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EFFECT OF DIET LEVELS OF LEUCINE, ISOLEUCINE AND VALINE

ON CHICK GROWTH RATE

by

Naiyana Nakhata

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Animal Science

Approved:



UTAH STATE UNIVERSITY
Logan, Utah

1975

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Naiyana Nakhata

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ABSTRACT

Effect of Diet Levels of Leucine, Isoleucine and Valine
on Chick Growth Rate

by

Naiyana Nakhata, Master of Science

Utah State University, 1975

Major Professor: Dr. J. O. Anderson

Department: Animal Science

Chick feeding tests were conducted to study the effects of dietary imbalances among the three branched chain amino acids on growth rate. All diets fed in these tests contained about 18% protein. The indispensable amino acids (IAA) were found in two of the diets fed in the proportions found by Dobson et al. (1964) to be well balanced. One of the diets had all IAA at 85% of these balanced levels while the other had them all at 125% of these levels. The tests involved reducing the isoleucine, leucine and valine levels in the high IAA diet to the low levels in all combinations.

Growth rates were similar with the diets containing all ten IAA at the low levels, all ten at the high levels, or seven at the high levels and isoleucine, leucine and valine at the low levels. Generally, when only one or two of these three were reduced to the low levels, growth rates were lower. Thus there appeared to be a three-way interaction among these amino acids with the reduction in weight gain being the result of an imbalance and not of a deficiency.

The changes in growth rate noted when the leucine level was reduced indicated that the leucine level in Dobson's balanced diet was relatively high; the isoleucine level appeared to be relatively low. The interaction between leucine and valine appeared to be more significant than the other two two-way interactions.

The effect of dietary level of these amino acids on the branched chain amino acid transaminase (BAT) activity in the liver and kidneys was determined. The differences noted were inconsistent. There tended to be a slightly higher activity in chicks fed the high levels of these amino acids, but the differences certainly were not as great as the changes in arginase activity reported by others when imbalanced diets were fed.

Chicks fed the diet low in valine and high in the other nine IAA were selected for fast or slow growth on this diet. They were raised to maturity and produced eggs that were hatched for feeding tests with the diets containing different levels of the branched chain amino acids. Only a limited number of chicks from the two strains were hatched. Performance of the chicks was similar to that of their parents when fed the low valine diet.

INTRODUCTION

This thesis reports studies on the subject of amino acid balance in chick nutrition.

It has been known for some time that the chick's arginine requirement increased as the levels of the other indispensable amino acids (IAA) increased. Prior to my starting the test report in this thesis, preliminary tests were conducted to determine whether the requirements for other IAA were affected in the same manner. Dobson et al. (1964) reported results of tests conducted to determine what IAA proportions make a well-balanced diet. The diets used in Dobson's work contained a total of 8.95% IAA and 18% protein (N x 6.25). The proportions were defined by adjusting the IAA levels in the diet until about the same reduction in growth rate was noted when a 20% reduction was made in the diet level of any of the 10 IAA.

In the preliminary tests, one diet was fed that contained the IAA at 85% of Dobson's balanced levels (low level), and another contained 125% of these levels (high level). Ten modifications of the high level diet also were fed. Each of these had one of the IAA reduced from the high to the low level, while the other nine were at the high level.

The relative gains and gain/feed ratios noted in these tests are given in Table 1. It may be noted that the greatest depressions in growth rate (about 15%) were noted with arginine and valine reductions. When all 10 IAA were

reduced to the low level, growth rate was as high as with the high level diet. This shows that Dobson's allowances for the IAA were beyond the minimum requirements. It also indicates that reduction in growth rate, resulting when the level of one amino acid was reduced from the high to the low level, can justifiably be termed the result of an imbalance and not of a deficiency. The depression in growth rate when arginine was reduced was expected, and it appeared that the valine requirement was increased as much as the arginine requirement by increasing the diet level of IAA.

Table 1. Results noted when the level of each essential amino acid in diet 2 was reduced to the level in diet 1

Diet	Relative gain	Relative gain/feed ratio
Diet 1	100	100
Complete Diet 2 (with leucine, iso, valine added)	98	100
Complete Diet 2 . . . low arginine	86.4	92.3
Complete Diet 2 . . . low histidine	94	99
Complete Diet 2 . . . low lysine	99	102
Complete Diet 2 . . . low leucine	91	100
Complete Diet 2 . . . low isoleucine	93.5	98
Complete Diet 2 . . . low valine	83.6	96
Complete Diet 2 . . . low phenylalanine and tyrosine	100	101
Complete Diet 2 . . . low methionine and cystine	90	95
Complete Diet 2 . . . low tryptophan	87	95
Complete Diet 2 . . . low threonine	92	98

Objectives

Since isoleucine, leucine and valine are structurally similar, and since there are several published reports indicating that there is an interaction among the three, it was felt that it would be worthwhile to conduct additional tests to study this three-way interaction. The situation here is more complicated than with an interaction such as the arginine-lysine interaction. Some of the possible interactions have not been clearly demonstrated.

An attempt was made to learn more about the mechanism involved in producing the depression to growth rate noted when the imbalanced diets are fed. The effect of diet level of these amino acids on the branched chain amino acid transaminase (BAT) activity was determined. Birds were selected for fast or slow growth when fed an imbalanced diet to determine if this ability to grow at a rapid rate on an imbalanced diet is inherited.

It should be pointed out that the amino acid additions used to produce the imbalances are not as great as those used by most who have conducted studies of this type. The imbalances found in the diets fed here could arise by using feedstuffs commonly used in poultry diets.

