[Frontiers In Bioscience, Landmark, 23, 640-655, January 1, 2018]

# Homeostatic regulation of plasma amino acid concentrations

**Shengfa F. Liao[1], Naresh Regmi[1], Guoyao Wu[2]**

*[1]Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS 39762-9815, USA, [2]Department of Animal Science, Texas A and M University, College Station, TX 77843-2471, USA*

## TABLE OF CONTENTS



## 1. ABSTRACT

One major goal of nutrition is to maximize the rate of muscle protein gain via provision of amino acids (AAs) through blood plasma. Comparing the plasma AA concentrations with the growth performance data can help to elucidate the metabolic mechanisms regulating plasma AA homeostasis, nutrient utilization, and intracellular protein turnover. Knowledge about the homeostatic regulation of plasma AA profile can aid in predicting dietary AA availabilities, the order of limiting AAs, and the whole body protein metabolism. Lysine, for example, is typically the first limiting AA in practical swine diets; however, our current knowledge is insufficient to draw a clear conclusion about the complex relationship between dietary lysine supply and plasma AA profiles. Thorough understanding of the effect of dietary AA supply on plasma AA profiles can help nutritionists to develop novel nutritional strategies to guide and improve dietary AA supplies. Further research is needed to study how different levels of dietary AAs, individually or in concert, affect the plasma concentrations of all AAs and related metabolites.

## 2. INTRODUCTION

The primary objective of swine production is to convert feedstuffs into edible pork for human consumption. Pork, the most consumed meat in the world, is a major source of high-quality food protein for human consumption (1). Pigs grow fast, offer more meat per breeding female and, therefore, are more prolific than other livestock species (2, 3). The predominant component of pork is skeletal muscle (interchangeably called muscle in this review), and in modern days the efficiency of pork production is measured by the efficiency of lean gain (i.e., the muscle gain) instead of the whole-body weight gain. Thus, knowledge about the growth and development of skeletal muscle in pigs is fundamentally important from both the economic and scientific standpoints (3).

Besides water which constitutes approximately 75% of the total muscle mass, the principal chemical component of muscle is protein that constitutes approximately 74% of the total muscle dry matter. All the other components, including lipids, non-protein nitrogenous substance, carbohydrates and minerals, compose approximately 26% of the dry matter in muscle (4). As is known, the protein molecules are polymers of amino acid (AA) residues linearly connected by peptide bonds (3, 5). Although there are more than 500 naturally occurring AAs (6), only about 20 of them are commonly found in plant and animal proteins, and these 20 AAs, called proteinogenic AAs (7), are alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine,

isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine (8, 9). Recently, a new AA called selenocysteine has been identified as a new AA naturally existing in certain proteins (10).

Traditionally AAs were classified as nutritionally essential or nonessential AAs for pigs. The essential AAs are those that need to be provided to the pigs through dietary supply because pigs cannot *de novo* synthesize them or cannot synthesize enough to meet their metabolic needs (3, 9). It was tactically assumed that pigs can synthesize enough amounts of nonessential AAs for use if enough dietary nitrogen is provided. Therefore, the U.S. National Research Council (NRC) has dietary requirement recommendations for the essential AAs but not for the nonessential AAs (9). Recently, a growing body of knowledge, however, showed that this classification is invalid, as pigs (as well as poultry and fish) do have dietary requirements for the so-called "nonessential AAs" to fulfill their genetic potential for maximum growth and reproduction performance, as well as for their optimal health and wellbeing (11). The long-standing term "nonessential AAs" is now considered as a long-standing misnomer in nutritional sciences. Beyond serving as the building blocks for protein synthesis, different AAs do have different biochemical functions, and this must be considered when formulating swine diets to improve the efficiency of nutrient utilization and animal well-being (11).

Skeletal muscle, the major reservoir of protein or AAs in the body, is actually a dynamic tissue. Different from the protein molecules in secreted milk or laid eggs, the protein molecules in muscle mass constantly undergo a normal intracellular life processes, which is called protein turnover − old or damaged proteins are degraded and new proteins are *de novo* synthesized (3, 5). This process continues throughout animal life even when the growth rate is zero or negative (12). Besides muscle, other tissues in animal body also possess intracellular protein turnover, but the rates of this metabolic cycle vary greatly among the tissues (13).

Constant muscle protein turnover requires a constant supply and removal of free AAs through extracellular and intracellular fluids. Blood circulation is connected to these fluids and is responsible for AA transportation and distribution within animal body. In order for animal muscle to grow or to grow fast, its protein biosynthesis rate must be much higher than the rate of protein degradation, which requires not only a maximal amount of AA supply (within the physiological range) but also the optimal ratios among the AAs at the site of protein synthesis (14). If one required AA is unavailable, the whole protein biosynthesis will be halted, and this halt will consequently lead to more muscle protein degradation, because the degradation process can release the lacking AA that is necessary for other more important organs (such as the brain and liver) to function (15, 16).

In swine production practice, it is crucial to constantly provide swine muscle cells with AAs in right amounts and at right ratios via blood circulation. As is known, the ultimate source of free AAs for the blood plasma comes from daily dietary intake of protein or AAs. Therefore, a thorough understanding of the relationship between the plasma AA profile and dietary AA supply is of ultimate importance for the nutritional management of pig production, such as diet formulation. This review was written to highlight some recent advances in this area of research.

## 3. PIVOTAL ROLES OF PLASMA FREE AMINO ACIDS

Besides the aforementioned muscle cells, virtually all other living cells within an animal's body involve the intracellular protein turnover process (Figure 1). For this process to proceed, the intracellular fluids need to receive and remove free AAs via extracellular fluids and blood circulation according to the metabolic status of the cells. The free AAs in the plasma with different concentrations (i.e., the AA profile) play pivotal roles during the protein turnover process (17). This is so because the free AAs are intermediates of the whole body protein turnover and nutrient metabolism, and they function as a type of circulating "currency" for these metabolic processes.

Blood circulation transports all metabolic products of protein digestion and absorption, which include peptides and free AAs, to other tissues in the body (Figure 1). Comparing the current plasma AA concentrations (after feed intake) with the reference plasma AA concentrations (at starvation) can give important information about the bioavailability of AAs in a diet (5). This type of comparison assay has been used for determining the limiting AAs in swine and poultry diets and to determine AA requirements of these animals (18, 19, 20). The profiles of the digestible or bioavailable AAs, especially those not *de novo* synthesizable in animal cells, in a diet is the single most important factor that determines the nutritional value of the dietary protein or the efficiency of dietary protein for metabolic utilization (21, 22).

In many circumstances, the cellular uptake and further metabolism of small peptides and free AAs depend on the concentrations of free AAs in the plasma (17, 23). If the required free AAs are readily supplied from the blood plasma, upon entering the cell they will rapidly join one another by peptide bonds to form peptides or proteins under the direction of corresponding cellular mRNAs. Therefore, except

