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Chapter

# Broiler Amino Acid Research: Then and Now

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## Abstract

The interconnected nature of the amino acids in broiler nutrition pose an interesting quandary for poultry nutritionists. Two primary antagonisms exist in poultry, that among lysine and arginine and the branched-chain amino acids. Throughout the discovery and investigation into these amino acids, researchers have questioned the existence of these antagonisms as well as their impact on practical formulation. The lysine-arginine antagonism was the first discovered in poultry and was largely solved when protein formulation shifted from crude protein requirements to formulation based on amino acid levels. In contrast, while branched-chain amino acid antagonism was discovered over 50 years ago, increased refinement of dietary amino acid profiles has allowed for this antagonism to become a reemerging concern. These antagonisms and the interplay of amino acids on dietary requirements will continue to challenge researchers for years to come and innovative formulation strategies will need to be developed in order to optimize broiler diets and production.

**Keywords:** arginine, lysine, amino acid antagonism, branched-chain amino acids, leucine

## 1. Introduction

The first amino acid to be isolated is credited to L.N. Vauquelin and P.J. Robiquet, who discovered asparagine in 1806 [1]. Isolation of the amino acids continued throughout the nineteenth and early twentieth century, with the last significant discovery occurring in 1935 with the identification of threonine as both a new and essential amino acid [2, 3]. During the time of which threonine was discovered, W.C. Rose conducted a series of experiments identifying the amino acids required for growth. At the conclusion of these experiments, Rose reported that 10 amino acids were indispensable and must be included in diets in order to support growth [4]. The evolution of indispensable amino acids based on the experimentation of Rose is presented in **Table 1**.

Post World War II, interest in animal nutrition increased exponentially, reaching a peak in the 1970s, as more importance was placed on food production [7]. Early poultry amino acid nutrition research mirrored the amino acid studies of the time, with the determination of essential amino acids in poultry [8–12]. These original studies were conducted in commercial-type type diets, with the first reports of the use of

| | 1932 | 1938 | 1948 |
|---|---|---|---|
| Alanine | Unknown | Dispensable | Dispensable |
| Arginine | Dispensable | Indispensable | Indispensable |
| Aspartic acid | Dispensable | Dispensable | Dispensable |
| Citrulline | Not listed | Dispensable | Dispensable |
| Cystine | Indispensable | Dispensable | Dispensable |
| Glutamic acid | Dispensable | Dispensable | Dispensable |
| Glycine | Dispensable | Dispensable | Dispensable |
| Histidine | Indispensable | Indispensable | Indispensable |
| Hydroxyglutamic acid | Dispensable | Dispensable | Not listed[2] |
| Hydroxyproline | Dispensable | Dispensable | Dispensable |
| Isoleucine | Unknown | Indispensable | Indispensable |
| Leucine | Unknown | Indispensable | Indispensable |
| Lysine | Indispensable | Indispensable | Indispensable |
| Methionine | Unknown | Indispensable | Indispensable |
| Norleucine | Unknown | Dispensable | Not listed[2] |
| Phenylalanine | Unknown | Indispensable | Indispensable |
| Proline | Dispensable | Dispensable | Dispensable |
| Serine | Unknown | Dispensable | Dispensable |
| Threonine | Not listed[3] | Indispensable | Indispensable |
| Tryptophan | Indispensable | Indispensable | Indispensable |
| Tyrosine | Dispensable | Dispensable | Dispensable |
| Valine | Unknown | Indispensable | Indispensable |

[1]*Adapted from Rose [4, 5] and Rose et al. [6].*
[2]*Removed from list of dispensable amino acids.*
[3]*Prior to identification.*

**Table 1.**
*Status of amino acids as indispensable or dispensable as reported by W.C. Rose[1].*

purified-type diets, with crystalline amino acids serving as the only source of protein, being that of Hegsted in 1944 [13]. The use of purified-type diet laid the foundation for accurate determination of amino acid requirements, as they allowed for researchers to know the exact dietary amino acid contents.

In the early phase of purified diets, researchers attempted to create reference diets that could produce similar growth performance to chicks fed commercial-type diets. Extensive work was done at the University of Illinois to construct the aforementioned reference diet [14–18]. In 1965, Dean and Scott [19] published a report detailing a reexamination of the findings of their previous work at the University of Illinois. In this set of experiments, it was found that after refinements had been made to the amino acid levels in the reference diet, earlier determined amino acid requirements were over estimated and could be reduced without negatively affecting performance. These findings indicated the interplay among the amino acids when determining requirement estimates.