REVIEW OF LITERATURE

General Aspects of Imbalance

The literature on the effects of feeding diets with an imbalance of amino acids is voluminous. This is not too surprising since almost any test involving the feeding of different levels of amino acids might be termed a study of amino acid balance. No attempt will be made here to review all of the published reports on this subject, but reports bearing on the mechanism involved in producing the decreased growth rate will be covered. More detailed reviews will be made on arginine-lysine and isoleucine-leucine-valine interactions. Harper et al. (1970) have published a rather lengthy review on dietary amino acid imbalances.

Reduction in food intake is one of the first changes noted when an imbalanced diet is fed. Dr. W. C. Rose (1938), a pioneer in this field, noted that there was a loss of appetite in rats when a threonine deficient diet was fed and a growth depression was a subsequent result. When threonine was added to the diet food intake was increased. He then stated "growing animals lose their desire to eat when food is not suitable for tissue synthesis, but regain it when all of the components required for anabolism are made available."

Together with the reduced appetite, reports by several groups (Almquist, 1954; Hill and Olson, 1963, 1963a; Harper and Rogers, 1965; Ellison

and king, 1968; and Peng and Harper, 1970) demonstrate that the plasma level of the first limiting amino acid drops drastically as the result of feeding an imbalanced diet. Dr. Harper and his coworkers have suggested that when the plasma level drops to a critical level the appetite control mechanism is stimulated to suppress food consumption to prevent further food consumption that might be hazardous to the animal. They suggested that when an imbalanced diet is fed, tissue protein synthesis proceeds until the plasma level of the most limiting amino acid is reduced to a low level.

Amino acid imbalance is not just a simple deficiency of an amino acid. Deshpande et al. (1958 and 1958a), and Kumta and Harper (1960) reported that the addition of small amounts of phenylalanine and methionine to a diet containing 6% fibrin reduces growth rate of rats. These two amino acids were calculated to be the most limiting amino acids in this diet. Rats fed the diet supplemented with the two amino acids showed loss of appetite, decreased nitrogen retention, increased liver fat, and growth reduction. The growth depression was overcome by adding a mixture of isoleucine, leucine, valine and histidine. Mixtures containing only three of these four amino acids did not overcome the depression.

Elvehjem (1956) has classified the amino acid additions that decrease growth rates in three groups or types as follows: (1) imbalances, the type noted where the addition of a relatively small quantity of one or a few amino acids causes the deficiency of the most limiting amino acid to be more evident; (2) antagonisms, the type where the addition of one amino acid increases the

requirement for another usually structurally related amino acid; (3) toxicities, the type produced by the addition of a relatively large quantity of one or a few amino acids. Harper, who was a student of Elvehjen and has done extensive research in this area, has continued to use this classification (Harper et al., 1970). Several have criticized it because they felt that many imbalances cannot clearly be placed in one of three types.

Sauberlich (1961) conducted tests on imbalances of the type that could be termed toxicities in the above classification. Weanling rats were fed diets with 5% added individual amino acids. These additions decreased growth to varying degrees and resulted in increased plasma level of the added amino acid. Methionine addition resulted in the most severe growth depression. With the exception of aspartic acid, the D-isomer was less toxic than the L-isomer. He noted that the depression produced by the 5% addition was related to the dietary need; those required by the rat in the highest quantity produced the least depression.

More recently, Lewis (1965), and Smith and Lewis (1966) proposed a concept of "target" and "agent" amino acids. The "agent" is an amino acid causing a growth depression when added at excessive levels, and the "target" is the amino acid which overcomes the growth depression. They look upon amino imbalances as being the result of interactions between pairs of amino acids.

Beyer et al. (1947), and Kamin and Handler (1951) have studied the effect of excessive levels of one amino acid on intestinal absorption and renal

reabsorption of other amino acids. Absorption and reabsorption rates of several amino acids were decreased when excesses of certain amino acids were present. Later it was proposed by Sauberlich (1956), Salmon (1958), and Florentino and Pearson (1962) that this might result in the wasting of an amino acid that was limiting in a diet and cause a more severe deficiency of that amino acid. This helps to explain why certain imbalances are detrimental. However, the excesses that were found to decrease absorption and reabsorption of others are not always those that have the greatest effect on depression of growth rate, and this factor is generally considered to be relatively unimportant here.

Leung et al. (1964, 1968, and 1969), in agreement with Sanahuja and Harper (1963), reported that rats chose a protein free diet over a diet with an amino acid imbalance when offered a choice. Addition of the limiting amino acid to the imbalanced diet produced a diet which was accepted. They found that force-feeding of the imbalanced diet overcame the growth depression as did the daily injection of cortisol. Rats injected with cortisol preferred the imbalanced diet over the protein free diet and the plasma level of the limiting amino acid was increased.

Morrison and Caldwell (1966) and Bowering and Morrison (1967) have reported other tests along this line. They found that rats fed an imbalanced protein twice daily and the remainder of the diet ad libitum did not show as much reduction in weight gain and feed consumption as those offered a mixture

of the two. The separate feeding seemed to result in the ingestion of larger quantities of the imbalanced protein.

