NUTRITIONAL INTERACTIONS OF AMINO ACIDS

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SUMMARY

Nutritional interactions of amino acids influence the efficiency with which limiting amino acids are used for growth and other productive purposes. They are responsible for the increase in requirement for essential amino acids as dietary protein increases and may confound the results of growth assays of amino acid availability. The mechanism by which these interactions occur is not fully elucidated. Imbalances, however, appear to depress food intake, thereby causing reduced performance. This is believed to be due to metabolic changes leading to altered amino acid profiles in blood which, when detected by the central nervous system, decrease appetite or feeding. The nature of the metabolic changes and the effects on the central nervous system are not known. Progress in research on these aspects of imbalance may lead to practical approaches to improving the efficiency of amino acid utilization by poultry and other monogastric species.

INTRODUCTION

Amino acid interactions have been recognized as a nutritional phenomenon since Harper 1956 first categorized amino acid interactions as imbalances, antagonisms, and toxicities. Decades of effort by several laboratories have already established the existence of the phenomenon and have provided some information on the mechanisms by which imbalances and specific antagonisms may occur. This report will review some aspects of amino acid interactions, emphasizing the mechanisms by which interactions occur, and identifying areas that merit further investigation.

PRACTICAL IMPORTANCE OF INTERACTIONS

Disregarding overt toxicities, which are unlikely to occur in practice because of the economic constraints on supplementation of practical diets with excessive levels of amino acids, imbalances and antagonisms are of practical importance. According to Harper's definition (Harper 1956; Harper et al. 1970), imbalances occur when a diet that is limiting in two amino acids is supplemented with the second limiting amino acid or with all essential amino acids, except the first limiting amino acid. This results in depression of growth (and other measures of performance) which can only be corrected by supplementation of the imbalanced diet with the first limiting amino acid. In theory this applies to diets first limiting in any essential amino acid. Thus imbalance is the most common form of interaction.

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Antagonisms have been defined as specific interactions in which the requirement for one essential amino acid, not necessarily the first limiting amino acid, is increased by the addition of a structurally related amino acid to the diet (Harper 1956; Harper et al. 1970). Two well-recognized examples are the lysine-arginine antagonism in which excess dietary lysine increases the requirement for arginine (Jones et al. 1964; O’Dell and Savage 1966; Lewis 1970) and the branched chain amino acid antagonism involving leucine, isoleucine and valine in which an excess of one (or two) branched chain amino acids increases the requirement for the other branched chain amino acid(s) (Spolter and Harper 1961). One other interaction that could be categorized as an antagonism involves methionine, glycine and arginine (Keshavarz and Fuller 1971). In this interaction excess arginine and glycine increase the requirement for methionine.

In general the occurrence of antagonisms is more limited than that of imbalances because these interactions are more specific, influencing the requirements for only a few amino acids. They are likely to occur mainly in practical diets of unusual composition. Diets based on corn, for example, contain relatively high proportions of leucine (National Research Council 1982), yet dietary levels of isoleucine or valine generally appear sufficient to prevent an antagonism. The branched chain amino acid antagonism in poultry and swine has been observed only when large supplements of leucine have been added to practical or semipurified diets (D’Mello 1974, 1975; Bray 1970; Tuttle and Balloun 1976; Henry et al. 1976). The lysine-arginine antagonism occurs readily in poultry, and it appears desirable to maintain the ratio of arginine to lysine at 1.0 or more. This antagonism may occur when ingredients of relatively low arginine content, such as milo, constitute a major portion of the dietary protein. The interaction of methionine, glycine and arginine may be of some consequence in practical diets, as methionine is frequently first limiting and the other two amino acids generally exceed the requirements.

Imbalances significantly impact upon nutrition in ways that are not always obvious. Grau and Kamei 1950 demonstrated that increased dietary protein increased the requirement for lysine and sulfur amino acids. This report in which the dietary requirement for lysine for chicks increased from 0.85% to 1.35% as the protein level increased from 20% to 40% provides a classic example of imbalance. Excess amino acids cause a growth depression that can only be overcome by providing a higher dietary level of the first limiting amino acid. Lysine imbalances were confirmed by Fisher et al. 1960. Thomas et al. 1975 determined whether lysine imbalance was of practical significance in broiler diets. They did not observe a marked shift in lysine requirement as the protein content of the diet was increased over a smaller range from 23 to 27%. Nonetheless their data suggest that the requirement increased. In practice, therefore, one should consider that the requirement for the most limiting amino acid will increase as the dietary protein content increases.

