demonstrate a significant linear trend in growth response to an increase in the protein level of the diet up to 26 percent. This is shown in Figure 1. A similar linear response in feed efficiency to increments of protein up to 26 percent of the diet is shown in Figure 2.

The data of Experiment IV as shown in Figures 1 and 2 demonstrate a significant quadratic response to increases

Table 7. Influence of dietary protein levels on chick growth, feed efficiency and protein efficiency

% dietary protein	Experiment II			Experiment III			Experiment IV		
	Av. % <sup>1</sup> daily gain	Feed <sup>2</sup> effi- ciency	Prot. <sup>3</sup> effi- ciency	Av. % <sup>1</sup> daily gain	Feed <sup>2</sup> effi- ciency	Prot. <sup>3</sup> effi- ciency	Av. % <sup>1</sup> daily gain	Feed <sup>2</sup> effi- ciency	Prot. <sup>3</sup> effi- ciency
14							7.23	2.19	.307
16	7.65	2.01	.322						
18	7.82	2.00	.360	7.78	1.89	.340	8.27	1.81	.328
20	8.08	1.88	.376	8.14	1.72	.344			
22	8.12	1.82	.399	7.94	1.76	.388	8.73	1.64	.361
24	8.30	1.77	.466	8.27	1.63	.392			
26				8.35	1.59	.414	8.87	1.60	.416
30							8.86	1.53	.459

<sup>1</sup>  $100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$

<sup>2</sup> Grams of feed per gram of gain.

<sup>3</sup> Grams of protein per gram of gain.

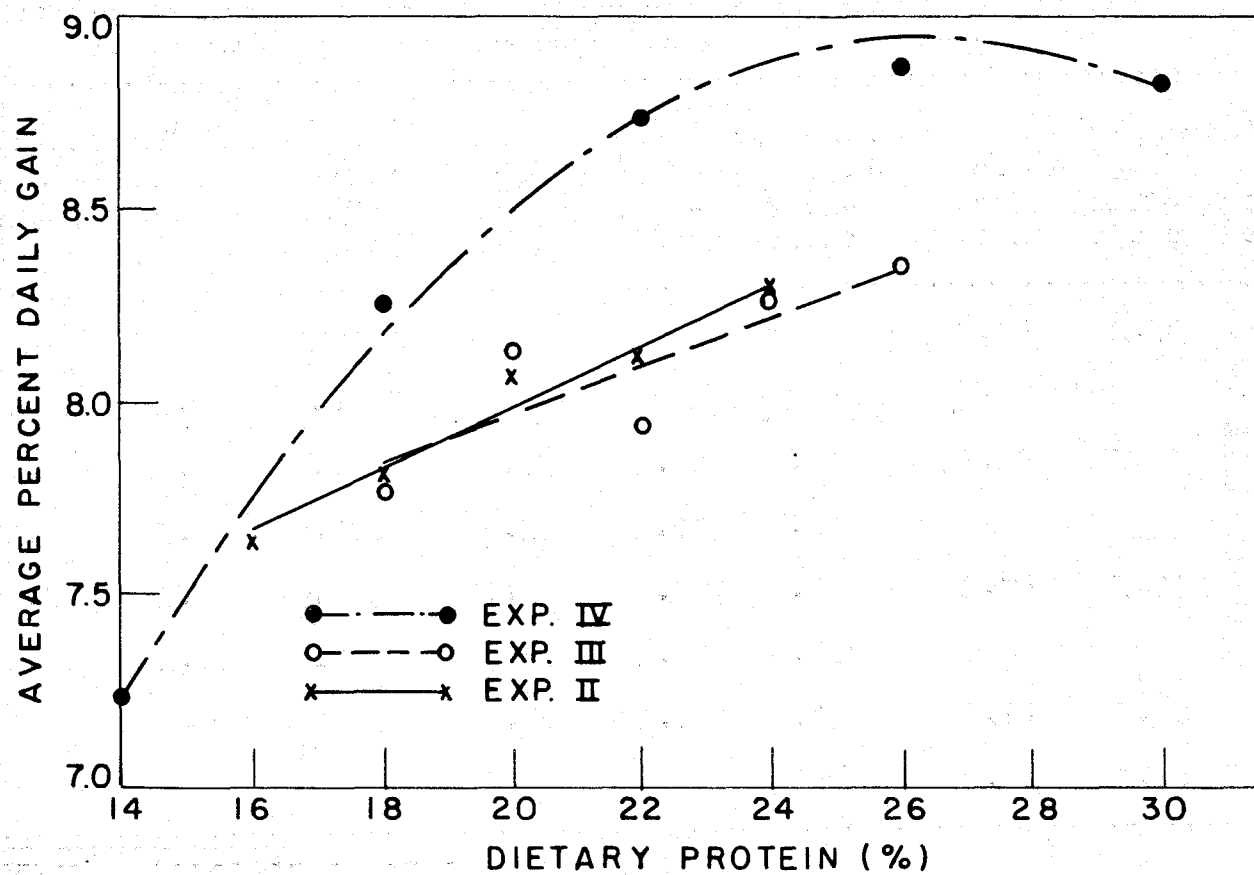


Figure 1. Influence of dietary protein levels on chick growth rates

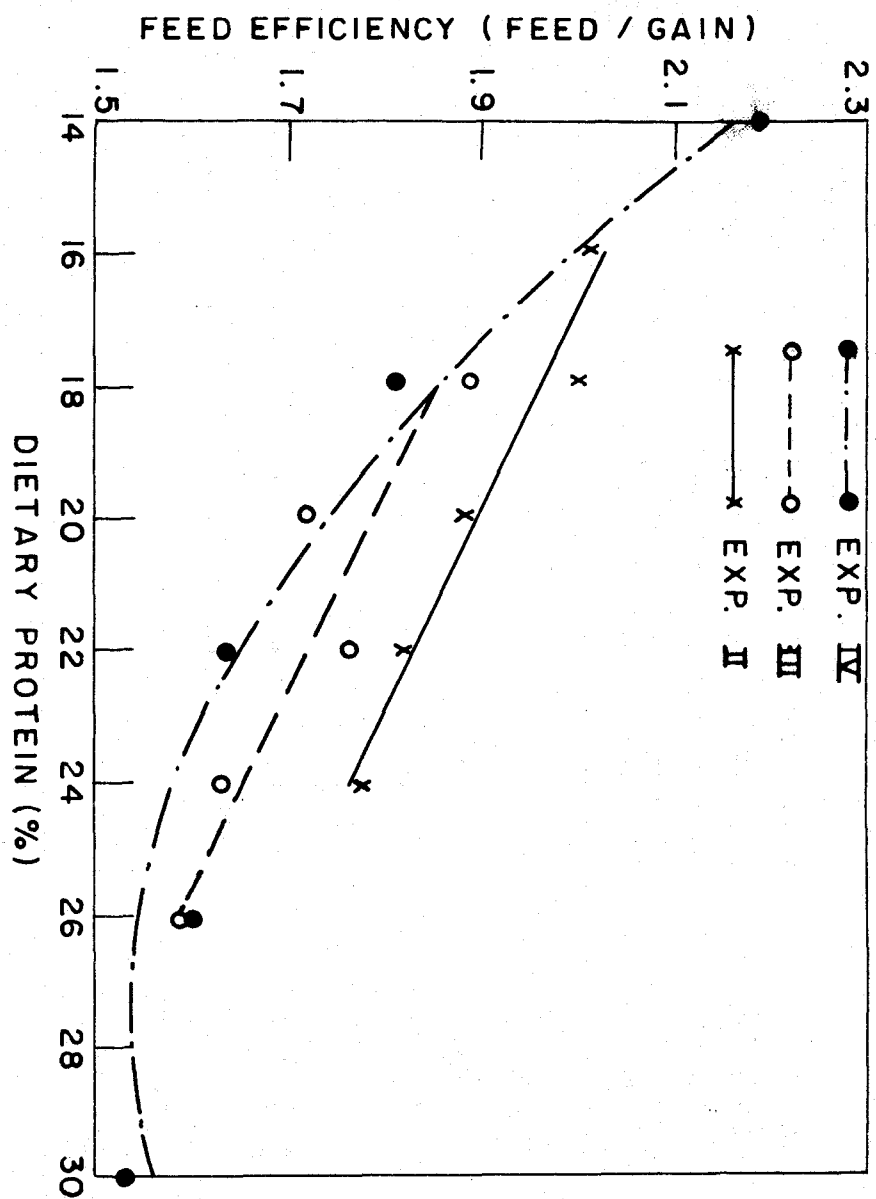


Figure 2. Influence of dietary protein levels on chick feed efficiency

in protein from 14 percent to 30 percent. The peak of the growth curve was reached at approximately the 26 percent level. The peak of the feed efficiency curve was reached at a slightly higher level of protein. The data in Table 7 demonstrate a decrease in protein efficiency as the level of dietary protein was increased.

### Experiment V

#### Objective

Experiment V was conducted to determine the effect of different protein sources upon the growth and feed efficiency of the chick. Four rations were formulated using different proteins and synthetic amino acids. Adjustments were made to satisfy the chick's requirements for all the essential amino acids.

The main difference between the diets was the source of protein so any variation in the availability of one or more of the essential amino acids in the proteins would be more readily detected. Since it was assumed that differences in protein availability would have a greater effect at a low level, 18 percent protein diets were used. A 20 percent soy protein diet was included as a comparison.

### Method

Two pens of ten male chicks and two pens of ten female chicks were assigned to each of the five experimental diets. The basal diet was similar to that used in Experiment I.

### Results

The data from Experiment V are given in Table 8. The results indicate a difference in growth response and feed efficiency due to the rations fed. The data suggest that Lot 3, fed a combination of isolated soy protein and casein as a source of protein, produced poorer growth and feed efficiency than any of the other lots. At the time this was believed to be due to the unavailability of one or more essential amino acids in casein but a later microbiological analysis of the casein source indicated a lower methionine content than had been calculated. The growth and feed efficiency depression in this experiment was probably due to a methionine deficiency.

Table 8. Chick growth as influenced by various protein combinations

Modification of basal diet (%)	Lot no.	Dietary protein (%)	Average percent daily gain <sup>1</sup>	Feed efficiency <sup>2</sup>
24.0 soy protein 0.5 DL-methionine	1	20 (19.9) <sup>3</sup>	8.49	1.78
21.5 soy protein 0.5 DL-methionine	2	18 (18.1)	8.41	1.84
10.8 soy protein 10.0 casein 0.21 glycine 0.20 DL-methionine	3	18 (18.0)	7.90	2.02
16.2 soy protein 4.8 gelatin 0.5 DL-methionine 0.1 DL-tryptophan	4	18 (18.2)	8.34	1.76
8.1 soy protein 7.5 casein 4.8 gelatin 0.42 DL-methionine 0.10 DL-tryptophan	5	18 (17.9)	8.54	1.71

<sup>1</sup>  $100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$

<sup>2</sup> Grams of gain per gram of feed.

<sup>3</sup> Figures in parentheses represent analyzed crude protein.

## Experiment VI

### Objective

The data from Experiment V seemed to indicate a growth depressing factor in the diet containing protein from a combination of isolated soy protein and casein. Experiment VI was conducted to determine if this depression could be due to a marked unavailability of one or more of the essential amino acids in the casein-soy protein combination.

The arginine content of a diet containing a casein-soy protein combination is marginal so a second objective of this experiment was to determine the effect on chick growth and feed efficiency by supplementing such a diet with L-arginine.

### Method

Two pens of ten male chicks each and two pens of ten female chicks each were assigned to each of the five rations. The excreta from Lots 3, 4 and 5 were analyzed microbiologically for nine of the essential amino acids, while the excreta from Lots 1 and 2 were analyzed for arginine only. The excreta from the males and females within a replicate were pooled for analyses. The basal diet was similar to

that used in Experiment I. The amino acid content of the diets for Lots 3, 4 and 5 is given in Table 9.

### Results

A statistical analysis indicated that no significant difference exists between growth rates as an effect of rations. However, an examination of the data (Table 10) shows that the growth rate of chicks fed the 18 percent protein ration, containing isolated soy protein as the only source of protein plus free amino acids (Lot 3), was somewhat less than that produced by rations containing similar levels of protein and using a combination of isolated protein plus casein as a protein source.

The feed efficiency data in Table 10 indicate that there is a significant difference among treatments. At the 18 percent level of dietary protein the difference between Lots 3 and 4 is non-significant while that between Lots 3 and 5 is significant.