Ambient temperature influences utilization of amino acids. Klain et al. (1962) and Kalin and Winder (1964) demonstrated that rats exposed to a cold environment readily consumed an imbalanced diet and there was less effect on growth than with rats at room temperature. Anderson et al. (1967 and 1969) found that the blood level of the most limiting amino acid was low in rats exposed to the cold environment, but the level of the other amino acids was higher. The elevated amino acid metabolism of the cold exposed rats, as measured by increased serine-threonine dehydratase activity, appears to be the factor that permits them to consume the imbalanced diet without changes in the blood amino acid pattern sufficient to induce a reduction in food intake. Similarly, pretreatment also affected the performance of the animals. Rats previously fed a low protein diet were more sensitive to the imbalanced diet than those fed a high protein diet. The conclusion is that changes in plasma concentration of amino acids, which exert an influence on the food intake regulatory mechanism, depend upon the amount and pattern of the amino acids ingested, and, the ability of the animal to degrade amino acids or utilize them in protein synthesis.

Whether or not the utilization of the first limiting amino acid is impaired is left in question. The demonstrations by Netke et al. (1969), Benevenga et al. (1968), Soliman and King (1969), Florentino and Pearson (1962), Wilson et al. (1962), and Yoshida et al. (1966) showed that the utilization

of the first-limiting amino acid is not impaired by an imbalance. In fact it enhanced incorporation into tissue protein. On the contrary, in many studies on the interactions among amino acids it was reported that an imbalance caused a reduction in utilization of the first limiting amino acid. Sugahara et al. (1968) conducted research to study the two points of view. They stated that both cases can be found since the response in animals is varied according to research condition and dietary type. They stated that "the pattern of amino acids in excess of that needed for protein synthesis dictates whether the effect of an amino acid imbalance will be superimposed on that of an amino acid deficiency." This could explain why diets equally deficient in a single amino acid do not necessarily result in equal gain.

Arginine-Lysine Interaction

The arginine-lysine interaction has been studied in greatest detail. Protein in the synthetic diets frequently used for nutritional research is provided by casein. It is a good source of all IAA except arginine and the sulfur amino acids, and it is an excellent source of lysine. The lysine/arginine ratio is the reverse of that found in cereal grains and many other foodstuffs which make up the large portion of poultry feed. These are universally deficient in lysine, while arginine is much less limiting (Almquist, 1972; Ewing, 1963; Schaible, 1970).

Extensive research has been reported on the mechanism involved in producing the high arginine requirement of chicks fed a diet based on casein.

It was known for some time that chick's arginine requirement is higher when fed a diet with casein as a source of protein than with a commercial-type diet (Weitlake et al., 1954; Snyder et al., 1956). Krautmann et al. (1958) suggested that the plant proteins used in commercial-type diets contain an unidentified factor that reduces the arginine requirement. There were few attempts to follow up this postulate because there were evidences of other factors being responsible for the phenomenon. O'Dell et al. (1958) sought to determine if it was because the arginine of casein was unavailable, but found no evidence to support this hypothesis.

Anderson and Dobson (1959) stated that amino acid balance in casein is responsible for the high arginine requirement. Growth rate produced by diets based on casein and those produced by diets based on plant protein were equal if the levels of the IAA were comparable. They further observed that protein level per se had little effect on arginine requirement, but arginine requirement increased as the total IAA level increased. When part of the casein in a casein-based diet was replaced with a mixture of dispensable amino acids, the high requirement for arginine was reduced. The procedure lowered the total IAA level while maintaining the protein level. The lower arginine requirement in plant protein-based diets was increased as the total IAA level was increased by adding IAA to the ration. Klain et al. (1959), and Fisher et al. (1960) also noted a high arginine requirement when an amino acid mixture simulating the composition of a casein diet was used. The experiments give no reason to conclude that natural ingredients contain an unidentified factor that

spares arginine. The chick's lysine requirement was found to increase as the dietary IAA level increased in the same manner, but to a much lesser extent.

According to Anderson and Dobson's (1959) observation, when a single amino acid level was increased from the plant protein diet level to the casein diet level, lysine increase resulted in the greatest growth depression. Jones (1964) observed that supplementing a casein-gelatin diet with excess lysine resulted in an increase in plasma lysine levels, but arginine levels were decreased. The symptoms were eliminated by supplementing the diet with arginine. Chicks receiving a soy protein ration (not limiting in arginine) were less affected by excess lysine although blood and tissue lysine levels were increased. They suggested that lysine reduced availability of arginine from casein or gelatin or from a combination of them. Dean and Scott (1966 and 1968) also observed that excess lysine decreased gains more in an arginine limiting diet than the diets limiting in other amino acids. They found that arginine was less effective when it was used to supplement a crystalline amino acid diet high in lysine. Whereas weight gain on an imbalance diet was improved with each increment of supplemental arginine up to the 0.73% level, the arginine efficacy progressively declined.

Allen et al. (1972) declared that the arginine efficacy decline in a linear regression with excess lysine. The growth was only 58.8% of normal when 1.84% excess lysine was presented. Further lysine addition did not induce a more negative response. The results were similar to those of D'Mello

and Lewis (1970). It was clear that arginine requirement was higher with a higher lysine level.

More information on the high arginine requirement came from the studies of its metabolism. O'Dell et al. (1958) showed a considerably greater excretion of urea by chicks fed free arginine. They suggested that high requirement was due in part to the cleavage of arginine by kidney arginase. Subsequent papers of the same authors (O'Dell et al., 1965 and 1966) demonstrated that chick's arginine requirement and kidney arginase activity were linearly correlated. The observation is supported by Shao and Hill (1969), Leach et al. (1970), Austic and Nesheim (1970), and Stutz et al. (1972).