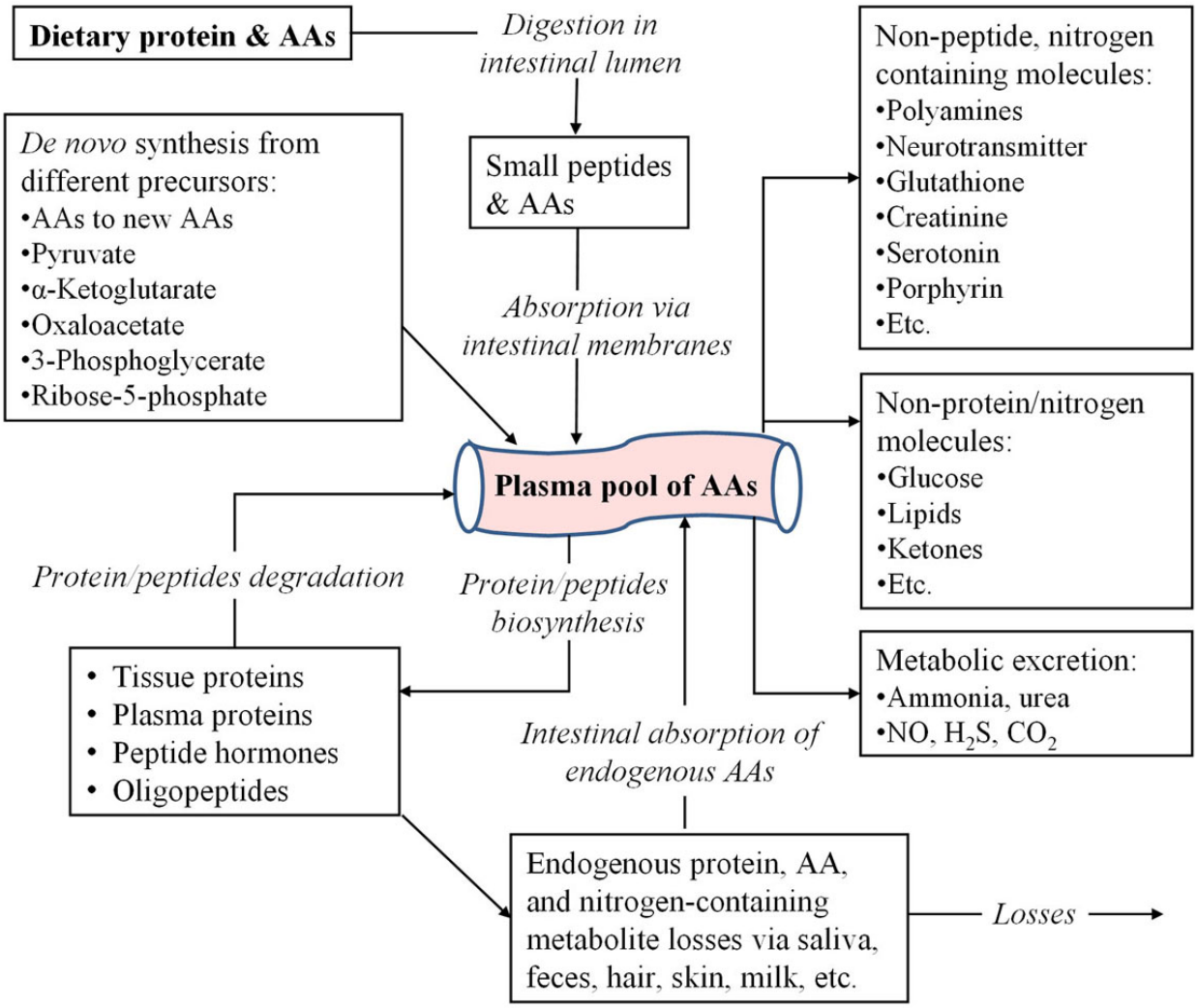


**Figure 1.** Homeostatic regulation of plasma free amino acid (AA) concentrations. The relationships among AA intake, AA *de novo* synthesis, the plasma pool of free AAs, AA catabolism, and protein and peptide turnover in animal body are outlined. Endogenous proteins and AAs are those coming from the inside of the body instead of from the dietary source (i.e., the exogenous source). The endogenous protein losses include the losses of swine tissue proteins and the intestinal microbial proteins and nitrogen-containing metabolites.

for taurine, glutamate, glutamine, aspartate, glycine, alanine, β-alanine, arginine, proline, and threonine, the concentrations of other free AAs in the skeletal muscle of young pigs usually remain low (< 0.5 mM) (5). Of note, the intramuscular concentrations of methionine, phenylalanine and tryptophan are only 0.076, 0.074, and 0.047 mM (5). Every particular type of cells has an upper limit with regard to the amounts of protein and AAs it needs or can store. After the cell has reached over its upper limit, the excess protein and AAs will be metabolized, via inter-organ cooperation, into other metabolic products (e.g., glycogen and lipids), or they will be used immediately as an energy source (3, 24, 25).

As new proteins are biosynthesized, many of the old or damaged proteins are catabolized within living cells and returned to the plasma in the form of small peptides or free AAs almost as rapidly as the speed of protein synthesis (5). Thus, there is a constant interchange or equilibrium between plasma AAs and the labile proteins in almost all the living cells (24). For instance, if a particular tissue requires more proteins, new proteins will be synthesized quickly using the plasma free AAs, and thereafter, the plasma AAs can be replenished via the diet or degradation of proteins in other tissues (24).

For an animal to have proper life processes and a healthy body, there is a need for a homeostatic plasma AA profile. Whenever the plasma AA concentrations fall below the homeostatic levels, and no or very little AAs come from diet at that particular time, more cellular proteins will be catabolized to provide

free AAs, which will be released and transported out of the cells into the plasma. In this way, the plasma AA profile is maintained at a reasonably constant or homeostatic status. Nevertheless, an animal body with a normal plasma AA profile or in a physiologically healthy state is not necessarily growing at a maximal or optimal rate. The optimal plasma AA profile for body health may not be the same as that for fast muscle growth.

## 4. FACTORS AFFECTING THE PLASMA FREE AMINO ACID PROFILE OF PIGS

### 4.1. The endogenous and exogenous sources of amino acids

In general, the plasma free AA profile of a pig at any given time is affected by the appearance rates ($R_a$) of various AAs to the blood and by their disappearance rates ($R_d$) from the blood at the same time (Figure 1). While the factors controlling $R_d$ include (i) the rate of AA withdrawal from the plasma by cells for syntheses of proteins, peptides, and non-peptide substances, (ii) the rates of plasma free AA degradation, and (iii) the rates of AA losses via urine, saliva, feces, and skin, those controlling $R_a$ include endogenous and exogenous sources (23, 26).

The endogenous sources of AAs are referred to those obtained from the inside of the body, whereas the exogenous sources of AAs are referred to those coming from the outside of the body. The endogenous AAs mainly include those that come from cellular proteolysis and *de novo* AA syntheses (Figure 1). In pigs, after secreted into the intestinal lumen some endogenous proteins are degraded into free AAs and, therefore, most of the AAs associated with those proteins are lost in feces (27, 28). At any given time, however, pigs can also recycle these endogenous AAs, and under some physiological situations, up to two-thirds of the total AAs needed for the whole body may come from the endogenous sources. For a pig to gain muscle, however, only using the endogenous AA $R_a$ to compensate the normal AA $R_d$ should be avoided, which means that the exogenous AAs should always be provided to the pig in order to maintain its plasma AA homeostasis, tissue health, and body growth (29).

The exogenous source of AAs basically is the dietary supply of AAs via mouth and gastrointestinal tract (23, 26), and this dietary source can be provided in the form of proteins, peptides, and/or free AAs. The form of proteins is the primary source of exogenous AAs in current swine production practice. The end products of the gastrointestinal digestion of dietary proteins and peptides are small oligopeptides (di- and tri-peptides) and free AAs, which can be actively absorbed into the blood stream via numerous facilitated system transporters anchored in the apical and basolateral membranes of the small intestinal epithelial cells (30). In addition, any endogenous proteins and peptides secreted into the gastrointestinal lumen are also subject to a similar digestion and absorption processes as that for the exogenous dietary proteins and peptides (28). Any factors that affect the activities of digestive enzymes (such as proteolytic enzymes) (24) and transport systems (31) will affect the rates and quantities of free AAs entering the blood stream.

### 4.2. The roles of gastrointestinal tract

First of all, besides being responsible for digestion of proteins and absorption of small peptides and AAs, animal gastrointestinal tract plays important roles in the synthesis, conversion and catabolism of AAs, which significantly affects the proportion of the absorbed AAs before being transported to the blood stream. Although the portal-drained viscera (intestines, pancreas, spleen, stomach) represents only about 5–7% of body mass, these tissues disproportionately account for 20–35% of whole-body protein synthesis, which means that the splanchnic tissues metabolize about 20–50% of dietary essential AAs (leucine, lysine, phenylalanine) in the first-pass (32, 33). With low-protein diets, this extraction of AAs can be as high as 70% of lysine intake (32). Based on the literature, Wu *et al*. (34) reported that most of glutamine and almost all glutamate and aspartate from the diet are catabolized by the small intestinal mucosa in the first-pass, and that the small intestinal mucosa degrades enteral arginine, ornithine, proline, branched-chain AAs and lysine, and perhaps methionine, phenylalanine, threonine, glycine, and serine, such that 30–50% of these dietary AAs do not enter the portal vein.

The small intestinal lumen of pigs harbors many species of microorganisms, which can degrade both exogenous and endogenous small peptides and free AAs and, thus, actively affect the luminal supply of free AAs to the blood stream. Dai *et al*. (35) reported high rates of the utilization of glutamine, lysine, arginine, threonine, leucine, isoleucine, valine and histidine by luminal bacteria in the small intestine. Except for the formation of glutamate and its metabolites (alanine and aspartate) from glutamine, and of ornithine from arginine, there was no net production of any AAs from glutamine, lysine, arginine, threonine, leucine, isoleucine, valine and histidine by pig intestinal microbes. Dai *et al*. (35) also found that small-intestinal bacteria, particularly *Streptococcus*, *M. elsdenii*, *Mitsuokella*, and *E. coli*, degraded AAs at different rates in a species-dependent manner and the protein synthesis was a major pathway for AA metabolism in all the bacteria studied. Of note, Yang *et al*. (36) found that bacteria in the small intestinal lumen of pigs degraded AAs, but bacteria that tightly adhered to the small intestinal epithelial wall might be able to synthesize AAs, suggesting variations in AA

metabolism by bacteria from different niches. The small intestinal bacteria also synthesize proteins from the luminal free AAs (37). Those free AAs that pass from the terminal ileum into the large intestine of pigs are catabolized by large intestinal microbial fermentation and lost with feces because large intestinal absorption of free AAs is very limited and only occurs during early postnatal development (33).

