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# EFFECTS OF METHIONINE ON FEED INTAKE vs. AMINO ACIDS IN PLASMA AND BRAIN IN THE CHICKEN

By

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ABSTRACT

EFFECTS OF METHIONINE ON FEED INTAKE vs.
AMINO ACIDS IN PLASMA AND BRAIN IN THE CHICK

By

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Growing S.C.W.L. male chicks and pullets of light and heavy breeds were used to study the relationship between feed intake and diets with different levels of methionine or total sulfur amino acids (TSAA). The experiments were conducted with diets containing practical or purified-type ingredients. The former diet with 16.6 or 21.2% protein and the latter diet with 13.1% protein level were formulated to be deficient, adequate or excessive in methionine (or TSAA).

Methionine was found to be the only limiting amino acid in the purified-type basal diet in which the only source of protein was isolated soy protein. The requirements of TSAA (or methionine) for maximum weight gain and feed intake were estimated to be 0.665 and 0.532% of diet, respectively, at 13.1% protein diet, and at a level between these two levels of TSAA, maximum feed efficiency was obtained.

The requirement of TSAA for optimum growth was lowered from 0.665 to 0.597% of diet with 13.1% protein when the proportion of methionine of TSAA was in the ranges of 46 to 57%.

A higher feed intake but no greater weight gain was obtained for pullets of light and heavy breeds fed practical-type diets deficient in methionine but with 16.6 or 21.2% levels of protein, respectively, compared to those fed the methionine adequate diets (0.094 or 0.120% added, respectively).

An improved feed efficiency was noticed by feeding as meals or by force the methionine excess diet (0.68% excess) with 13.1% protein. This diet fed ad libitum, or as meals reduced or did not reduce, respectively, feed intake and weight gain, as compared to meal-feeding the diet adequate in methionine. The methionine deficient diet fed as meals caused less feed intake and weight gain than the same diet fed ad libitum. However, force-feeding this diet alleviated the depressed feed intake and weight gain and produced an enlarged liver. The depressed growth rate caused by methionine deficiency was partly due to decreased feed intake and partly due to the deficiency itself.

Crop emptying rate was slower with the diets deficient in methionine regardless of the dietary protein levels.

Plasma and brain amino acids were determined, and it was found that when the level of methionine was low in the diet with 13.1% protein, the level of plasma lysine was increased and vice versa.

The diet with 13.1% protein but deficient in methionine tended to increase the levels of lysine, threonine and serine in plasma and brain; decreases of cysteine or methionine in plasma and brain were not consistent. The diet with excess

methionine usually increased the level of methionine or cysteine but decreased threonine and serine in plasma and brain.

A dramatic increase of methionine in plasma or brain of chicks meal-fed the diet with excess methionine at 13.1% protein level was not associated with decreases of feed intake and weight gain. Thus, the changes in the concentrations of individual amino acids in plasma and brain may not be directly effective in regulating feed intake.

The levels of total free amino acids (TFAA), essential amino acids (EAA) and EAA/TFAA in plasma tended to be increased by the diets deficient or with excess methionine. Negative correlation with a coefficient of -0.67 was noticed between the amount of feed intake and the plasma EAA/TFAA ratio.

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## ABBREVIATIONS

| | |
|---|---|
| ALA | Alanine |
| ARG | Arginine |
| ASN | Asparagine |
| ASN | Aspartic acid |
| CYS | Cysteine |
| EAA | Essential amino acids |
| FAA | Free amino acids |
| GLN | Glutamine |
| GLU | Glutamic acid |
| HIS | Histidine |
| ILE | Isoleucine |
| LEU | Leucine |
| LH | Lateral hypothalamus |
| LYS | Lysine |
| MA | Methionine adequate |
| MD | Methionine deficient (or deficiency) |
| ME | Methionine excess |
| MET | Methionine |
| MMD | Moderate methionine deficient |
| NEAA | Nonessential amino acids |
| PHE | Phenylalanine |
| PRO | Proline |
| SAA | Sulfur amino acids |
| SCWL | Single Comb White Leghorn |
| SER | Serine |
| TFAA | Total free amino acids |
| THR | Threonine |
| TSAA | Total sulfur amino acids |
| TYR | Tyrosine |
| VAL | Valine |
| VMH | Ventromedial hypothalamus |

# I. INTRODUCTION

Food intake by animals is an act of a complex phenomenon under the influence of external and internal stimuli. An understanding of its mode of action has long been a sought after goal, one difficult to establish within the framework of a single solution. Each step forward in an understanding of how it operates has added complexity to the overall picture. Our knowledge gained has resulted in many hypotheses on how animals respond to food as a stimulus. One concept considers the control of food intake under the influence of a craving for energy (Kleiber, 1961). Another considers that the responses are governed in two hypothalamic centers of the brain, ventromedial and lateral hypothalamus (Brobeck et al., 1943; Teitelbaum and Stellar, 1954). Within this concept various biochemical factors have been considered as signals for responses by the hypothalamic centers; such factors being glucose (Mayer, 1955), fat metabolites (Kennedy, 1953), and amino acids (Mellinkoff et al., 1956).

Poultry have received limited attention in regard to the role of the biochemical factors influencing food intake. Blood glucose levels were shown not to play a major role in the control of feeding by chickens (Lepkovsky et al., 1965;

Richardson, 1970b). Presumably, fat metabolites or fatty deposits supposedly play a role through a concept hypothesized as a set-point theory by Lepkovsky and Furuta (1971). Their chickens were made obese by force-feeding and as a result ceased to eat. They began to eat and food intake eventually reached normal, while the fat in the adipose tissues decreased toward or to a normal level; this was hypothesized to be within the concept of a set-point theory. However, Polin and Wolford (1973) reject this body fat-related set-point theory on the basis that laying hens force-fed at 150% level did not completely stop feeding on an ad libitum basis, and that obese laying birds continue to eat and become more obese even though they have very high levels of lipid in plasma, abdomen and liver. The aminostatic theory proposed by Mellinkoff et al. (1956) has received considerable attention on its role as a mechanism for regulating the food intake of mammals (Harper et al., 1970; Harper, 1976). Particularly, the free amino acid pattern in rat brain was reported to provide a signal triggering the central mechanism for a change in food intake (Leung and Rogers, 1969). The anterior prepyriform cortex area in rat brain was proposed as the site containing a central receptor sensitive to the concentration of the growth-limiting amino acids (Rogers and Leung, 1973). There are also many other reports showing that an elevation of total free amino acids in rat plasma had a correlation to the amount of feed intake (Anderson et al., 1969; Peng and

Harper, 1970). Peng et al. (1969) observed a high quantitative relationship (-0.98) between depression of food intake and elevation of concentration of indispensable amino acids.

No attention has been paid to the relationship of altered amino acids in the body fluids of poultry and the effect on food intake. Nevertheless, there are several studies to show that dietary deprivation of certain indispensable amino acid, do influence food intake (Fisher and Shapiro, 1961; Fisher et al., 1960; Solberg et al., 1971; Wethli et al., 1975), indicating that the aminostatic theory may be operating in the bird as well as the mammal.

This study was to determine which part of the altered pattern of amino acids in plasma and brain from chickens was effective in the control of feed intake and the relationship between feed intake and growth rate by using a meal-feeding or a force-feeding technique. The emptying rate of crop was also examined as one of the possible mechanisms responsible for the reduction of feed intake by diets with a deficiency or excess of amino acids.

## II. REVIEW OF LITERATURE

### A. General mechanisms of food intake control

The understanding of the mechanisms of food intake control has been a challenge for many years to nutritionists, physiologists and psychologists. Animals are known to control their food intake to keep their body weight relatively constant. They overeat in response to dilutions of diets with inert materials (Adolph, 1947; Peterson et al., 1954; Hill and Dansky, 1954), reduce food intake following experimental obesity in normal animals (Cohn and Joseph, 1962) and exhibit compensatory overeating during the first few days of refeeding after food deprivation (Adolph, 1947). In the chicken, Lepkovsky and Furuta (1971) showed that after cessation of forced-feeding, the food intake remained very low for 6-10 days until body weight returned to normal.

Brobeck et al. (1943) observed that the obesity following ventromedial hypothalamic lesions arose from the onset of hyperphagic behavior and that the adiposity which occurred was due to increased food intake and not to a disturbance of metabolism. They suggested that this area of the ventromedial hypothalamus (VMH) was concerned in the inhibition of food ingestion so that, when its influence was removed, the animal exhibited hyperphagic behavior.

In 1951, Anand and Brobeck described the converse of this condition - an aphagia in rats having lesions of the hypothalamus just lateral of the above mentioned operation. Rats with this type of lesion showed complete aphagia and death from starvation. Teitelbaum and Stellar (1954) confirmed this observation and noted that animals with lateral hypothalamic (LH) lesions may recover their feeding behavior if they are kept alive by tube feeding for a few days or weeks.

The original experiments in rats have been extended to other animals including chickens. Aphagia was reported in the chicken as a result of selective damage in the lateral hypothalamus (Feldman et al. 1957). Smith (1969) also showed that there were a hyperphagic and an aphagic center located in close proximity to each other in the anterior hypothalamus of the bird. Both were physiologically similar to the ventromedial-lateral hypothalamic system of mammals. Thus, the hypothalamic center was divided into a lateral "feeding center" and a medial "satiety center" with the two centers reciprocally responsive to innervation, but with the medial center being dominant since it overrode feeding (Brobeck, 1955).

Baile et al. (1970) could not find any change in food intake when they destroyed the ventromedial nuclei with precision in sheep. This finding agreed with the others that the ventromedial nucleus of the hypothalamic constellation of neuronal nuclei was not the anatomical locus of the

physiological entity designated as the ventromedial hypothalamic satiety center (Bell, 1971). The region of the lateral hypothalamus also lacked cellular uniformity and the numerous neurons showed no aggregation so that they could be regarded only as part of the neuropil or the bed nucleus of tracts (Bell, 1971).

Though there were some problems of anatomic identifications of feeding center and satiety center, the existence of hypothalamic glucoreceptors has been described by several different groups in a variety of species (Oomura et al., 1974; Anand et al., 1964). In addition to that, various biochemical influences were considered which could trigger the hypothalamic centers. These factors included the availability of glucose in body fluids (glucostatic theory by Mayer, 1955), the concentration of fat metabolites in body fluids (lipostatic theory by Kennedy, 1953), and the concentration of serum amino acids in body fluids (aminostatic theory by Melinkoff et al., 1956).

Mayer (1955) proposed that glucoreceptors, probably situated in the VMH, mediate short-term control of food intake. To account for the hyperphagia of diabetic animals, he postulated that transport of glucose into the receptors was dependent on insulin. As evidence for the existence of glucoreceptors in brain, some investigators showed that gold-thio-glucose, a glucose antimetabolite, damaged several areas of the brain, including VMH, and led to overeating and obesity (Baile et al., 1970; Smith and Britt, 1971). Also,

the infusion of 2-deoxy-D-glucose, a competitive inhibitor of glucose utilization in mammalian tissue, into the carotid but not peripheral veins produced an increased food intake in rats and rabbits (Houpt and Hance, 1971).

In contrast to the results in rats, blood glucose level does not play a major role in the control of feeding in ruminants (Baile and Mayer, 1969) and chickens (Lepkovsky et al., 1965; Richardson, 1970b). As for the chicken, hypoglycemia produced by insulin did not cause a hyperphagia, and hyperglycemia produced by oral or intravenous administration of glucose solution did not lower the food intake (Lepkovsky et al., 1965; Richardson, 1970b). Furthermore, Carpenter et al. (1969) did not obtain any effects from gold-thio-glucose injection on food intake, fat deposition and reproductive performance in Japanese quail. Also, no structural differences were detected between control and experimental brains. The probable lack of glucose receptors in the brains of birds or ruminants has been suggested as the reason for the lack of response of these animals to the injection of gold-thio-glucose (Baile et al., 1970).