To confirm the postulate that the alteration of arginase activity is a major factor in variations in arginine requirement of chicks, two strains of chickens have been developed by genetic selection. The two strains differ markedly in their arginine requirement (Hutt and Nesheim, 1966 and 1968; Nesheim et al., 1967). The high-arginine requirement chicks had higher arginase activity and tolerated excess lysine less well. The arginase activity, after feeding excess lysine, was increased more in the high requirement chicks than in the low-requirement type (Nesheim, 1968; Hutt and Nesheim, 1968; Austic and Nesheim, 1970). Accordingly the induction of kidney arginase by excess lysine has been implied to be one of the mechanisms of arginine-lysine antagonism.

The arginine-lysine interaction was proposed to be a specific antagonism (Jones, 1964). Reports by Huston and Scott (1968), and D'Mello and Lewis

(1970) support the suggestion. The latter group fed diets that were first limiting in an amino acid other than arginine and second limiting in arginine. It was arginine and not the amino acids calculated to be first limiting that had the greatest effect in overcoming the growth depression caused by lysine addition. Supplementing with both arginine and the first limiting amino acid exerted more effect. They concluded that in chick nutrition lysine and arginine are involved in a unique interaction.

Despite the postulation of specificity of lysine-arginine antagonisms, Boorman and Fisher (1966) lend support to the view expressed by Snetsinger and Scott (1961), that arginine is a non-specific detoxifying amino acid. Arginine addition to the diet can alleviate the growth depression induced by excess lysine or several other amino acids as well. The role of arginine in detoxifying the excesses of amino acids was first studied in rats (Gullino et al., 1956; and Winitz et al., 1956). It was postulated that arginine was needed to accelerate the metabolization of the excess nitrogen by way of the urea cycle. The arginine was termed to have "protective effect" against ammonia toxicity resulting from the ingestion of excess amino acids. But, when research with chicks was conducted, no protective effect of arginine was observed (Snetsinger and Scott, 1961). The difference noted in the two species may be due to the fact that chicks lack the ability to synthesize arginine.

Excess lysine was demonstrated to impair arginine utilization and efficacy, but the studies on the influence of excess arginine on lysine requirement are limited. Using sesame or zein as protein sources in the diet,

Fisher et al. (1960) determined the lysine utilization and efficiency in promoting growth when an imbalance was induced. A decrease in gain was observed when a low-lysine or lysine-free essential amino acid mixture was added to the experimental diets which were already deficient in lysine. Growth depression and reduced feed consumption were observed, but there was little evidence that the excess arginine impaired lysine utilization. Considering gains per gram of lysine consumed, excess arginine provided in an amino acid mixture resulted in less depression of lysine efficacy than excess lysine on arginine efficacy as demonstrated by Allen and Baker (1972), and D'Mello and Lewis (1970). Also Fisher et al. (1960) reported that the depression caused by excess arginine in a lysine was greater in a high protein diet than with a low protein diet.

O'Dell and Savage (1966) conducted research to study the effect of excess arginine on lysine utilization. Excess arginine was added to a sesame meal diet deficient in lysine. The effect of potassium acetate was observed too. Potassium acetate had been shown to stimulate growth of chicks fed an arginine deficient diet imbalanced by excess lysine. Excess arginine in the presence of added potassium acetate depressed growth with a low lysine diet but not when lysine was adequate. They suggested that there exists a metabolic antagonism between arginine and lysine.

The effects of excess lysine and arginine on the degradation of lysine was studied later by Wang et al. (1973). It was demonstrated that the activity of the lysine degradative enzyme, the lysine-ketoglutarate reductase in liver,

was affected by dietary lysine level but not arginine. Kidney arginase activity was affected by both excess lysine and arginine.

The two strains of chickens that differed in arginine requirement were studied. When fed the basal diet, the high arginine requirement strain had a higher level of the kidney arginase but a lower liver lysine-ketoglutarate reductase than the low requirement strains. Excess lysine induced an increase of lysine-ketoglutarate reductase in the low-arginine requirement strain but had less effect in the high requirement strain. Arginase activity in the high requirement strain was greatly increased by excess lysine, but much smaller changes were observed in the low requirement strain. The authors proposed that the rate of lysine oxidation in vivo may be regulated by the lysine pool at low levels of dietary lysine, and by the level of degradative enzyme at high levels of dietary lysine.

Lysine utilization was shown to differ in different strains (Enos and Moreng, 1965). Griminger and Fisher (1962) attempted to develop strains with a high or a low lysine requirement, but they did not find as much difference in requirement as with the strains selected for high or low arginine requirement.

Leucine-Isoleucine-Valine Interaction

The tests being reported in this thesis were conducted to study the interaction among the branched-chain amino acids. Several reports of work with rats appear in the literature. Harper et al. (1954), reported that the

addition of leucine to a diet containing 9% casein, 0.1% DL-tryptophan, and 0.3% DL-methionine, depressed growth rate. Isoleucine addition overcame the depression. Later papers (Harper et al., 1965; and Benton et al., 1956) reported that the addition of tryptophan, valine, or threonine to the diet with added leucine had no effect in correcting the imbalance. A combination of valine and isoleucine was required to completely overcome the depression caused by the addition of 3% leucine. Isoleucine-valine, phenylalanine-isoleucine, and phenylalanine-valine antagonisms were demonstrated.

Spolter and Harper (1961) reported that the addition of 5% leucine to a diet based on casein caused a severe growth retardation. A similar retardation was noted with a diet based on an amino acids mixture, indicating that the action is not by interfering with some phase of digestion. The addition of only 0.16% isoleucine and 0.15% valine overcame the retarded growth as much as any higher levels. The injection of insulin overcame the appetite depressing effect of the excess leucine in some rats.