### 4.3. Inter-organ exchange of amino acids

Besides the gastrointestinal tract, other internal organs, such as liver, kidney, and muscle, also affect the plasma AA profile through inter-organ AA metabolism. Although the uptake of basic AAs (e.g., arginine and lysine) by liver is low due to the near absence of the $y^+$ AA transport system (5), the liver can degrade most AAs with only a few exceptions (e.g., the branched-chain AAs including leucine, isoleucine and valine) and plays a central role in determining the plasma concentrations of AAs (38). Although a large quantity of rapidly exchangeable proteins can be synthesized in it, liver is the major organ responsible for AA catabolism and disposal and, along with the enterocytes, is capable of synthesizing urea from ammonia (39). If the liver is injured or diseased, the total plasma AA concentration will be increased (40). In addition to AA catabolism, the liver also has an ability to synthesize certain AAs and some important compounds from plasma AAs. For instance, with a few exceptions (e.g., citrulline and arginine), the so-called nonessential AAs can all be synthesized in the liver (41). As is known, the plasma proteins have many physiological functions, including transport of lipids, hormones, vitamins, and metals in the circulatory system and regulation of acellular activities (24). Many blood proteins act as enzymes, antibodies, complement components, protease inhibitors, or kinin precursors. Most of the plasma proteins, with the exception of some gamma globulins, are synthesized from free AAs by the hepatic cells (24).

The kidney, as another metabolically important organ, is able to finely filter the plasma and tissue pools of free AAs and also to synthesize some AAs (e.g., glycine from hydroxyproline; arginine from citrulline; and homoarginine from arginine and lysine) (5, 42–44). Therefore, the kidney also plays a major role in the homeostatic control of plasma free AAs through filtration, reabsorption, synthesis, degradation, and urinary excretion of free AAs and peptides (45, 46). In a renal failure situation, nearly all plasma or tissue AA levels are within roughly 60% and 150% of the normal range of levels, with the exception of citrulline, cysteine, and methylhistidines (47). Detailed descriptions about renal handling of individual AAs can be found in an excellent review paper of Kuhlmann and Kopple (47).

The skeletal muscle, without doubt, has a very critical role in affecting the plasma AA profile, since it is the largest single tissue in the body (comprising up to 40–45% of the body mass), which contains a paramount portion of the body proteins (48). Certainly, the skeletal muscle takes up free AAs from the plasma for its own protein synthesis when it is in an anabolic status, and releases free AAs to the plasma when in a catabolic status. Although the skeletal muscle does not catabolize most AAs such as lysine, serine, proline, threonine, methionine, cysteine, phenylalanine, histidine, tyrosine, and tryptophan, it is the primary tissue for catabolism of three branched-chain AAs in mammals, and it is also very active in the net synthesis of others, specifically, alanine and glutamine (48).

As previously alluded to, the plasma free AA profile is influenced by a variety of nutritional and physiological factors. Of particular note, some AAs can be endogenously synthesized by the swine (49, 50) and each AA has its own characteristics in the inter-organ metabolism (5). However, much evidence shows that the plasma concentrations of free AAs are predominantly affected by the exogenous dietary AA supply via the gastrointestinal tract (51–53). The current knowledge regarding the relationship between the plasma free AA profile and dietary AA supply is reviewed as follows.

## 5. RELATIONSHIP BETWEEN PLASMA AMINO ACID CONCENTRATION AND DIETARY AMINO ACID INTAKE

The plasma AA concentrations are often measured to gain insight into the status of whole-body AA metabolism in response to AA intake, not only because of the pivotal metabolic roles of the free AAs but also because of the blood accessibility (54). The relationship between the plasma AA profile and various aspects of AA nutrition can provide a comprehensive vision about (i) the availability of individual AAs, (ii) the deficiency, the limiting, or the excess order of AAs in various sources of dietary protein (55), (iii) the pattern of AA absorption from the small intestine into the blood, and (iv) the AA balance or imbalance phenomena. If the dietary provisions of AAs, energy, vitamins and minerals are adequate, one could use the plasma or serum AA profile as an indicator of the whole-body protein nutrition status (16, 56–58).

For increasing the rate of muscle protein biosynthesis in pigs, the knowledge of the free AA profile of blood plasma has more direct implications than the knowledge of an AA profile of a diet, because free AAs in the plasma are the immediate source of AAs for utilization by the muscle. Nevertheless, most swine nutritionists or diet formulators are not always concerned about the availability of AAs coming from a diet to the blood stream for muscle protein biosynthesis.

The plasma AA profile of a pig is not only affected by the exogenous source but also by the endogenous source of AAs, as described above. Because the endogenous source of AAs originally also came from the diet and pigs cannot readily utilize the microbial proteins resulting from the intestinal fermentation, it can be stated that pigs solely depend on their diets for the supply of non-synthesizable AAs. While the influence of dietary AAs on the plasma AA profile is not easy to be understood (56), numerous studies were conducted in the past to determine the relationship between plasma AA concentrations and dietary AA supply (54, 59–61). Although the published results did not always support each other, different researchers have tried different sources and compositions of dietary AAs with hopes to find out the alteration patterns of AA concentrations in the blood plasma.

As mentioned before, the plasma AA profile is predominantly affected by the dietary supply of AAs, so there may be some kinds of pattern or relationship between the plasma and dietary AA concentrations (16), although the plasma AA concentrations do not always parallel the dietary AA levels (53, 62). It should be born in mind that the time of blood sampling relative to animal's last meal also greatly influences the results (61).

### 5.1. Dietary change in crude protein levels

With a few exceptions, for those AAs that are not synthesized by animal cells, their plasma profile generally reflects their provision from a diet, which means that the plasma AA concentrations increase after a protein meal (45) and decrease after the animal is deprived of dietary protein, with a pattern for individual AAs being related to that in the dietary protein (63, 64). The AAs that are not limiting in a diet (as compared to the animal requirements) tend to increase in the plasma with the increasing level of dietary protein. But, when the increase in the level of dietary protein is associated with a substantial increase in the rate of body weight gain, the increase in plasma AA levels is of a lesser magnitude or nil (51).

Increases in the plasma concentrations of some AAs (e.g., leucine and glycine) may be an indicator of an inadequate intake of dietary proteins, resulting from increased degradation of intracellular proteins, rather than an excessive intake of dietary protein that is abundant in these AAs (58, 65). On the other hand, intake of excessive dietary protein actually can cause a decrease in the plasma concentrations of certain AAs (e.g., threonine). This phenomenon has been referred to as the "dietary protein paradox". The complexity of this paradox was supported by Frame (45), who found that the increase in plasma AA concentrations after a protein meal did not parallel the relative AA composition of the food, but Frame (45) failed to correctly postulate any theory behind it.

In humans, Adibi and Mercer (62) found that the total concentrations of free AAs in blood plasma significantly increased after a protein rich meal. On other hand, a protein-free meal failed to elevate the concentrations of all individual free AAs. In addition, they also observed a relationship between the magnitudes of increases in plasma AA levels and the amounts of AAs in dietary proteins. Of note, these authors failed to establish a precise relationship because of the complexity of AA transport and metabolism in the small intestine and other tissues. However, it was clear that the AAs that were the most and least abundant in the dietary protein exhibited the greatest and smallest rises in the plasma, respectively. Gustafson *et al*. (16) measured the serum AA concentrations of male rats after they were fed seven graded levels of dietary casein. In general, the serum essential AA concentrations increased, while the non-essential AA concentrations decreased with the increasing in dietary casein concentrations. However, the relationships between serum concentrations of individual AAs and dietary casein levels were quite varied.

As mentioned above for humans (62), the concentrations of individual free AAs in the blood plasma of pigs can also be increased by a protein rich meal (66, 67). Davey *et al*. (52) performed a study in pigs to determine the influence of dietary protein level, animal age, and time of sampling on the plasma concentrations of essential AAs. Their results showed that the concentrations of valine, isoleucine, and leucine were significantly higher in plasma from the pigs fed a 20% crude-protein diet compared to the pigs fed a 13% crude-protein diet, while the reverse was found for threonine. No differences were found for methionine, phenylalanine, lysine, histidine, arginine, and total AAs. Fuller *et al*. (68) reported that the blood leucine concentration of the growing pigs was significantly higher with the high-protein diet than with the low-protein diet, and the lysine supplementation reduced the blood leucine concentration with both diets. The blood concentrations of other AAs were not studied.

### 5.2. Dietary change in the levels of multiple amino acids

Generally, after a protein-rich meal the changes in the plasma AA concentrations of an animal follow a simple rule – the concentrations of plasma AAs will increase after the dietary intake and the magnitudes of the increase are proportional to the levels of the respective AAs in the diet (54). For example, in the case of some AAs, such as leucine, isoleucine or valine, there is usually a linear relationship between

dietary level and plasma concentration. The reduced concentrations of arginine and proline in the plasma of neonatal pigs are fairly sensitive and can be used as indicators for their low intake from the diet (54).

These simple correlations, however, are not always true, and the plasma concentrations of most AAs are not linearly related to or parallel their dietary intake. The relationship between plasma and dietary AA concentrations could be influenced by the interactions or antagonism between structurally related AAs competing for intestinal absorption, because similar AAs ride the same transporters (30, 69). The complex interactions among AAs can alter not only the expected release of AAs from the diet into the blood, but it is also possible that the excess of one AA may act as competitive antagonist, reducing the utilization of other structurally similar AAs.