## 2. Identification of classic amino acid antagonisms in the chick

### 2.1 Lysine and Arginine

In early work concerning the arginine requirement for poultry, Almquist and Merritt [20] found that the arginine requirement increased at a constant rate as crude protein was increased, citing requirement values of 0.9, 1.2, and 1.8% of the diet for arginine at crude protein levels of 15, 20, and 25%, respectively. Taking this into account, Anderson and Dobson [21] noticed that the arginine requirement fluctuated in diets containing similar crude protein levels [22], and postulated that amino acid balance was the more important variable than crude protein in general at the conclusion of their own experimentation. Furthermore, Anderson and Dobson [21] indicated that a relationship between arginine and lysine might be expected due to similarities in their chemical structure and potentially their metabolism. Likewise, Fisher et al. [23] indicated that the amino acid content of casein, used in purified diets to determine amino acid requirements, likely increased the arginine requirement compared to diets containing soybean meal, which contains approximately a third the lysine of casein. This disparity between purified and commercial-type diets had been previously discovered by Krautmann et al. in 1957 [24], but Krautmann et al. [24] had failed to make the connection of amino acid content and instead proposed an "unidentified factor of plant origin" was to blame for the disparity in arginine requirements among diet types.

Due to the extreme variation of requirement estimates that had been published at the time, Lewis et al. [25] attempted to establish to an arginine requirement based on commercial-type diets used in the United Kingdom. Lewis et al. [25] not only investigated the effects of varying crude protein levels, but also examined the influence of an amino acid imbalance induced by excess lysine based on the previous work of Anderson and Dobson [21]. The results of these studies indicated that under practical conditions using commercial-type diets it was unlikely that an arginine deficiency would occur unless excess lysine was introduced into the diet [25]. Despite these findings, work continued using purified diets in order to understand the mechanisms behind the lysine-arginine antagonism.

Jones [26] studied the antagonism between lysine and arginine using diets containing casein and gelatin as the protein contributing ingredients. In this study, Jones [26] indicated that excess lysine depressed the utilization of arginine in purified diets containing these protein sources. Boorman and Fisher [27] then reported that the antagonism was not reciprocal, indicating that excess levels of arginine did not result in further growth depressions when lysine was deficient. Boorman and Fisher [27] went further to indicate that a lysine-arginine antagonism did not exist, but that the results of their experiment showed a response of a general amino acid toxicity.

A major step in the identification of a mechanism behind the lysine-arginine antagonism was reported by Jones et al. in 1967 [28]. First, Jones et al. [28] showed that both control and excess lysine fed chicks were able to effectively digest and absorb arginine, dispelling the theory that lysine reduces the utilization (i.e., digestion and absorption) of arginine. Secondly, Jones et al. [28] proposed three potential mechanisms behind the antagonism, of which the primary effect of lysine was indicated to be increased catabolism of arginine or a reduction in renal tubular resorption of arginine. The increase in dietary lysine was associated with an increase in kidney arginase activity, but as this was a delayed response, Jones et al. [28] did not believe it

to be the primary cause of the increased arginine catabolism. Boorman et al. [29] later showed that intravenous infusions of lysine resulted in increased plasma lysine levels and inhibited renal reabsorption of arginine in cockerels.

Nesheim [30] studied the influence of lysine on chickens selected for high and low arginine requirements. In these studies, Nesheim [30] found that excess lysine had a greater growth depressing effect on chickens selected for high arginine requirements compared with those selected for low requirements. Despite the larger effect observed in the high arginine birds, Nesheim [30] observed growth depressing effects of lysine on the low arginine requirement birds, seemingly independent of kidney arginase levels. Neisheim [31] also observed an increase in urinary arginine loss when high levels of lysine were fed. Conversely, Austic and Nesheim [32] observed two to four-fold increases in arginase activity when excess lysine, histidine, tyrosine, and isoleucine were fed with and without arginine. These responses were determined to occur in concert with the depressions in body weight gain through the implementation of time-course studies. Therefore, Austic and Nesheim [32] concluded that arginase activity was a major factor in the variation of the arginine requirement, in stark contrast to previous research.

In 1970, D'Mello and Lewis [33] published the first of their series of papers on amino acid interactions in chick nutrition, focusing on the lysine-arginine antagonism. As previous researchers had challenged the existence of a lysine-arginine antagonism [27], D'Mello and Lewis [33] utilized a basal diet limiting in methionine and only marginally adequate in arginine. When excess lysine was added to the diet, depressions in chick performance could not be corrected with additional methionine, but only when arginine was added. These responses suggested a restructuring of the order of limitation in the basal diet and confirmed a direct relationship between lysine and arginine. The third paper in the D'Mello and Lewis [34] series defined the arginine requirement when diets contained excess lysine. When arginine was titrated at four lysine levels, D'Mello and Lewis [34] reported a linear increase in the arginine requirement (**Figure 1**).