Tannous et al. (1966) reported that the addition of leucine to a diet based on 9% casein caused a marked reduction in the isoleucine and valine levels in the plasma. Blood plasma levels of leucine were markedly increased. The amino acid pattern in muscle was similar to that in plasma. Liver levels, however, resembled those of the intestinal contents. In a later publication (Rogers et al., 1967), an attempt was made to calculate how much the addition of 5% leucine to a 9% casein diet affected the body pool levels of five amino acids. Their calculations were based on analyses of tissues comprising about

50% of the body weight. They calculated that the leucine level in the body pool when 5% leucine was added to the diet, was 161% of that with the basal diet. The isoleucine, valine, lysine and histidine levels were 80%, 64%, 44%, and 73%, respectively. Thus the effect on the isoleucine and valine levels were not unique.

Harper et al. (1970) summarized work conducted to study the effect of dietary level of these three amino acids on certain enzymes involved in the metabolism of the acids. They concluded that the level of the branched-chain amino acid transaminase (BAT) was not altered to any great degree by changing the dietary level of these three amino acids. They did note that the activity of the branched-chain keto-acid dehydrogenases were increased by feeding any of the three amino acids.

Studies also have been conducted with chicks on the interaction among these three amino acids. Mathieu and Scott (1968) found that the addition of leucine to their reference standard amino acid mixture did not depress growth. But when leucine was added to a diet with slightly lower levels of isoleucine, valine or arginine growth was depressed. D'Mello and Lewis (1970) demonstrated what they felt were rather specific interactions between isoleucine and leucine and valine and leucine. The chick's isoleucine requirement was found to be 0.58% when the diet contained 1.4% leucine, 0.62% when the diet contained 2.15% leucine, and 0.65% when the diet contained 2.9% leucine. The valine requirement was found to be 0.77% when the diet contained 1.4% leucine, 0.89%

when the diet contained 2.4% leucine, and 1.01% when the diet contained 3.4% leucine.

Allen and Baker (1972) have determined what they termed the "efficacy" of dietary isoleucine or valine in diets with leucine levels graded from 0 to 6% above that required for normal growth. They found that the valine efficacy was reduced to 75% at the level of 4.5% added leucine. The isoleucine efficacy was reduced about 6.5% for each increase of 1% leucine in the diet up to a level of 3% added leucine.

A practical aspect of this interrelationship is reported by Bray (1970). He found that diets containing high levels of corn required more isoleucine and valine in order to allow maximum egg production in pullets.

The present studies reported in this thesis were conducted to more clearly demonstrate all of the interactions among the three branch-chain amino acids. The studies cited in the review concentrated on the increases in valine and isoleucine requirements when the leucine level of the diet was increased. This is because excess leucine is the most common type of imbalance noted with the feedstuffs used in formulating rations. Corn and sorghum proteins are very rich sources of leucine, and it is difficult to find a protein source that is deficient in leucine. Some attempt was made in the work being reported to learn more about the mechanism involved in these interactions.

MATERIALS AND METHODS

Animals and Diets

Table 2 gives the composition of the four semi-purified basal diets. The calculated amino acids levels in these diets are presented in Table 3. All basal diets contain 126.5 grams casein to provide most of the amino acids. This amount of casein provided leucine at the level of 85% of Dobson's balance level. Additions of sodium glutamate, potassium glutamate, asparagine and glutamic acid were varied to provide approximately equal levels of sodium, potassium, and nitrogen in all rations. The nitrogen level is equivalent to that of an 18% protein diet.

Experiment 1. Diet 1 was fed along with eight modifications of diet 2. Diet 1 had the IAA at 85% of Dobson's balanced level. Diet 2 had seven indispensable amino acids at the level of 125% of the balanced levels. The test with diet 2 involved increasing leucine, isoleucine and valine from the 85% to the 125% levels in difference combinations.

Experiment 2. Diets 3 and 4 were used. Except for the increasing of isoleucine and methionine-cystine levels, diets 3 and 4 contained the same levels of IAA as diet 1 and diet 2, respectively. The feeding tests conducted were similar to those with diets 1 and 2.

Chicks were placed in electrically heated batteries with a wiremesh floor immediately after removal from the incubator. They were fed a practical

Table 2. Composition of the basic diets fed in the tests

Ingredient	Diet			
	1	2	3	4
	gm/kg			
Casein	126.5	126.5	126.5	126.5
Constant ingredients	498.45 ^a			
Arginine HCl	7.7	13.88	7.7	13.9
Histidine HCl·H ₂ O	.3	2.65	.3	2.62
Lysine HCl	.55	6.3	.54	6.27
Leucine	0	0 (5.25) ^b	0	0 (5.18) ^b
Isoleucine	.46	.46 (3.65) ^b	.72	.72 (4.04) ^b
Valine	.12	.12 (3.95) ^b	.12	.12 (3.92) ^b
Phenylalanine	.54	3.4	.53	3.05
Tyrosine		2.25		2.5
Tryptophan	.09	.9	.09	.89
Methionine	.38	2.0	.25	1.7
Cystine	2.28	3.55	2.58	4.12
Threonine	1.69	4.8	1.68	4.8
Glycine	10	10	10	10
Sodium glutamate	8	17	8	16.1
Potassium glutamate	5		5	
Asparagine	5		5	
Glutamic acid	52.5		59.1	
Other minerals	5.8	3.3	5.8	3.3
Glucose monohydrate to	1000			

^aThis included starch, 300; vitamin mix, 100; oil, 40; Alphacel, 20; trace mineral mix, 0.75; magnesium sulfate, 3.5; dibasic potassium phosphate, 7; sodium chloride, 1; Tricalcium phosphate, 24.4; calcium carbonate, 1.8 grams per kilogram of diet. The composition of the vitamin and trace mineral mixtures is given by Dobson *et al.* (1964).