The data in Table 11 indicate that there is no significant difference in the utilization of arginine at the two levels of dietary protein. There is an increase in the utilization of the total dietary arginine when synthetic L-arginine is added to the diet. An analysis of the data (Table 12) shows that the arginine from isolated soy protein

Table 9. Amino acid content of diets used in Experiment VI

Lot	Arg. <sup>1</sup>	Gly. <sup>2</sup>	Lys. <sup>1</sup>	Cys. <sup>2</sup>	Met. <sup>1</sup>	Try. <sup>2</sup>	Hist. <sup>2</sup>	Isoleu. <sup>2</sup>	Leu. <sup>1</sup>	Phen. <sup>1</sup>	Thre. <sup>1</sup>	Val. <sup>2</sup>
3	1.46	0.86	0.96	0.10	0.75	0.17	0.46	1.14	1.32	0.88	0.64	0.97
4	1.15	0.86	1.20	0.09	0.73	0.28	0.49	1.25	1.57	0.91	0.73	1.21
5	1.53	0.86	1.20	0.09	0.73	0.28	0.49	1.25	1.57	0.91	0.73	1.21

<sup>1</sup>Microbiological assay.

<sup>2</sup>Calculated.

Table 10. Chick growth on diets of various protein combinations

Modification of basal diet (%)	Lot no.	Dietary protein (%)	Average percent daily gain <sup>1</sup>	Feed efficiency <sup>2</sup>
23.7 soy protein 0.58 DL-methionine 0.20 glycine	1	20 (20.0) <sup>3</sup>	7.20	1.80
11.8 soy protein 11.6 casein 0.36 DL-methionine 0.61 glycine	2	20 (20.4)	7.53	1.76
21.3 soy protein 0.52 DL-methionine 0.14 glycine	3	18 (17.4)	6.20	2.02
10.6 soy protein 10.4 casein 0.32 DL-methionine 0.45 glycine	4	18 (18.4)	7.16	1.88
10.6 soy protein 10.4 casein 0.32 DL-methionine 0.45 glycine 0.38 L-arginine	5	18 (18.8)	7.67	1.75

<sup>1</sup>  $100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$

<sup>2</sup> Grams of feed per gram of gain.

<sup>3</sup> Figures in parentheses represent analyzed crude protein.

Table 11. Amino acid availability factors<sup>1</sup>

	Lot 1		Lot 2		Lot 3		Lot 4		Lot 5	
Arginine	2.39 2.45	2.42	2.65 2.60	2.62	2.62 3.12	2.87	2.24 2.36	2.30	2.00 2.24	2.12
Histidine					4.61 5.41	5.01	4.73 4.69	4.71	5.28 4.94	5.11
Isoleucine					5.56 5.45	5.50	5.90 6.45	6.18	6.19 6.24	6.22
Leucine					6.80 6.68	6.74	3.99 4.04	4.02	4.04 4.41	4.20
Lysine					5.52 5.38	5.45	4.54 4.58	4.56	5.03 4.82	4.92
Methionine					1.80 1.67	1.74	2.25 2.30	2.28	2.38 2.05	2.22
Phenylalanine					5.11 5.36	5.24	3.10 3.16	3.13	3.68 3.66	3.67
Threonine					10.12 9.86	9.99	8.35 7.94	8.14	8.55 8.53	8.54
Valine					6.86 6.88	6.87	5.96 6.17	6.06	6.45 6.16	6.30

<sup>1</sup> $\frac{\text{Percent amino acid in excreta}}{\text{Percent amino acid in diet}} \times 10$

Table 12. Differences between mean utilization factors of arginine from five diets

Lot arginine utilization factor means (ranked) <sup>1</sup>						
	Lot means	5 2.12	4 2.30	1 2.42	2 2.62	3 2.87
	3 2.87	.75*	.57 .60			
Lot	2 2.62	.50 .60				
arginine	1 2.42					
utilization	4 2.30					
factor	5 2.12					
means						
(ranked) <sup>1</sup>						

\*Significant difference at  $P = 0.05$ .

<sup>1</sup>Ranked according to decreasing availability of arginine to the chick.

(Lot 3) and that from a casein-isolated soy protein combination (Lot 4) are utilized equally well.

An analysis of variance of the amino acid utilization factors of Lots 3, 4 and 5 reveals a significant difference between lots and between amino acids. The analysis also indicates a significant interaction between lots and amino acids. The biological inferences from these statistical

operations suggest that the amino acids are not of the same relative availability in the three diets. Further analyses of the amino acid availability within a diet were carried out on Lot 3 for the soy protein and Lot 4 for the casein-soy protein combination.

An examination of the sequential range test of the mean amino acid utilization factors, as shown in Tables 13 and 14, demonstrates the basis for the above mentioned interaction. In soy protein, isoleucine is significantly more available than threonine, valine and leucine while in the casein-soy protein combination it is significantly more available than threonine only. In fact, in this diet it is less available than leucine.

It should be noted that the high availability of methionine in all diets is due to the presence of supplemental synthetic DL-methionine which is readily available to the chick. The synthetic DL-methionine comprised 74.3 percent and 45.7 percent of the total dietary methionine in Lots 3 and 4, respectively.

Figure 3 depicts the amino acid utilization factors for soy protein and the casein-soy protein combination.

Table 13. Differences between mean utilization factors of amino acids in soy protein

		Amino acids (ranked) <sup>1</sup>								
$\bar{x}$		Meth. 1.74	Arg. 2.87	Hist. 5.01	Phen. 5.24	Lys. 5.45	Isoleu. 5.50	Leu. 6.74	Val. 6.87	Thre. 9.99
Amino acids (ranked) <sup>1</sup>	Thre. 9.99	8.25*	7.12*	4.98*	4.75*	4.54*	4.49*	3.25*	3.12*	
		1.03	1.00	.96	.92	.87	.80	.72	.58	
	Val. 6.87	5.13*	4.00*	1.86*	1.63*	1.42*	1.37*	0.13		
		1.00	.96	.92	.87	.80	.72	.58		
	Leu. 6.74	5.00*	3.87*	1.73*	1.50*	1.29*	1.24*			
		.96	.92	.87	.80	.72	.58			
	Isoleu. 5.50	3.76*	2.63*	.49						
		.92	.87	.80						
	Lys. 5.45	3.71*	2.58*							
		.87	.80							
	Phen. 5.24	3.50*	2.37*							
		.80	.72							
	Hist. 5.01	3.27*	2.14*							
		.72	.58							
	Arg. 2.87	1.13*								
		.58								
	Meth. 1.74									

\*Significant difference at  $P = 0.05$ .

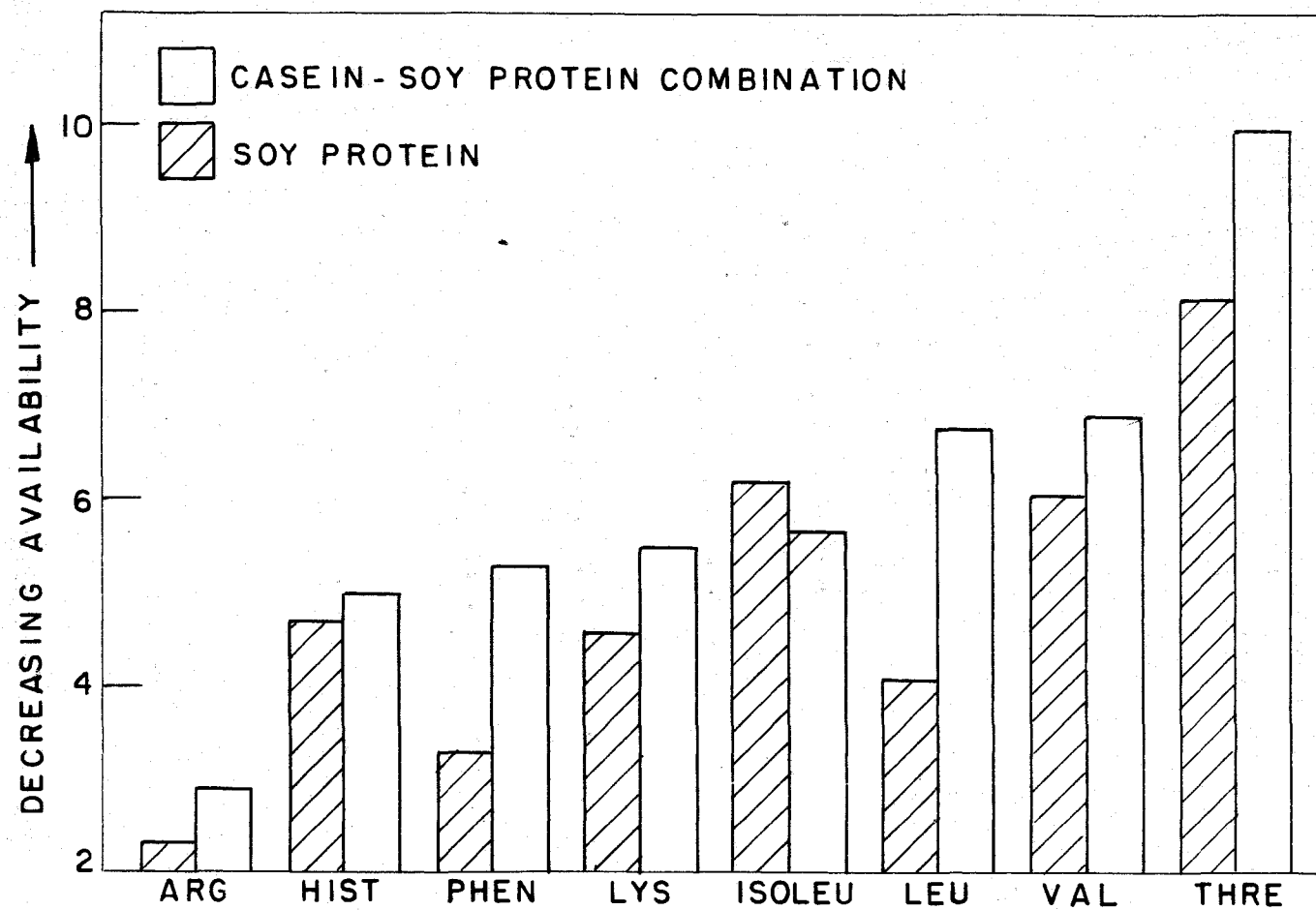
<sup>1</sup>Ranked according to decreasing availability to the chick.

Table 14. Differences between mean utilization factors of amino acids in a 10.4% casein-10.6% soy protein combination

		Amino acid means (ranked) <sup>1</sup>								
		Meth.	Arg.	Phen.	Leu.	Lys.	Hist.	Val.	Isoleu.	Thre.
		2.28	2.30	3.13	4.02	4.56	4.71	6.06	6.18	8.14
Amino acid means (ranked) <sup>1</sup>	Thre.	5.86*	5.84*	5.01*	4.12*	3.58*	3.43*	2.08*	1.96*	
	8.14	.72	.70	.67	.64	.61	.56	.50	.41	
	Isoleu.	3.90*	3.88*	3.05*	2.16*	1.62*	1.47*	.12		
	6.18	.70	.67	.64	.61	.56	.50	.41		
	Val.	3.78*	3.76*	2.93*	2.04*	1.50*	1.35*			
	6.06	.67	.64	.61	.56	.50	.41			
	Hist.	2.43*	2.41*	1.58*	.69*	.15				
	4.71	.64	.61	.56	.50	.41				
	Lys.	2.28*	2.26*	1.43*	.54*					
	4.56	.61	.56	.50	.41					
Leu.	1.74*	1.72*	.89*							
4.02	.56	.50	.41							
Phen.	.85*	.83*								
3.13	.50	.41								
Arg.	.02									
2.30	.41									
Meth.										
2.28										

\*Significant difference at P = 0.05.

<sup>1</sup>Ranked according to decreasing availability to the chick.



**Figure 3. Amino acid utilization factors**

## Experiment VII

### Objective

A diet deriving its main source of protein from meat scraps generally contains adequate lysine, but may be sub-marginal in methionine, with respect to the chick's requirement. Yet March, Biely and Young (1950) and Patrick (1953) found improved chick growth resulted when such a diet was supplemented with lysine. Results were variable when methionine was added. The objectives of Experiment VII were two fold: to determine the influence of synthetic amino acid supplementation on (1) the relative availability of the bound amino acids in meat scraps protein and (2) the growth rate and feed efficiency of the chick.

The meat scraps used in this experiment were of the expeller type, obtained on the open market. All lots purchased were mixed into one composite sample before use in this experiment.

### Method

Two replicates, each comprising ten treatments, were conducted at different times. Twelve New Hampshire chicks, six male and six female, were randomly assigned to each of

the ten treatments in Replicate I and 20 New Hampshire chicks, ten male and ten female, were similarly assigned in Replicate II.

Excreta were collected, stored and analyzed microbiologically for arginine, lysine and methionine. The livers from two males and two females in each replicate were used for fat determinations.

The basal diets are given in Table 15.

### Results

The growth rate and feed efficiency of birds fed the 20 percent protein diets were superior to those fed 18 percent protein as shown in Table 16. The addition of synthetic L-arginine, DL-lysine, DL-methionine, DL-tryptophan and glycine to the basal diets had no effect on growth rates at either protein level. Supplementation of the 18 percent protein basal with L-arginine, DL-lysine or DL-methionine singly, or in combinations of two, had no beneficial effect on growth rate or feed efficiency.