Allen et al. [35] titrated arginine in diets containing dietary lysine levels of 0.55, 0.95, 1.35, 1.95, and 2.55%. These titrations allowed for the comparison of growth curves to show the declining efficiency of arginine to promote weight gain. Arginine efficacy decreased linearly to 58.8% of control levels as lysine was increased to 1.84%.

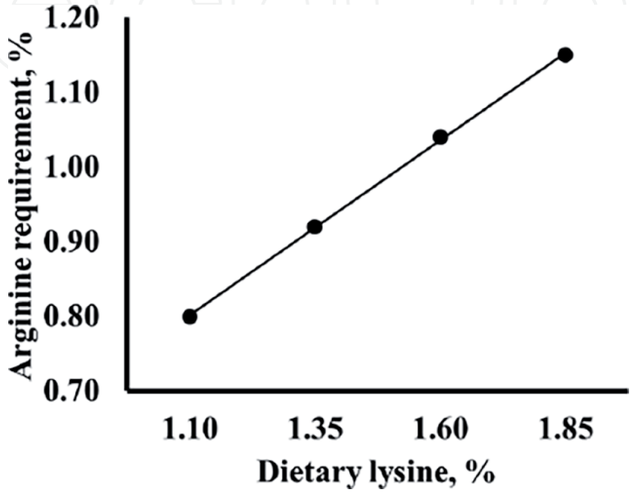



**Figure 1.**
*Influence of dietary lysine level on the determined arginine requirement. Adapted from D'Mello and Lewis [34].*

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Further increases of dietary lysine had no effect on arginine efficiency. Based on these observations, Allen et al. [35] concluded that the lysine-arginine antagonism was based on lysine magnifying the effects of an arginine deficiency. Allen and Baker [36] then determined the arginine requirement when dietary lysine levels were 0.95 and 1.95%. The required arginine level increased by 52 and 37% for body weight gain and feed conversion, respectively (**Figure 2**).

Wang et al. [37] investigated the influence of excess dietary lysine and arginine on the enzyme activity of lysine-ketoglutarate reductase and arginase. Increased supplementation of L-lysine HCl, ranging from 0 to 1.0%, resulted in a in an approximate two and five-and-a-half-fold increase in lysine-ketoglutarate reductase and arginase, respectively. Conversely, supplementing L-arginine from 0 to 2.0% resulted in an approximate two-fold increase in kidney arginase activity, but arginine supplementation had no effect on lysine-ketoglutarate reductase activity.

Kadirvel and Kratzer [38] examined the intestinal uptake of L-arginine and L-lysine when excesses of lysine, leucine, and glycine in vitro. Focusing on arginine and lysine, it was discovered that arginine absorption was reduced when lysine was added to the solution, but progressive amounts of lysine had no further influence on arginine absorption, indicating that limited competition between lysine and arginine exists during absorption. Kadirvel and Kratzer [38] then displayed the effects of feeding the aforementioned amino acids to broilers and evaluated their effects on bird performance. During the in vivo study, only excess lysine resulted in the appearance of arginine deficiency symptoms, which Kadirvel and Kratzer [38] interpreted to indicate that lysine-arginine antagonism is mediated through a metabolic effect as opposed to competitive absorption. Robbins and Baker [39] revisited the influence of amino acid excess on kidney arginase activity. They found that not only did lysine, arginine, and histidine influenced arginase activity, in agreement with Austic and Nesheim [32], but also an effect of total nitrogen that exceeded that of individual amino acids. Robbins and Baker [39] concluded that total nitrogen level was equally important in the activity of arginase as dietary arginine and lysine.

Based on research evaluating lysine-arginine antagonism from its discovery until the early 1980s several conclusions can be drawn characterizing the antagonism. First, a specific antagonism exists among arginine and lysine that appears to be

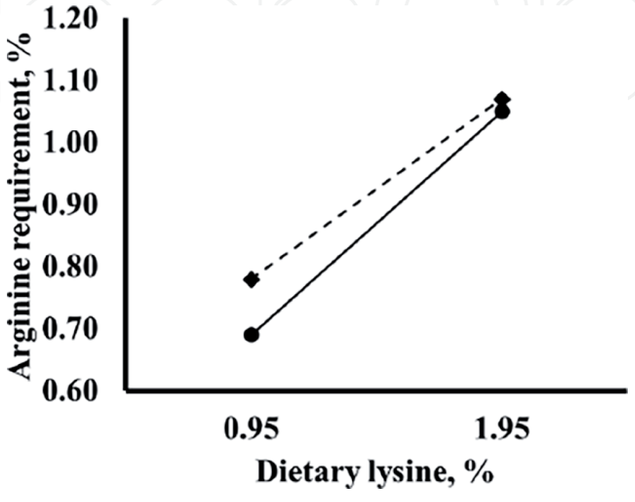



**Figure 2.**
*Influence of dietary lysine level on the arginine requirement for body weight gain (solid line) and feed conversion (dashed line). Adapted from Allen and Baker [35].*