^bDiets 2 and 4 contained the high level of all IAA except isoleucine, leucine and valine. The feeding tests involved increasing the levels of these three amino acids to the high level. The value in parentheses indicates how much of the given amino acid was added to increase the amino acid to the high level. When a diet contained a low level of one of these amino acids, the nitrogen level in the diet was maintained by the addition of glutamic acid.

Table 3. Calculated amino acid levels in the basal diets

Amino Acid	Dobson's Balanced Level (%)	Level in Diet	
		1	2
Arginine	1.28	1.088	1.60
Histidine	0.43	0.366	0.54
Lysine	1.15	0.979	1.439
Leucine	1.30	1.107	1.632
Isoleucine	0.80 (0.83) ^a	0.69 (0.706) ^a	1.00 (1.04) ^a
Valine	0.95	0.808	1.19
Phenylalanine and tyrosine	1.33	1.161	1.67
Tryptophan	0.20	0.17	0.25
Methionine and Cystine	0.73 (0.75) ^a	0.35 0.27 (0.638) ^a	0.512 0.398 (0.938) ^a
Threonine	0.78	0.663	0.974

^aValues in parentheses for isoleucine and the sum of methionine and cysteine are the levels used in formulation diets 3 and 4. The other essential amino acids were found in diets 3 and 4 at the levels in diets 1 and 2, respectively.

type diet, ad libitum, for 4 days. Then they were distributed to experimental groups in a way that the groups were of comparable weight and sex. The experimental diets were fed ad libitum to duplicate groups of five chicks each. Water was provided ad libitum. After 2 weeks of age, chicks were killed for branched chain amino acid transaminase (BAT) assay by using chloroform. The carcasses were stored in a deep freezer until the assays were conducted.

Unless otherwise indicated the chicks used in the feeding tests were produced from a cross between White Mountain males and Hubbard females. This cross is used extensively in commercial broiler production. In some places in this thesis, chicks of this strain are referred to as strain C. A few tests were conducted with chicks from two other matings. Strain A chicks were

hatched from eggs produced by birds that had grown relatively fast as chicks when fed the diet low in valine and high in the other nine IAA. Strain B chicks were from birds that grew relatively slowly when fed the same diet.

Analytical Procedure

Samples of liver and kidney were homogenized in 9 and 19 volumes, respectively, of 0.05 M potassium phosphate buffer pH 7.0. The homogenates were then centrifuged at 34,000 g for 20 minutes in a cold room. Clear supernatant fluid was used as the enzyme source. The enzyme activities in the crude enzyme were determined by measuring the amount of the hydrazone of alpha-ketioisocaproate produced as described by Taylor and Jenkin (1966). Enzyme activity is expressed as micromole of alpha-ketioisocaproate formed in 10 minutes at 37 C per milligram of protein. The soluble protein content of the enzyme preparation was determined by the modified biuret procedure followed Gornall et al. (1949). Bovine serum (fraction V) was used as a standard. Chemicals used in analytical determination were reagent grade and were purchased from commercial sources.

RESULTS

Summaries of the results of three feeding tests (a total of four replications) conducted with diets 1 and 2 in 1972 and three similar tests (four replications) conducted in 1974 are given in Table 4. The results of the two series of tests differ to some extent. The average gain with diet 2 was relatively low in 1974. This lower average is primarily the result of poor performance with this diet in only one of the three tests. The isoleucine level in diet 2 appeared to be more critical in the 1974 tests. In fact, isoleucine addition to these diets made more difference in performance than did valine addition. Different lots of casein were used in the two series of tests. It is possible that the casein used in 1974 contained a slightly lower level of isoleucine.

In the statistical analysis made on these data, the treatment sums of squares was partitioned into components corresponding to individual degrees of freedom by using two different sets of orthogonal coefficients (Snedecor and Cochran, 1973). In both sets 1 degree of freedom compared the performance of chicks fed diet 1 with the overall performance of chicks fed the eight modifications of diet 2. The one set of coefficients partitioned the remaining sum of squares in the usual manner for a 2^3 factorial test; that is, 1 d. f. for each of the three main effects, 1 for each of the three two-way interactions, and 1 for the three-way interaction. The differences associated with only 2 d. f. were of interest in the analyses with the second set of orthogonal coefficients. One compared the performance of chicks fed diet 2 with none or all of

Table 4. Summary of chick performances noted when valine, isoleucine and leucine were added to basal diet 2

Amino acid increased in Diet 2 ^a	1972 Series		1974 Series Rel. gain
	Rel. gain	Rel. gain/feed	
None	99	102	95
Valine	99	102	96
Isoleucine	100	101	102
Leucine	90	95	92
Valine and isoleucine	94	101	99
Valine and leucine	96	96	90
Isoleucine and leucine	93	98	96
All three	100	100	101

Ration 1 (low IAA diet)	100	100	105

^aThis increased the level of the name amino acid(s) from 85% to 125% of Dobson's (1964) balanced level.