To propose the lipostatic theory, Kennedy (1953) showed the VMH lesions increased the level at which body fat was regulated. Both Kennedy (1953) and Mayer (1955) have accounted for longer term regulation of energy balance by suggesting that feeding is regulated so that the proportion of fat to total body weight remains constant. Hoebel and Teitelbaum (1966) performed an experiment supporting this

theory. Before introducing lesions into the VMH, the rats were made obese by the injection of protamine zinc insulin. After placement of the lesions, the rats gained little weight because they were already obese. This experiment suggested that in the regulation of food intake, the hypothalamus regulates the amount of fat in the adipose tissues, not by regulating appetite but by control of the set-points in the adipose tissues.

Lepkovsky and Furuta (1971) made a test of the set-point hypothesis in the chicken. Obesity was induced in W. Leghorn cockerels by force-feeding; thus without hypothalamic lesions and presumably without a change in the set-point in the control system of the adipose tissues. The cockerels were force-fed approximately twice their ad libitum food intake, and they soon ceased to eat. With the cessation of force-feeding, the birds did not eat for 7 to 10 days. They lost weight rapidly and only after the fat in the adipose tissues decreased toward normal did the birds begin to eat. Food intake reached normal when the fat in the adipose tissues decreased to normal level which was required by the set-point. However, Polin and Wolford (1973) reject this body fat-related set-point theory on the bases that laying hens force-fed at 150% level did not completely stop feeding on an ad libitum basis, and that those birds had very high levels of lipid in plasma, abdomen and liver. Also, they contend that the crop in the chicken played a role in the regulation of feed intake. Hungry birds force-fed water,

cellulose or food can be almost immediately satiated for a short period of time (Polin and Wolford, 1973). Thus, they postulate the upper part of the digestive tract of the chicken to be a prime area from which, and into which, messages, neural and humoral, are sent to regulate feed intake.

There is a little argument about the idea of independent existence of the receptors for these two nutrients, i.e. glucose and lipids in those animals in which such receptors exist. Based on the classical observation that animals eat for calories (Adolph, 1947), Panksepp (1974) has suggested an energostatic hypothesis. When equicaloric amounts of the individual macronutrients - fats, proteins, and carbohydrates - were administered directly into stomachs of the rats having free access to food, voluntary food intake decreased in proper measure to the number of administered calories. However, Le Magnen (1976), after studying the relations between the two controlling mechanisms in acute versus chronic conditions of feeding, has come to the suggestion of a different action of the glucostatic and lipostatic mechanism in two different conditions of food deprivation. Meanwhile, he still admitted that glucose and lipid interactions govern the time of the meal onset and thereby the day-to-day energy balance.

B. Aminostatic mechanism of food intake control

Since the first statement of the aminostatic theory of food intake control by Mellinkoff et al. (1956), the role of dietary and plasma amino acids in the control of food intake has been examined in many studies (Harper et al., 1970; Harper, 1976).

Earlier in 1947, Frazier et al. showed in rats that the omission of each of several indispensable amino acids including methionine led to a marked loss of weight, coincident with a prompt loss of appetite. When, however, the missing amino acid was added to the ration, the rats quickly recovered the lost appetite and rapidly regained the lost weight. Almquist (1954) examined the literature on the relationship between amino acid intake and amino acid pattern of the blood of chicks and noted that the amino acids present in excess in the diet tended to accumulate in blood, and that those in deficit in the diet tended to decrease to low concentrations. He also suggested that these deviations in the blood amino acid pattern probably lead to an automatic impairment of appetite to curtail the further ingestion of protein.

According to Mellinkoff et al. (1956), the total serum amino acid concentration per se was not an important determinant of appetite, instead the changes in blood amino acid patterns appeared to be more closely related to changes in feed intake. They also suggested that the amino acid patterns of extracellular fluids, modified by intestine and

liver may influence the desire for food. This suggestion was proved by Harper et al. (1964), who showed that the alterations in the feeding behavior of animals ingesting amino acid imbalanced diets have been attributed directly or indirectly to the changes in the plasma amino acid pattern. They also noted that the depressed food intake of rats fed amino acid imbalanced diets was rapid, occurring within 5-12 hours. This decrease in food intake was shown to be associated with a decline in the most limiting amino acid in the plasma of animals fed the imbalanced diets.

Leung and Rogers (1969) suggested that if some basic mechanism regulating food intake was affected by an amino acid imbalance, then the decrease of the most limiting amino acid in the blood plasma could provide a signal triggering the central mechanism for a change in food intake which may ultimately curtail the voluntary intake of the diet. They provided evidence that the food intake regulatory mechanism sensitive to the change in the blood plasma acted from a central and not peripheral location to influence the feeding behavior of the animals ingesting amino acid imbalanced diets. The infusion of the growth limiting amino acid into the carotid artery which leads directly to the brain alleviated the deleterious effect of an amino acid imbalance; whereas, the same amount of the most limiting amino acid infused into the jugular vein, which leads to the heart and thus equally to all parts of the body, had no effect on food intake of animals ingesting the imbalanced diet. There were

other reports demonstrating comparable alterations of amino acid concentrations between plasma and brain, though the elevations were less in brain than in plasma (Peng et al., 1972; Roberts, 1968; Daniel and Waisman, 1969a).

Electrolytic lesions were placed in certain brain areas to induce hyperphagia to determine which neurostructures might be involved in mediating the depressing effect of an amino-acid imbalanced diet on food intake (Scharrer et al., 1970; Leung and Rogers, 1970). These investigators showed that the lesioned hyperphagic rats fed an amino acid imbalanced diet decreased their food intake. Also, they found that aphagic rats allowed to recover and then fed an imbalanced diet decreased their feed intake. Krauss and Mayer (1965) demonstrated the same effects in animals lesioned in the VMH by using diets with excess leucine or protein. Thus an intact VMH was not required to mediate a depression in food intake caused by an amino acid imbalance, an amino acid excess, or a high protein diet.

In 1971, Leung and Rogers proposed that the anterior prepyriform cortex (APC) was the site of a central receptor for the regulation of food intake of rats fed diets deficient or imbalanced in amino acids. A lack of depression in food intake was observed in animals with lesions in certain areas of the APC when fed the amino acid imbalanced or devoid diet. These animals also altered their dietary choice, compared to the intact animals, by choosing the imbalanced diet instead of the protein-free diet. The APC which may

contain areas sensitive to the concentration of the growth-limiting amino acids, was proposed to send an inhibitory signal to the lateral hypothalamic feeding area to curtail food intake (Rogers and Leung, 1973).

However, Russek (1971) did not agree with the above mechanisms of food intake control involving the plasma and brain amino acid pattern. He suggested the presence of hepatic glucoammonium receptors that monitor the glucose and ammonia from perhaps excess amino acids which were received from the portal circulation.

Many studies showed that there was a relationship between the intake of an amino acid and the response of food intake and growth rate. Peng et al. (1973) produced severe depressions in growth and feed intake in rats by the ingestion of a large amount of methionine, tryptophan, leucine or phenylalanine (large neutral amino acids). However, they failed to produce the same severe depressions by the ingestion of a large amount of lysine (basic amino acid), threonine (small neutral amino acid) or glutamic acid (acidic amino acid). Sauberlich (1961) measured growth responses of weanling rats fed 5% of individual amino acids supplemented in a 6% casein diet. He found that, on the basis of growth depression, methionine was the most toxic indispensable amino acid followed in decreasing order by tryptophan, histidine, phenylalanine, leucine, valine, isoleucine, arginine, lysine, and threonine.

Neame (1968) reported the results of *in vivo* and *in vitro* studies indicating that there were at least four amino acid transport systems in rat brain, one for acidic amino acids, one for basic amino acids, one for small neutral amino acids including threonine, and one for large neutral amino acids (valine, leucine, isoleucine, phenylalanine, tryptophan, and methionine). Thus, one should note that the indispensable amino acids which were relatively toxic compete for a common transport system.

Peng *et al*. (1973),therefore, indicated that the effects of individual amino acids in excess on depletion of the other amino acids were primarily limited to those within the same transport group. Harper (1976) examined the competition by surplus amino acids for their ability to compete against histidine, as the limiting amino acid, for its uptake by brain slices *in vitro*. He showed that basic amino acids compete relatively little with histidine, also a basic amino acid, for such entry. Threonine, a small neutral amino acid, exerted some inhibition, but the aromatic, the branched-chain amino acids, and methionine were by far very effective inhibitors of histidine uptake. The *in vitro* experimental results appeared to correspond well with an *in vivo* experiment in which the basic amino acids of lysine and arginine at the level of 4.5% in a diet low in histidine did not depress food intake and growth to any significant extent. Also, the same amount of a mixture of large neutral amino acids caused a marked depression of food

intake and growth. These observations indicated that the competition between the amino acids for entry into brain tissue was not limited to those within the same transport group. Despite these facts, Harper (1976) still admits that the competition for amino acid transport can not explain the situations of amino-acid imbalanced diets with the limiting and competing amino acids being in different transport groups.

C. Adverse effects of methionine deficiency

The concept of amino acid balance was introduced by Osborne and Mendel (1915) from the observations that proteins varied greatly in amino acid compositions and that the nutritional value of a protein depended upon the proportions of the various indispensable amino acids it contained.

Since many different adverse effects have been caused by the ingestion of diets containing disproportionate amounts of amino acids, Elvehjem (1956) proposed three terms to describe the adverse effects in animals resulting from the ingestion of such diets. They are a) toxicities, b) antagonisms, and c) imbalances. The term "amino acid toxicity" includes adverse effects of varying degrees resulting from ingestion of large quantities of individual amino acids. Amino acid antagonisms are demonstrated when depressions of growth caused by ingestion of excessive amounts of an amino acid are alleviated by supplements of structurally similar amino acids. Amino acid imbalances are detected as adverse effects caused by a surplus of essential amino acids other

than the one that is limiting for growth or maintenance. To create imbalances, no single amino acid would be included in the diet in an amount that would be considered toxic (Harper, 1974a). The distinction between imbalances and deficiencies is that investigations of deficiencies deal with the effects of an inadequate intake of an amino acid, whereas imbalances deal with the effects of surpluses of amino acids on the limiting amino acids. However, Fisher et al. (1960) and Fisher and Shapiro (1961) stated that the word, imbalance, is not being used properly and insisted that imbalance may be considered as an exaggeration of a specific amino acid deficiency.

In common dietary practices the amino acids that are most likely to be limiting in animals as well as in chicken, are lysine, methionine and tryptophan. The omission of each of these indispensable amino acids leads to a marked loss of weight, coincident with a prompt loss of appetite. When, however, the missing amino acid was added to the ration, the rats quickly regained their appetite and their weight (Frazier et al., 1947).

Amino acid deficiencies affect liver enzyme activities. Williams et al. (1949) showed that force-feeding of a methionine deficient diet reduced liver succinate dehydrogenase activity slightly and completely reduced liver xanthine oxidase activity. The activity of succinate dehydrogenase was centered mainly in the liver mitochondria. Though protein starvation lowered the riboflavin content of the

liver, this could not explain such a marked loss in that flavin enzyme activity (Williams et al., 1949).

Since the liver plays such an important role in general nitrogen metabolism, the free amino acid contents of this organ and that of brain were determined in rats to observe whether any correlation existed between their abilities to retain protein and their free amino acid contents during a methionine deficiency (Denton et al., 1950). The concentrations of arginine and methionine were decreased considerably in the liver of the methionine-deficient group, while those of isoleucine, leucine and phenylalanine appeared to be decreased only slightly. Lysine was the only amino acid whose concentration in the liver was increased by the methionine deficiency. Although the concentrations of tryptophan and valine were decreased in the brain, they did not change in the liver. The content of histidine in the brain increased greatly over that of the control group, while in the liver its content did not change. But most of all, methionine content in the brain of the methionine-deficient group was the same as that of controls which was in contrast to the decrease of methionine found in the liver.

Nakagawa and Masana (1967) have observed that the withdrawal of methionine from the diet of men resulted in a decrease of concentrations of plasma arginine and alanine, and in an increase of threonine. There were no explanations about the possible causes of this change. According to Sanchez (1969), plasma arginine and ornithine were elevated

by a feeding of sulfur-amino acid deficient diet in adult male rats, though this diet caused no alterations in plasma methionine but a decrease in cystine. Liver threonine, serine, and ornithine were increased, but aspartate was decreased by the dietary sulfur-amino acid deficiency. This diet also caused decreases in activities of threonine dehydrase and glutamate oxaloacetate transaminase and an increase in tyrosine transaminase activity in liver tissue. The activity of choline oxidase in chicken liver, also, appeared to be reduced by low methionine or low lysine diets (Garanca and Cielens, 1971).