Imbalances also influence the assessment of amino acid availability by conventional growth assay. These assays involve a comparison of the growth of rats or chicks fed a basal diet limiting in the amino acid to be tested, the basal diet containing graded levels of the amino acid being tested (reference diets), or the basal diet supplemented with
graded levels of the test feedstuff. Amino acid availability is calculated from the relative slopes of the response curves for animals fed the reference and test diets. The relative performance is greatly affected by the other amino acids in the test feedstuff. When these amino acids are also included as free amino acids in the reference diet they depress the response curve of the animals fed these diets, thereby improving the relative response of animals fed the test feedstuff. The depression is due to amino acid imbalance. Baker 1978 has demonstrated the marked effect that this phenomenon has on the determination of amino acid availability by growth assay. Other examples of the impact of imbalance in practical nutrition may be cited, but the above-mentioned examples will suffice to indicate the practical importance of amino acid interactions.

**METABOLIC BASIS OF INTERACTIONS**

The metabolic nature of amino acid imbalances and antagonisms are not completely understood. However, considerable progress has been made in research on this aspect of amino acid interactions. For the purpose of this review, this research will be divided into three basic areas: (1) catabolism of the limiting amino acid, (2) studies on food intake, and (3) metabolic events leading to altered food intake.

**Role of amino acid catabolism**

Since amino acid interactions increase the requirements for amino acids, increased catabolism of the affected amino acid is one possible explanation for the altered requirement. Davis 1980 reviewed eight published studies that were conducted to test this possibility in rats or pigs subjected to imbalances. Although the specific details of the experiments differ, they are generally similar in that animals were fed control or imbalanced diets containing the limiting amino acid with a carbon-14 label. Respiratory $^{14}\text{CO}_2$ was collected from the animals fed both diets. Most of these studies involved imbalances of histidine, tryptophan or threonine. In only one of these studies was any evidence of increased catabolism of the limiting amino acid obtained. In two experiments involving threonine (Davis and Austic 1982) and isoleucine (Esteve and Austic unpublished) imbalance in chicks, no change in $^{14}\text{CO}_2$ production from $^{14}$C-threonine due to imbalance was found, and a significant, but numerically small increase in $^{14}\text{CO}_2$ production from $^{14}$C-isoleucine was observed. We conclude, therefore, that if imbalances lead to increased catabolism of the limiting amino acid, the loss of amino acid by these pathways is very small at best.

In contrast to the results with amino acid imbalances, antagonisms appear to increase the loss of the affected amino acid. Jones et al. 1967 demonstrated that chicks fed excess lysine had increased activity of kidney arginase. Austic and Nesheim 1970 confirmed this finding and demonstrated that the loss of arginine, as measured by urea excretion, was markedly increased when dietary lysine was present in excess. The loss of arginine represented nearly 30% of the ingested arginine in some instances. By use of other amino acids such as threonine or d-aminoiso-butyric acid which prevented the increase in arginase activity, and promoted growth of chicks fed excess lysine these investigators concluded that the enzyme was a major factor in the lysine-arginine antagonism. Two other metabolic factors also were found to contribute to the antagonism: depressed creatine synthesis (Jones et al. 1967, Austic and Nesheim 1971) and urinary losses of arginine (Jones et al. 1967; Nesheim 1968). Depressed creatine formation was deduced from the
reduction of liver glycine amidinotransferase activity in liver and the observation that supplementation of the diet with creatine improved the growth of chicks that were fed excess lysine. Urinary loss of arginine was presumed due to the fact that both lysine and arginine compete for a common transport system for reabsorption in the renal tubules (Webber et al. 1961; Boorman 1971). When lysine is present in large excess, up to 8-10% of the arginine intake is represented by urinary losses of arginine. With an antagonism of less severity, urinary losses appeared virtually nil (Austic and Scott 1975).

In the branched chain amino acid antagonism in which excess leucine increases the requirement for isoleucine and valine by rats and chicks, increased activities of enzymes responsible for branched chain amino acid catabolism were reported. The activity of branched chain alpha-ketoacid dehydrogenase in the liver of rats was significantly increased due to leucine (Wohlheuter and Harper 1970). In chicks, this enzyme was unaffected, but muscle branched chain amino acid transaminase was significantly increased in chicks subjected to the antagonism (Smith and Austic 1978). When diets contained l-14C-isoleucine or l-14C-valine, the amount of respiratory 14CO2 was significantly increased by excess leucine (Smith and Austic 1978; Calvert et al. 1982). Therefore this antagonism, as in the case of the lysine arginine antagonism, involves increased catabolism of the affected amino acid.