non-reciprocal, displaying only effects of lysine on arginine metabolism. Secondly, the reason for the discovery of said antagonism lies in the amino acid contents of specific protein sources that were used in the diets of the period used to characterize amino acid requirements, namely casein due to its low arginine content relative to lysine. Lastly, the mechanism behind the lysine-arginine antagonism has not been cleanly defined but it does appear to be linked to the reduced capacity for the renal tubes to reabsorb arginine. While the role of arginase, and lysine's effect on it, is still debated, the findings of Robbins and Baker [39] combined with the findings of Keene and Austic [40] twenty years later may potentially explain the conflicting reports on arginase activity. Keene and Austic [40] found that catabolic enzymes are stimulated more by dietary protein than by the single amino acid targeted by the enzyme. The response of arginase by multiple amino acids is likely the response of increased dietary nitrogen, or in the case of Robbins and Baker [39] a balanced amino acid mixture, as opposed to the individual amino acids.

### 2.2. Branched-chain amino acids

Knowledge of the branched-chain amino acids concerning poultry started in much the same way as arginine with the determination of essentiality for poultry in 1944 [12, 13], and the first data set outlining their requirements was published two years later in 1946 [41]. The original requirement values were established to be 0.5, 1.5, and 0.7% of the diet for isoleucine, leucine, and valine, respectively, and varied little during the years of experimentation contributing to crystalline amino acid diets [14, 16, 19, 42].

In 1960, Laksesvela [43] reported that that deletions of isoleucine resulted in a 27% reduction in the "combinative protein value" of herring solubles, whereas additions of leucine resulted in a 16% reduction in the aforementioned metric. The implications of this discovery would not be fully appreciated until 1968 when Mathieu and Scott [44] reported that feeding excess leucine in diets containing isoleucine and valine near adequacy resulted in depressions in body weight. This report started investigations into branched-chain amino acid antagonism, as the existence of amino acid antagonisms were known (i.e., the existing work on the lysine-arginine antagonism) as well as its previous discovery in rats [45].

The second interaction investigated in the early 1970s by D'Mello and Lewis in a series on amino acid interactions in chick was that among the branched-chain amino acids [46]. As with lysine and arginine, D'Mello and Lewis sought to confirm the existence of the former amino acid antagonism in chick nutrition. Through the course of five experiments, D'Mello and Lewis [46] isolated and definitively showed the existence of an antagonism between leucine and isoleucine and leucine and valine, but theorized that up to six antagonisms existed among the branched-chain amino acids. D'Mello and Lewis [46] further defined that this antagonism is most prevalent when valine and isoleucine are included in the diets at adequate levels but could present itself if valine was not the limiting amino acid in the basal diet, in the case of a leucine-valine antagonism.

In their next series of experiments, D'Mello and Lewis [34] determined the influence of excess leucine on the requirements of valine and isoleucine. Excess leucine shifted the requirements of both valine and isoleucine in order to obtain maximal body weight gain (**Figure 3**). Of particular interest, D'Mello and Lewis [46] had indicated that the leucine × valine interaction was probably of more practical importance to broiler production and later showed that adjusting valine to maximize growth at higher leucine levels improved average daily gain above control levels whereas

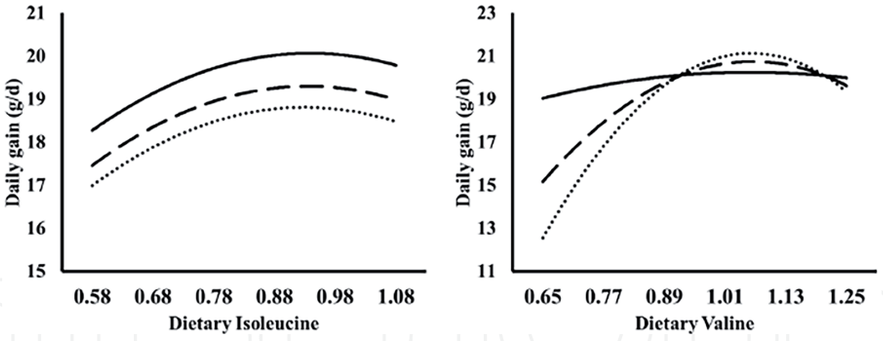



**Figure 3.**
*Influence of titrating isoleucine and valine on average daily gain at low (solid line), medium (dashed line), and high leucine (dotted line). Adapted from D'Mello and Lewis [34].*

increasing isoleucine resulted in a decrease in optimal average daily gain [34]. In their final study, D'Mello and Lewis [47] established the existence of a metabolic mechanism behind the branched-chain amino acid antagonism by pair feeding diets containing adequate and excessive leucine, as depressions in body weight gain were still observed in birds fed diets containing excess leucine compared with consuming an equal amount of a control diet.