The effects of methionine deficiency (MD) on nitrogen absorption from the intestinal tract of chickens and on carcass nitrogen content of birds were studied by Pisano et al. (1959) and Fisher and Shapiro (1961), respectively. Both of the groups agreed that the methionine deficient chicks were not limited in their ability to digest and absorb the relatively low amount of protein they consumed.

However, Solberg et al. (1971) showed that a MD diet caused a decreased nitrogen retention on the basis of percentage ingested nitrogen, and increased uric acid excretion in chicks. Liver xanthine dehydrogenase (or xanthine oxidase) activity increased in relation to the more active state of uric acid synthesis. This group of investigators also confirmed in their study the fact (Carew and Hill, 1961) that a higher level of dietary methionine was required to produce maximum growth. Carew and Hill (1961) observed that a moderate methionine-deficiency increased feed intake, and thus

led to a higher consumption of metabolizable energy. As a result, the chicks consumed more energy and had a greater concentration of calories per gram of body weight, but it did not appear as additional weight gain because of the change in body composition. In contrast, Shoji et al. (1966) found that methionine deficiency decreased energy deposition in the carcass and decreased the efficiency of metabolizable-energy utilization as a result of increased heat production. Baldini (1961) also observed that methionine deficiency caused increased heat production. However, Davidson et al. (1964) stated that the two possibilities that the extra energy ingested resulted in both increased energy deposition and increased heat production were not mutually exclusive. The excess energy ingested gave rise to an increased rate of heat loss to the environment and to an increase in deposition of body fat at the expense of body water.

D. Adverse effects from methionine excess

"The importances of studying the effects of excessive intakes of each indispensable amino acid are to increase the understanding of inborn errors of amino acid metabolism to provide the nutritional background for studies of metabolic adaptations to alterations in dietary amino acid pattern; and to provide background for biochemical studies on the significance of alternate pathways of amino acid metabolism in the intact animal." (Harper et al., 1966).

Excessive intake of one indispensable amino acid in animals fed a low protein diet usually produced depressions in growth and feed intake (Brown and Allison, 1948). Methionine is the most toxic of the nutritionally important amino acids (Russell et al., 1952; Sauberlich, 1961). According to Harper et al. (1970), consumption of methionine at four times its requirement resulted in growth depression and tissue damage when incorporated into a diet low in protein. Tryptophan, which is considered to be the second most toxic amino acid, was added at levels in excess of tenfold of its requirement before adverse effects were noted.

The effects of excessive intake of methionine; in addition to depressions in growth and feed intake, are an increased excretion of creatinine (Brown and Allison, 1948), splenic hemosiderosis (Van Pilsum and Berg, 1950), pancreatic damage (Kaufman et al., 1960), fatty liver (Harper et al., 1954 a, b; Williams and Hurlebaus, 1965), hypoglycemia accompanied by a progressive fall in hepatic ATP (Hardwick et al., 1970) and the induction of hepatic serine-threonine dehydrase (Girard-Globa et al., 1972). Chicks, when fed a high methionine diet, showed poor feather development, hock joint disorder, discoloration of the eye and shank and curled toe paralysis. After the high methionine diet was changed to normal diet, the chicks showed complete remission of all deformities (Tamimie, 1970).

Klavins (1965) has demonstrated that the administration of excess methionine (4.5% of diet) caused an increased

concentration of methionine, α-aminobutyric acid, lysine, and glutathione in rat serum, and a decrease of threonine, valine and leucine. He also noted that the effect of methionine in lowering the serum amino acid concentration was more evident in those amino acids which are known to have lower affinity for intestinal transport system, with the exception of lysine, and which are normally in serum at higher concentrations than methionine. Daniel and Waisman (1969b) observed that the presence of a high methionine environment induced a disruption of the normal balance of free amino acid pools. Hepatic levels of aspartic acid, threonine, serine, glutamine, glutamic acid, glycine, and alanine were depressed, while levels of taurine, cystathionine, methionine, lysine, and ornithine were markedly elevated after excessive intake of methionine in rats. Brain levels of aspartic acid, threonine, serine, glutamic acid, glycine, alanine, and γ-aminobutyric acid were markedly depressed, and increased levels of cystathionine, methionine, lysine, and glutamine were observed. They also noted that the alterations in liver free amino acids induced by dietary excess methionine were not accompanied by similar changes in serum, that serum levels of amino acids, except methionine, remained relatively constant and were probably removed from plasma by rapid excretion as a nitrogenous waste product. Most of all, they found that liver is influenced to a greater degree than brain by an excess of methionine in the diet.

Sanchez and Swendseid (1969) have shown that, in rats fed diets containing excess methionine, plasma and liver histidine, alanine and α-aminobutyric acid were increased, glycine was decreased in liver and muscle, and glutamic acid, aspartic acid, glutamine, citrulline, ornithine, arginine and lysine were significantly lower in concentrations in tissues. The supplementation of excess methionine caused the increased activities of threonine dehydrogenase, tryptophan pyrrolase and tryosine transaminase. The alterations of plasma amino acid pattern in adult male chickens were studied by Ohno et al., (1972). When dietary methionine was increased to 0.56% of diet, arginine, serine, alanine, lysine, isoleucine, threonine, and aspartic acid were increased, and valine, tyrosine, leucine, histidine, tryptophan, phenylalanine, cystine, proline, glutamic acid and glycine remained constant.

Although numerous theories were proposed, the underlying mechanism by which excess methionine exerts its adverse effects is not well understood. Greenstein and Winitz (1961) studied the toxicity of the individual amino acids. They observed that rats injected with lethal doses of most of the individual amino acids died with elevated blood ammonia concentration. However, Harper et al. (1970) suggested that the magnitude of amino acid influx obtained by injection could not occur in animals fed excessive amounts of amino acids because the ingested food is metered to the intestine by the various mechanisms that regulate stomach emptying, and

ingested amino acids pass initially to the liver, where deamination and urea synthesis occur in close proximity. Finally, food intake is depressed by a dietary load of an individual amino acid, a reaction that in itself tends to protect the animal against severe toxicity.

Roberge and Charbonneau (1968) and DuRuisseau et al. (1956) have noted that the injection of methionine does not lead to an elevation of blood ammonia, and moreover, Iles and Hamilton (1976) have observed that the injection of L-methionine intravenously to mice at a dosage level of 200 mg./Kg. body weight per 15 minutes leads to a slight decrease of blood ammonia concentration.

According to a hypothesis by Cohen and Berg (1951), the detoxification of excess methionine was accomplished by supplying the proper substances to utilize the methyl groups. Glycine and arginine as precursors of creatine are very effective in detoxifying methionine; the detoxification is accomplished by using up excess methyl groups to form creatine. The urinary creatine was increased when glycine and arginine were fed. This explanation, however, appeared untenable because rats supplemented in this way did not excrete enough extra creatine to explain the beneficial effects observed and also because methionine toxicity was not alleviated by including guanidino-acetate directly in the diet (Cohen et al., 1958).

Benevenga and Harper (1967) report that in rats fed diets supplemented with 3% L-methionine or an equivalent

amount of DL-homocystine, glycine and serine both alleviate but do not prevent, the growth depressions caused by methionine and homocystine. Glycine appears to be more effective than serine in alleviating methionine toxicity but serine is more effective in alleviating homocystine toxicity. The specificity of glycine and serine in alleviating methionine toxicity may be explained by the ready metabolic interconversion of these 2 molecules and by the need for an adequate supply of serine for conversion of the homocysteine derived from methionine to cystathionine as shown in following metabolic pathway (Figure 1). The Figure 1 is a slight modification of that presented by Baker (1976). If serine were more effective than glycine in preventing growth depressions due to excess homocystine and methionine then this explanation would appear to be adequate. But since glycine is superior to serine in protecting against methionine toxicity, the metabolism of the labile methyl group of methionine has been proposed as a means by which methionine toxicity is exerted (Benevenga, 1974). He suggested that a pathway which is competitively inhibited by S-methyl-L-cysteine accounts for the majority of the methionine catabolized when high levels of methionine are fed.

Meanwhile, Katz and Baker (1975) observed that the relative toxicities of homocysteine and methionine are conflicting in different studies (Cohen et al., 1958), Benevenga and Harper, 1967) and have provided evidence that homocysteine accumulation in plasma and tissues is one of the several

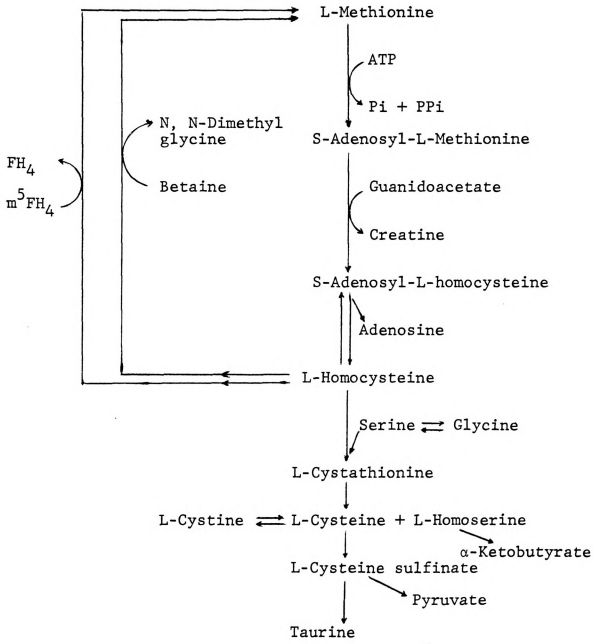


Figure 1. Scheme of Methionine Metabolic Pathway

possible factors responsible for lesions associated with methionine toxicity in chicks. They refuted the hypothesis of the labile methyl group of methionine suggested by Benevenga (1974) on the basis of the findings that homocysteine and homocystine are as toxic as methionine. The metabolism of the methyl group of methionine precedes the formation of homocysteine and thus could not be involved in the toxicity resulting from ingestion of high levels of homocysteine. Also, hydroxy-methionine (Ca) contains a labile methyl group similar to the one in methionine, yet it appears to be much less toxic than methionine.

As mentioned earlier, there is the other hypothesis that the toxicity of the amino acids and, in particular, methionine, may be due to their competitive effect on amino acid transport (Peng et al., 1973; Harper, 1976). Evidence that at least part of the adverse effects of excessive levels of dietary methionine may be due in part to their effect on transport is shown by the experiments of Webber (1962). The infusion with 0.88 mMole of methionine/min. markedly affected the reabsorption of alanine, serine, glycine, and histidine in dogs and had a lesser effect on the reabsorption of valine and phenylalanine. Moreover, Webber's experiment with alanine may shed some light on the effect of methionine on kidney function. Even at the lowest level of alanine infusion, a marked effect on the reabsorption of serine, threonine and histidine was apparent. At higher levels of alanine infusion, reabsorption of these amino acids was further

reduced without an apparent effect on kidney function. L-alanine, even at high levels (5% to 10%) in the diet, is not toxic and does not result in an altered rate of growth (Harper, 1964). Thus, the toxic effect of methionine may not necessarily be related to its effect on the reabsorption of amino acids by the kidney (Benevenga, 1974).

The above discussed three major theories can be summarized as follows in terms of their causes of adverse effects by excess methionine; 1) excessive labile methyl group (Cohen and Berg, 1951; Benevenga, 1974), 2) homocysteine accumulation (Katz and Baker, 1975), and 3) competitive transport of amino acids (Peng et al., 1973). In spite of other numerous theories, the mechanisms by which excess methionine exerts its adverse effects on the alterations of the other plasma amino acid concentrations and the liver lipid metabolism are still far from being clear.

E. Force-feeding and meal-feeding of various levels of amino acid diet.

Since food intake and growth of rats fed an amino acid imbalanced diet could be stimulated by the administration of insulin (Kumta and Harper, 1961), or exposure to a cold environment (Harper and Rogers, 1966), the depression in food intake has been postulated as the primary effect causing the growth depressions of rats ingesting the imbalanced diet.