One classical example of AA interactions is the arginine-lysine antagonism. As is known, lysine is the first-limiting AA in most swine diets while arginine is present in great excess. This leads to the possibility that excess arginine interferes with lysine absorption, and as such, adversely affects pig performance. In an experiment with young pigs, excess dietary lysine alone increased plasma lysine and histidine concentrations, without altering the concentrations of other AAs (70). When dietary lysine and arginine were added together, plasma lysine and histidine concentrations decreased, relative to the situation when dietary lysine was added alone, which implicated the arginine-lysine interaction (70). Hagemeier *et al*. (71) conducted two experiments with weaned piglets to study the effects of excess arginine on plasma AA levels and growth performance, and found that the plasma lysine concentration was reduced by excess arginine in pigs fed a high level (1.26%) of dietary lysine, but not in pigs fed a lower level (1.03%) of dietary lysine. While the plasma concentrations of other essential AAs were not affected, the plasma concentrations of threonine and methionine were reduced by excess arginine in both experiments. Nonetheless, dietary supplementation with 0.8% arginine to a basal diet containing 12% crude protein (including 0.7% arginine and 0.57% lysine) did not result in antagonism among basic AAs in gestating gilts (72–74).

Vice versa, research with poultry (75–78) has demonstrated that excess lysine has an adverse effect on arginine utilization, and increases chicken's requirement for arginine. Yun *et al* (79) fed three different levels of dietary lysine, 0.6%, 1.2% and 1.8%, to broilers. The serum concentration of arginine decreased with a higher inclusion rate of dietary lysine. This result is consistent with the previous findings reported by Dean and Scott (80). Although some research data from chicks, humans, and other monogastric animals were cited in this paper for supporting the concept, readers should keep in mind that there are some species differences in terms of AA metabolism and physiological plasma AA concentrations. However, the detailed comparison of plasma AA profiles among these animals is not an aim of this review paper.

A study conducted by Anderson *et al*. (81), however, showed that the adverse effects of excess arginine on nitrogen utilization in young pigs was not alleviated by dietary supplementation of crystalline lysine, although the plasma lysine concentration was reduced by adding dietary arginine and increased by adding dietary lysine. Edmonds and Baker (82) found evidence against lysine-arginine antagonism in pigs. Addition of graded levels of lysine to a basal diet resulted in a quadratic elevation in plasma lysine and its metabolite, α-aminoadipic acid. Plasma levels of arginine, ornithine and histidine remained essentially unchanged in pigs which were fed excess lysine. Thus, it was stated that there was little evidence for dietary lysine-arginine antagonism. Also, Henry *et al*. (83) observed that the plasma content of free arginine was increased with dietary lysine supplementation. This might be because of higher expression of cationic AA transporter CAT-1 in the small intestine (84). Taken together, it is evident that pigs can tolerate very high levels of arginine in the diet (57, 85), because this AA is extensively degraded by the small intestine (86).

Besides the arginine-lysine antagonism, another classical example of AA interactions is the interaction amongst three branched-chain AAs (87, 88), and this interaction could further affect lysine absorption (84) and methionine metabolism (89). Garcia-Villalobos *et al*. (84) tried to explain some complexity in the absorption and serum concentrations of AAs. They assumed that typical swine diets formulated to meet the requirement of lysine contain excess leucine, which may depress the absorption of lysine, causing depression in the performance of pigs. The principle behind this assumption was that both the levels and the sources of leucine in practical swine diets affect the expression of cationic AA transporters and hence the availability of cationic AAs for the animal. They hypothesized that reducing the dietary protein content, coupled with adequate supplementation of crystalline lysine, threonine, and methionine may correct the leucine excess. In the study of Garcia-Villalobos *et al*. (84), the serum concentrations of lysine and threonine were around 24- and 4-fold higher, respectively, in pigs fed the supplemental diet (with a lower crude protein level and supplemented with crystalline lysine, threonine, and methionine) than in pigs fed the basal diet. Their results indicated that the dietary lysine : leucine ratio affects the expression of cationic AA transporters and, therefore, affects the serum AA profile.

### 5.3. Dietary change in the level of single amino acid

When a diet is deficient in one AA, a dietary addition in the amount of that AA does not always increase the plasma concentration of that AA. The previous studies by Morrison *et al*. (90) with rats, Zimmerman and Scott (91) with chicks, and Mitchell *et al*. (19) with pigs have shown that when an AA is added in graded levels to a diet deficient in that AA, the plasma concentration of that AA remains rather low and constant until its dietary requirement is reached. A rapid and approximately linear increase in the plasma concentration of one AA can only be achieved when a higher or super-optimal level of that AA is fed.

Lysine is usually the most deficient AA in typical cereal based diets for rats, chicks, and swine. Dietary lysine alone can alter the overall plasma AA profile (61, 79, 90), which can have a series of interconnected consequences in animal health and productivity. Morrison *et al*. (90) measured the plasma free lysine concentrations in growing female rats after giving them a lysine-deficient diet supplemented with graded levels of lysine. The plasma free lysine level, after an initial lag, rose rapidly in response to the addition of lysine to the diet, and reached a maximum at a dietary concentration of approximately 1.0%. The plasma free threonine concentration showed a reciprocal relationship with that of lysine. It was concluded that the alleviated concentration of plasma threonine during higher lysine supply was due to the higher demand of threonine for protein synthesis during lysine adequacy. The lysine : threonine ratios for the lysine deficient diet, the lysine adequate diet, and the lysine surplus diet were found to be less than 1, around 1, and more than 1, respectively. Thus, the measurement of the ratio between plasma free lysine and free threonine concentrations may provide a sensitive indication of dietary lysine adequacy.

Zimmerman and Scott (91) reported that a dietary deficiency of lysine in chicks resulted in increased plasma concentrations of several AAs, notably, threonine, histidine, tyrosine, phenylalanine, leucine, and isoleucine. The plasma lysine started to accumulate at a rapid rate only when the level of dietary lysine was approximately 10% in excess of that required to maximize weight gain. This was probably due to the inability of the body to further metabolize the excess lysine. The plasma pattern of arginine and valine concentrations reflected the same basic characteristics as observed for lysine.

In the experiment of Dean and Scott (80), diets containing suboptimal levels of lysine resulted in a marked decrease in lysine and increases in most other AAs in chicken plasma. An excess of dietary lysine resulted in a striking increase in plasma lysine concentration and relatively small changes in other AAs. However, arginine, glutamic acid, and a mixture of glutamine and asparagine appeared to be decreased as a result of the excess lysine. Similar results were also obtained by Mitchell *et al*. (19) from studies involving baby pigs, in which the plasma free lysine started to increase greatly when the level of dietary lysine provided the highest nitrogen retention.

Also, in the experiment of Corzo *et al*. (92), who fed different levels of dietary lysine to male broilers, increasing the level of dietary lysine increased plasma concentration of lysine, but almost all other AAs tended to remain at constant concentrations in plasma except for arginine and threonine, which were found to be decreased upon increasing the lysine level. This result, however, was not supported by other findings, almost all of which showed direct changes in many more AAs due to dietary lysine (80, 90, 91). More recently, Mahdavi *et al*. (93) demonstrated a directly proportional relationship between dietary lysine supply and plasma lysine concentration in broilers, and Regmi *et al*. (61) also demonstrated a similar relationship in finishing pigs (Figure 2).

Braude *et al*. (53) studied growing pigs fed diets based on cereals and groundnut meal and supplemented with graded levels of lysine. They found that the concentration of plasma lysine increased linearly over a wide range of dietary lysine contents, but the concentrations of most other AAs were largely unaffected, except for arginine and leucine. The plasma concentrations of arginine and leucine were positively correlated with the dietary lysine contents. Roy *et al*. (60) studied the plasma AA profiles of growing barrows fed diets that were deficient, adequate, and excess in lysine, and found that the plasma lysine concentration linearly increased as the dietary lysine level increased, while the plasma concentrations of isoleucine, taurine, threonine, and valine linearly decreased. In pigs fed a diet either deficient in or with an excess of lysine, the plasma concentration of histidine decreased, but that of serine increased. The plasma concentrations of all other AAs were unaffected. This finding supported the result of Zimmerman and Scott (91) and indicated a distinct pattern of AA concentration change in plasma in response to dietary lysine level.