An examination of the data in Table 16 reveals a noticeable reduction in percent liver fat when the basal ration is supplemented with a combination of all five amino acids tested at both protein levels. These differences proved to be non-significant.

Table 15. Composition of basal diets for Experiment VII

Ingredients	Percent	
	18% protein	20% protein
Yellow corn, ground	75.5	72.0
Meat scraps	22.0	27.0
Bone meal, steamed	1.25	--
Oyster shell, ground	0.25	--
Iodized salt	0.50	0.50
MnSO <sub>4</sub>	0.006	0.006
The following antibiotic and vitamins were added per pound:		
Penicillin, mg.	4	4
Vitamin A, I.U.	2000	2000
Vitamin D <sub>3</sub> , I.C.U.	300	300
Vitamin E, I.U.	4	4
Choline, mg.	591	591
Niacin, mg.	62	62
Calcium pantothenate, mg.	23	23
Riboflavin, mg.	12.5	12.5
Vitamin B <sub>12</sub> , mcg.	6	6
Menadione, mg.	0.6	0.6
% crude protein <sup>1</sup>	18.2	20.6
% calcium <sup>2</sup>	2.6	2.6
% phosphorus <sup>2</sup>	1.3	1.3

<sup>1</sup>By analysis.<sup>2</sup>Calculated.

Table 16. Amino acid supplementation to a corn-meat scraps diet

Modification of basal diet (%)	Dietary protein (%)	Average percent daily gain <sup>1</sup>	Feed efficiency <sup>2</sup>	Liver fat (%)
Basal	20 (20.6) <sup>3</sup>	6.44	2.28	12.42
Amino Acid Supp.No.1 <sup>4</sup>	20	6.23	2.21	11.00
Basal	18 (18.2)	5.94	2.76	12.63
0.16 L-arginine-HCl	18	6.08	2.66	12.52
0.7 DL-lysine	18	5.91	2.52	12.92
0.6 DL-methionine	18	6.08	2.42	12.57
0.16 L-arginine-HCl) 0.7 DL-lysine )	18	5.93	2.63	11.86
0.16 L-arginine-HCl) 0.6 DL-methionine )	18	5.89	2.62	14.46
0.7 DL-lysine ) 0.6 DL-methionine)	18	5.39	2.64	12.28
Amino Acid Supp.No.1 <sup>4</sup>	18	5.82	2.42	10.62

<sup>1</sup>  $100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$

<sup>2</sup> Grams of feed per gram of gain.

<sup>3</sup> Figures in parentheses represent analyzed crude protein.

Amino Acid Supp.No.1	Percent of total diet
L-arginine-HCl	0.16
DL-lysine	0.7
DL-methionine	0.6
DL-tryptophan	0.2
glycine	0.3

The influence of amino acid supplementation on the utilization of arginine, lysine and methionine is shown in Table 17. The data as illustrated in Figure 4 indicate a marked improvement in the utilization of each of these amino acids when its respective synthetic form was added to the basal diet. Also, the addition of each of these amino acids to the basal resulted in an improvement in the utilization of the other two amino acids.

The present experiment does not indicate whether or not increased utilization of the total dietary content of a particular amino acid, when a portion is supplied in the synthetic form, is due only to the better utilization of the synthetic form or to an enhanced use of the protein-bound form. Thus calculations were made on the basis of the protein-bound amino acid content of the diet only and the results are illustrated in Figure 5. These calculations indicate that the synthetic L-arginine and DL-methionine were not 100 percent utilized, or they were 100 percent utilized but their presence caused a slight reduction in the utilization of the bound amino acid. In the case of lysine the synthetic form appeared to be completely utilized but there was no marked effect on the utilization of the bound lysine.

Synthetic DL-lysine and DL-methionine each improved the utilization of both free and bound L-arginine. Free L-arginine appeared to inhibit free lysine utilization

Table 17. Amino acid utilization factors<sup>1</sup>

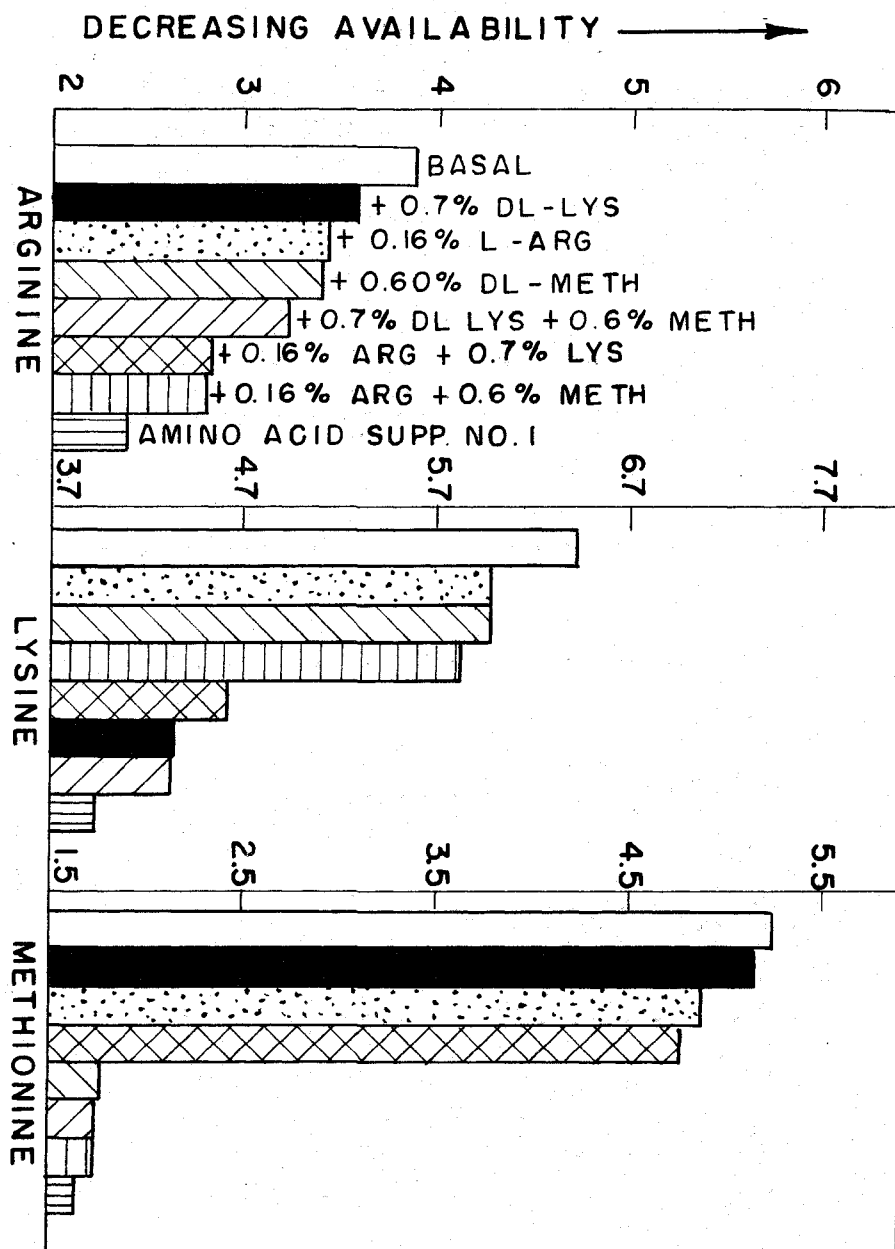
Modification of basal diet (%)	Dietary protein (%)	Arginine	Lysine	Methi- online
Basal	20 (20.6) <sup>2</sup>	4.07	6.07	4.83
Amino Acid Supp.No.1 <sup>3</sup>	20	2.80	4.50	1.76
Basal	18 (18.2)	3.90	6.42	5.25
0.16 L-arginine-HCl	18	3.46	5.98	4.89
0.7 DL-lysine	18	3.60	4.34	5.15
0.6 DL-methionine	18	3.44	5.98	1.75
0.16 L-arginine-HCl) 0.7 DL-lysine )	18	2.84	4.61	4.78
0.16 L-arginine-HCl) 0.6 DL-methionine )	18	2.83	5.82	1.73
0.7 DL-lysine ) 0.6 DL-methionine)	18	3.24	4.32	1.74
Amino Acid Supp.No.1 <sup>3</sup>	18	2.41	3.91	1.63

<sup>1</sup> $\frac{\text{Percent amino acid in excreta}}{\text{Percent amino acid in diet}} \times 10$

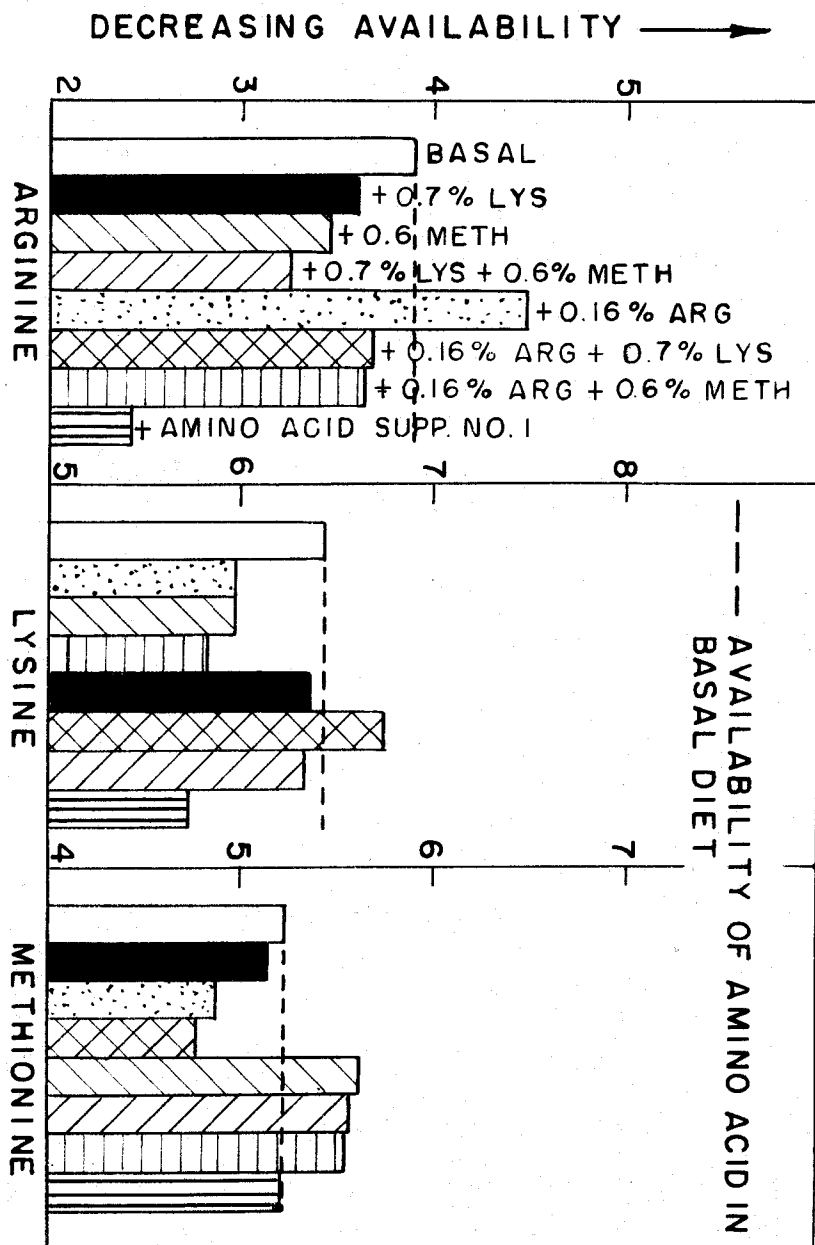
<sup>2</sup>Figures in parentheses represent analyzed crude protein.

<sup>3</sup> Amino Acid Supp.No.1	Percent of total diet
L-arginine-HCl	0.16
DL-lysine	0.7
DL-methionine	0.6
DL-tryptophan	0.2
glycine	0.3

Figure 4. Effect of amino acid supplementation on dietary amino acid utilization by the chick



**Figure 5. Effect of amino acid supplementation on protein-bound amino acid utilization**



slightly while free DL-methionine exerted little influence. The combination of L-arginine, DL-lysine, DL-methionine, DL-tryptophan and glycine enhanced utilization of both the bound and free lysine to the greatest extent.

Synthetic L-arginine at a low level (0.16 percent) improved the utilization of bound methionine to a greater extent than did synthetic DL-lysine. A combination of the two was even more beneficial. The addition of free L-arginine or DL-lysine to the DL-methionine supplemented diet had practically no beneficial effect on total methionine utilization over a basal supplemented with only DL-methionine. The combination of the five synthetic amino acids resulted in improved free methionine utilization up to 100 percent.