Harper et al. (1964) has reported that force-feeding of an imbalanced casein diet to rats yielded the same growth as force-feeding similar levels of the balanced diet. Leung et al. (1968) obtained as much weight gain from rats force-fed

a threonine-imbalanced diet as that of rats fed the same amount of control diet. This provides the evidence that depression of food intake is the primary cause of growth depression. Benton (1964) also showed that rats force-fed the 9% casein diet with 3% of L-Leucine grew at the same rate and retained the same amount of nitrogen as rats force-fed the unsupplemented diet. When such diets were fed ad libitum, there were great differences in growth and nitrogen retention.

Kumta et al. (1958) and Leung et al. (1968) used meal-eating as a means to overcome the depression in food intake of rats fed amino acid imbalanced diets. Both groups of investigators found that, in general, the growth depression was less for rats on meal feeding than for rats fed ad libitum. These observations along with those of a cold exposure and of an insulin administration, indicate that an imbalanced diet can support growth or alleviate the adverse effect on growth if food intake, and thus the intake of the most limiting amino acid in the diet, is increased.

However, Leung et al. (1968) also observed that there is a point at which the balance between the concentration of the most limiting amino acid and that of the surplus of indispensable amino acids is such that the food intake of rats ingesting an imbalanced diet can not be stimulated by insulin injection, cold exposure or force feeding.

F. Effect of amino acids on stomach emptying rate.

Gastric emptying is a dynamic interaction of a wide assortment of nervous and endocrine influences. A delayed

rate of stomach emptying has been considered as one possible reason for the depression in food intake of animals fed diets having amino acid imbalances.

Harper and Kumta (1959) and Kumta and Harper (1961) measured the stomach emptying rate of rats fed a diet with excess amino acids. No effect from the additions of excess methionine and phenylalanine on stomach emptying rate was detected within 7 hours from the beginning of the feeding period, whereas a depression in food intake was detected within 4 hours.

Notwithstanding these earlier observations, Benevenga and Harper (1970) have observed in rats a delayed stomach-emptying-rate on a high-methionine diet (3% of diet). The supplementation of the high methionine diet with glycine or serine returned the rate of stomach emptying toward normal, followed by an increase in methionine catabolism and a lowering of plasma levels of methionine. Also, a delayed emptying of stomach has been demomstrated in rats fed a high-protein diet; whereas in rats fed a diet with an amino acid (threonine) imbalance, only minor and inconsistent effects on stomach emptying were observed (Leung and Rogers, 1971; Peng et al., 1972). More recently, Stephens et al. (1975) found that tryptophan injected at levels from a physiological to pharmacological level slowed gastric emptying significantly (60% inhibition) in a dose-related response. Other essential amino acids (including methionine and lysine) had no effect at very high concentrations (pharmacological level). They suggested that the release of cholecystokinin may not be the

only mechanism by which tryptophan acts. Later, Cooke and Ward (1976) showed that tryptophan slows gastric emptying by exciting a receptor in the intestine and not by direct effect on the stomach or brain or via its major metabolites.

According to Harper (1974b), the regulation of stomach emptying is explained as one of the homeostatic mechanisms of the animal which undergoes adaptation that enables it to adjust to the high protein intake. As intake increases above the amount required to maintain the various body structures in their standard state, the excess of amino acid is degraded and used as a source of energy. If intake increases enough, then the capacity of the organism for degradation of amino acids may be exceeded, and amino acid will accumulate in body fluids. As a result, entry of amino acids into the body may be slowed by a reduction in the rate of stomach emptying. If the protein content of the diet is high enough, entry may be further decreased by a reduction in voluntary food intake.

Fisher and Weiss (1956) reported that cropectomy in chickens did not give any ill effects on subsequent growth and feed consumption, and concluded that the crop did not play a major role in controlling feed consumption. Richardson (1970a) and Feigenbaum et al. (1962), have observed that food intake and body weight gain were at least equal and sometimes higher for cropectomized birds than for controls when given unlimited access to food. However, when the feeding period was restricted to 2 hours or less per day, a lower feed intake was observed in cropectomized birds.

These data indicate the important role of the crop as a regulator of feed intake (Feigenbaum et al., 1962). Polin and Wolford (1973) postulate the upper part of the digestive tract of the chicken to be a prime area to regulate feed intake. Three types of regulating activities govern the crop's response to food: rate of fill, capacity, and rate of discharge. These regulating activities are controlled by neural and humoral messages.

Chickens have slightly different structures in the digestive system compared to that of mammals. The crop, which is essentially the same structure as the esophagus, undergoes contractions which vary considerably in rhythm and amplitude. Hunger produces restless and irregular crop activity in normal birds and those whose cerebral hemisphere has been removed. When the gizzard and crop are full, crop contractions may cease. The motility of the esophagus and crop is under nervous control. These organs receive parasympathetic excitatory fibers from the vagus, and also both excitatory and inhibitory fibers from the sympathetic system (Sturkie, 1976).

## III. EXPERIMENTAL PROCEDURES

### A. Introduction

#### Experiment I

Experiments I A and I B were conducted to determine in young chicks the most limiting amino acid in a low protein basal diet. A purified-type diet (Table 1) containing 15% of isolated-soy-protein to provide a 13% level of dietary protein was used as the basal diet. The reason for the use of the low dietary protein level was to enhance any amino acid deficiency, an effect previously obtained in young animals on a low protein diet (Harper et al., 1970). Also a 13% dietary protein level was reported to be high enough to permit growth, and sufficiently high to distinguish between the amino acid requirements for maintenance and growth of young chicks (Summers and Fisher, 1961).

The durations for experiments I A and I B were from 28 days old to 36 days of age and from 7 days old to 23 days of age, respectively.

#### Experiment II

Experiments II A and II B were designed to determine the requirement of methionine for optimum growth of young chicks fed a diet of 13% protein level. Since methionine

alone can meet all requirements of sulfur-containing amino acids for optimum growth (Baker, 1976), the requirement of cystine was not determined separately in the present experiment. In experiment II B, free amino acid compositions in plasma and brain were analyzed to detect the relationship between the amino acid patterns and feed intake.

The chicks of 3 weeks of age were fed diets for 1 week or 3 days, respectively, in experiments II A or II B.

Experiment III

The relationship between methionine and cystine in terms of the ability of cystine to spare methionine was reported early in 1941 by Womack and Rose. Though methionine can be converted to cysteine, the reverse reaction does not take place, presumably because the cystathionine synthase-catalyzed reaction can not be reversed (Baker, 1976). The conversion of methionine to cysteine was not efficient enough for the prevention of nutritional muscular dystrophy in vitamin E-deficient chicks (Hathcock and Scott, 1966). Baker (1976) reports that, in chicks, a dietary requirement for TSAA expressed in terms of either milligrams or concentration would be lower when a proper combination of methionine and cystine is fed than when methionine alone is used to meet the requirement. Graber et al. (1971) showed approximately 50% of the TSAA requirement for growth of chicks can be met by cystine. Byington et al. (1972) reported that a rat diet with sulfur-containing amino acids in the ratio of 70% from methionine and 30% from cystine was superior to that of

30% from methionine and 70% from cystine. The ratio between methionine and cystine significantly influenced feed intake, weight gain and nitrogen deposition in rats (Byington et al., 1972) and the specific activity of cystathionase in rat hepatic tissue (Shannon et al., 1972).

The present experiment was designed to study the effects of different proportions of methionine and cystine and different levels of TSAA in young chicks fed diets of 13% protein level on feed intake and body weight gain. The diets were fed to chicks of 3 weeks of age for a period of 1 week.

Experiment IV

Experiments IV A, IV B and IV C were conducted to determine the effects of methionine deficiency (MD) or methionine excess (ME) on hunger using a feeding of meals each with different intervals of time.

A reduced dietary energy intake produces a desire for food and triggers the feeding center in LH to stimulate animals to consume more food (Mayer, 1955). However, a diet deficient in amino acid(s) or with an excess amount of amino acid(s) depressed feed intake and weight gain in rats (Mellinkoff et al., 1956; Leung and Rogers, 1969; Rogers and Leung, 1973; Peng et al., 1973; Benevenga and Harper, 1967). The depression in food intake has been considered as a primary effect causing a growth depression of rats ingesting an amino-acid imbalanced diet (Kumta and Harper, 1961; Harper and Rogers, 1966). Meal feeding which would stimulate the desire

to eat food, was postulated, in the present experiment, to stimulate the consumption of an increased amount of the MD or the ME diets in a given period of time and thereby to reduce the adverse effects of those diets on weight gain.

In experiments IV B and IV C, free amino acids in plasma and brain were analyzed to determine their relationships with feed intake in both programs of ad libitum feeding or meal-feeding.

Chicks, 3 weeks of age, were put on trials for a period of 72 hours in each of the present experiments.

Experiment V

Experiments V A and V B were designed to study the effects of force-feeding the MD or the ME diets on body weight gain in young chicks. The force-feeding method was adopted to equalize the amount of feed intake.

Three-week old chicks were force-fed the diets for a period of 3 days in each trial.

Experiment VI

Experiments VI A and VI B were conducted to study a relationship between the dietary methionine levels and a crop-emptying rate by using a purified-type diet or a practical-type diet, respectively.

The regulation of the stomach-emptying rate has been known as one of the homeostatic mechanisms of an animal which undergoes adaptation that enables it to adjust to a high protein intake (Harper, 1974b). In the chick, the crop plays

a comparable role in regulating feed intake (Polin and Wolford, 1973). A positive relationship between the emptying rate and an excess amino acid intake was shown with methionine or tryptophan in rats or in dogs (Benevenga and Harper, 1970; Stephens et al., 1975). However, some studies were reported without any responses in the emptying rate to the addition of excess methionine, or phenylalanine by Harper and Kumta (1959), Kumta and Harper (1961) in rats, and Stephens et al. (1975) in dogs. Studies on the effect of methionine deficiency on stomach emptying rate were not available at all.

Experiment VII

A diet deficient in amino acid(s) usually has produced a reduction in feed intake and a consequent decrease of body weight gain in rats (Leung and Rogers, 1969; Rogers and Leung, 1973). From a practical point of view, a restriction of feed intake has been considered as an effective way of raising replacement pullets because a restricted feed intake is able to delay sexual maturity (Connor and Burton, 1971). A delayed sexual maturity and a higher rate of egg production were observed from heavy-breed pullets fed a low lysine diet during their growing period (Couch and Trammell, 1970). When Roberson and Trujilo (1975) fed a diet moderately deficient in TSAA to a group of pullets as basal, or with 0.07% DL-methionine added to the basal diet, no differences were found in feed intake and weight gain.

The present experiment was conducted to determine the effects of methionine-deficient practical-type diets on feed intake and weight gain in two breeds of pullets, i.e. a light breed (SCWL) and a heavy breed (Hubbard White Mountain). The pullets were fed experimental diets during a period from 8 weeks old through the end of the 19th week of age.

B. General Procedure

Male and female, SCWL chicks (Shaver) were obtained from the Rainbow Trail Hatchery, Inc., St. Louis, Michigan, and female heavy breed chicks (Hubbard White Mountain) from the Fairview Hatchery, Inc., Remington, Indiana.

Growing male SCWL chicks were used for experiments I to VI. For experiment VII, light breed (SCWL) and heavy breed (Hubbard White Mountain) pullets were used. In all experiments, the chicks were raised on a practical-type diet up to an age at which they were individually weighed and assigned to treatments so that groups were of equal average weight.

The purified-type basal diet (Table 1) used in experiment I A was slightly modified in experiment I B. Mono-Na-glutamate was removed from the formulation (Table 1) and instead the level of glucose was increased from 49% to 50%. The isolated-soy-protein (from "General Biochemicals") was used for experiment I. It was replaced by the soy-protein whose trade name is "Promine-D" (from "Central Soya Co.") for experiments II, III, IV, V and VIA. This modified formulation of the basal diet from Table 1 was used as the purified-type basal diet for the remaining experiments where needed.