The following year, Allen and Baker [48] conducted a series of experiments to determine the efficacy of isoleucine and valine when leucine was fed in excess. The ability of isoleucine to sustain body weight gain was linearly reduced to 80% of control levels when leucine was increased from 0 to 3% of the diets, conversely the ability of valine to support body weight gain was quadratically reduced to 74% of control levels as leucine supplementation was increased to 6%. Due to the difference in leucine excesses employed in the isoleucine and valine experiments, the minimal efficacy values do not allow for a direct comparison. Equalizing leucine inclusion levels to 3% displays an average efficacy of 81 and 79 for isoleucine and valine, respectively, agreeing with the postulations of D'Mello and Lewis [46] that the valine × leucine interaction would likely have more impact on poultry production.

Due to the similarities in chemical structure and common enzymes used in the transamination and decarboxylation steps of catabolism [49, 50], researchers believed that the antagonism among the branched-chain amino acids was linked to increased catabolism brought about by excessive leucine. Researchers at the University of Nottingham tested this hypothesis by monitoring the activity of aminotransferase for leucine and valine [51], as well as the catabolism of C14 labeled valine [52]. Both studies failed to observe any influence on the rate of catabolism of valine when leucine were fed in excess. Conversely, Smith and Austic [53] observed a small increase, approximately 2% of ingested levels, in the catabolism of C14 labeled valine and isoleucine when leucine reached 2.25% of the diet. Similarly, Calvert et al. [54] observed a 50 and 43% increase in isoleucine and valine, respectively, when leucine was fed at 5%. In addition, Calvert et al. [54] pair fed chicks diets containing 1.2 or 5.0% leucine to gauge the effect of reduced feed intake on branched-chain amino acid antagonism responses. Calvert et al. [54] found that growth depressions associated with excess leucine persisted when feed intake was equalized, agreeing with the previous findings of D'Mello and Lewis [47]. Based on their overall findings, Calvert et al. [54] proposed that 70% of the negative effects associated with branched-chain amino acid antagonism is linked to feed intake as opposed to a primary effect of metabolic changes.

Jackson and Potter [55] reported that the classic responses of branched-chain amino acid antagonism observed in poultry also occurred in turkeys. Branched-chain amino acid antagonism had previously observed in turkey poults [56, 57], but Jackson and Potter [55] also discovered that a reciprocal antagonism between isoleucine and valine that could result in depressions in body weight when either was fed at adequacy while the other was fed in excess. Mendonca and Jensen [58] latter confirmed the existence of isoleucine and valine antagonism in chickens, when it was found that supplementing isoleucine reduced performance, whereas a concomitant addition of isoleucine and valine had no effect.

Unlike with lysine-arginine antagonism, less is know about the mode of action behind the branched-chain amino acids with many theories lacking critical evidence to definitively prove. Despite this, cornerstone data were generated during the research conducted from the 1960s to 1980s. Firstly, the branched chain amino acid antagonism is a reciprocal antagonism, in that it can present itself by targeting both valine and isoleucine, and subsequent interactions between valine and isoleucine. Secondly, leucine appears to be the primary antagonist, but apparent performance gains can be made if proper supplementation of valine is made to account for the antagonism. Lastly, the largest piece of information that can be gleaned from classic research is that the antagonism is most apparent when isoleucine and valine are at adequacy levels, indicating that negative effects are likely to occur in reduced crude protein diets.

## 3. Evolution of poultry amino acid nutrition

In the 1990s, poultry amino acid nutrition reports placed more emphasis on practical aspects than those of previous decades. Large advancements in least-cost formulation strategies for broiler integrators, brought about by linear programing and personal computers that could conduct it, occurred in the mid 1980s, allowing nutritionists to rapidly produce mock formulas [59]. Lack of experience with this technology caused a distrust with feed-grade amino acids limiting their use, which would later be overcome with the widespread adoption of L-threonine in the 1990s [59]. This allowed for dietary crude protein to settle on the 4th limiting amino acid which varied depending on the ingredients included in broiler diets [60]. Therefore, research during the 1990s and early 2000s largely shifted to the determination of amino acid requirements, although some antagonism work remained.