### Experiment VIII

#### Objective

Hill and Dansky (1950) state that the protein requirement for maximum chick growth when expressed as a percentage of the diet is related to the productive energy level. Experiment VIII was conducted to determine the protein requirement for maximum chick growth and feed efficiency at a low (720 kilocalories per pound) and a high (1055 kilocalories per pound) dietary productive energy level. Pure

cellulose was used as inert material to reduce the energy value of the ration. Unfortunately the varying bulk of the rations was confounded with the varying energy content.

### Method

Two pens of ten male chicks and two pens of ten female chicks were assigned to each of the experimental diets. Livers were collected and stored for analysis. The experimental diets are given in Table 18.

### Results

The results of Experiment VIII given in Tables 19 and 20 indicate that at all protein levels tested, chicks receiving the higher productive energy per pound of diet grew at a faster rate and utilized feed more efficiently.

On the higher energy diet there was a significant quadratic response in growth rate to increments in the dietary protein level, reaching a maximum at approximately the 26 percent level (Figure 6).

An analysis of the feed efficiency data in Table 19 indicates a significant linear response (Figure 7) and non-significant quadratic response to increasing dietary protein levels up to 30 percent but an examination of the

Table 18. Composition of experimental diets

	Dietary protein level									
	14	18	22	26	30	14	18	22	26	30
	720 kilocal./lb.					1055 kilocal./lb.				
Glucose (1030 kilocal./lb.) <sup>1</sup>	52.33	47.63	42.93	38.33	33.53	66.38	61.33	56.18	51.13	45.98
Soy protein (1000 kilocal./lb.) <sup>1</sup>	16.40	21.20	26.10	30.90	35.80	16.40	21.20	26.10	30.90	35.80
Soybean oil (2000 kilocal./lb.) <sup>1</sup>	1.00	1.00	1.00	1.00	1.00	10.00	10.25	10.50	10.75	11.00
Cellulose	23.05	22.95	22.75	22.55	22.45	--	--	--	--	--
DL-methionine	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral mix <sup>2</sup>										
Vitamin-antibiotic mix <sup>3</sup>										

<sup>1</sup>Calculated.

<sup>2</sup>Minerals were supplied at levels indicated in Appendix B.

<sup>3</sup>Vitamins and antibiotic were supplied at levels indicated in Appendix A.

Table 19. Effect of high energy diet on response to protein level increments

Level of protein in diet (%)	Average percent daily gain <sup>1</sup>	Feed efficiency <sup>2</sup>	Liver fat (%)
14	6.30	2.12	13.35
18	6.81	1.94	13.76
22	6.89	1.77	11.26
26	7.66	1.60	10.57
30	6.77	1.73	10.20

<sup>1</sup>  $100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$

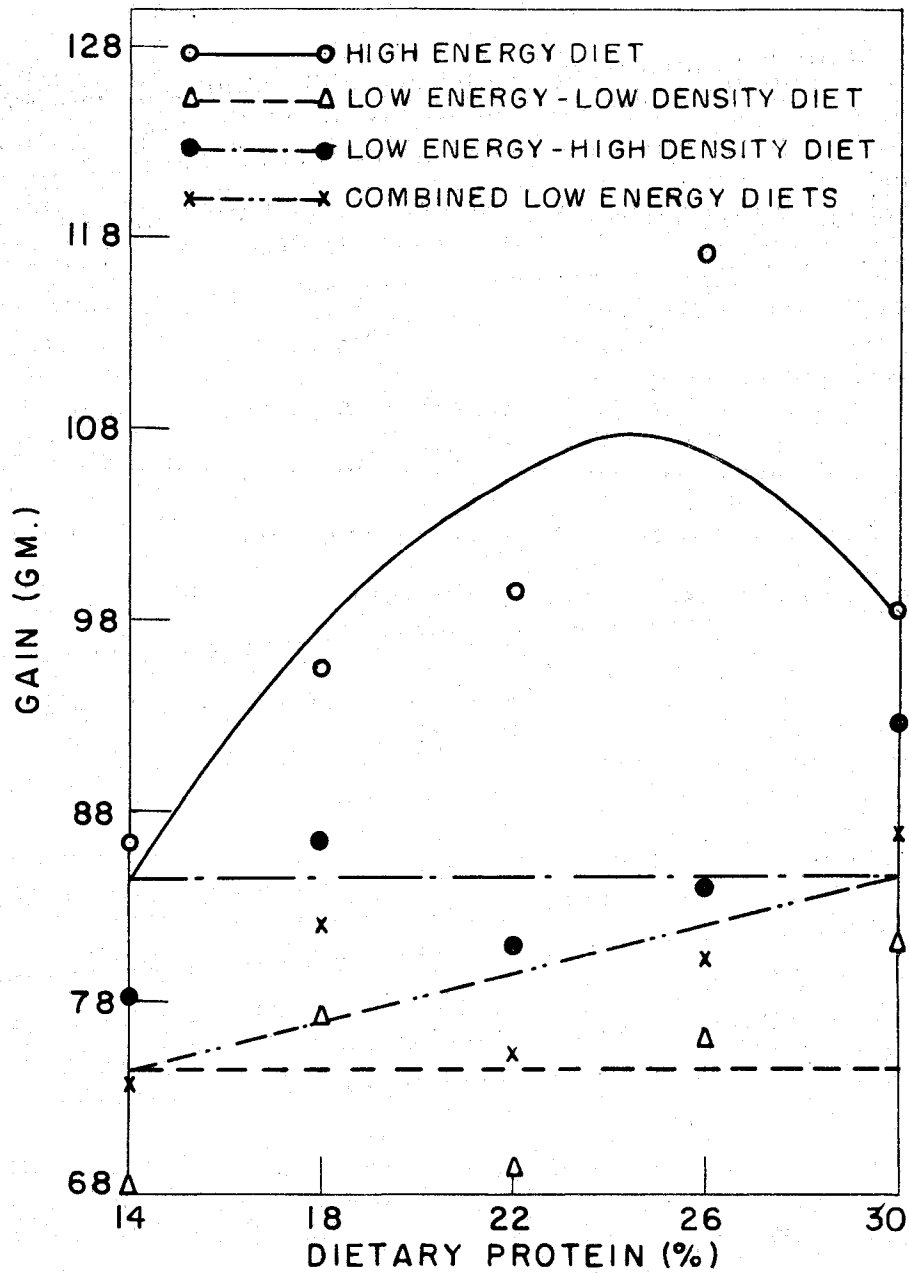
<sup>2</sup> Grams feed per gram gain.

Table 20. Effect of low energy diet on response to protein level increments

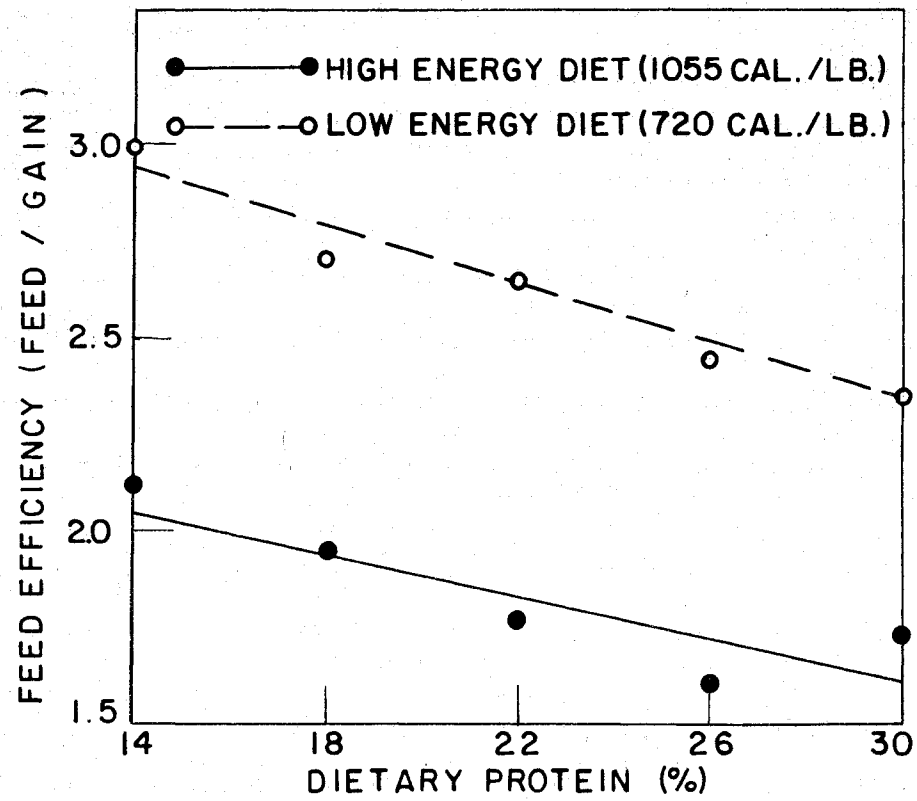
Level of protein in diet (%)	Average percent daily gain <sup>1</sup>	Feed efficiency <sup>2</sup>	Liver fat (%)
14	5.47	3.01	12.35
18	5.79	2.71	13.58
22	5.46	2.66	13.24
26	5.78	2.46	11.79
30	6.09	2.38	10.54

<sup>1</sup>  $100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$

<sup>2</sup> Grams feed per gram gain.



**Figure 6. Effect of high and low energy diets on growth response to protein level increments**



**Figure 7. Effect of high and low energy diets on feed efficiency response to protein level increments**

data suggests a quadratic response with a maximum reached at approximately 26 percent. It is possible that the protein requirement for maximum feed efficiency is greater than for maximum growth. It should be noted that total feed consumption was markedly reduced at the highest protein level (Table 21).

At the 720 kilocalorie level of productive energy there was no significant growth rate response to increments in the dietary protein level. There was a significant linear response in feed utilization to protein increments. Apparently the chick consumed more total feed (Table 22) at the lower protein levels but utilized it less efficiently so growth rate was about the same at all protein levels.

The results in Tables 21 and 22 show that the birds on the low energy diet consumed on the average 8.4 percent more feed but had on the average a 25.8 percent lower caloric intake than those on high energy diets.

The energy level of the diet apparently has no marked effect on the average chick liver fat content. Tables 19 and 20 show that the overall average fat content on both energy levels was about the same. The data indicate that the amount of fat in the liver is related to the protein level of the diet, being less at the higher levels as shown in Figure 8. On low energy diets, where increments in the protein level elicited no growth response, there was a

Table 21. Effect of high energy diet on chick feed consumption in relation to protein level increments

Level of protein in diet (%)	Average feed consumed	Average protein consumed	Average kilocalories consumed
	gm./bird	gm./bird	kilocal./bird
14	182	25	422
18	185	33	431
22	174	38	404
26	187	49	434
30	<u>165</u>	<u>50</u>	<u>384</u>
Overall average	179	39	415

Table 22. Effect of low energy diet on chick feed consumption in relation to protein level increments

Level of protein in diet (%)	Average feed consumed	Average protein consumed	Average kilocalories consumed
	gm./bird	gm./bird	kilocal./bird
14	207	31	328
18	206	37	326
22	184	40	292
26	185	48	294
30	<u>190</u>	<u>57</u>	<u>301</u>
Overall average	194	43	308

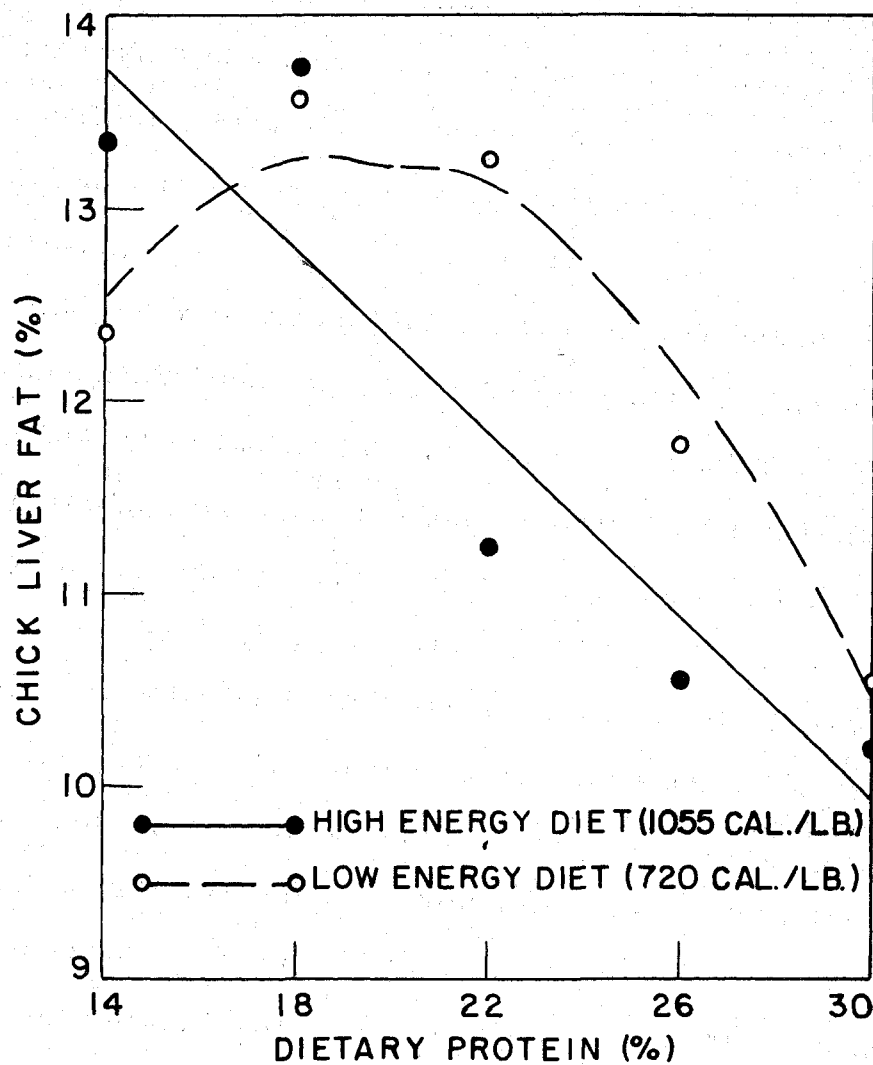


Figure 8. Effect of high and low energy diets on liver fat content with relation to protein level increments

significant difference in the percent liver fat.