With the exception of experiment VII, all experiments were conducted under identical environmental and housing conditions. Chicks were kept in electrically heated battery brooders with wire-floored pens and the room was lighted for 24 hours a day and had a temperature of 21 $\pm$ 2°C during the experimental periods. The lighting period during the growing stage was 13 hours a day. The chicks for experiment VII were raised in electrically-heated battery brooders up to 3 weeks of age. Then, they were transferred into cages, 57 cm. wide x 40 cm. high x 61 cm. deep, as a group of 10 birds, in a windowless, gas-heated house in which they were kept until 7 weeks of age.

C. The Experiments

1. Experiment I

Experiment I A. One hundred and twenty chicks, 4 weeks of age, were divided into 24 groups with 5 birds each per replication and 3 replications per treatment. The initial average body weight of the chicks was 261 grams.

The experimental design (Table 7) was a completely randomized block design with 8 treatments. To determine the most deficient amino acid in the basal diet (Table 1), each individual amino acid such as DL-methionine, L-lysine HCl, L-tryptophan and L-threonine, or a mixture of different combinations of each amino acid was added to the basal diet at the expense of mono-Na-glutamate ("General Biochemicals") on a weight for weight basis. Thus, all diets were isonitrogenous. The levels of amino acids supplemented were to be

Table 1. Composition of purified - type basal diet (Experiment I)

| Ingredients | % of Diet |
|---|---|
| Isolated Soy Protein[1] | 15.00 |
| Starch, corn | 20.00 |
| Corn oil, stabilized[2] | 4.00 |
| Cellulose ("Sulkafloc)[3] | 3.50 |
| Choline - Cl (50%)[4] | 0.35 |
| Vitamin mixture[5] | 0.50 |
| Salt mixture[6] | 6.24 |
| Mono-Na-glutamate | 1.00 |
| Glucose monohydrate ("Clintose")[7] | up to 100 |
| Calculated analysis | |
| Crude protein (%) | 13.05 |
| Crude fat (%) | 4.00 |
| Metabolizable Energy (Kcal./g.) | 3.42 |
| Calcium:Phosphorus | 1.0:0.6 |

[1]Isolated Soy Protein (87% protein), General Biochemicals, Laboratory Park, Charin Falls, Ohio. In experiment II A, Isolated Soy Protein was replaced by Promine-D (Central Soya, North Laramie Avenue, Chicago, Illinois).

[2]Stabilized with ethoxyquin at levels of 125 mg./kg. diet.

[3]Brown Company, Berlin, New Hampshire

[4]Cholfeed-S, N.V. Chemische Industrie Randstad, Soest, Holland

[5]Supplied the following per kg. of diet: Vitamin A, 10,000 I.U.; Vitamin $D_3$, 1,000 I.C.U.; Vitamin E, 10 I.U.; Vitamin K, 2.0 mg.; Thiamin, 3.0 mg.; Riboflavin, 10.0 mg.; Pantothenic acid, 15.0 mg.; Niacin, 100 mg.; Pyridoxine, 6.0 mg.; Biotin, 0.15 mg.; Folacin, 3.0 mg.; Vitamin $B_{12}$, 0.015 mg.

[6]Supplied the following per kg. of diet: $CaCO_3$, 18.0 g.; $CaHPO_4 \cdot H_2O$, 25.0 g.; $K_2HPO_4$, 9.0 g.; $MnSO_4 \cdot H_2O$ 169.23 mg.; MgO, 828.9 mg.; $FeSO_4 \cdot 7H_2O$, 398.2 mg.; $CuCl_2 \cdot 2H_2O$, 10.73 mg.; $ZnSO_4 \cdot H_2O$, 137.25 mg.; KI, 0.46 mg.; $Na_2MoO_4 \cdot 2H_2O$, 9.84 mg.; $Na_2SeO_4 \cdot IOH_2$, 0.47 mg.; $CoSO_4 \cdot 7H_2O$, 1.0 mg.; $H_3BO_3$, 9.0 mg.; NaCl, 8.8 mg.

[7]"Clintose", Clinton Corn Processing Co., Clinton, Iowa.

Table 2. Comparison of essential amino acids in the basal diet with the specifications by NAS-NRC and Scott et al. (1969), for growth of chicks, (Experiment I).

| | 65% of amino acid requirements[1] equivalent to 13% protein | % of amino acid requirements of protein | % of amino acid in basal diet[2] | % of amino acid of protein in basal diet | B/A |
|---|---|---|---|---|---|
| | % | A | % | B | |
| Arginine | 0.780 | 6.0 | 0.885 | 6.8 | 1.13 |
| Histidine | 0.260 | 2.0 | 0.315 | 2.4 | 1.20 |
| Isoleucine | 0.488 | 3.7 | 0.585 | 4.5 | 1.22 |
| Leucine | 0.910 | 7.0 | 1.035 | 8.0 | 1.14 |
| Lysine | 0.715 | 5.5 | 0.735 | 5.7 | 1.04 |
| Methionine + Cystine | 0.488 | 3.75 | 0.345 | 2.65 | 0.71 |
| Phenylalanine + Tyrosine | 0.845 | 6.5 | 1.185 | 9.1 | 1.40 |
| Threonine | 0.455 | 3.5 | 0.435 | 3.3 | 0.94 |
| Tryptophan | 0.130 | 1.0 | 0.098 | 0.75 | 0.75 |
| Valine | 0.553 | 4.2 | 0.600 | 4.6 | 1.10 |

[1] NAS-NRC (1971), Nutrient requirements of poultry.

[2] Calculated based on the data from Scott et al. (1969).

in excess of NAS-NRC requirements (1971) for young chicks. Therefore, the levels of each amino acid were provided at 80% of the NAS-NRC requirement (1971) as shown in Table 7, though the protein level of the basal diet (13.05%) was equivalent to 65% of the requirement (20%). Actually, calculations (Table 2) based on the data from Scott et al. (1969) showed that only methionine (TSAA) and tryptophan were limiting, with methionine most limiting. Threonine was only borderline deficient if at all. Lysine was added to be certain of its adequacy. Sources of each amino acid were Calbiochem (DL-methionine, B grade), ICN Pharmaceuticals, Inc. (L-lysine·HCl), General Biochemicals (L-tryptophan) and Nutritional Biochemicals, Inc. (L-threonine).

The basal diet (Table 1) was a purified-type diet with a protein content of 13.15% and a calculated metabolizable energy of 3.42 kcal./g. Glucose monohydrate ("Clintose") and corn starch were the major sources of dietary energy. Corn oil stabilized with ethoxyquin was added at 4% level to provide a sufficient amount of essential fatty acids. Choline chloride ("Cholfeed-S") as a 50% active compound was added to the diet at a level of 0.35%. The basal diet contained 0.165% methionine and 0.180% cystine, according to calculations based on data published by Scott et al. (1969).

Experiment I B. At one week of age, 240 chicks with an average body weight of 59.5 grams were alloted into 10 birds per replication and 4 replications per treatment. For this

experiment, mono-Na-glutamate was omitted from the basal diet (Table 1) because it was reported to cause damage to various areas of the brain (Robinzon et al., 1975). Amino acids were replaced at the expense of "Clintose" on the same weight basis, instead of replacing mono-Na-glutamate.

A different approach from that of experiment I A was used in the present experiment to determine the most limiting amino acid in the basal diet. For a particular treatment, an amino acid was omitted from a mixture of four amino acids (Table 8). The levels of each amino acid supplemented were the same as in experiment I A.

For both experiments I A and I B, feed intake and body weight gain were measured on a group basis. Chicks were fasted for 8 hours before starting the experiments. The differences among the means were tested using Duncan's Multiple Range Test according to Little and Hills (1975).

2. Experiment II

Experiment II A. One hundred and thirty five chicks of relatively uniform body weight (average 188 gram) were randomly assigned to 27 pens (3 replications x 9 treatments) as groups of five birds.

Various levels of DL-methionine (Table 8), i.e. 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, and 0.50% of diet, were supplemented to the basal diet at the expense of glucose on a weight for weight basis. One treatment involved feeding the unsupplemented basal.

The formulation of the basal diet was the same as in Table 1 except that no mono-Na-glutamate was added, and the level of glucose was increased to 50% instead of 49%. Also the soy-protein (trade name, "Promine-D") from "Central Soya Co." replaced the isolated-soy-protein from "General Biochemicals" used for previous experiments.

Experiment II B. The same experimental design as in experiment II A was used except that the levels of DL-methionine supplemented by replacing glucose on a weight basis were 0, 0.15, 0.20, 0.25, 0.30, 0.35, and 0.50% (Table 10). The basal diet was as described in experiment II A.

One hundred and sixty eight chicks with average body weight of 166 grams were randomly assigned as 6 birds/replication and 4 replications/treatment for a 3 day experiment.

At termination of the experiment, 9 birds from each of the following treatment groups, i.e. 0, 0.20, and 0.30% levels of DL-methionine supplementation, were sacrificed to obtain plasma and brain samples. The samples were later analyzed for free amino acid patterns. The procedures for collection of the samples and preparation for analysis were as described in Analytical Procedure.

Daily feed intake and beginning and final body weight were measured on a group basis in both of the experiments.

Analysis of variance using a completely randomized design was employed to test treatment differences and Duncan's Multiple Range Test (Little and Hills, 1975) was used to test the differences among the means. Curves were fitted to the data by the least squares method.

Experiment III. One hundred and eighty, 3 weeks old, male chicks were divided into 30 groups with 6 birds per group and 3 replications per treatment. The experiment was designed as a 3 x 3 factorial design with 3 levels of TSAA, i.e. 0.531, 0.597, and 0.663% of diet, and 3 ratios of methionine to cystine, i.e. 46, 52 and 57% of methionine of TSAA (Table 3). In addition to these 9 treatments, a control diet containing 0.665% of TSAA with DL-methionine (0.32%) added to the basal diet as the only sulfur amino acid (SAA) was adopted for a comparison. Thus, the percent methionine of TSAA in the control diet was 73%. The supplementations of the amino acids to the basal diet were made at the expense of glucose on a weight for weight basis. Since the basal diet contained originally 0.165% of methionine and 0.180% of cystine, the differences in each amino acid between the designed and the intrinsic levels were added to the basal diet. The basal diet was a purified-type diet modified from Table 1 as described in experiment II A.

The chicks were fed ad libitum the experimental diets with a continuous lighting during a period of 7 days.

The data were analyzed by a 3 x 3 factorial design and were tested with L.S.D. test. The data of the control group were compared with those of the others by one-way analysis following Duncan's Multiple Range Test.

3. Experiment IV

Experiment IV A. Two hundred and sixteen 3 weeks old chicks were allotted to pens of 6 birds per pen with 3 replications per treatment.

Table 3. Experimental design[1] (Experiment III)

| % TSAA of diet | % methionine of TSAA | % methionine of diet | % cystine of diet |
|---|---|---|---|
| 0.531 | 46 | 0.245[2] (0.080)[3] | 0.256[2] (0.106)[3] |
| | 52 | 0.275 (0.110) | 0.256 (0.076) |
| | 57 | 0.304 (0.139) | 0.227 (0.047) |
| 0.597 | 46 | 0.276 (0.111) | 0.321 (0.141) |
| | 52 | 0.309 (0.144) | 0.288 (0.108) |
| | 57 | 0.342 (0.177) | 0.255 (0.075) |
| 0.663 | 46 | 0.306 (0.141) | 0.357 (0.177) |
| | 52 | 0.343 (0.178) | 0.320 (0.140) |
| | 57 | 0.381 (0.219) | 0.282 (0.102) |
| 0.665 | | 0.485 (0.320) | 0.180 (0) |

[1]6 birds/rep. x 3 replications/treatment.

[2]Indicates designed levels. Thus, it includes methionine (0.165%) or cystine (0.180%) originally present in the basal diet.

[3]The amount of each amino acid actually added to the basal diet to increase each of them up to the designed levels.

[4]Control diet with DL-methionine added to the basal as the only sulfur amino acid. This diet has been adopted as the methionine adequate diet for most part of this study.