By the 1990s research evaluating the lysine-arginine antagonism had largely come to an end. Mendes et al. [61] failed to observe any response to variations in dietary lysine or the arginine to lysine ratio when feeding broilers three to six weeks of age. The classic responses observed were largely the result of the ingredients used in non-practical diets (i.e., casein) and not something that would typically occur in poultry production. Similarly, studies determining the arginine requirement began to produce relatively consistent requirement estimates, likely resulting from the constraints placed on lysine during formulation (**Table 2**). In addition to arginine's role in animal growth, research into its influence on animal health gained popularity and was added to requirement parameters [67–70].

Conversely for the branched-chain amino acids, Farran and Thomas [71] implemented central-composite, rotatable design to model the branched-chain amino acids

| Reference | Strain | Sex | Age, day | Requirement estimate[1] |
|---|---|---|---|---|
| Corzo and Kidd [62] | Ross × Ross 308 | Male | 0–18 | 101 |
| Cuca and Jensen [63][2] | Peterson × Arbor Acres | Male | 0–21 | 106 |
| Chamruspollert et al. [64][3] | Ross × Ross 208 | Mix | 7–21 | 105 |
| Mack et al. [65] | Ross | Male | 20–40 | 112 |
| Corzo and Kidd [62] | Ross × Ross 508 | Female | 21–35 | ND |
| Mendes et al. [61] | Ross × Ross | Male | 21–42 | 110 |
| Corzo et al. [66] | Ross × Ross 308 | Male | 42–56 | 115 |

[1]*Ratio of arginine to lysine.*
[2]*Select trials used due to experimental design.*
[3]*Non-heat stressed.*

**Table 2.**
*Estimations of the arginine requirement for broiler chickens of various age, strain, and sex.*

and determine the requirements of the three simultaneously. Farran and Thomas [71] found significant interactions between valine and isoleucine, but were unable to identify any effect of leucine, differing from historic data. Due to the lack of effect of leucine, Farran and Thomas [71] eliminated leucine from their model, only determining requirements for valine and isoleucine, and began working with valine instead of continuing antagonism work [72, 73].

Also in the early 1990s, Burnham et al. [74] implemented a dilution technique in order to assess the effects of increasing isoleucine at different dietary valine and leucine levels. Burnham et al. [74] found that valine had no effect on the isoleucine requirement, and that leucine only depressed body weight when isoleucine was at the lowest tested levels. These findings resulted in Burnham et al. [74] postulating that the negative influences of leucine would not be an issue in practical diets if the ingredients used contained adequate amounts of isoleucine. Barbour and Latshaw [75] also evaluated the influence of valine and leucine on broiler isoleucine requirements but implemented practical type diets. No influence of valine nor leucine were observed on the isoleucine requirement. Barbour and Latshaw [75] indicated that the lack of a response was due to their experimental design in which not only were basal diets formulated with practical ingredients but adjustments in valine and leucine were brought about by practical ingredients available to the broiler industry. The final experiment of this era was conducted by Waldroup et al. [76]. Similar to the design of Barbour and Latshaw [75], Waldroup et al. [76] tested the effect of excess leucine by varying the amount of corn gluten meal in the diet. No negative effects were observed as a result of the excessive leucine levels, reaching over 3.5% of the diet. Waldroup et al. [76] indicated that the lack of response was driven by the increasing levels of isoleucine and valine that accompanied the excess leucine levels as a result of using intact protein sources to drive the leucine level. These universal excesses among the branched-chain amino acids allowed for the bird to account for potential losses of valine and isoleucine associated with the antagonism. Waldroup et al. [76] concluded their report theorizing that as more feed-grade amino acids entered poultry formulation, branched-chain amino acid antagonism may become a practical concern due to the elimination of excess valine and isoleucine in broiler diets.

## 4. The return of antagonism research

The doctoral work of I.C. Ospina-Rojas, resulted in three papers investigating interactions between valine and leucine [77–80]. To evaluate the relationship between valine and leucine and its influence on live performance and carcass traits, Ospina-Rojas [77] conducted two 5 × 5 factorials, after which results were displayed via response surface graphs to allow for visual observations of trends. During a 1–21 day starter phase, Ospina-Rojas et al. [80] observed valine × leucine interactions for fed intake and feed conversion. Ospina-Rojas et al. [80] was able to determine leucine and valine to lysine requirement values of 104 and 77 and 102 and 73 for feed intake and feed conversion, respectively. Feed intake was most severely impacted when valine levels were low and leucine levels were high, whereas feed conversion spiked when both amino acids were fed at low levels.