### Experiment IX

#### Objective

Chicks regulate their energy intake in relation to their energy needs according to Hill and Dansky (1954). Since chicks on the low energy diet in Experiment VIII consumed approximately 26 percent less productive energy than the chicks on the high energy rations, Experiment IX was undertaken to determine whether the chicks would maintain a high caloric intake on a low-energy, high-density diet.

#### Method

Two pens of ten male chicks and two pens of ten female chicks were assigned to each of five experimental rations. The birds were started on experiment when they were ten days of age and the experiment was continued for 20 days.

A diet identical to the low energy one used in Experiment VIII was formulated except that the pure cellulose of low density (specific gravity 0.19 gm./cc.) was substituted by a high density mixture of oat hulls<sup>1</sup> and washed sand

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<sup>1</sup>Cleaned oat hulls were obtained from the Quaker Oats Company. The energy content was assumed to be approximately zero.

(specific gravity 0.71 gm./cc.). The chicks in Experiment VIII had a ten-hour day by artificial light. In this experiment the period was increased to 14 hours in order to allow the birds more time in which to consume feed.

### Results

Analyses of the data given in Table 23 indicate no significant differences in growth rate or feed efficiency

Table 23. Effect on chick growth and feed efficiency in relation to protein level increments on low-energy, high-density diet

Level of protein in diet (%)	Average percent daily gain <sup>1</sup>	Feed efficiency <sup>2</sup>
<u>11 to 20 day period</u>		
14	7.30	2.45
18	7.74	2.32
22	7.41	2.26
26	7.64	2.34
30	8.00	2.21
<u>21 to 30 day period</u>		
14	6.01	2.65
18	6.15	2.47
22	5.76	2.58
26	5.64	2.34
30	6.16	2.34

<sup>1</sup>  $100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$

<sup>2</sup> Grams feed per gram gain.

during the 11 to 20 or the 21 to 30 day periods when the diet contained 720 kilocalories of productive energy per pound.

The results in Table 24 demonstrate that total feed consumption was not markedly increased above that of the birds in Experiment VIII by increasing the density of the diet and increasing the number of daylight hours for the birds through artificial lighting.

Table 24. Effect on feed consumption in relation to protein level increments on low-energy, high-density diet

Level of protein in diet (%)	Average feed consumed	Average protein consumed	Average kilocalories consumed
<u>11 to 20 day period</u>			
	gm./bird	gm./bird	kilocal./bird
14	192	27	308
18	199	26	316
22	183	40	291
26	193	50	308
30	<u>200</u>	<u>60</u>	<u>318</u>
Overall average	193	41	308

## Experiment X

### Objective

Experiment X was conducted to determine whether or not the type of carbohydrate incorporated into the diet would affect the utilization of protein or the protein requirement of the chick. A monosaccharide (glucose) was compared with a polysaccharide (starch) as a source of carbohydrate.

A further objective of this experiment was to examine the protein requirements during the one to ten and 21 to 30 day periods of the chick's life cycle. Experiments II-IV demonstrated that for maximum growth and feed efficiency during the 11 to 20 day period a dietary protein level of approximately 26 percent was required.

### Method

Eleven female plus 11 male day-old chicks were assigned to each of 12 experimental rations in each of two replicates. Replicate I was conducted in the battery room where the other experiments in this series were conducted while Replicate II was conducted in a building nearby.

The basal rations were similar to those used in Experiments II-IV except that in one series starch replaced

glucose. A 26 percent protein basal was fed during the 11 to 20 day period. The carbohydrate portion of this ration consisted of 50 percent glucose and 50 percent starch.

The protein levels fed during each experimental period are given in Table 25. The birds were assigned to each treatment in the second phase of this experiment upon the basis of their gain and their one to ten day treatment.

Table 25. Influence of dietary protein level on average percent daily gain<sup>1</sup> with two types of carbohydrates

Level of protein in diet (%)			1 - 10 days		21 - 30 days	
			Glucose	Starch	Glucose	Starch
16	--	(16.7) <sup>2</sup>	--	--	4.60	4.80
19	(18.3) <sup>3</sup>	(19.2)	8.32	7.10	5.40	5.30
22	(21.7)	(22.0)	7.08	9.62	5.54	5.63
25	(24.5)	(24.7)	9.80	8.62	5.08	5.02
28	(27.4)	(27.7)	8.34	8.26	5.22	4.72
31	(30.9)	(30.8)	7.84	7.02	5.12	4.79
34	(34.1)	--	7.80	7.24	--	--

<sup>1</sup>  $100(W_2 - W_1) / (1/2)(W_2 - W_1)(\text{No. days})$

<sup>2</sup> Figures in parentheses represent analyzed crude protein for 21 - 30 day period diets.

<sup>3</sup> Figures in parentheses represent analyzed crude protein for 1 - 10 day period diets.

## Results

The data from Replicate II were discarded because of the influence of variables beyond control confounding the results. A period of sub-zero weather occurred while Replicate II was conducted and the heating system in the building failed to function properly. Abnormal mortality and depressed growth rates of the chicks occurred, especially in the lower decks of the batteries.

The results of Replicate I are presented in Tables 25 and 26. They indicate that during the first ten days after

Table 26. Influence of dietary protein level on feed efficiency<sup>1</sup> with two types of carbohydrates

Level of protein in diet (%)	1 - 10 days		21 - 30 days	
	Glucose	Starch	Glucose	Starch
16	--	--	2.48	2.14
19	1.69	1.76	1.97	1.96
22	1.78	1.45	2.07	1.88
25	1.46	1.47	1.93	2.35
28	1.56	1.49	2.01	2.21
31	1.56	1.55	2.04	2.14
34	1.57	1.61	--	--

<sup>1</sup>Grams feed per gram gain.

hatching the type of carbohydrate in the diet may influence the protein requirement of the chick. Maximum growth and feed efficiency occurred on a 25-26 percent protein diet with glucose while with starch a 22 percent protein diet appeared to be optimum as illustrated in Figures 9 and 10. During the 21 to 30 day period the effect of the type of dietary carbohydrate on the protein requirement of the chick apparently disappears. The chick appears to require a dietary protein level of approximately 22 percent for maximum growth and feed efficiency during the 21 to 30 day period with either type of carbohydrate.

An examination of the data in Table 25 reveals that during both periods chicks on high levels of protein were able to grow at a faster rate when glucose was the dietary carbohydrate, but only during the second period was the feed efficiency (Table 26) better on the glucose diet.

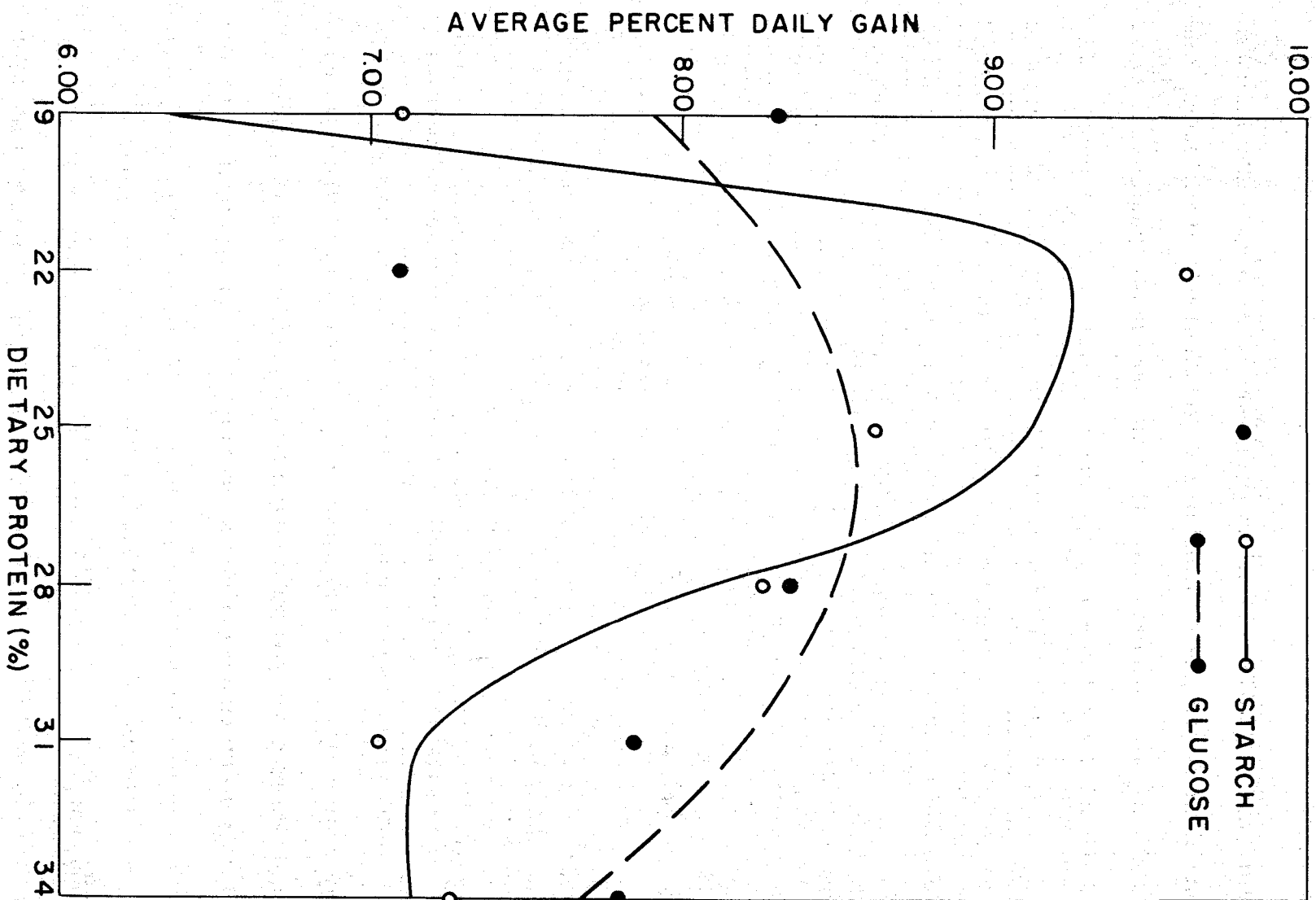
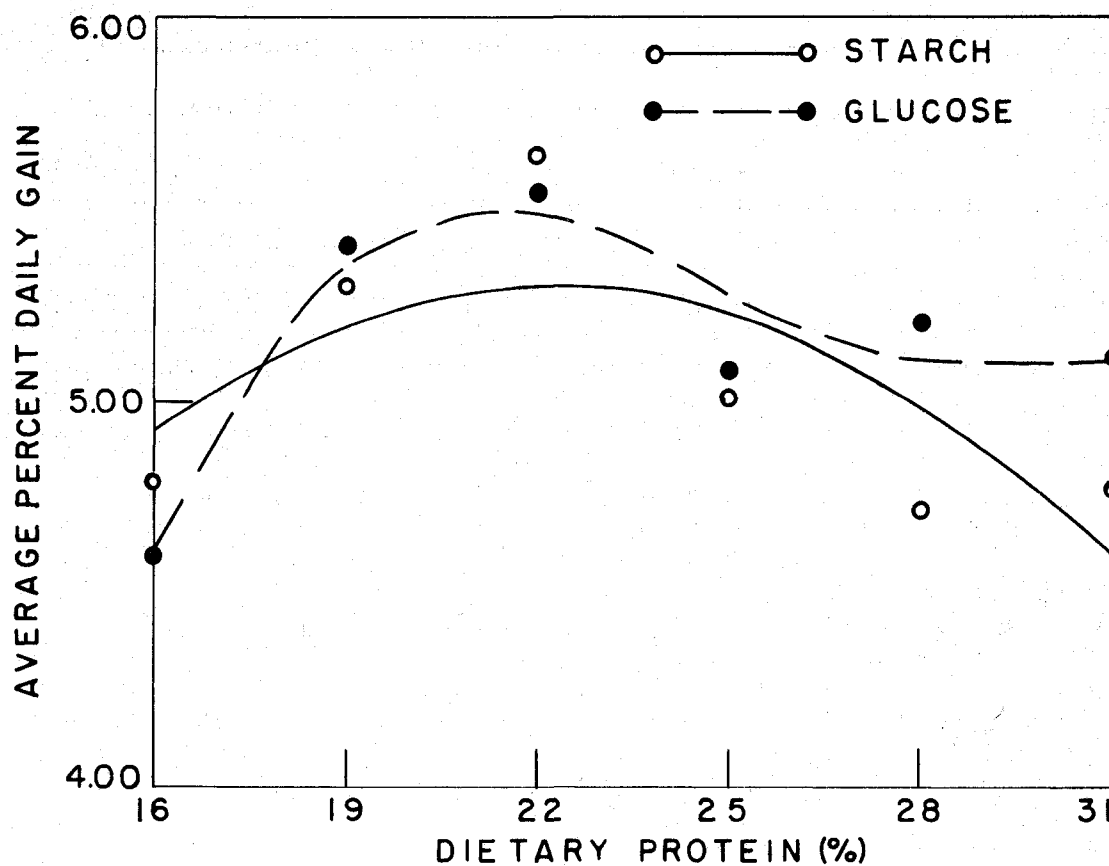