More recently, Zeng *et al*. (94) studied the serum concentrations of various AAs of growing pigs fed three different diets with 0.65%, 0.95% and 1.25% lysine. It was observed that the serum lysine concentration was higher in the pigs fed the 0.95% than the 1.25% lysine; meanwhile the glutamic acid concentration was higher in pigs fed the 0.95% than the 0.65% lysine. In contrast, the serine concentration was lower with the 0.65% lysine diet than with the other diets, and the threonine concentration was lower with the 0.95% than with the 0.65% lysine diet. The 1.25%

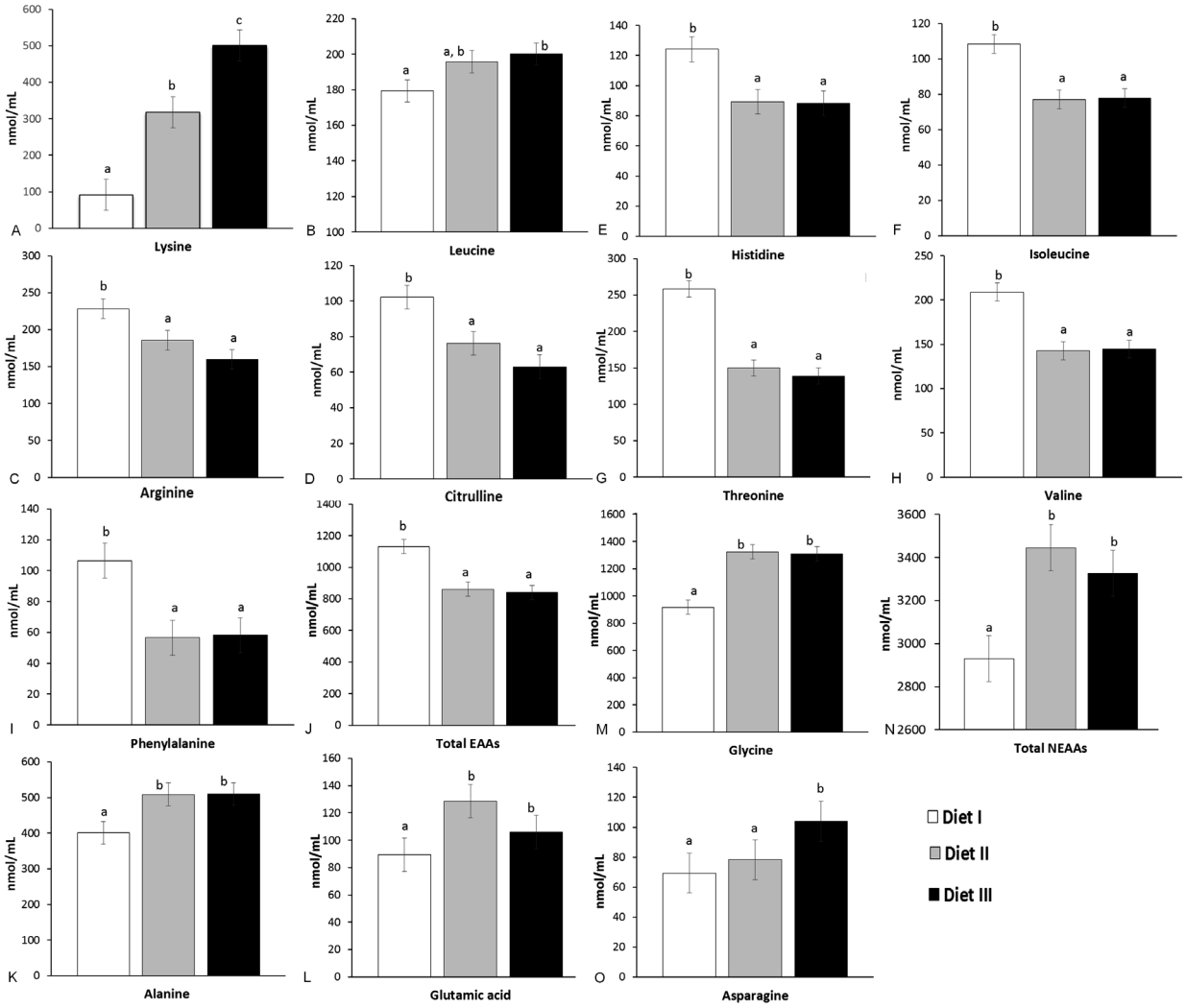


**Figure 2.** Patterns of blood plasma amino acid (AA) concentration change in finishing pigs in response to different levels of dietary lysine in a corn and soybean meal based diet. Diets I, II, and III were lysine-deficient, lysine-adequate, and lysine-excess diets, respectively. The total lysine concentrations in diets I, II, and III were 0.43%, 0.71%, and 0.98% (as-fed basis), respectively. EAAs = traditionally classified essential AAs, and NEAAs = traditionally classified nonessential AAs. The plasma concentrations of 11 other AAs (methionine, tryptophan, aspartic acid, β-alanine, cysteine, glutamine, ornithine, proline, serine, tyrosine and, taurine) were not statistically different (data not shown) among the three pig groups fed diets I, II, and III. Bars labelled without same letters (i.e., a, b, and c) differ ($P < 0.05$). Data were adopted from Regmi *et al*. (61). A. Lysine, B. Leucine, C. Arginine, D. Citrulline, E. Histidine, F. Isoleucine, G. Threonine, H. Valine, I. Phenylalanine, J. total EAAs, K. Alanine, L. Glutamic acid, M. Glycine, N. total NEAAs, and O. Asparagine.

lysine group exhibited a lower tyrosine concentration compared to the other two groups. The concentrations of most other AAs were lower in the 0.95% lysine group than in the other two groups, with the exception of alanine, aspartic acid, histidine, and leucine.

A similar type of plasma AA pattern was observed by Morales *et al*. (95) when they fed two different levels of dietary lysine (lysine deficient and lysine adequate) to growing pigs. They observed the effect of different levels of dietary lysine on plasma AA concentrations and found serum lysine to be higher and serum valine to be lower in the pigs fed the lysine adequate diet. In a study with late-stage finishing pigs, Regmi *et al*. (61) observed similar results in their studies of the alteration of the plasma AA profile in pigs fed different levels of dietary lysine. Specifically, the concentrations of arginine, citrulline, histidine, isoleucine, threonine, valine, phenylalanine, and total non-synthesizable AAs were decreased with the lysine-adequate diet (probably because of the increased tissue protein synthesis), but were not further decreased with the lysine-excess diet when compared to the lysine-deficient group (Figure 2), whereas the plasma concentrations of 3 synthesizable AAs, alanine, glutamic acid, and glycine, as well as the plasma concentrations of total synthesizable AAs, were increased in the lysine-adequate or -excess group (Figure 2). However, contrary to the findings of Morrison *et al*. (88) who observed an initial lag

in plasma lysine, Regmi *et al*. (61) found that the plasma lysine increased almost linearly without any initial lag as dietary lysine was increased. A possible reason for the discrepancy might be that at least 5 levels of dietary lysine were fed to rats by Morrison *et al*. (90), whereas only 3 levels of dietary lysine were fed to pigs by Regmi *et al*. (61), which could make it difficult to identify the initial lag in the pigs. They also demonstrated that most of the essential AAs showed a reciprocal relationship with dietary lysine, which is in agreement with findings of Dean and Scott (80), Morrison *et al*. (90), and Zimmerman and Scott (91). However, in terms of plasma concentration of glutamic acid, the findings of Regmi *et al*. (61) are not consistent with those of Dean and Scott (80), in that the former found increased plasma concentration with increased dietary lysine, whereas the latter found a decrease. Compared to the findings of Zeng *et al*. (94), Regmi *et al*. (61) observed similar patterns for plasma glutamic acid, threonine, and most of other AAs except for serine, the concentration of which was the lowest in the lysine-deficient group. This may be because nearly all of the dietary glutamic acid is extensively degraded in the small intestine of pigs (96). Thus, dietary supplementation with 0.5 to 4% monosodium glutamate does not appreciably affect glutamate concentrations in the plasma of pigs (97).

Why the concentrations of some AAs, but not others, are affected by lysine deficiency or excess could be attributed to multiple factors. Theoretically, when one AA is deficient in a diet, the metabolic demand for that AA cannot met. Thus, that AA would be present at a low concentration in the plasma until its dietary requirement is met. While gradually increasing the level of that AA, a stage will come when the plasma concentration of that AA starts to increase and that of other AAs to decrease (due to the increased rate of protein synthesis) (5). This corresponding dietary level should be considered as an optimum level of that AA for utilization by the animal. In principle, every AA should have an optimum level in a diet. It is expected that beyond this optimum level, the plasma concentrations of many but not all AAs would rise in the plasma until a certain time point when the excess AA is all catabolized.

## 6. CONCLUSIONS

One ultimate task of swine nutritionists is to maximize the rate of muscle protein accretion via maintaining an optimal plasma AA profile through dietary AA supplies. Comparing the plasma AA concentrations with the growth performance data of pigs can help to elucidate the metabolic mechanisms regarding the regulation of the plasma AA homeostasis, the nutrient utilization for muscle growth, as well as the cellular protein turnover process. Knowledge about plasma AA profile that is regulated by a single AA, or by multiple AAs in concert, can offer useful references to predict the availabilities of AAs and the order of limiting AAs in a diet, and to provide a useful insight into pig's whole body status of protein metabolism.