The methionine-glycine-arginine interaction in chicks appears to result in increased creatine formation, and this interaction therefore may reflect loss of methionine due to increased demand for methyl groups in the conversion of guanidinoacetic acid to creatine (Keshavarz and Fuller 1971).

**Food intake**

There is convincing evidence that amino acid imbalances and antagonisms depress food intake. Rogers et al. 1967 reported that rats given a choice of a diet containing excess leucine or a protein-free diet would consume the protein-free diet almost exclusively. When rats were offered a choice of threonine imbalanced diet or protein-free diet (Leung et al. 1968a), they selected the protein-free diet. These studies demonstrate that amino acid interactions markedly affect appetite: given a choice animals select a diet that will not support growth or maintenance rather than consume an imbalanced or antagonized diet, which despite the existence of an amino acid interaction, can support substantial growth.

How much of the depressed performance of animals fed imbalanced diets is due to reduced food consumption? Rats force-fed diets imbalanced in isoleucine or threonine grow as well as rats fed the appropriate control diet lacking the imbalancing mixture of amino acids (Leung et al. 1968b). Similarly, chicks that are force-fed threonine imbalanced diets grew at rates comparable to chicks force-fed the control diet (Davis and Austic 1982). Thus it appears that amino acid imbalances strongly depress food intake, and that when the depression is prevented, growth is normal. This is consistent with the studies of amino acid catabolism, which indicate little if any loss of the limiting amino acid due to catabolism.

Amino acid antagonisms result in significant losses of the affected amino acid. Therefore it is not surprising that chicks that are force fed a diet containing excess lysine (Austic and Scott 1975) or excess...
branched chain amino acids (Calvert et al. 1982) do not exhibit growth comparable to their counterparts which are force fed the basal diet which has not been supplemented with these amino acids.

(i) Role of the central nervous system
Several studies have been undertaken to determine the role of the central nervous system in the depression of food intake caused by imbalances. Leung and Rogers 1969 provided evidence of the involvement of the brain when they fed rats having cannulated jugular veins and carotid arteries diets imbalanced with regard to isoleucine and threonine. Feed intake was markedly increased when the carotid artery was infused with a minute amount of the first limiting amino acid. However food intake was unaffected when the same amount of each amino acid was infused via the jugular vein, or when saline only was infused in either blood vessel. Tobin and Boorman (1979) also reported a similar finding in chickens fed an imbalanced diet. These studies established the probable role of the brain in detecting amino acid imbalances. Subsequent experiments by Leung and Rogers 1971 demonstrated that rats which received electrosurgical lesions in the prepyriform cortex of the brain did not decrease food intake when fed amino acid imbalanced diets. Although further studies are needed to establish the specific areas of the brain involved, these studies strongly suggest that alterations in the profile of amino acids in plasma cause reduced food intake through their effects on the central nervous system.

(ii) Changes in plasma amino acids
It appears possible that decreased plasma level of the most limiting amino acid causes the depressed food intake in amino acid imbalances. Decreased level of the first limiting amino acid is a consistent feature of imbalances (Harper et al. 1970; Rogers 1976). There are relatively few experiments on the metabolic basis of this change. Ip and Harper 1974 and Noda et al. 1975 injected or fed rats 14C-labelled amino acid, and observed increased radioactivity in liver of rats fed diets containing imbalancing mixtures of amino acids. These investigators suggest that the decreased blood level of the limiting amino acid may be due to increased uptake of amino acids from blood by liver, this uptake decreasing the relative level of the first limiting amino acid. Esteve and Austic (unpublished data) using radiolabeling technique to measure the fractional synthetic rate of protein (FSR) in liver, have observed an increased FSR in chicks fed isoleucine imbalanced diets. Changes in liver protein synthesis is an attractive hypothesis to explain the change in the blood level of the limiting amino acid. However changes in protein synthesis or degradation in other tissues could counterbalance the change in liver. Therefore more research is needed to test this hypothesis using a more complete assessment of protein synthesis and turnover in several tissues and organs.

CONCLUSION

Amino acid imbalances and antagonisms, particularly the former, are important nutritional interactions. The negative consequences of amino acid imbalances may be due to depression in the blood level of limiting amino acids which cause reduced appetite and depressed food intake. More research on the metabolic basis of these interactions may provide methods of preventing the depression of food intake through intervention at the level of appetite regulating centers in the brain or by preventing the metabolic changes that lead to altered levels of the limiting amino acid in blood. These methods would have significant benefit in the practical feeding of poultry, swine and other monogastric species.
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