Figure 9. Effect of dietary protein level on chick growth with two types of carbohydrate (1 - 10 days)



**Figure 10.** Effect of dietary protein level on chick growth with two types of carbohydrate (21 - 30 days)

## DISCUSSION

## The Protein Requirements of the Chick

The protein requirement of the chick for maximum growth has been shown, under the conditions of these experiments, to be influenced by the age of the chick, the energy content of the diet and the type of carbohydrate in the ration. The growth rate at various protein levels was also shown to be related to the amino acid sufficiency of the diet.

A high energy, semi-purified diet of glucose and an isolated soy protein, containing an ample supply of all vitamins and minerals known to be required by the chick, was used to establish the protein requirements in three phases of the first 30 days of the chick's life. The protein requirement for maximum growth is apparently greater during the first 20-day period (25-26 percent) than during the 21 to 30-day period (22 percent). This may be due to the fact that as the bird becomes larger a greater portion of the diet is used for purposes requiring less protein and more energy than for growth. In this connection it is well to note that the growth rate and the ability to utilize feed is very similar during the first and second ten-day periods of the chick's life. Percent average daily gain in these

two periods was 9 and 8.5, respectively. Feed required per unit gain was 1.47 and 1.57. There then appears to be a rather marked decrease in growth (5.5) and an increase in the amount of feed required per unit of gain (1.90) during the third ten-day period.

The productive energy content of the diet appears to have considerable influence upon the dietary level of protein required by the chick for maximum growth. Using high levels of productive energy (1055 and 975 kilocalories per pound) the chick required between 24 and 26 percent protein for maximum growth. On a low productive energy diet (720 kilocalories per pound) the chick was apparently able to consume enough protein when the ration was as low as 18 percent protein. This is in line with the work of Peterson, Grau and Peek (1954). They found that by adding cellulose to a diet low in protein, chicks consumed more total feed in an effort to increase their energy intake and thus consumed more total protein.

The response to the low energy diet in Experiment VIII was rather irregular and the chick growth rate was somewhat below the growth rate on the high energy diet. In Experiment IX the exact formulation for the low energy diet of Experiment VIII was used except that a mixture of sand and oat hulls replaced the cellulose, thus increasing the density of the diet considerably. There was no difference in average

feed consumption between the experiments although the birds fed the more dense diet grew better and utilized their diet better.

Hill and Dansky (1954) stated that the feed consumption of chicks in their experiments was determined primarily by the energy level of the ration but that some other unaccountable factor was also involved. The present work indicates that on these low energy diets the birds did not appreciably increase their total feed consumption to meet their energy needs. Even increasing the density of the diet resulted in no marked increase in consumption. This appears to agree with the work of Fisher, Singsen and Matterson (1953) who reported that at both high (968 kilocalories per pound) and low (683 kilocalories per pound) productive energy levels the total feed consumption by weight was approximately the same and the birds on the lower energy level weighed less at the end of the experimental period. They suggested that on a well-balanced, fortified diet the birds on a high energy level will consume the maximum amount of feed which they are physiologically capable of handling and thus the birds on the low energy ration will not be able to offset their dietary caloric deficiency by increased consumption. This is in opposition to the work of Hill and Dansky (1954). They reported that birds on a low energy diet (623 kilocalories per pound) grew at the same rate as

birds on a high energy diet (975 kilocalories per pound). This was due to a marked increase in feed consumption in the opinion of these writers.

A combined analysis of the low energy diets used in Experiments VIII and IX demonstrate a significant linear response to increases in the dietary protein levels. In effect more protein was used for building body tissues with the increased protein intake. As seen from the decreased protein efficiency more protein also was being utilized for energy purposes due to the imbalance in the calorie-protein ratio in the diet. This analysis is not entirely valid as the diets were identical except for the type of inert material, but as the protein level times experiment interaction was not significant the resulting information may be useful.

The replacement of glucose with starch apparently enhances protein utilization during the first ten days of the chick's life cycle but as the chick increases in age the difference disappears. Dreisbach and Nasset (1954) reported that in rats glucose is absorbed more rapidly than starch, reaching a maximum at two and three hours, respectively, after ingestion. The amount of carbohydrate remaining in the intestine of the sacrificed animal was used as their criteria. Rations containing polysaccharides have been found by Monson, Dietrich and Elvehjem (1950) to pass

through the digestive tract of chicks more slowly than those containing disaccharides. Thus time may play an important part in carbohydrate absorption in the young chick. The ration containing starch may be delayed a little longer in the small intestine, thus permitting more time for enzymatic hydrolysis of the protein. Also, the delayed absorption of starch may allow a more even distribution of carbohydrate absorption along with amino acid absorption.

There appears to be no carbohydrate influence in the utilization of moderate levels of protein in the ration of the older chick. Dreisbach and Nasset (1954) reported a better utilization of protein by rats when diets contained starch instead of glucose. These workers used casein as a source of protein while an isolated soy protein was used in this study. Denton and Elvehjem (1954) have demonstrated in vivo with dogs a difference in rate of absorption of amino acids from different protein sources.

Chicks apparently are able to grow better on high levels of protein with glucose as the carbohydrate source instead of starch. This appears to be true during the one to ten day and 21 to 30 day periods of the chick's life cycle. Feed and protein efficiency was better with the glucose diet only in the older chicks fed high levels of protein.

Amino acid supplementation and availability

An examination of the amino acid composition of Drackett Assay Protein C-1 (an isolated soy protein) in comparison to the chick's estimated requirements (Table 5) shows methionine to be very deficient, glycine to be sub-marginal and tryptophan to be marginal. Methionine appears to be the only one of these three amino acids which is limiting with respect to chick growth under the conditions of the experiments reported here. Evidence is also presented that the threonine in the soy protein is the least available of the nine essential amino acids tested. This may not make it a limiting amino acid, however, as the isolated soy protein contains approximately 33 percent threonine in excess of the chick's requirements.

Isolated soy protein, a combination of 16.2 percent soy protein plus 4.8 percent gelatin and a combination of 8.1 percent soy protein plus 7.5 percent casein plus 4.8 percent gelatin produced similar growth rates and feed utilization. These diets were properly supplemented to meet the chick's amino acid requirements. A combination of 10.8 percent soy protein and 10.0 percent casein produced poorer growth and feed efficiency than the other protein sources due to a methionine deficiency in the diet.

In Experiment VI a diet containing isolated soy protein

supplemented with methionine and glycine produced growth and feed efficiency results similar to one containing a combination of 11.8 percent soy protein and 11.6 percent casein supplemented with methionine and glycine at a 20 percent dietary level of protein.

Reducing the protein level in the diet to 18 percent resulted in no significant differences in growth rate between the diets utilizing the different sources of protein. Supplementing the isolated soy protein-casein diet with synthetic L-arginine so that the total dietary arginine was well above the chick's requirement produced no increased growth or feed efficiency.

At the 18 percent protein level, feed efficiency on the soy protein ration was less than on the soy protein-casein combination. A contributing factor may have been that although soy protein contains an excess of threonine, its high unavailability may make it a marginal amino acid for optimum feed efficiency at low protein levels. This may be true even though sufficient available threonine may be present for optimum growth. That the chick may have a higher amino acid requirement for maximum feed efficiency than for maximum growth has been suggested for methionine by Hill (1953) and for glycine by Fisher, Scott and Johnson (1955). Also, that a marginal amino acid in a protein may become limiting at a lower protein level in the diet

has been intimated by Ewing (1951, p. 159) where he stated:

. . . that when a protein is not too severely deficient in an amino acid it is sometimes possible to meet the needs of the animal by simply feeding a higher percentage of the protein in the diet.

Grau and Kamei (1950) postulated that the amino acid requirement of the chick for maximum growth expressed as a percent of the protein for lysine and methionine plus cystine, decreases as the protein level in the diet increases.

These data show that, in the case of arginine, amino acid availability from dietary protein remains constant with a variation in the protein level of the diet. If this is true for other amino acids then it indicates that the increased protein efficiency obtained by lowering the dietary protein level (Experiments II-IV) is due to a better utilization of the absorbed amino acids and not to an increase in the percentage of amino acids absorbed.

The biological inferences drawn from the statistical analysis of the data in Table 9 suggest a significant difference in the overall availability of amino acids from the different protein sources. The main effects of the types of protein on the amino acid availability to the chick are confounded as indicated by the diet-amino acid interaction. This interaction indicates that the relationship among amino acids according to their availability is different within each ration. In other words, one amino acid may be the

least available of all the amino acids in one diet and the most available of all the amino acids in the other diet. This is probably due to the differences in the relationship between amino acids in soy protein and casein. The utilization factors for amino acids given in Table 9 can be calculated so as to remove the soy protein contribution to the total amino acids excreted by chicks fed the ration containing the casein-soy protein combination. The resulting utilization factors for eight of the essential amino acids in casein were calculated and used in Figure 11. Such a procedure may not be entirely valid as the combining of two protein sources may result in either a synergistic or an antagonizing effect with respect to the availability of their constituent amino acids which would then confound the results. Yet such calculations may reveal some worth while information if one keeps in mind the imposed limitations.

A ranking of the amino acids in casein according to their utilization factors demonstrates a marked similarity to their rank calculated from the in vitro studies of Denton and Elvehjem (1953). The data of these workers are expressed as percent of the total amino acid freed by enzymatic digestion. The data from the present work are expressed as utilization factors and do not represent the total availability of the amino acids. Reciprocals of the data of Denton and Elvehjem (1953) were used in Figure 11 so as to