Table 4. Experimental design[1] (Experiment IV A)

| Feeding Methods | Levels of DL-methionine supplemented[2] 0% | 0.32% | 1.0% |
|---|---|---|---|
| Ad libitum feeding | 18 birds | 18 birds | 18 birds |
| Meal feeding | | | |
| 6 hr.interval-2 hr.feeding | 18 | 18 | 18 |
| 14 hr.interval-2 hr.feeding | 18 | 18 | 18 |
| 22 hr.interval-2 hr.feeding | 18 | 18 | 18 |

[1]6 birds/replication x 3 replications/treatment.

[2]Each level of methionine supplemented represents methionine deficient diet (0%),methionine adequate diet (0.32%) or methionine excess diet (1.0%).

The experimental design is shown in Table 4. Birds were fed ad libitum or 2-hour meals at time-intervals of 6, 14 and 22 hours each producing a varying degree of hunger (Table 4). The same basal diet modified from Table 1, as described in experiment II A, was used. DL-methionine was supplemented to the basal diet at levels of 0, 0.32, and 1.0% to form 3 different levels of methionine diets at the expense of glucose on a weight for weight basis. During the growing period between 2 and 3 weeks of age, chicks to be put on a meal-feeding program were trained for it by allowing two 2-hour meals during day-time with overnight-fasting. Thus, the initial average body weight for chicks on the ad libitum program or those on the meal-feeding was 175 grams or 154 grams, respectively. Feed intake was measured daily for birds on the ad libitum feeding program or at the end of each meal for those on the meal-feeding program.

Experiment IV B. One hundred and fifty chicks were divided into 30 groups with 5 birds/replication x 3 replications/treatment. The treatments were two different feeding programs, i.e. ad libitum feeding and meal-feeding, and 5 different diets , i.e. methionine deficient diet (MD diet), MD diet + 0.32% DL-methionine, MD diet + 1.32% DL-methionine, MD diet + 0.32% DL-methionine + 1.0% L-glutamate, and MD diet + 1.32% L-glutamate (Table 15). Because the amino acids were added to the basal diet at the expense of glucose on a weight for weight basis, the last three diets were isonitrogenous. L-glutamate (Sigma Chemical Co.) was

added to the diet as a source of α-amino nitrogen. In the present experiment, a meal feeding program of 6 hours of time-interval with a 2 hour-meal was used to compare the effect of meal-feeding with that of ad libitum feeding. The birds trained for meal-feeding as described in experiment IV A had an average body weight of 193 g.; whereas the initial body weight for the ad libitum group was 222 g.

Experiment IV C. All the methods and procedures were the same as described before, except that, in the present experiment, the meal-feeding interval was set at 14 hours instead of 6 hours to stimulate a greater hunger. The level of DL-methionine in the methionine excess diet was set at 1.0% of diet as in experiment IV A. One hundred and eight chicks were divided into 6 birds/replication and 3 replications/treatment. Diets of 3 different levels of DL-methionine, i.e. 0, 0.32, and 1.0%, added to the basal diet by replacing glucose on the same weight basis were fed to chicks in ad libitum feeding or meal-feeding programs (Table 16). The chicks for meal-feeding and ad libitum feeding showed initial average body weight of 171 g. vs. 181 g. respectively.

For experiments IV A, IV B and IV C, chicks were fasted for 8 hours prior to feeding the experimental diets. Each experiment ran for a period of 72 hours with a continuous lighting. The length of lighting period in the room during the training for meal-feeding was 13 hours. Final body weights were measured 6 hours after the last feeding for each program. In experiments IV B and IV C, feed intake of the

ad libitum feeding group was measured at the same time as that of the meal-feeding group to which it was compared. Three hours after the measurement of final body weight, in experiments IV B and IV C, plasma and brain samples were collected from 3 birds in each pen to determine free amino acids as described in Analytical Procedure.

4. Experiment V

Experiment V A. A group of SCWL male chicks were raised on a commercial chick starter up to 5 weeks of age. Forty of them with average body weight of 390 g. were divided into 5 birds per group and 2 groups per treatment. The same basal diet modified from Table 1 as described in experiment II A was used as a methionine deficient (MD) diet. To this MD diet, 0.32% level of DL-methionine was added at the expense of glucose on a weight for weight basis to make a methionine adequate (MA) diet. The chicks on the ad libitum feeding program were allowed access to the MD or the MA diets 12 hours earlier than those to be force-fed the same diets. Before feeding the diets, the chicks to be on an ad libitum feeding or a force-feeding program were fasted for 8 hours or 20 hours, respectively. The amount of the MD or the MA diets to be force-fed was the same amount as consumed by the chicks fed ad libitum the MA diet. The frequency of force-feeding was 5 times a day with 3 hours of time-interval. The diets used in the force-feeding were mixed with water so that each ml. of the liquid diet contained

approximately 0.52 g. of dry diet. The liquid diet was intubated and the crop contents were determined as described in Analytical Procedure.

Experiment V B. Sixty chicks, 5 weeks old (average body weight 368 g.), were alloted into 12 groups with 5 birds/replication and 2 replications/treatment. All the procedures were the same as in experiment V A, except that a group fed a methionine-excess (ME) diet was added to the treatments. The ME diet was prepared by supplementing 1.0% level of DL-methionine to the basal diet at the expense of glucose on the same weight basis. The birds were force-fed 4 times a day with 4 hours of time-interval.

For experiments V A and V B, a free access to water and a continuous lighting were allowed during each 3 days of the experiment. Final body weights of individual chicks were measured 8 hours after removal of the diets to eliminate the influence of feed remaining in gastrointestinal tracts on weight gain. All birds were sacrificed with chloroform and liver weights were measured as % of body weight.

5. Experiment VI

Experiment VI A. Fifty-four SCWL male chicks, 5 weeks old, were allocated to groups of 9 birds for each treatment. Body weights of the chicks were between the ranges of 350-360 g. per bird. The treatments were 3 different levels of DL-methionine, i.e. 0, 0.32 and 1.0%, added to the basal diet at the expense of glucose on a weight for

weight basis and 2 different time-intervals, i.e. 2 and 4 hours, for collection of crop contents after administration of liquid diet. The basal diet was the same purified-type diet modified from Table 1 as described in experiment II A. A liquid diet of each dietary treatment was prepared and administered into the crop as described in Analytical Procedures. A volume of 30 ml. of liquid diet contained 16.83 g. of dry diet. Crop contents were collected individually and determined as described in Analytical Procedures.

The chicks were allowed free access to water during the experiment.

Experiment VI B. This experiment was carried out with a practical-type diet of the formulation as shown in Table 5. The basal diet was formulated to be deficient in only methionine at 73% of the requirement for normal growth (NAS-NRC, 1971). Ground wheat was the major source of dietary energy and soybean oil meal was the major protein source. Methionine content of the basal diet was 1.43% of dietary protein and 0.237% of diet. Three different levels of DL-methionine, i.e. 0, 0.094 and 1.0%, were provided to make MD, MA and ME diets, respectively, by replacing yellow corn with methionine on a weight for weight basis.

Fifty-four, 5 weeksold, male chicks were assigned into 6 groups of 9 birds each. The 6 treatments were two different intervals for collection of crop contents and 3 different levels of methionine diets. Most of the procedures were the same as in experiment V A except that the collection

Table 5. Composition of practical-type basal diet (Experiment VI B)

| Ingredients | % of diet |
|---|---|
| Yellow corn, ground | 12.0 |
| Wheat, ground | 40.2 |
| Oats, ground | 15.0 |
| Wheat middlings | 13.5 |
| Corn oil | 3.0 |
| Soybean oil meal, 48% | 10.0 |
| Meat and bone meal | 2.0 |
| Dicalcium phosphate | 2.2 |
| Limestone | 0.8 |
| NaCl | 0.3 |
| Vitamin mixture[1] | 0.5 |
| Mineral mixture[2] | 0.5 |
| Total | 100.0 |

Calculated Analysis:

| | |
|---|---|
| Crude Protein, % | 16.59 |
| M.E. Kcal./g. | 2.91 |
| TSAA, % of protein | 3.09 |
| Methionine, % of protein | 1.46 |

[1]Supplied the following per kg. of diet: The same as in Table 1.

[2]Supplied the following per kg. of diet: $MnSO_4 \cdot H_2O$, 169.23 mg.; MgO, 914.07 mg.; $FeSO_4 \cdot 7H_2O$, 398.20 mg.; $CuCl_2 \cdot 2H_2O$, 10.74 mg.; $ZnSO_4 \cdot H_2O$, 219.60 mg.; $Na2SeO_4 \cdot 10H_2O$, 0.468 mg.

of crop contents was done at 3 and 6 hours after the oral administration of the liquid diet. The liquid diets were prepared to contain 13.94 g. of dried diet in a 30 ml. volume.

Experiment VII. Two different breeds of female chicks, i.e. a light breed (SCWL) female and a heavy breed (Hubbard White Mountain) female, were raised on a conventional starter diet for each breed to 7 weeks of age from day-old. At the end of 7 weeks of age, they were individually weighed and 108 chicks of each breed with average body weight of 585 g. and 1,569 g. for light breed and heavy breed, respectively, were selected. They were distributed in 41 cm. wide x 36 cm. high x 81 cm. deep cages to have 6 birds per cage and 6 cages per treatment.

Two practical type-basal diets (Table 6) were prepared for each breed for the first stage of the experiment. They were formulated to be deficient only in methionine (or TSAA) by using wheat as the major dietary energy source and soybean meal as the major protein source. Thus, the levels of crude protein, metabolizable energy and methionine of each diet were 16.59%, 2.91 kcal./g. of diet and 1.43% of protein level, respectively, for the light breed, and 21.19%, 2.91 kcal./g. of diet and 1.43% of protein level, respectively, for the heavy breed.

For the second stage of the experiment, two different formulations of practical-type diets (Table 6) deficient in methionine were prepared for each breed by using wheat and

Table 6. Compositions of basal diets (MD diets) and the design for experimental diets (Experiment VII)

| | Diets for light breed | | Diets for heavy breed | |
|---|---|---|---|---|
| | Grower[1] | Developer[2] | Grower[1] | Developer[2] |
| Yellow corn, ground | 12.0 | 16.0 | 14.0 | 12.0 |
| Wheat, ground | 40.2 | 26.0 | 40.2 | 40.2 |
| Oats, ground | 15.0 | 27.0 | - | 15.0 |
| Wheat middlings | 13.5 | 14.1 | 15.0 | 13.5 |
| Corn oil | 3.0 | 4.0 | 3.0 | 3.0 |
| Soybean meal, 48% | 10.0 | 7.0 | 21.0 | 10.0 |
| Meat and bone meal | 2.0 | 2.0 | 3.0 | 2.0 |
| Dicalcium phosphate | 2.2 | 2.0 | 2.0 | 2.2 |
| Limestone | 0.8 | 0.9 | 0.5 | 0.8 |
| NaCl | 0.3 | 0.3 | 0.3 | 0.3 |
| Vitamin mixture[3] | 0.5 | 0.5 | 0.5 | 0.5 |
| Mineral mixture[4] | 0.5 | 0.5 | 0.5 | 0.5 |
| Total | 100.0 | 100.3 | 100.0 | 100.0 |
| Calculated analysis: | | | | |
| Cr. protein % | 16.59 | 14.89 | 21.19 | 16.59 |
| M.E. kcal./g. | 2.91 | 2.92 | 2.91 | 2.91 |
| TSAA, %[5] | 3.09 | 3.34 | 3.05 | 3.09 |
| Methionine, %[5] | 1.46 | 1.46 | 1.43 | 1.43 |
| Design for experimental diets: | | | | |
| %DL-methionine added to basal (MD diets) | | | | |
| MMD diet, % | 0.047 | 0.040 | 0.060 | 0.047 |
| MA diet[6], % | 0.094 | 0.080 | 0.120 | 0.094 |

[1]Fed from 8th week of age through 13th week of age.

[2]Fed from 14th week of age to first egg (19th week).