Lysine, for example, is usually the first limiting AA in almost all practical swine diets; however, the currently available knowledge is still not sufficient to draw a clear conclusion about the complex relationship between dietary lysine supply and the plasma AA profile. Thorough understanding of the effect of dietary AA supply on the plasma AA concentrations can help animal nutritionists to develop novel nutritional strategies to manage swine plasma AA profile via dietary AA supplies. In this regard, further research is still needed to study how different levels of dietary AAs, individually or in concert, affect the plasma concentrations of all AAs and the related nutrient metabolites.

## 7. ACKNOWLEDGMENTS

This material is based upon the research work supported by a Hatch/Multistate Project (under No. 1007691) funded through the USDA National Institute of Food and Agriculture, by two Agriculture and Food Research Initiative Competitive Grants (2014-67015-21770 and 2015-67015-23276) funded by the USDA National Institute of Food and Agriculture, and by the Texas A&M AgriLife Research (H-8200). Some technical assistance during manuscript preparation from Md Shamimul Hasan, a graduate student in the Department of Animal and Dairy Sciences, Mississippi State University, is highly appreciated.

## 8. REFERENCES

1. Wu, G., F. W. Bazer, and H. R. Cross: Land-based production of animal protein: impacts, efficiency, and sustainability. *Ann NY Acad Sci* 1328, 18–28 (2014) DOI: 10.1111/nyas.12566

2. Adesehinwa, A. O. K., E. O. Akinfala, A. J. Omole, B. A. Makanjuola: Mitigating challenges in pig production and marketing: proceeding of the 1st Nigerian institutional pig summit. Nigerian Institute of Animal Science, Abuja, Nigeria (2010).

3. Liao, S. F., T. Wang, and N. Regmi: Lysine nutrition in swine and the related monogastric animals: muscle protein biosynthesis and beyond. *SpringerPlus* 4, 147 (2015) DOI: 10.1186/s40064-015-0927-5

4. Aberle, E. D., J. C. Forrest, D. E. Gerrard, and E. W. Mills: Principles of meat science – Structure and composition of animal tissues. Kendall/Hunt, Dubuque, Iowa (2001)

5. Wu, G.: Amino acids: biochemistry and nutrition, CRC Press, Boca Raton, FL (2013) DOI: 10.1201/b14661

6. Wagner, I., and H. Musso: New naturally occurring amino acids. *Angew Chem Int Ed Engl* 22, 816–828 (1983) DOI: 10.1002/anie.198308161

7. Wu, G., H. R. Cross, K. B. Gehring, J. W. Savell, A. N. Arnold, and S. H. McNeill: Composition of free and peptide-bound amino acids in beef chuck, loin, and round cuts. *J Anim Sci* 94, 2603–2613 (2016) DOI: 10.2527/jas.2016-0478

8. Lewis, A. J.: Amino acids in swine nutrition. In: Swine Nutrition. Eds: AJ Lewis, LL Southern. CRC Press, Boca Raton, FL. 131–150 (2001)

9. NRC: Proteins and amino acids. In: Nutrient Requirements of Swine, 11th rev. ed., The National Academies Press, Washington, DC 15–44 (2012)

10. Johansson, L., G. Gafvelin, and E. S. J. Arnér: Selenocysteine in proteins – properties and biotechnological use. *Biochim Biophys Acta, Gen Sub* 1726, 1–13 (2005) DOI: 10.1016/j.bbagen.2005.05.010

11. Hou, Y., and G. Wu: Nutritionally nonessential amino acids: a misnomer in nutritional sciences. *Adv Nutr* 8, 137–139 (2017) DOI: 10.3945/an.116.012971

12. Reeds, P. J., A. Cadenhead, M. F. Fuller, G. E. Lobley, and J. D. McDonald: Protein turnover in growing pigs. Effects of age and food intake. *Br J Nutr* 43, 445–455 (1980) DOI: 10.1079/BJN19800112

13. Waterlow, J. C: Whole-body protein turnover in humans – past, present, and future. *Annu Rev Nutr* 15, 57–92 (1995) DOI: 10.1146/annurev.nu.15.070195.000421

14. Wu, G., F. W. Bazer, Z. L. Dai, D. F. Li, J. J. Wang, and Z. L. Wu: Amino acid nutrition in animals: protein synthesis and beyond. *Annu Rev Anim Biosci* 2, 387–417 (2014) DOI:10.1146/annurev-animal-022513-114113

15. Waterlow, J. C.: The assessment of protein nutrition and metabolism in the whole animal, with special reference to man. In: Mammalian Protein Metabolism (III). Eds: H. N. Munro, Academic Press, New York, NY. 326–390 (1969) DOI: 10.1016/B978-1-4832-3211-9.50014-0

16. Gustafson, J. M., S. J. Dodds, R. C. Burgus, and L. P. Mercer: Prediction of brain and serum free amino acid profiles in rats fed graded levels of protein. *J Nutr* 116, 1667–1681 (1986)

17. Abumrad, N. N., and B. Miller: The physiologic and nutritional significance of plasma-free amino acid levels. *J Parenter Enteral Nutr* 7, 163–170 (1983) DOI: 10.1177/0148607183007002163

18. McLaughlan, J. M., and W. I. Illman: Use of free plasma amino acid levels for estimating amino acid requirements of the growing rat. *J Nutr* 93, 21–24 (1967)

19. Mitchell, J. R., D. E. Becker, A. H. Jensen, B. G. Harmon, and H. W. Norton: Determination of amino acid needs of the young pig by nitrogen balance and plasma free amino acids. *J Anim Sci* 27, 1327–1331 (1968) DOI: 10.2527/jas1968.2751327x

20. Fernandez-Figares, I., C. Prieto, R. Nieto, and J. F. Aquilera: Free amino acid concentrations in plasma, muscle, and liver as indirect measures of protein adequacy in growing chickens. *Ani Sci* 64, 529–539 (1997) DOI: 10.1017/S1357729800016167

21. Hou, Y. Q., Y. L. Yin, and G. Wu: Dietary essentiality of “nutritionally nonessential amino acids” for animals and humans. *Exp. Biol. Med* 240, 997–1007 (2015) DOI: 10.1177/1535370215587913

22. Hou, Y. Q., K. Yao, Y. L. Yin, and G. Wu: Endogenous synthesis of amino acids limits growth, lactation and reproduction of animals. *Adv. Nutr* 7, 331–342 (2016) DOI: 10.3945/an.115.010850

23. Cynober, L. A.: Plasma amino acid levels with a note on membrane transport: characteristics, regulation, and metabolic significance. *Nutrition* 18, 761–766 (2002) DOI: 10.1016/S0899-9007(02)00780-3

24. Guyton, A. C., and J. E. Hall: Textbook of Medical Physiology. 11th ed. Elsevier Saunders, Philadelphia, PA (2006)

25. Li, P., Y. L. Yin, D. F. Li, S. W. Kim, and G. Wu: Amino acids and immune function. *Br. J. Nutr* 98, 237–252 (2007)
DOI: 10.1017/S000711450769936X

26. Shikata, N., Y. Maki, Y. Noguchi, M. Mori, T. Hanai, M. Takahashi, and M. Okamoto: Multi-layered network structure of amino acid (AA) metabolism characterized by each essential AA-deficient condition. *Amino Acids* 33, 113–121 (2007)
DOI: 10.1007/s00726-006-0412-0

27. Nyachoti, C. M., C. F. M. deLange, B. W. McBride, and H. Schulze: Significance of endogenous gut nitrogen losses in the nutrition of growing pigs: A review. *Can J Anim Sci* 77, 149–163 (1997)
DOI: 10.4141/A96-044

28. Bergen, W. G., and G. Wu: Intestinal nitrogen recycling and utilization in health and disease. *J Nutr* 139, 821–825 (2009)
DOI: 10.3945/jn.109.104497

29. Wu, G., Z. L. Wu, Z. L. Dai, Y. Yang, W. W. Wang, C. Liu, B. Wang, J. J. Wang and Y. L. Yin: Dietary requirements of "nutritionally nonessential amino acids" by animals and humans. *Amino Acids* 44, 1107–1113 (2013)
DOI: 10.1007/s00726-012-1444-2

30. Matthews, J. C: Amino acid and peptide transport systems. In: Farm Animal Metabolism and Nutrition. Eds: J. P. F. D'Mello, CAB International, Wallingford, UK. 3–23 (2000)
DOI: 10.1079/9780851993782.0003

31. Yen, J. T., B. J. Kerr, R. A. Easter, and A. M. Parkhurst: Difference in rates of net portal absorption between crystalline and protein-bound lysine and threonine in growing pigs fed once daily. *J Anim Sci* 82, 1079–1090 (2004)
DOI: 10.2527/2004.8241079x

32. Bertolo, R. F. P., P. B. Pencharz, and R. O. Ball: Role of intestinal first-pass metabolism on whole-body amino acid requirements. In: Biology of Metabolism of Growing Animals. Eds: DG Burrin, HJ Mersmann. New York, NY, Elsevier. 127–156 (2005)
DOI: 10.1016/S1877-1823(09)70013-X