When varying valine and leucine levels were fed during a 21–42 day period, Ospina-Rojas [78] observed significant valine by leucine interactions for feed intake and body weight gain. Unlike with the previous growth phase, requirement values could not be estimated for maximal feed intake as a ridge occurred for feed intake between valine to lysine ratios of 82 and 91 for the entire range of leucine. Feed intake values remained relatively constant across leucine levels but it was again minimized when valine levels were low and leucine levels were high. For body weight gain, a requirement estimate was determined at a valine and leucine ratio to lysine of 111 and 83, respectively. As with feed intake, body weight gain was lowest when dietary valine was low and leucine was high.

Zeitz et al. [81] evaluated the influence of excess leucine on broiler performance and carcass traits when branched-chain amino acid levels were either fixed [82] or allowed to drop in relation to leucine level [81]. When branched-chain amino acid ratios were fixed, no differences were observed in growth performance over a 1–35 day period, but breast yields were decreased when leucine was increased by approximately 60%. However, no differences were observed for a 1–34 day period nor day 34 carcass traits when levels valine and isoleucine ratios in relation were allowed to drop when leucine increased.

Ospina-Rojas et al. [83] evaluated the influence of high leucine levels on the valine and isoleucine requirements for a starter (1–14 day), grower (14–28 day), and finisher periods (28–42 day) through the implementation of central-composite, rotatable design. Ospna-Rojas et al. [83] observed consistent influence of branched-chain amino acids on feed conversion across all three feeding phases, but body weight gain was not affected until the finisher phase. Unlike previous experiments, Ospina-Rojas et al. [83] did not generate response surface graphs, but did report regression equations. The lack of response surface graphs was due to the significant effect of three factors that cannot be displayed on a three-dimensional graph. Requirements estimates needed for optimal body weight gain reported by Ospina-Rojas et al. [83] generally showed that valine and isoleucine requirements decrease as the bird ages, but leucine needs increase.

A pair of studies published in 2021 implemented the use of Box-Behnken design to characterize the broilers response to various branched-chain amino acid levels [84, 85]. The studies were completed as part of a ring study and followed the same experimental design, with the only difference being the type of birds used (i.e., strain and sex). Maynard et al. [85] found significant interactions between valine and isoleucine for body weight gain, feed conversion, and breast meat yield when branched-chain amino acid levels were varied in diets fed to Cobb MV × 500 broilers. The effect of leucine was

limited to an interaction between leucine and valine on breast meat yield. Maynard et al. [85] came to a similar conclusion to that of Farran and Thomas [71] that leucine may not be a significant factor under practical conditions and eliminated it from their model, replacing it with glycine + serine due to the potential limitation of glycine or nonessential nitrogen in the reduced crude protein diets implemented. When leucine was removed from the model, the interactions between valine and isoleucine were virtually eliminated, indicating that the "real" effect of leucine may be a "shadow effect" that does not present as a traditional significant response Maynard et al. [85]. Kidd et al. [84] focused on the branched-chain amino acids in their study but conducted it in male and female Lohman Indian River broilers. Contrary to the findings of Maynard et al. [85], Kidd et al. [84] did observe significant influence of leucine, citing interactions between leucine and isoleucine for body weight gain and feed conversion and leucine × valine interactions for carcass and breast meat yield. Furthermore, Kidd et al. [84] found that female broilers were more responsive to branched-chain amino acid supplementation than males.

Maynard [86] followed up the findings from the Maynard et al. [85] studies through the implementation of factorial designs meant to confirm the modeling responses. The first factorial study conducted by Maynard et al. [86] sought to determine the shift in the valine requirement when high and low levels of isoleucine and leucine were fed in practical type diets. Interestingly, a three-way interaction was observed for feed conversion but two sub interactions, valine × leucine and valine × isoleucine, were observed for body weight gain. The body weight gain responses observed by Maynard [86] (**Figure 4**) closely resembled those observed by D'Mello and Lewis [34] 50 years ago, without using purified diets or large swings in dietary leucine. Maynard [86] then attempted to characterize the sub interactions, valine × leucine and valine × isoleucine, observed in the larger study but failed to get a response. This lack of response again highlighted and expanded upon the observations Maynard et al. [85], that investigations into the branched-chain amino acid antagonism require testing of all three due to the complex nature of the antagonism.