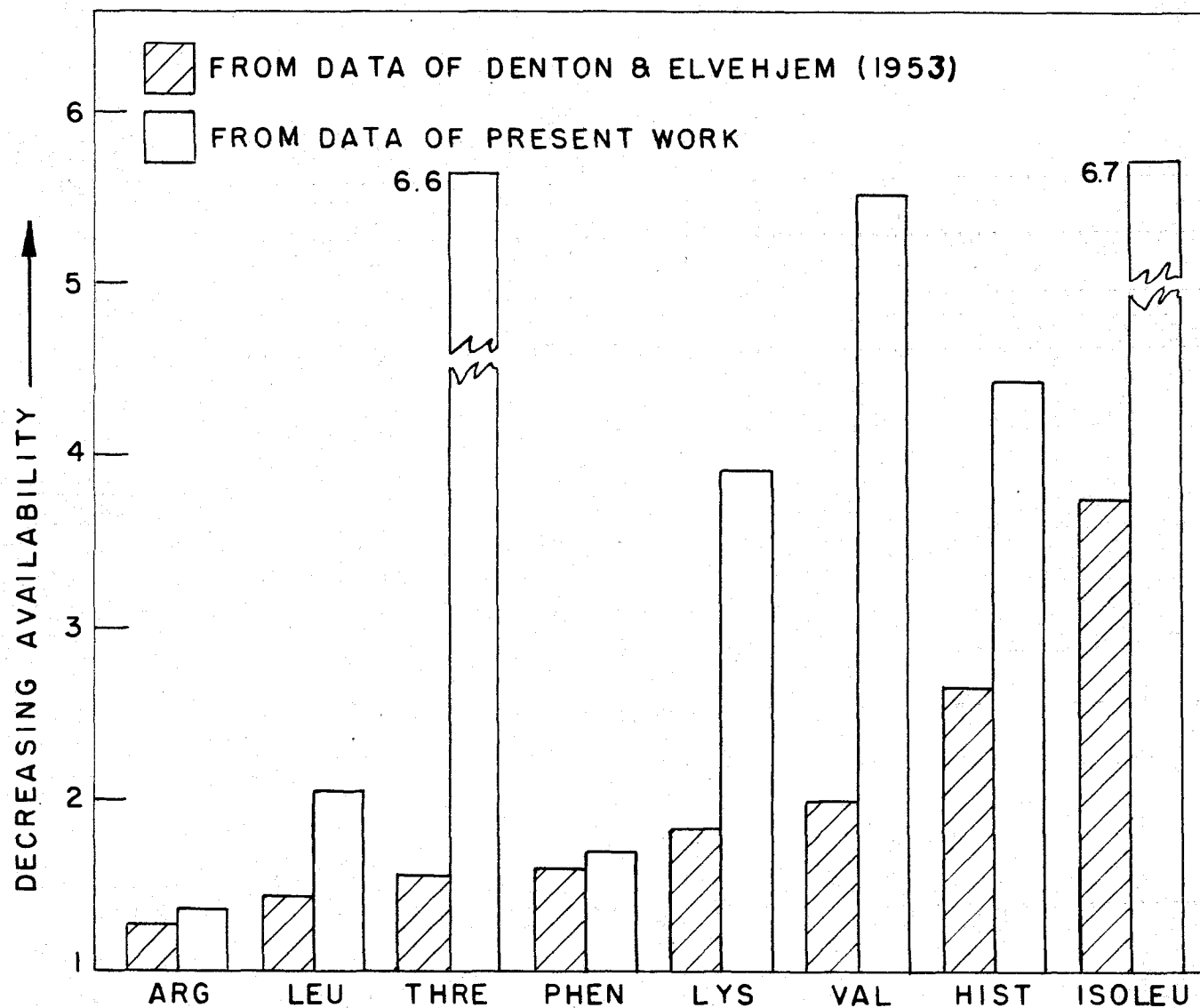


Figure 11. Comparison between the relative availabilities in vitro and in vivo of the amino acids in casein

afford a basis with which to compare them with the present work.

Availability to the chick of the threonine, lysine, valine, histidine and isoleucine in casein appears to be less when compared to leucine, arginine and phenylalanine, than a similar comparison made from the data of Denton and Elvehjem (1953). This was not unexpected as other workers have not been able to completely confirm the above mentioned in vitro work with in vivo studies. Geiger, Courtney and Geiger (1952) showed that valine in zein is highly unavailable to the rat while valine ranks second among the amino acids of zein with an availability of 96.7 percent according to the in vitro work of Denton and Elvehjem (1953).

Threonine is apparently the least available of the amino acids tested in soy protein and probably in casein, also. Arginine appears to be the most readily available in both.

The results of these experiments lead one to postulate that one or more of the essential amino acids in soy protein are involved in some enzyme-resistant linkage. Threonine and possibly leucine and valine would be the most likely amino acids involved in such linkages. Geiger, Courtney and Geiger (1952) reported the presence of possible peptides in an enzymatic digest of zein which released threonine and proline upon acid hydrolysis.

The effect observed in these experiments of supplementing an expeller-extracted meat scraps-corn ration with DL-lysine, DL-tryptophan, DL-methionine, L-arginine and glycine are not in agreement with those of Patrick (1953) and March, Biely and Young (1950). These workers found that although their meat scraps rations contained ample lysine to meet the chick's requirements, supplemental synthetic L-lysine added to the basal diet improved the growth of chicks. It might be well to note that Patrick used a low-protein basal diet (17.2 percent) and he added lysine to a basal ration without adjusting the nitrogen content of the diet. Thus, his lysine-supplemented diet contained a 17.6 percent protein equivalent based on dietary nitrogen content. The 0.4 percent added protein equivalent would probably be beneficial in a low protein diet.

March, Stupich and Biely (1949) showed that, on the basis of the protein quality index, amino acid assays and chick biological tests, meat meals varied considerably. Rohlf (1954), however, found no significant differences in nutritive value for swine among 11 different commercially produced meat scraps and solvent soybean oil meal.

It is obvious that lysine availability in meat scraps is dependent upon the processing treatment. Evans and Butts (1948) and Hanks et al. (1948) reported that lysine is one of the amino acids most readily affected by high

temperatures.

With 18 or 20 percent protein diets, supplementing the basal diet with synthetic L-arginine, DL-lysine, DL-methionine, DL-tryptophan and glycine improved the utilization of the total dietary arginine, lysine and methionine.

Figure 5 illustrates that low level supplementation of a single synthetic amino acid enhanced the utilization of other protein-bound amino acids in the diet. Adding combinations of two synthetic amino acids resulted in better utilization of other amino acids in the protein-bound form than that due to either alone, although the improvement did not appear to be additive.

It is apparent that free amino acids added to the diet are utilized to a much greater extent than those supplied in the protein-bound form. This is probably because the free amino acids are available for absorption at the moment they enter the duodenum while the protein-bound amino acids must have their peptide linkages hydrolyzed by the intestinal and pancreatic proteolytic enzymes. The time taken for such hydrolysis differs with the protein as is evidenced by the report of Denton and Elvehjem (1954). These authors showed that one hour after feeding casein to a dog there was an increase in blood plasma concentration of all ten amino acids measured, while not until five hours after feeding zein was there an increase in plasma amino acid concentra-

tion. Heating proteins also affects the rate of enzymatic liberation of constituent amino acids as was shown by Pader, Melnick and Oser (1948).

One might speculate further as to the increased utilization of free amino acids. Agar (1954) has suggested that L-amino acids are absorbed through the intestinal wall by an active process of transfer. The amino acid is thus probably absorbed via specific enzymatic mechanisms, such as the concept of the phosphorylation of glucose during absorption. If such is the case, then the rate of the absorption of an amino acid would depend upon the velocity of its enzymatic reaction. The enzyme concentration in the intestinal cells is probably rather constant, therefore, at low amino acid concentrations the initial velocity of the enzyme-amino acid reaction would be proportional to the initial amino acid concentration. Consequently free amino acids in the diet would tend to cause an increased rate of transfer of amino acids into the blood stream.

The present work suggests that adding a low level of a synthetic amino acid to a chick's diet enhances the utilization of other protein-bound amino acids. At first thought such a concept appears to conflict with the work of Kamin and Handler (1952). They demonstrated with rats that the presence of an excess of an amino acid almost invariably inhibited the intestinal absorption of another amino acid.

In their experiments these workers used combinations of only two amino acids in solution, with one of the two being two to five times the molar quantity of the other, thus presenting rather abnormal conditions. Yet, such data would tend to eliminate a theory suggesting that the present results were due to an amino acid directly enhancing the rate of absorption of another. An alternate explanation might be that free amino acids in general may, by their ready availability, enhance proteolytic enzymatic activity in the intestine through some possible catalytic action. It is of interest to note that Elkins-Kaufman and Neurath (1948) reported that the presence of L-phenylalanine had no effect on the activity of carboxypeptidase.

Microorganisms within the small intestine might also be enhanced to predigest the protein thus making the protein more available to the proteolytic enzymes. A similar action would be the priming effect a little free sugar has on the microorganisms within the rumen which enables them to better digest cellulose.

Many nutritionists have wondered why low level methionine supplementation to practical diets seems to improve growth and feed efficiency of chicks to a greater extent than one would expect as the result of increasing a slightly submarginal amino acid to a marginal level. Saxena and McGinnis (1952) have suggested that the addition of

DL-methionine to poultry rations may exert a beneficial effect by supplying some special property not associated with protein need. This view is shared by Couch (1953). The present work indicates that possibly the effect of supplemental DL-methionine may be two-fold. First it brings the dietary methionine content up to meet the chick's requirement and secondly it may enhance the chick's utilization of other amino acids in the diet. This may help explain why free amino acid supplementation enhances feed efficiency and not growth rate under some conditions as shown by Hill (1953) and Fisher, Scott and Johnson (1955).

The data illustrated in Figure 5 indicates that when large quantities of a free amino acid are added to the diet there is a somewhat smaller improvement in the utilization of the bound form of that amino acid obtained by adding other synthetic amino acids. The effect of synthetic amino acids on enhancing the utilization of bound amino acids in the diet is not a simple phenomenon. Apparently the particular amino acid, the stereochemical form and the amount added may be important considerations. Certain D-amino acids inhibit carboxypeptidase activity according to Elkins-Kaufman and Neurath (1948). When five amino acids were added the beneficial effect on bound-amino acid utilization was greatest for arginine and least for methionine.

A review of the literature reveals that adding low

levels of synthetic methionine is more beneficial than higher levels (Reed, Quisenberry and Couch, 1954 and Bolin et al., 1952). Russell, Taylor and Hogan (1952) showed high levels of methionine to be detrimental. An adverse effect due to high level free amino acid supplementation was also demonstrated in this work. In Experiment VI a high level of L-arginine (0.36 percent) was added and there appeared to be a slight decrease in the utilization of the other bound amino acids.

#### Lipotropic effect of protein

Chicks consuming a high fat diet (ten percent) accumulated no more fat in their livers than those consuming a low fat diet (one percent). This suggests that dietary fat per se was not deposited to any great extent in the livers of the chicks. Harper et al. (1954c) reported similar effects in rats.

The present work reveals that over a specific range there is an inverse relationship between the level of protein in the diet and the amount of fat deposited in the chick's liver. These results are in agreement with those of Lucas and Ridout (1955) with rats. The diets in the present study were sufficient in all nutrients known to be required by the chick and these as well as the energy content of the diet were held approximately constant over the range of

dietary protein levels tested. In most of the previous rat work such rigid control over extraneous variables has not been practiced.

It is apparent from the present work that protein exerts a lipotropic effect through means not directly involving its choline or choline precursor content.

Aside from improving the amino acid balance of a ration all compounds which have been shown to exert a lipotropic effect are directly connected in some manner with choline synthesis, except threonine. These compounds are effective in the absence of choline while threonine is not. The lipotropic effect of threonine has been demonstrated by most workers by using casein. The present work suggests that threonine in casein is not readily available to the chick and this may hold true for other species. Thus lipotropic action of supplemental threonine may be due principally to its function in improving the dietary amino acid balance.

Threonine appears to be biologically unavailable from many protein sources. Pecora and Hundley (1951) demonstrated its relative unavailability in rice with rats. Geiger, Courtney and Geiger (1952) have suggested its unavailability in zein and Sure (1953b) has reported an advantage to threonine supplementation of wheat and corn protein for rat growth. The present work has demonstrated the

relative unavailability of threonine in isolated soy protein and has suggested the same for casein.

## SUMMARY

The protein requirements of the chick for maximum growth and feed efficiency and factors influencing these requirements have been studied. As an integral part of this evaluation, amino acid availability in certain proteins and the influence of the dietary protein level on liver fat content were also studied.

The chick's protein requirement on a semi-purified, glucose-soy protein diet was found to be approximately 25 to 26 percent during the first 20 days after hatching. During the next ten-day period the protein requirement was shown to be approximately 22 percent.

The type of carbohydrate in the diet appears to influence the chick's protein requirement during its first ten days of life. When starch was used as the carbohydrate during this period the chick's protein requirement for maximum growth was approximately 22 percent. Substituting a monosaccharide, glucose, for the starch raised the requirement to about 26 percent. By the time the chick was 21 to 30 days of age the influence of carbohydrate source on the protein requirement had disappeared.

The chick's protein requirement for maximum growth was about the same (26 percent) during the 11 to 20 day age period on a diet containing either 975 or 1055 kilocalories

of productive energy per pound. On a diet containing 720 kilocalories per pound there appeared to be a slight increase in growth with increments in dietary protein levels from 12 to 30 percent. Increasing the density of the diet and the length of the feeding day did not enable the chicks to increase their total feed consumption enough to meet their energy needs.

Methionine appears to be the only limiting amino acid in soy protein of the three amino acids tested (methionine, glycine and tryptophan).

The essential amino acids, with the exception of tryptophan and glycine, in soy protein and in a casein-soy protein combination were tested for their relative utilization by the chick. The availability of these amino acids was based upon the ratio of the content of each amino acid in the diet to the content of each corresponding amino acid in the excreta. The amino acid contents were determined by microbiological analysis.

These amino acids differed in their availability within and between protein sources. Arginine appeared to be the most readily available and threonine the least available in both the soy protein and the casein-soy protein combination.

Free arginine, lysine and methionine when added to a corn-meat scraps diet were more completely utilized by the

chick than the corresponding protein-bound amino acids. The addition of each of these three synthetic amino acids increased the chick's ability to utilize protein-bound amino acids.

The chick's liver fat content was not affected by the energy level of the diet but increasing the dietary protein level from 14 to 30 percent caused a decrease in liver fat.