[3]Supplied the following per kg. of diet: Vitamin A, 10,000 I.U.; Vitamin $D_3$, 1,000 I.C.U.; Vitamin E, 10 I.U.; Vitamin K., 2.0 mg.; Thiamin, 3.0 mg.; Riboflavin, 10.0 mg.; Pantothenic acid, 15.0 mg.; Niacin, 100 mg.; Pyridoxine, 6.0 mg.; Biotin, 0.15 mg.; Folacin, 3.0 mg.; Vitamin $B_{12}$, 0.015 mg.

[4]Supplied the following per kg. of diet: $MnSO_4 \cdot H_2O$, 169.23 mg.; MgO, 914.07 mg.; $FeSO4 \cdot 7H_2O$, 398.20 mg.; $CuCl_2 \cdot 2H_2O$, 10.74 mg.; $ZnSO_4 \cdot H_2O$, 219.60 mg.; $Na_2SeO_4 \cdot 10H_2O$, 0.468 mg.

[5]Expressed as % of dietary protein.

[6]The requirement of methionine was calculated as 2.0% of dietary protein level.

soybean meal as the major sources for energy and protein, respectively. The levels of crude protein, metabolizable energy and methionine of each diet were 14.89%, 2.92 kcal./g. of diet and 1.46% of protein level, respectively, for the light breed, and 16.59%, 2.91 kcal./g. diet and 1.43% of protein level, respectively, for the heavy breed.

To the basal diets (MD) for the first stage of the present experiment, two different levels of DL-methionine were added, at the expense of yellow corn (W/W basis), to make a moderate methionine-deficient diet (MMD) and a methionine adequate diet (MA). The levels of the amino acid added to the basal diets of each breed were 0.047 and 0.094% of diet for the light breed, and 0.060 and 0.120% of diet for the heavy breed, respectively, for the MMD diet and the MA diet. For the second-stage experiment, the following levels of DL-methionine were added to the basal diet (Table 6) by replacing yellow corn on W/W basis. The levels of the amino acid for the light breed were 0.040 and 0.080% of diet, and those for the heavy breed were 0.047 and 0.094% of diet, respectively, for the MMD diet and the MA diet.

The first stage of the experiment was from the beginning of 8 weeks of age to the end of the 13th week of age, at which time the chick-grower diets were replaced with developer diets (Table 6) for the second stage of the experiment. At the time of feeding the developer diets, one bird from each cage (6 birds per treatment) was sacrificed to determine liver lipid-content. The total liver-lipid was analyzed as described in Analytical Procedures.

The birds were under controlled lighting with the lighting time gradually decreased to 7 hours a day by the time they were 12 weeks of age and then kept constant until termination of the experiment. Body weight and feed intake were measured at 3 week intervals and the final measurements were made at the end of the 19th week of age. Feeds were fed ad libitum and a free access to water was allowed.

D. Analytical Procedures

1. Determination of plasma free amino acids

Two ml. of blood were taken from each chick by heart puncture with a heparinized syringe and pooled together with the blood samples of the same group in a heparinized test tube.

Immediately after collection, samples were centrifuged at 1,500 rpm. for 20 min. to separate the plasma from the red cells. Two ml. of plasma were added with 0.2 ml of internal standard containing 1 mM of nor-leucine and 1 mM of S-β-(4-pyridylethyl)-L-cysteine into a 16 x 125 mm. screw-capped test tube. This was followed by the addition of 0.1 ml. sulfosalicylic acid (50% by W/V) per ml. of plasma to precipitate plasma proteins. The mixture was vibrated on a Vortex mixer, and placed in an ice bag for at least 30 min. Then the mixture was centrifuged at 25,000 x g. for 15 minutes. The supernatant was removed with a Pasteur pipette and stored in a freezer until analyzed.

The plasma free amino acid concentrations were analyzed with a Technicon TSM-1 Auto Amino Acid Analyzer (Bergen and Potter, 1975). Tryptophan was not determined.

2. Determination of brain free amino acids.

The chicken's head was severed directly into liquid nitrogen and stored in a frozen state at -29°C. The cerebral hemispheres in which such neuro-structures as ventromedial and lateral hypothalamus and anterior prepyriform cortex are located, were collected following the exposure of brain and pooled together with the samples of the same group. They were homogenized with deionized water at the rate of 1 g. cerebrum plus 3 ml. deionized water. To 2 ml. of brain homogenate was added 0.2 ml. of internal standard solution containing 1 mM of nor-leucine and 1 mM of S-β-(4-pyridylethyl)-L-cysteine followed by the addition of 0.5 ml. of 15% (W/V) sulfosalicylic acid (Peng et al., 1973). The mixture was centrifuged at 25,000 x g. for 15 minutes. The supernatant was removed with a pasteur pipette and stored at -29°C until analyzed.

The brain free amino acid concentrations were analyzed with a Technicon TSM-1 Auto Amino Acid Analyzer. Tryptophan was not determined.

3. Determination of total liver lipid

The assay technique was a modification of the method described by Folch et al. (1957).

The whole livers of the pooled group were minced while removing connective tissues and blended well before sampling. Three grams of tissue sample were weighed into a tared 100 ml. beaker. Thirty ml. of chloroform: methanol,

2:1(V/V), were added to the sample for extraction of lipids and homogenized for 3 min. with a Tekmar high speed homogenizer. The contents were filtered into a 125 ml. separatory funnel through glass wool followed by a rinse with 30 ml. of the extraction mixture. Twelve ml. (0.2 volume) of distilled water containing 0.04% of $CaCl_2$, 0.034% of $MgCl_2$ and 0.58% of NaCl was added to the chloroform:methanol extract. This mixture was mixed gently and allowed to separate into 2 layers. The upper phase was removed by careful siphoning and the interface was rinsed 3 times with upper phase solvent (chloroform : methanol : water, 3 : 48 : 47 by volume). Methanol was added to the lower phase until it became miscible with the residual rinse solvent forming one layer. One spoonful of anhydrous $Na_2SO_4$ was added to the separatory funnel. After standing for 15 min., the solvent was filtered into a tared 100 ml. beaker through glass wool, and the funnel was washed with chloroform. The filtrate was evaporated in a drying oven at 50°C. The total crude lipids recovered were weighed and expressed as a percentage of the initial sample weight.

4. Determination of dried weight of crop contents

A liquid diet was prepared by mixing the diet with a volume of water so that the diet of liquid-form would contain a proper density (for example, 30 ml. volume of liquid diet to contain approximately 17.0 g. of a dry purified-type diet). This liquid diet was orally administered into the crop by using a plastic syringe (50 ml. volume) attached

with a flexible plastic tube (0.6 cm. O.D.). The plunger of the plastic syringe was pressed down to a predetermined level to deliver a measured weight of diet after inserting the tip of the plastic tube into the crop. Chicks were fasted approximately 18 hours prior to the administration of liquid diet. A complete empty crop was observed at 12 hours after fasting. With 2 to 6 hour time-intervals after the administration of the diet, 9 birds on each level of methionine diet were killed by I.V. injection of excess amount of Na-pentobarbiturate (30 mg./ml.) solution. Immediately after the injection, the upper part of the neck was tied tightly to prevent involuntary outflow of the crop contents, and the junction between the crop and lower esophagus was pinched with a hemostat. The contents in the crop and the upper esophagus were collected carefully in a pre-weighed tare and dried in a drying oven at 80°C. until constant weight was obtained.

E. Statistical Analysis

All experimental data were subjected to analysis of variance. The data for experiment II A and II B were fitted into linear lines by the least squares method. The data for experiment III were statistically analyzed by a 3 x 3 factorial design and means between the treatments were tested using L.S.D. test. Duncan's Multiple Range Test (Little and Hills, 1975) was used whenever applicable for comparisons of the differences between the treatment means.

# IV. RESULTS

## A. Experiment I

The results from the experiments I A and I B are shown in Tables 7 and 8, respectively. The data in both Tables indicate that methionine (or sulfur amino acids) is (are) the only limiting amino acid(s) in the basal diet for optimum growth of chicks. Therefore, for further experiments in this study, only methionine was added to the basal diet to balance the amino acids compositions.

## B. Experiment II

Requirements of methionine (or TSAA) for maximum weight gain and feed intake.

The effects on weight gain and feed intake of young chicks fed various levels of supplemental DL-methionine in a diet with 13% protein, are shown by the data presented in Table 9 and Figure 2.

A linear response in weight gain to supplemental methionine was observed and further supplementation failed to produce any significant increase (Figure 2). The requirement of DL-methionine was estimated as the point at which the growth-response curve intersected a line representing the plateau for maximum weight gain. The dose-response line

Table 7. Effect of various amino acids on body weight gain and feed intake of young chicks fed a purified-type diet with supplemental amino acids (Experiment I A)

| Diets | Weight gain[1] g./bird/day | Feed intake[1] g./bird/day |
|---|---|---|
| Basal diet | 0.03±1.1[3]a[4] | 22.7±2.2[3]a[4] |
| " + DL-MET (0.26%)[2] | 14.00±0.9 b | 38.1±1.8 b |
| " + L-TRY (0.7%) | -0.13±0.7 a | 22.0±2.6 a |
| " + L-THR (0.13%) | -0.55±0.9 a | 20.4±1.8 a |
| " + L-LYS·HCl (0.19%) | -0.70±0.4 a | 22.4±1.2 a |
| " + L-THR+L-LYS·HCl | -0.12±0.7 a | 22.6±0.8 a |
| " + DL-MET + L-TRY | 13.90±0.8 b | 37.4±1.7 b |
| " + DL-MET + L-TRY + L-THR + L-LYS·HCl | 14.60±0.3 b | 38.7±0.5 b |

[1]Mean of 5 birds/rep. x 3 replications/treatment.

[2]Amount of amino acids added to the basal diet.

[3]Mean ± S.E.

[4]Means not carrying the same subscript in each column are significantly different ($P < 0.01$) in accordance with Duncan's Multiple Range Test.

Analysis of variance of weight gain and feed intake

| Source of variation | d.f. | Mean Square | |
|---|---|---|---|
| | | Weight gain | Feed intake |
| Total | 23 | | |
| Treatment | 7 | 168.56** | 208.92** |
| Error | 16 | 1.73 | 8.66 |

**$P < 0.01$.

Table 8. Effects of various amino acids on body weight gain and feed intake of young chicks fed a purified-type diet with or without supplemental amino acids (Experiment I B)

| Supplemental amino acids[1] | | | | Weight gain[2] | Feed intake[2] |
|---|---|---|---|---|---|
| 0.26% DL-MET | 0.07% L-TRY | 0.13% L-THR | 0.19% L-LYS·HCl | g./bird/day | g./bird/day |
| - | - | - | - | 1.3±0.1[3]a[4] | 8.1±0.7[3]a[4] |
| + | + | + | + | 8.1±0.2 b | 18.1±0.5 b |
| - | + | + | + | 1.1±0.1 a | 7.2±0.3 a |
| + | - | + | + | 8.0±0.2 b | 18.1±0.1 b |
| + | + | - | + | 8.4±0.4 b | 18.5±0.5 b |
| + | + | + | - | 8.5±0.2 b | 18.4±0.2 b |

[1]Purified-type diet (Table 1) supplemented with the amino acids at the indicated levels.

[2]Mean of 10 birds/rep. x 4 replications/treatment.

[3]Mean ± S.E.

[4]Means not carrying the same subscript in each column are significantly different ($P < 0.01$).

Analysis of variance of weight gain and feed intake

| Source of variation | d.f. | Mean Square | |
|---|---|---|---|
| | | Weight gain | Feed intake |
| Total | 23 | | |
| Treatment | 5 | 53.26** | 120.36** |
| Error | 18 | 0.20 | 0.72 |

**$P < 0.01$

for growth was Y = 27.5 X + 2.3 and the horizontal line representing the plateau portion for growth was Y = -1.4 + 11.3, where Y = weight gain in grams/bird/day, and X = % of supplemental DL-methionine. That point corresponded to 0.311% of supplemental DL-methionine. Thus, the estimated TSAA requirement for maximal growth at a 13% protein level, taking into account the methionine (0.165%) and cystine (0.180%) supplied by isolated-soy-protein, was 0.650% of diet or 5.01% of dietary protein.