33. Stoll, B. and D. G. Burrin: Measuring splanchnic amino acid metabolism *in vivo* using stable isotopic tracers. *J Anim Sci* 84 (Suppl.), E60-E72 (2006)
DOI: 10.2527/2006.8413_supplE60x

34. Wu, G., D. A. Knabe, and N. E. Flynn: Amino acid metabolism in the small intestine: biochemical bases and nutritional significance. In: Biology of Metabolism of Growing Animals. Eds: DG Burrin, HJ Mersmann. New York, NY, Elsevier. 107–126 (2005)
DOI: 10.1016/S1877-1823(09)70012-8

35. Dai, Z. L., G. Wu, and W. Y. Zhu: Amino acid metabolism in intestinal bacteria: links between gut ecology and host health. *Front Biosci* 16, 1768–1786 (2011)
DOI: 10.2741/3820

36. Yang, Y. X., Z. L. Dai, and W. Y. Zhu: Important impacts of intestinal bacteria on utilization of dietary amino acids in pigs. *Amino Acids* 46, 2489–2501 (2014)
DOI: 10.1007/s00726-014-1807-y

37. Dai, Z. L., J. Zhang, G. Wu, and W. Y. Zhu: Utilization of amino acids by bacteria from the pig small intestine. *Amino Acids* 39, 1201–1215 (2010)
DOI: 10.1007/s00726-010-0556-9

38. Brosnan, J. T: Interorgan amino acid transport and its regulation. *J Nutr* 133, 2068S-2072S (2003)

39. Wu, G: Urea synthesis in enterocytes of developing pigs. *Biochem J* 312, 717–723 (1995)
DOI: 10.1042/bj3120717

40. Yuan, Y., X. Wu, Q. Ou, J. Gao, B. C. Tennant, W. Han, and Y. Yu: Differential expression of the genes involved in amino acids and nitrogen metabolisms during liver regeneration of mice. *Hepatol Res* 39, 301–312 (2009)
DOI: 10.1111/j.1872-034X.2008.00456.x

41. Wu, G., and S. M. Morris, Jr: Arginine metabolism: nitric oxide and beyond. *Biochem J* 336, 1–17 (1998)
DOI: 10.1042/bj3360001

42. Wang, W. W., Z. L. Wu, Z. L. Dai, Y. Yang, J. J. Wang, and G. Wu: Glycine metabolism in animals and humans: implications for nutrition and health. *Amino Acids* 45, 463–477 (2013)
DOI: 10.1007/s00726-013-1493-1

43. Yang, Y., Z. L. Wu, S. C. Jia, S. Dahanayaka, S. Feng, C. J. Meininger, C. J. McNeal, and G. Wu: Safety of long-term dietary supplementation with L-arginine in rats. *Amino Acids* 47, 1907–1920 (2015)
DOI: 10.1007/s00726-015-1992-3

44. Hou, Y. Q., S. D. Hu, S. C. Jia, G. Nawaratna, D. S. Che, F. L. Wang, F. W. Bazer, and G. Wu: Whole-body synthesis of L-homoarginine in pigs and rats supplemented with L-arginine. *Amino Acids* 48, 993–1001 (2016) DOI: 10.1007/s00726-015-2145-4

45. Frame, E. G: The levels of individual free amino acids in the plasma of normal man at various intervals after a high-protein meal. *J Clin Invest* 37, 1710–1723 (1958) DOI: 10.1172/JCI103763

46. Garibotto, G., A. Sofia, S. Saffioti, A. Bonanni, I. Mannucci, and D. Verzola: Amino acid and protein metabolism in the human kidney and in patients with chronic kidney disease. *Clin Nutr* 29, 424–433 (2010) DOI: 10.1016/j.clnu.2010.02.005

47. Kuhlmann, M. K., and J. D. Kopple: Amino acid metabolism in the kidney. *Semin Nephrol* 10, 445–457 (1990)

48. Goldberg, A. L., and T. W. Chang: Regulation and significance of amino acid metabolism in skeletal muscle. *Fed Proc* 37, 2301–2307 (1978)

49. Rezaei, R., W. W. Wang, Z. L. Wu, Z. L. Dai, J. J. Wang, and G. Wu: Biochemical and physiological bases for utilization of dietary amino acids by young pigs. *J Anim Sci Biotechnol* 4, 7 (2013) DOI: 10.1186/2049-1891-4-7

50. Wu, G: Dietary requirements of synthesizable amino acids by animals: A paradigm shift in protein nutrition. *J Anim Sci Biotechnol* 5, 34 (2014) DOI: 10.1186/2049-1891-5-34

51. Windels, H. F., R. J. Meade, J. W. Nordstrom, and W. L. Stockland: Influence of source and level of dietary protein on plasma free amino acid concentrations in growing swine. *J Anim Sci* 32, 268–276 (1971) DOI: 10.2527/jas1971.322268x

52. Davey, R. J., J. W. Phelps, and C. H. Thomas: Plasma free amino acids of swine as influenced by diet protein level, animal age and time of sampling. *J Anim Sci* 37, 81–86 (1973) DOI: 10.2527/jas1973.37181x

53. Braude, R., R. J. Fulford, K. G. Mitchell, and A. W. Myres: Performance and blood plasma amino acid and urea concentrations in growing pigs given diets of cereals and groundnut meal and supplemented with graded amounts of L-lysine. *Livest Prod Sci* 1, 383–400 (1974) DOI: 10.1016/0301-6226(74)90068-2

54. Johnson, D. J., and G. H. Anderson: Prediction of plasma amino acid concentration from diet amino acid content. *Am J Physiol Physiol* 243, R99–103 (1982)

55. Typpo, J. T., R. J. Meade, J. W. Nordstrom, and W. L. Stockland: Influence of time of fast on concentrations of free amino acids in plasma and total amino acids in liver protein of young swine. *J Anim Sci* 31, 885–893 (1970) DOI: 10.2527/jas1970.315885x

56. Stockland, W. L., R. J. Meade, M. E. Tumbleson, and B. W. Palm: Influence of site of sampling and stage of fast on concentrations of all free amino acids in the plasma and liver in the young pig. *J Anim Sci* 32, 1143–1152 (1971) DOI: 10.2527/jas1971.3261143x

57. Hu, S. D., X. L. Li, R. Rezaei, C. J. Meininger, C. J. McNeal, and G. Wu: Safety of long-term dietary supplementation with L-arginine in pigs. *Amino Acids* 47, 925–936 (2015) DOI: 10.1007/s00726-015-1921-5

58. Sun, Y. L., Z. L. Wu, W. Li, C. Zhang, K. J. Sun, Y. Ji, B. Wang, N. Jiao, B. B. He, W. W. Wang, Z. L. Dai, and G. Wu: Dietary L-leucine supplementation enhances intestinal development in suckling piglets. *Amino Acids* 47, 1517–1525 (2015) DOI: 10.1007/s00726-015-1985-2

59. Figueroa, J. L., A. J. Lewis, P. S. Miller, R. L. Fischer, and R. M. Diedrichsen: Growth, carcass traits, and plasma amino acid concentrations of gilts fed low-protein diets supplemented with amino acids including histidine, isoleucine, and valine. *J Anim Sci* 81, 1529–1537 (2003) DOI: 10.2527/2003.8161529x

60. Roy, N., H. Lapierre, and J. F. Bernier: Whole-body protein metabolism and plasma profiles of amino acids and hormones in growing barrows fed diets adequate or deficient in lysine. *Can J Anim Sci* 80, 585–595 (2000) DOI: 10.4141/A98-057

61. Regmi, N., T. Wang, M. A. Crenshaw, B. J. Rude, G. Wu, and S. F. Liao: Effects of

dietary lysine levels on plasma free amino acid profile in late-stage finishing pigs. *SpringerPlus* 5, 888 (2016)
DOI: 10.1186/s40064-016-2463-3

62. Adibi, S. A., and D. W. Mercer: Protein digestion in human intestine as reflected in luminal, mucosal, and plasma amino acid concentrations after meals. *J Clin Invest* 52, 1586–1594 (1973)
DOI: 10.1172/JCI107335

63. McLaughlan, J. M: Relationship between protein quality and plasma amino acid levels. *Fed Proc* 22, 1122–1125 (1963)

64. Munro, H. N., and J. B. Allison: Mammalian Protein Metabolism (I). Academic Press, New York, NY (1964)

65. Wang, W. W., Z. L. Dai, Z. L. Wu, G. Lin, S. C. Jia, S. D. Hu, S. Dahanayaka, and G. Wu: Glycine is a nutritionally essential amino acid for maximal growth of milk-fed young pigs. *Amino Acids* 46, 2037–2045 (2014)
DOI: 10.1007/s00726-014-1758-3

66. He, L. Q., L. Wu, Z. Q. Xu, T. J. Li, K. Yao, Z. J. Cui, Y. L. Yin, and G. Wu: Low-protein diets affect ileal amino acid digestibility and gene expression of digestive enzymes in growing and finishing pigs. *Amino Acids* 48, 21–30 (2016)
DOI: 10.1007/s00726-015-2059-1