The most recent study evaluating the branched-chain amino acids was a central-composite, rotatable design presented by Corzo and Silva [87]. Corzo and Silva [87] observed significant three-way interactions for body weight gain, feed conversion, carcass yield, and breast meat yield. General trends showed that increased isoleucine and valine were needed when leucine was fed in excess, but potentially more important, that the negative effects of leucine could be overcome for all parameters, except

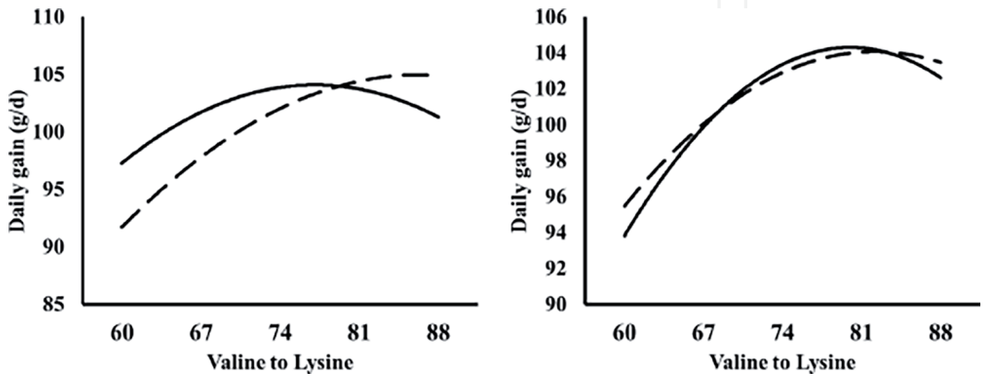



**Figure 4.**
*Influence of titrating valine on average daily gain at high (solid line) and low (dashed line) leucine (left) and isoleucine (right). Adapted from Maynard [86].*

carcass yield, when leucine levels continued to increase with proper isoleucine and valine supplementation.

These recent studies have shown that unlike the lysine-arginine antagonism, branched-chain amino acid antagonism presents in practical type diets and will continue to be an issue for practical broiler production as crude protein levels decrease. The work of Corzo and Silva [87] is promising as it appears the effects of this antagonism could be turned from a negative to a positive. Maynard et al. [88] indicated that in the future this phenomenon may be referred to as the branched-chain amino acid synergism based on a meta-analysis conducted on branched-chain amino acid research conducted from 2000 to 2021.

## 5. Future research

In the modern era, broiler amino acid research is centered around more complex problems as opposed to the simple strategies of the past. Titration studies used to determine amino acids requirements will remain the gold standard, as laid out by Lewis [89], but further refinement of these requirements will require researchers to consider test diet nutrient profiles compared to those observed in commercial practice. With the present known, and potentially unknown, antagonisms influencing amino acid requirements, generated values from test diets may not accurately represent those that produce optimal performance under commercial or practical conditions. Likewise, differentiated responses to branched-chain amino acid levels were observed when broiler strain or sex was changed under similar experimental conditions.

The double-edged sword of evaluating these complex interactions is the need for larger research facilities to achieve necessary experimental unit and replication. Another more manageable approach is the use of modeling. Previous researchers have shown that modeling research (i.e., Box-Behnken design) can be used in order to reduce the treatments necessary to characterize large scale interactions [90]. By effectively halving the number of treatments necessary to test a 3 × 3 × 3 interaction, the number of replicates can be doubled without increasing the number of necessary pens. This approach can be used over a broad range of inclusion levels in order to map general responses, then if a significant response is observed, treatment ranges can be reduced to reflect those observed in commercial practice to allow for a targeted approach. While Maynard [86] largely failed in the attempt to follow this strategy, the larger valine titration factorial was successful in observing a shift in valine requirements.

It is important to note that the collective work of Kidd et al. [84], Maynard [86], and Maynard et al. [85] used P-values $\geq$0.10 to identify significant interactions due to repeated observance of these levels. Originally, Kidd et al. [84] and Maynard et al. [85] set significance levels at $P \geq 0.10$ due to the modeling approach used in their studies, but subsequent work by Maynard [86] observed similar P-values in their factorial approaches. P-values for the three-way interaction observed by Maynard [86] for feed conversion were found to be between 0.05 and 0.10, but when the data was broken into the individual titrations, P-values were found to be highly significant (i.e., $P < 0.01$). Relative consistency in responses to the branched-chain amino acids have been historically observed and noted by previous researchers [91].

While the current body of literature does not allow for concrete formulation strategies, promising studies have been recently conducted and the prevalence of this

style of research is increasing. The original observations of these antagonisms were brought about through the use varying ingredients, which changed the amino acid profiles of the diets implemented. The implications of how these discoveries were made are still relevant today with the ability of nutritionists to simply monitor the levels of nutrients in diets through the addition of nutrients to formulation software. While requirement minimums or formulation constraints will not necessarily be added for these nutrients, their inclusion in matrices allow for monitoring that can be reevaluated if negative performance or responses are observed in the field.

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