Table 5. Effect of adding combinations of valine, isoleucine and leucine to diet 4

Amino acid increased in Diet 4	Relative gain	Relative gain/feed
None	101	105
Valine	98	103
Isoleucine	95	98
Leucine	89	98
Valine and isoleucine	96	107
Valine and leucine	98	105
Isoleucine and leucine	91	103
All three	99	103

Ration 3 (low IAA diet)	100	100

the three amino acids involved at the high level (balanced diets) with the performance of those fed diet 2 with only one or two of these amino acids at the high level (imbalanced diets). The other compared performance of groups with one of the three amino acids increased to that with two increased. Table 6 presents the results of the analyses.

The weight gains of chicks fed diet 1 were significantly greater than the overall gains made with the eight modifications of diet 2 ($P < 0.01$). Since the gains made with diet 2 and those made when all three amino acids were increased to the high level were not greatly different than those made with diet 1, the imbalance produced by adding one or two amino acids were detrimental. The only statistically significant main effects were those produced by the addition of isoleucine in the 1974 series. Leucine addition decreased gains in both the 1972 and 1974 series. While no single difference was statistically significant, it does suggest that the leucine level in Dobson's balanced diet was relatively high.

The increase in gain noted with isoleucine addition in the 1974 series with diets 1 and 2 indicated that the isoleucine level was relatively low. This is why the isoleucine level in the set of balanced levels was increased from 0.8 to 0.83% for a second series of tests conducted in 1974. The results of these tests are summarized in Table 5.

The analysis of variance on these results were similar to those on the data in Table 4. The gain with diet 3 was higher than the overall gains made by chicks fed the eight modifications of diet 4, but this was not a

Table 6. Results noted when the treatment sum of squares for gain was partitioned to components corresponding to individual degrees of freedom by the ANOVA^a

Ind. d. f.	1972 Series Diets 1 and 2 ^b		1974 Series Diets 1 and 2 ^b		1974 Series Diets 3 and 4 ^c	
	M. S.	F. Val.	M. S.	F Val.	M. S.	F. Val.
Low IAA vs. High	40.5	2.0	1645	6.4*	567	2.33
Isol.	6.1		2090	8.14**	165	
Leucine	78.1	3.87	662	2.58	776	3.19
Valine	28.1		2		1036	4.26*
I x L	50	2.48	38		487	2.0
I x V	12.5		26		0	
L x V	180.5	8.94**	67		1989	8.18**
I x L x V	36.1	1.79	47		150	

0 & 3 addn. vs. 1 & 2	96	4.75*	196		1815	7.46**
1 addn. vs. 2	30		4		60	

Error	20.2 (21 d. f.)		257 (24 d. f.)		243 (40 d. f.)	

^aThe analyses of variance were done on the actual gains in the two 1974 series and on the relative gains in the 1972 series.

*P < 0.05.

**P < 0.01.

^bThe experiments were conducted with four replications.

^cThe experiments were conducted with six replications.

statistically significant difference. The gains with diet 4 and diet 4 with all three amino acids increased from the low to the high level were greater than those obtained with only one or two increased. Valine addition produced a significant increase in gain in these tests ($P < 0.05$), and leucine addition produced a decrease in gain that approached statistical significance. The valine-leucine interaction was the only statistically significant interaction ($P < 0.01$).

The gain/feed ratios were determined for about half of the groups of chicks in these tests. Weight gains and gain/feed ratios were positively correlated. The gain/feed ratios and the gains can be used to calculate feed consumption. When this was done it was found that chicks fed the imbalanced diets consumed less feed. This is similar to results reported by many others as noted in the review of literature.

Table 7 is a summary of the results of feeding tests with chicks of strains A and B. Only a limited number of chicks from the two strains were available for these tests. The number was very limited with strain B because of poor hatchability. Only three tests were conducted with chicks of strain B, while six were conducted with strain A.

Chicks of strain B gained less than those of strain A regardless of the diet fed. The difference was greatest when the low valine diet was fed, but rather large differences were noted with the low leucine and isoleucine diets. There appears to be a strain-ration interaction here with a genetic basis.

Table 7. Results of feeding diets low in valine, isoleucine or leucine to chicks of strains A and B

Diet	Relative Weight		
	Str. A ^b	Str. B ^b	Str. A ^a
2 with all IAA at high levels	102	98	101
2 only valine at low level	91	70	87
2 only isoleucine at low level	96	84	92
2 only leucine at low level	101	82	97
1 all IAA at low level	104	96	99

^aThis column is a summary of the results of six tests with strain A chicks. Only three tests were conducted with strain B chicks, and these are summarized in the first two columns. The average weight of chicks fed diet 1 and diet 2 with all IAA at the high level was given a value of 100.

^bStrain A are birds selected to grow fast with low valine diet. Strain B are birds selected to grow slow with low valine diet.

Table 8 gives a summary of the BAT activities found in chicks fed diet 1 and the eight modifications of diet 2. These determinations were made on chicks of strains A and C. Strain A chicks are from birds that grew relatively fast as chicks when fed the low valine diet, and strain C is a commercial broiler chick unselected for this factor. The BAT activity in the kidneys was higher than in the liver. This agrees with the reports of Featherston and Horn (1973), and Shiflet and Haskell (1969).

The BAT activity was greater in chicks of strain C than strain A. Thus, there appears to be an inverse relationship between the growth rate of

Table 8. Summary of kidney and liver branched chain amino acid transaminase activity^a found in chicks fed diets 1 and 2

Amino acid increased in Diet 2	Kidney		Liver	
	Strain A ^b	Strain C ^b	Strain A	Strain C
None	5.8	8.5	3.1	2.5
Valine	7.7	9.2	2.2	2.5
Isoleucine	6.1	6.8	1.6	2.5
Leucine	4.5	9.0	1.4	2.0
Valine and isoleucine	8.3	8.3	1.7	3.0
Valine and leucine	5.6	8.7	1.9	2.6
Isoleucine and leucine	6.8	8.8	2.1	2.9
All three	6.1	9.8	2.2	2.9

Diet 1	4.0	8.0	1.4	2.7

^aActivity is expressed as micromole of α -keto-isocaproate/10 min/mg protein.