67. Liu, Y., X. Kong, F. Li, B. Tan, Y. Li, Y. Duan, Y. Yin, J. He, C. Hu, F. Blachier, and G. Wu: Co-dependence of genotype and dietary protein intake to affect expression of amino acid/peptide transporters in porcine skeletal muscle. *Amino Acids* 48, 75–90 (2016)
DOI: 10.1007/s00726-015-2066-2

68. Fuller, M. F., P. J. Reeds, A. Cadenhead, B. Seve, and T. Preston: Effects of the amount and quality of dietary protein on nitrogen metabolism and protein turnover of pigs. *Br J Nutr* 58, 287–300 (1987)
DOI: 10.1079/BJN19870096

69. Harper, A. E., N. J. Benevenga, and R. M. Wohlheuter: Effects of ingestion of disproportionate and amount of amino acids. *Physiol Rev* 50, 428–558 (1970)

70. Southern, L. L., and D. H. Baker: Performance and concentration of amino acids in plasma and urine of young pigs fed diets with excesses of either arginine or lysine. *J Anim Sci* 55, 857–866 (1982)
DOI: 10.2527/jas1982.554857x

71. Hagemeier, D. L., G. W. Libal, and R. C. Wahlstrom: Effects of excess arginine on swine growth and plasma amino acid levels. *J. Anim. Sci* 57: 99–105 (1983)
DOI: 10.2527/jas1983.57199x

72. Li, X. L., F. W. Bazer, G. A. Johnson, R. C. Burghardt, D. W. Erikson, J. W. Frank, T. E. Spencer, I. Shinzato, and G. Wu: Dietary supplementation with 0.8% L-arginine between days 0 and 25 of gestation reduces litter size in gilts. *J Nutr* 140, 1111–1116 (2010)
DOI: 10.3945/jn.110.121350

73. Li, X. L., F. W. Bazer, G. A. Johnson, R. C. Burghardt, J. W. Frank, Z. L. Dai, J. J. Wang, Z. L. Wu, I. Shinzato, and G. Wu: Dietary supplementation with L-arginine between days 14 and 25 of gestation enhances embryonic development and survival in gilts. *Amino Acids* 46, 375–384 (2014)
DOI: 10.1007/s00726-013-1626-6

74. Mateo, R. D., G. Wu, F. W. Bazer, J. C. Park, I. Shinzato, and S. W. Kim: Dietary L-arginine supplementation enhances the reproductive performance of gilts. *J Nutr* 137, 652–656 (2007)

75. Jones, J. D: Lysine–arginine antagonism in the chick. *J Nutr* 84, 313–321 (1964)

76. Jones, J. D., S. J. Petersburg, and P. C. Burnett: The mechanism of the lysine-arginine antagonism in the chick: effect of lysine on digestion, kidney arginase, and liver transamidinase. *J Nutr* 93, 103–116 (1967)

77. Boorman, K. N., and H. Fisher: The arginine–lysine interaction in the chick. *Br Poult Sci* 7, 39 (1966)
DOI: 10.1080/00071666608415604

78. D'Mello, J. P. F., and D. Lewis: Amino acid interactions in chick nutrition. I. The interrelationship between lysine and arginine. *Br Poult Sci* 11, 299 (1970)
DOI: 10.1080/00071667008415820

79. Yun, C. H., I. K. Han, Y. J. Choi, B. C. Park, and H. S. Lee: Effects of lysine and sodium on the growth performance, bone parameter, serum composition and lysine–arginine

antagonism in broiler chicks. *Asian-Aust J Anim Sci* 4, 53–360 (1991)
DOI: 10.5713/ajas.1991.353

80. Dean, W. F., and H. M. Scott: Use of free amino acid concentrations in blood plasma of chicks to detect defeciencies and excesses of dietary amino acids. *J Nutr* 88, 75 (1966)

81. Anderson, L. C., A. J. Lewis, E. R. Peo, and J. D. Crenshaw: Effects of excess arginine with and without supplemental lysine on performance, plasma amino acid concentrations and nitrogen balance of young swine. *J Anim. Sci* 58, 369–377 (1984)
DOI: 10.2527/jas1984.582369x

82. Edmonds, M. S., and D. H. Baker: Failure of excess dietary lysine to antagonize arginine in young pigs. *J Nutr* 117, 1396–1401 (1987)

83. Henry, Y., Y. Colléaux, and B. Sève: Effects of dietary level of lysine and of level and source of protein on feed intake, growth performance, and plasma amino acid pattern in the finishing pig. *J Anim Sci* 70, 188–195 (1992)
DOI: 10.2527/1992.701188x

84. Garcia-Villalobos, H., A. Morales-Trejo, B. A. Araiza-Pina, J. K. Htoo, and M. Cervantes-Ramirez: Effects of dietary protein and amino acid levels on the expression of selected cationic amino acid transporters and serum amino acid concentration in growing pigs. *Arch Anim Nutr* 66, 257–270 (2012)
DOI: 10.1080/1745039X.2012.697351

85. Wu, G., F. W. Bazer, T. A. Cudd, W. S. Jobgen, S. W. Kim, A. Lassala, P. Li, J. H. Matis, C. J. Meininger, and T. E. Spencer: Pharmacokinetics and safety of arginine supplementation in animals. *J Nutr* 137, 1673S-1680S (2007)

86. Wu, Z. L., Y. Q. Hou, S. D. Hu, F. W. Bazer, C. J. Meininger, C. J. McNeal, and G. Wu: Catabolism and safety of supplemental L-arginine in animals. *Amino Acids* 48, 1541–1552 (2016)
DOI: 10.1007/s00726-016-2245-9

87. Taylor, S. J., D. J. A. Cole, and D. Lewis: Amino acid requirements of growing pigs: 5. The interaction between isoleucine and leucine. *Anim Prod* 38, 257–261 (1984)
DOI: 10.1017/S0003356100002257

88. Wiltafsky, M. K., M. W. Pfaffl, and F. X. Roth: The effects of branched-chain amino acid interactions on growth performance, blood metabolites, enzyme kinetics and transcriptomics in weaned pigs. *Br J Nutr* 103, 964–976 (2010)
DOI: 10.1017/S0007114509992212

89. Langer, S., and M. F. Fuller: Interactions among the branched-chain amino acids and their effects on methionine utilization in growing pigs: effects on nitrogen retention and amino acid utilization. *Br J Nutr* 83, 43–48 (2000)
DOI: 10.1017/S0007114500000088

90. Morrison, A. B., E. J. Middleton, and J. M. McLaughlan: Blood amino acid studies: II. Effects of dietary lysine concentration, sex, and growth rate on plasma free lysine and threonine levels in the rat. *Can J Biochem Physiol* 39, 1675–1680 (1961)
DOI: 10.1139/o61-185

91. Zimmerman, R. A., and H. M. Scott: Interrelationship of plasma amino acid levels and weight gain in chicks as influenced by suboptimal and super optimal dietary concentrations of single amino acids. *J Nutr* 87, 13 (1965)

92. Corzo, A., E. T. Moran, and D. Hoeler: Lysine needs of summer-reared male broilers from six to eight weeks of age. *Poult Sci* 82, 1602–1607 (2003)
DOI: 10.1093/ps/82.10.1602

93. Mahdavi, A., M. Shivazad, F. Alemi, M. Zaghari, H. Moravej, and B. Darabighane: Digestible lysine requirement of broilers based on practical diet. *Ital J Anim Sci* 11, e13 (2012)
DOI: 10.4081/ijas.2012.e13

94. Zeng, P. L., H. C. Yan, X. Q. Wang, C. M. Zhang, C. Zhu, G. Shu, and Q. Y. Jiang: Effects of dietary lysine levels on apparent nutrient digestibility and serum amino acid absorption mode in growing pigs. *Asian-Aust J Anim Sci* 26, 1003–1011 (2013)
DOI: 10.5713/ajas.2012.12555

95. Morales, A., H. García, N. Arce, M. Cota, R. T. Zijlstra, B. A. Araiza, M. Cervantes: Effect of L-lysine on expression of selected genes, serum concentration of amino acids, muscle growth and performance of growing pigs. *J Anim Physiol Anim Nutr* 99, 701–709 (2015)
DOI: 10.1111/jpn.12267

96. Wu, G: Intestinal mucosal amino acid catabolism. *J Nutr* 128, 1249–1252 (1998)

97. Rezaei, R., D. A. Knabe, C. D. Tekwe, S. Dahanayaka, M. D. Ficken, S. E. Fielder, S. J. Eide, S. L. Lovering, and G. Wu: Dietary supplementation with monosodium glutamate is safe and improves growth performance in postweaning pigs. *Amino Acids* 44, 911–923 (2013)
DOI: 10.1007/s00726-012-1420-x

**Key Words:** Amino acid, Blood plasma, Homeostatic regulation, Dietary supply, Swine, Review

**Send correspondence to:** Shengfa F. Liao, Department of Animal and Dairy Sciences, Mississippi State University, Mississippi State, MS 39762-9815, USA, Tel: 662-325-7318, Fax: 662-325-8873, E-mail: s.liao@msstate.edu