## CONCLUSIONS

1. The addition of free arginine, lysine or methionine to the diet enhances the chick's utilization of protein-bound amino acids.
2. The level of dietary protein, within limits (18 to 20 percent), does not appear to influence the utilization of protein-bound arginine.
3. Free arginine, lysine and methionine are more completely utilized by the chick than the corresponding amino acids in the protein-bound form.
4. Most of the essential amino acids in soy protein differ in their availability to the chick. Arginine is the most readily available and threonine is the least available of the eight amino acids tested. The availability of the amino acids is based upon the ratio of the amino acid content in the diet to the amino acid content in the excreta.
5. The chick requires approximately 25 to 26 percent dietary protein for maximum growth from the time of hatching to 20 days of age when fed a semi-purified, glucose-soy protein diet.
6. Approximately 21 to 22 percent dietary protein is needed for maximum growth from 21 to 30 days of age under these conditions.

7. The level of dietary protein required by the chick under these conditions for maximum feed efficiency from 11 to 20 days of age seems to be slightly higher than that required for maximum growth.
8. The chick appears to have a lower dietary protein requirement for maximum growth and feed efficiency from one to ten days of age when starch replaces glucose in a semi-purified diet.
9. The dietary protein requirement of the chick from 21 to 30 days of age is approximately the same for maximum growth and feed efficiency when either glucose or starch makes up the carbohydrate portion of a semi-purified diet.
10. During the 11 to 20 day age period the chick's dietary protein requirement is approximately the same (25-26 percent) when fed a diet containing either 975 or 1055 kilocalories of productive energy per pound.
11. When fed a low energy diet (720 kilocalories per pound) the chicks were not able to increase their total feed consumption enough to meet their caloric needs and did not grow as well as birds on a high (1055 kilocalories per pound) energy diet.
12. The level of energy in the diet does not appear to affect the level of fat in the chick's liver.

13. The liver fat content decreases as the dietary protein is increased from 14 to 30 percent.

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APPENDICES

APPENDIX A  
VITAMIN AND ANTIBIOTIC PREMIX

<u>Vitamin or antibiotic</u>	<u>Quantity contributed by the premix to one pound of complete ration</u>
Vitamin A, I.U.	4000
Vitamin D <sub>3</sub> , I.C.U.	500
Alpha tocopherol, mg.	7
Menadione, mg.	.6
Choline chloride, mg.	868
Inositol, mg.	455
Niacin, mg.	45
Calcium pantothenate, mg.	10
Riboflavin, mg.	3.6
Pyridoxine hydrochloride, mg.	2.7
Thiamine hydrochloride, mg.	1.8
Folic acid, mg.	1.4
Para-aminobenzoic acid, mg.	.9
Biotin, mcg.	90
Vitamin B <sub>12</sub> , mcg.	6
Penicillin, mg.	4

APPENDIX B  
MINERAL PREMIX

<u>Mineral</u>	<u>Quantity contributed by the premix to one pound of complete ration</u>
Sodium chloride, %	.5
Calcium, %	1.6
Phosphorus, %	.88
Zinc, mg.	1.08
Potassium, gm.	1.3
Manganese, mg.	33.3
Iron, mg.	30.6
Cobalt, mg.	.6
Copper, mg.	1.2
Magnesium, mg.	300
Iodine, mg.	5

## APPENDIX C

## ANALYSES OF VARIANCE

## 1. Experiment I

<u>Source of variation</u>	<u>d.f.</u>	Average percent daily gain
		<u>M.S.</u>
Reps.	1	2.3329
Treatments	4	0.7754
Interaction	<u>4</u>	0.2781
Total	9	

## 2. Experiment II

<u>Source of variation</u>	<u>d.f.</u>	Average percent daily gain	Feed efficiency
		<u>M.S.</u>	<u>M.S.</u>
Reps.	1	0.4682	0.0470
Protein levels	4	0.2646	0.0462*
Linear component	1	1.0240*	0.1756**
Quadratic component	1	0.0120	0.0000
Cubic component	1	0.0010	0.0062
Remainder	1	0.0261	0.0033
Interaction	4	0.0785	0.0058
Sex	1	0.3125*	0.0054
Sex X protein levels	4	0.0413	0.0097
Experimental error	<u>5</u>	0.0205	0.0049
Total	19		

## 3. Experiment III

<u>Source of variation</u>	<u>d.f.</u>	Average percent daily gain	Feed efficiency
		<u>M.S.</u>	<u>M.S.</u>
Reps.	1	0.2311	0.0205
Protein levels	4	0.0538	0.0537**
Linear component	1	0.6376**	0.1836**
Quadratic component	1	0.0000	0.0019
Cubic component	1	0.0360	0.0058
Remainder	1	0.1924	0.0238*
Interaction	4	0.0276	0.0024
Sex	1	1.3992**	0.0231*

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\*Significant difference at  $P = 0.05$ .

\*\*Significant difference at  $P = 0.01$ .

Sex X protein levels	4	0.0218	0.0029
Experimental error	<u>5</u>	0.0403	0.0018

Total	19
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## 4. Experiment IV

<u>Source of variation</u>	<u>d.f.</u>	<u>Average percent daily gain M.S.</u>	<u>Feed efficiency M.S.</u>
Reps.	1	0.4561	0.0003
Protein levels	4	1.9271**	0.2808**
Linear component	1	5.9985**	0.9364**
Quadratic component	1	1.6354**	0.1629**
Cubic component	1	0.0740	0.0230*
Remainder	1	0.0004	0.0010
Interaction	4	0.0137	0.0022
Sex	1	0.0232	0.0039
Sex X protein levels	4	0.0659	0.0082
Experimental error	<u>5</u>	0.0437	0.0032
Total	19		

## 5. Experiment V

<u>Source of variation</u>	<u>d.f.</u>	<u>Average percent daily gain M.S.</u>
Reps.	1	0.2326
Treatments	4	1.1915*
Rep. X treatment	4	0.2070
Pens/blk./treat.	10	0.3276
Sex	1	1.6040*
Sex X treatments	4	0.0701
Experimental error	<u>15</u>	0.2158
Total	39	

Least significant difference at  $P = 0.05$  is 1.33.

## 6. Experiment VI

a. Growth and feed efficiency data

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\*Significant difference at  $P = 0.05$ .  
 \*\*Significant difference at  $P = 0.01$ .

<u>Source of variation</u>	<u>d.f.</u>	<u>Average percent daily gain M.S.</u>	<u>Feed efficiency M.S.</u>
Reps.	1	0.7840	0.0397
Treatments	4	2.6433	0.1058**
Interaction	4	0.6565	0.0018
Total	9		

Least significant difference at  $P = 0.05$  is 0.189.

b. Arginine utilization

<u>Source of variation</u>	<u>d.f.</u>	<u>M.S.</u>
Reps.	1	0.0758
Treatments	4	0.1689*
Interaction	4	0.0221
Total	9	

c. Amino acid utilization in Lots 3, 4 and 5

<u>Source of variation</u>	<u>d.f.</u>	<u>M.S.</u>
Reps.	1	0.0147
Lots	2	3.8986**
Lots X reps.	2	0.0298
Amino acids	8	25.6537**
Lots X amino acids	16	0.8258**
Experimental error	24 <sup>1</sup>	0.0440
Total	53 <sup>1</sup>	

d. Amino acid utilization in Lot 3

<u>Source of variation</u>	<u>d.f.</u>	<u>M.S.</u>
Reps.	1	0.0364
Amino acids	8	11.2446**
Interaction	8	0.0632
Total	17	

\*Significant difference at  $P = 0.05$ .

\*\*Significant difference at  $P = 0.01$ .

<sup>1</sup>The degrees of freedom are 23 and 52 for error and total respectively due to one missing value.

## e. Amino acid utilization in Lot 4

<u>Source of variation</u>	<u>d.f.</u>	<u>M.S.</u>
Reps.	1	0.0221
Amino acids	8	8.6875**
Interaction	8	0.0310
Total	17	

## 7. Experiment VII

<u>Source of variation</u>	<u>d.f.</u>	<u>Average percent daily gain M.S.</u>	<u>Feed efficiency M.S.</u>
Reps.	1	2.9568	0.1901
Treatments	9	0.6087*	0.0642*
Interaction	9	0.1510	0.0164
Total	19		

<u>Source of variation</u>	<u>Average percent liver fat M.S.</u>
Reps.	0.0442
Treatments	2.2260
Interaction	1.4430

## 8. Experiment VIII

## a. Combined energy levels

<u>Source of variation</u>	<u>d.f.</u>	<u>Average percent daily gain M.S.</u>
Reps.	1	0.3202
Energy levels	1	13.6420 <sup>2</sup>
Interaction	1	0.5810
Protein levels	4	0.7600 <sup>1</sup>
Protein levels X reps.	4	0.1649
Protein levels X energy levels	4	0.4772 <sup>2</sup>

\*Significant difference at  $P = 0.05$ .

\*\*Significant difference at  $P = 0.01$ .

<sup>1</sup>Significant difference at  $P = 0.10$ .

<sup>2</sup>Significant difference at  $P = 0.20$ .

Interaction	4	0.1344
Sex	1	3.1134
Sex X energy level	1	0.1257
Sex X protein level	4	0.2514
Experimental error	<u>14</u>	0.5256
Total	39	

## b. High energy level (1055 Calories per lb.)

<u>Source of variation</u>	<u>d.f.</u>	<u>Average percent daily gain M.S.</u>	<u>Feed efficiency M.S.</u>
Reps.	1	0.8820	0.0594
Protein levels	4	0.9701*	0.1604*
Linear component	1	1.2816*	0.4928*
Quadratic component	1	1.2903*	0.1063
Cubic component	1	0.6175	0.0378
Remainder	1	0.6910	0.0047
Interaction	4	0.1504	0.0246
Sex	1	2.2445	0.0069
Sex X protein levels	4	0.0820	0.0101
Experimental error	<u>5</u>	0.5862	0.0567
Total	19		

## c. Low energy level (720 Calories per lb.)

<u>Source of variation</u>	<u>d.f.</u>	<u>Average percent daily gain M.S.</u>	<u>Feed efficiency M.S.</u>
Reps.	1	0.0192	0.0304
Protein levels	4	0.2730	0.2469**
Linear component	1	0.6002	0.9333**
Quadratic component	1	0.1080	0.0236
Cubic component	1	0.1626	0.0058
Remainder	1	0.2212	0.0249
Interaction	4	0.1489	0.0058
Sex	1	0.9946	0.1037
Sex X protein levels	4	0.4992	0.1854
Experimental error	<u>5</u>	0.6211	0.0820
Total	19		

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\*Significant difference at  $P = 0.05$ .

\*\*Significant difference at  $P = 0.01$ .

## d. Fat level in livers

<u>Source of variation</u>	<u>d.f.</u>	Low energy diet	High energy diet
		<u>M.S.</u>	<u>M.S.</u>
Reps.	1	9.5453	8.0103
Protein levels	4	2.9364*	5.3162*
Linear component	1	5.8536**	18.0500*
Quadratic component	1	5.2462**	0.0096
Cubic component	1	0.6266	2.0801
Remainder	1	0.0192	1.1250
Interaction	<u>4</u>	0.2282	0.3910
Total	9		

## 9. Experiment IX

<u>Source of variation</u>	<u>d.f.</u>	<u>11 - 20 day period</u>	
		Average percent	Feed
		<u>daily gain</u>	<u>efficiency</u>
		<u>M.S.</u>	<u>M.S.</u>
Protein levels	4	0.3015	0.0319
Linear component	1		0.0837
Quadratic component	1		0.0062
Cubic component	1		0.0297
Remainder	1		0.0079
Interaction	5	0.2922	0.0996
Sex	1	0.0115	0.1022
Sex X protein levels	4	0.2268	0.1010
Experimental error	<u>5<sup>1</sup></u>	0.1774	0.0506
Total	19 <sup>1</sup>		

<u>Source of variation</u>	<u>21 - 30 day period</u>
	<u>Feed efficiency</u>
	<u>M.S.</u>
Protein levels	0.0818
Linear component	
Quadratic component	
Cubic component	
Remainder	

\*Significant difference at  $P = 0.05$ .

\*\*Significant difference at  $P = 0.01$ .

<sup>1</sup>Degrees of freedom for error and total are 3 and 17 respectively in the case of the 11 to 20 day period feed efficiency due to missing values.

Interaction	0.1109
Sex	0.0065
Sex X protein levels	0.0279
Experimental error	0.0984

10. Combined analysis of the low energy diets of Experiments VIII and IX

<u>Source of variation</u>	<u>d.f.</u>	Average percent daily gain
		<u>M.S.</u>
Experiments	1	72.0481
Protein levels	4	1.1381
Linear component	1	2.5301*
Quadratic component	1	0.2972
Cubic component	1	0.9394
Remainder	1	0.7855
Protein levels X experiments	4	0.0109
Experimental error	<u>10</u>	0.4152
Total	19	

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\*Significant difference at  $P = 0.05$ .

\*\*Significant difference at  $P = 0.01$ .