^bStrain A are birds selected to grow fast with low valine diets. Strain C are non-selected birds.

the chicks and BAT activity. The differences in BAT activities found in chicks fed the different diets were inconsistent, and there is no obvious relationship between gain and BAT activity among birds fed the different modifications of diet 2. In general, the activity was higher among the birds fed diet 2 than among those fed diet 1.

DISCUSSION

Overall of the tests summarized in Tables 4 and 5, it was noted that increasing the levels of only one or two of the amino acids in diet 2 or 4 decreased growth rate. But, when all three were increased, the gains were comparable to the gains made by chicks fed diets 1 or 3. Thus it is felt that an amino acid imbalance among three branched chain IAA and not a deficiency is the cause of the lower gains. Adding one of them seemed to disturb the equilibrium among the three.

The results of the preliminary tests and results found in several reports found in the literature provided some indication as to what was to be expected in these tests. If there is a three-way interaction among the amino acids leucine, isoleucine and valine, increasing the dietary level of only one or two of these should decrease growth rate. It seems reasonable to expect that if the levels of the three in the basic diet are well balanced, about the same decrease in growth rate would be noted when any one of the three is increased from 85 to 125% of the basic diet level. The addition of any two of the three should decrease gains; some combination of two might decrease the gain more than either one alone. However, when all three are increased, rate of gain should be at least as high as with the low IAA diet or the diet with none of the three at the high level.

Under the above assumptions the statistical analysis carried out on these results should have partitioned the treatment (diet) sum of squares into components corresponding to individual degrees of freedom in a certain pattern. That is, in the first partitioning, the components corresponding to the three main effects should be rather low. Most of the treatment sum of squares should go into the three two-way interactions. In the other partitioning, the component based on the comparison of performance with one or two additions vs. none or three additions should be high.

In these tests there were some significant main effects. For example, in the 1974 series with diet 2, isoleucine addition resulted in a high significant increase in gain. This is why the basic isoleucine level was increased in making diets 3 and 4. In all three series the addition of leucine decreased gain. While this component was not statistically significant at the 5% level in any one series, it was consistently near this level. This indicates that the leucine level is relatively high in Dobson's (1964) balanced diet.

In the 1972 series with diet 2 and the 1974 series with diet 4, the components of the treatment sum of squares corresponding to the two-way interactions were large. So was the component from the comparison of none and three additions with one and two additions. The component resulting from the comparison between one and two additions was very small. These differences indicate that there is a three-way interaction among the three amino acids. The leucine-valine interaction was the most significant of the three

interactions. This suggests that the need for one of these two is affected more by the dietary level of the other than by the isoleucine level.

An attempt was made to elucidate the mechanism of the interaction by observing changes in BAT activity as the dietary levels of isoleucine, leucine and valine were changed. The influence of the dietary level of these amino acids is not clear because of the inconsistency in the results. There may have been a slightly higher activity among chicks fed the diets with the high levels of these three amino acids, but there was not a change comparable to the increase of arginase activity reported by the Cornell workers. The increase in arginase activity is believed to be responsible for the increase in arginine requirement when a high lysine diet is fed (Austic and Nesheim, 1970; and Austic and Nesheim, 1971). A change in BAT activity appears not to be responsible for the interactions noted in these tests.

The results with chicks of strains A and B indicate that low and high valine requirement strains might be developed. Whether the birds selected for a high valine requirement also had high isoleucine and leucine requirements was not completely clear. More information on the relationships among the requirements may help us understand the mechanism of this interaction.

SUMMARY

Chick feeding tests were conducted to study the effects of dietary imbalances among the three branched chain amino acids on growth rate. All diets fed in these tests contained about 18% protein. The indispensable amino acids (IAA) were found in two of the diets fed in the proportions found by Dobson et al. (1964) to be well balanced. One of the diets had all IAA at 85% of these balanced levels while the other had them all at 125% of these levels. The tests involved reducing the isoleucine, leucine and valine levels in the high IAA diet to the low levels in all combinations.

Growth rates were similar with the diets containing all ten IAA at the low levels, all ten at the high levels, or seven at the high levels and isoleucine, leucine and valine at the low levels. Generally, when only one or two of these three were reduced to the low levels, growth rates were lower. Thus there appeared to be a three-way interaction among these amino acids with the reduction in weight gain being the result of an imbalance and not of a deficiency. The changes in growth rate noted when the leucine level was reduced indicated that the leucine level in Dobson's balanced diet was relatively high; the isoleucine level appeared to be relatively low. The interaction between leucine and valine appeared to be more significant than the other two two-way interactions.

The effect of dietary level of these amino acids on the branched chain amino transferase activity in the liver and kidneys was determined. The differences noted were inconsistent. There tended to be a slightly higher activity in chicks fed the high levels of these amino acids, but the differences certainly were not as great as the changes in arginase activity reported by others when imbalanced diets were fed.

Chicks fed the diet low in valine and high in the other nine IAA were selected for fast or slow growth on this diet. They were raised to maturity and produced eggs that were hatched for feeding tests with the diets containing different levels of the branched chain amino acids. Only a limited number of chicks from the two strains were hatched. Performance of the chicks was similar to that of their parents when fed the low valine diet.

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