When the data on feed intake were plotted against the level of supplemental DL-methionine (Figure 2), a linear response was observed. The regression analysis revealed that this line was characterized by the equation, Y = 67.7 X + 16.6, where Y = feed intake in grams/bird/day and X = % of supplemental methionine. The point of intersection with the horizontal line, Y = 1.8 X + 28.4, where Y = feed intake in grams/bird/day and X = % of supplemental methionine, was at 0.18% of supplemental DL-methionine. Therefore, the requirement of TSAA for maximum feed intake was estimated to be 0.525% of diet or 4.01% of dietary protein, considering the methionine (0.165%) and cystine (0.180%) from isolated-soy-protein. Thus, the requirement of TSAA for optimal growth was 25% higher than that for optimal feed intake.

Gain/feed ratio (Table 9) appeared to become more efficient as the supplemental levels of DL-methionine were increasing up to about 0.3% and, thereafter, remained constant.

The results of weight gain, feed intake and gain/feed ratio for experiment II B are in Table 10. The growth and feed intake response curves are depicted in Figure 3. The data in this experiment were not as definitive in assessing optimum methionine requirement for feed intake and growth as in the first experiment. Variability was greater in the groups on the plateau portion of the response lines. The estimate made for maximum growth appeared to be between 0.2 and 0.3% with the intersection occurring at 0.305% as in Figure 3. The two equations for the growth responses were $Y = 3.6 X + 10.7$ for the plateau portion and $Y = 21.3 X + 5.3$ for the growing portion, where Y = average weight gain in grams/bird/day and X = % of supplemental DL-methionine.

The estimated optimum level of supplemental methionine to obtain maximum feed intake was 0.192%, as in Figure 3. The two equations for plateau and response were $Y = 1.9 X + 27.5$ and $Y = 47.2 X + 18.8$, respectively, where Y = average feed intake in grams/bird/day, and X = % of DL-methionine supplemented in the diet.

Thus, again considering the levels of methionine and cystine from isolated-soy-protein, the TSAA requirements for maximum growth and feed intake were 4.96% and 4.10%, of dietary protein, respectively.

The gain/feed ratio (Table 10) was plateaued at a point between 0.20% and 0.25% of DL-methionine level which was somewhat lower than the level of about 0.3% in experiment II A.

Table 9. Estimation of requirement for DL-methionine to obtain optimum growth of young chicks fed a purified-type diet containing a level of 13% protein (Experiment II A). Also see Figure 2.

| Levels of DL-methionine added to basal, % | Weight gain[1] g./bird/day | Feed intake[1] g./bird/day | Gain/Feed |
|---|---|---|---|
| 0 | $-1.2 \pm 1.3^{2}a^{3}$ | $16.5 \pm 2.2^{2}e^{3}$ | - |
| 0.10 | $4.8 \pm 0.1$ b | $23.5 \pm 0.9$ f | $0.21 \pm 0.01^{2}h^{3}$ |
| 0.15 | $6.8 \pm 0.1$ bc | $26.6 \pm 1.5$ fg | $0.26 \pm 0.02$ i |
| 0.20 | $7.9 \pm 0.3$ cd | $28.3 \pm 1.2$ g | $0.28 \pm 0.01$ ij |
| 0.25 | $9.2 \pm 1.2$ d | $29.8 \pm 2.1$ g | $0.31 \pm 0.03$ j |
| 0.30 | $10.5 \pm 0.7$ d | $28.6 \pm 1.7$ g | $0.37 \pm 0.01$ k |
| 0.35 | $10.8 \pm 0.7$ d | $28.6 \pm 0.8$ g | $0.38 \pm 0.02$ k |
| 0.40 | $10.8 \pm 0.6$ d | $29.6 \pm 1.5$ g | $0.37 \pm 0.01$ k |
| 0.50 | $10.6 \pm 0.5$ d | $29.2 \pm 0.3$ g | $0.36 \pm 0.01$ k |

[1]Means of 5 birds/rep. x 3 replications/treatment.

[2]Mean $\pm$ S.E.

[3]Means not carrying the same subscript in each column are significantly different ($P < 0.05$) in accordance with Duncan's Multiple Range Test.

Analysis of variance of weight gain, feed intake and gain/feed

| Source of variation | d.f. | Mean Square: Weight gain | Mean Square: Feed intake | d.f. | Mean Square: Gain/Feed |
|---|---|---|---|---|---|
| Total | 26 | | | 23 | |
| Treatment | 8 | 47.18* | 55.86* | 7 | 0.012* |
| Error | 18 | 1.55 | 6.50 | 16 | 0.0007 |

*$P < 0.05$

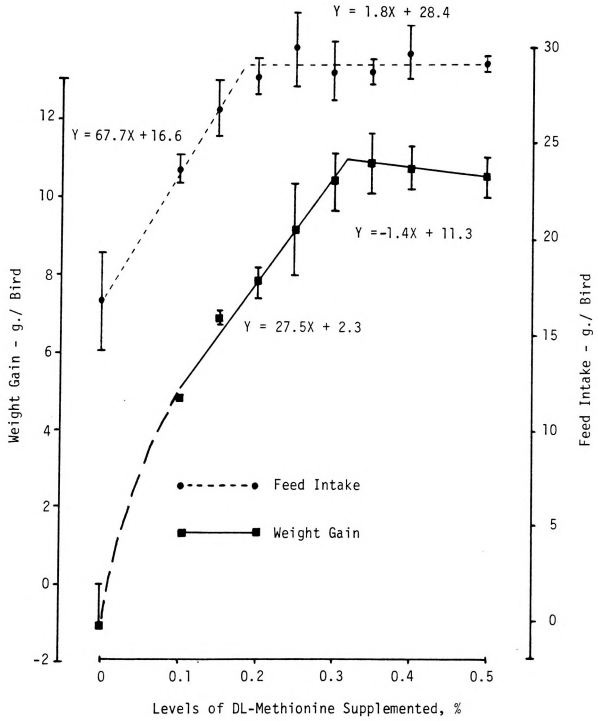


Figure 2.--Chick weight gain and feed intake as a function of dietary sulfur amino acids concentrations. The basal diet contained 0.165% methionine and 0.180% cystine (Experiment II A).

Table 10. Weight gain, feed intake and gain/feed ratio of young chicks fed diets of various levels of DL-methionine (Experiment II B). Also see Figure 3.

| Level of DL-methionine added to basal diet, % | Weight gain[1] g./bird/day | Feed intake[1] g./bird/day | Gain/Feed |
|---|---|---|---|
| 0 | $1.8 \pm 1.1^{2}a^{3}$ | $18.8 \pm 1.4^{2}f^{3}$ | $0.14 \pm 0.02$ h |
| 0.15 | $8.5 \pm 0.4$ b | $26.0 \pm 0.7$ g | $0.33 \pm 0.02$ i |
| 0.20 | $9.7 \pm 0.8$ bc | $28.2 \pm 2.1$ g | $0.34 \pm 0.01$ j |
| 0.25 | $10.5 \pm 0.3$ cd | $27.4 \pm 0.9$ g | $0.38 \pm 0.02$ j |
| 0.30 | $11.8 \pm 0.5$ de | $28.8 \pm 1.0$ g | $0.41 \pm 0.01$ j |
| 0.35 | $10.6 \pm 0.3$ cd | $26.6 \pm 0.6$ g | $0.40 \pm 0.01$ j |
| 0.50 | $12.5 \pm 0.3$ e | $28.3 \pm 1.5$ g | $0.44 \pm 0.01$ k |

[1]Means of 6 bird/rep. x 4 replications/treatment.

[2]Mean $\pm$ S.E.

[3]Means not carrying the same subscript in each column are significantly different ($P < 0.05$).

Analysis of variance of weight gain, feed intake and gain/feed

| Source of variation | d.f. | Mean Squares | | |
|---|---|---|---|---|
| | | Weight gain | Feed intake | Gain/Feed |
| Total | 27 | | | |
| Treatment | 6 | 50.49* | 47.33* | 0.033* |
| Error | 21 | 1.25 | 6.49 | 0.001 |

* $P < 0.05$

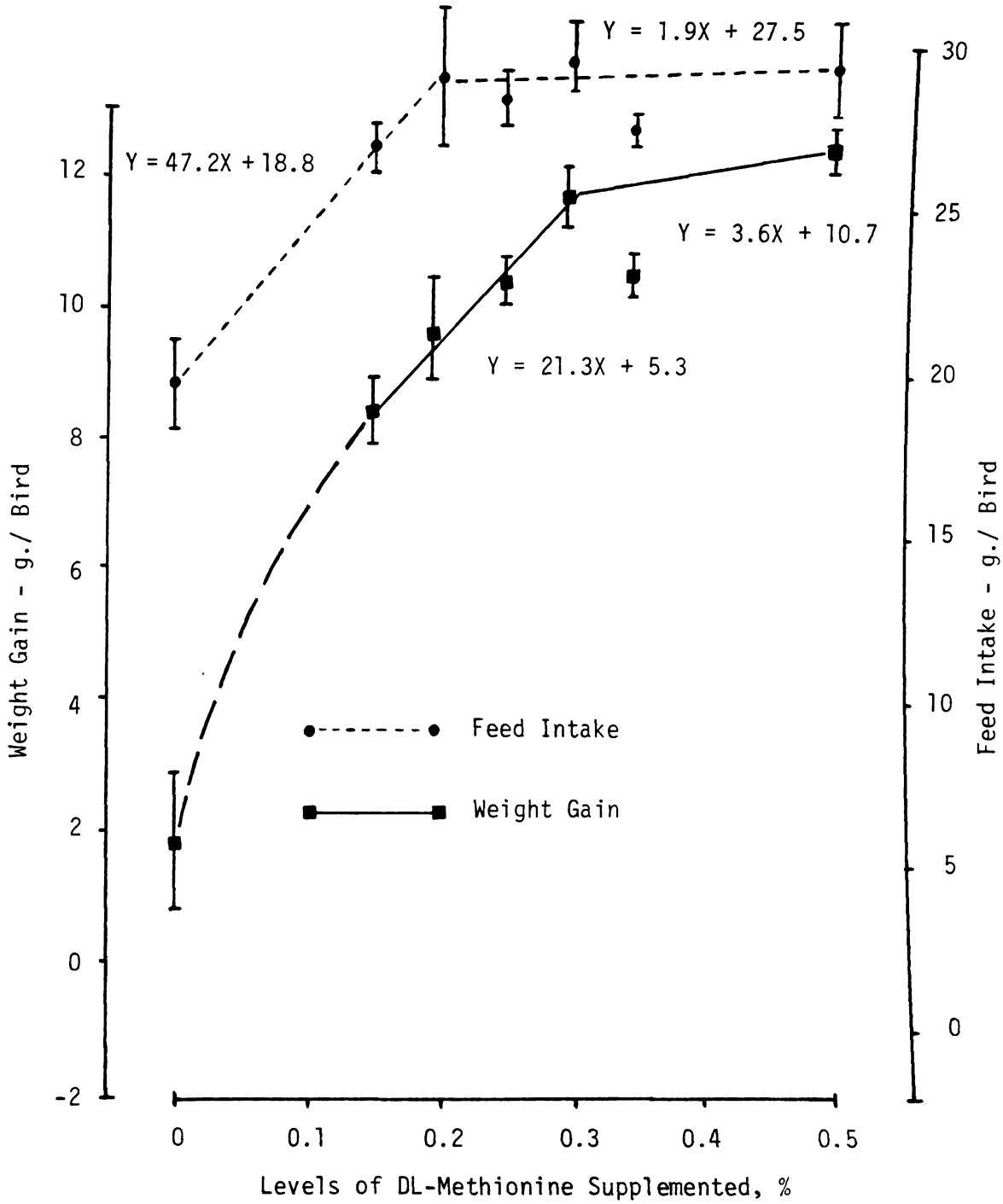


Figure 3.--Chick weight gain and feed intake as a function of dietary sulfur amino acids concentration. The basal diet contained 0.165% methionine and 0.180% cystine (Experiment II B).

Concentrations of free amino acids in plasma and brain of chicks fed the diets adequate or deficient in methionine

The relationship between feed intake and free amino acid concentrations in plasma and brain was determined in chicks fed diets to which were added none, 0.2 or 0.3% levels of DL-methionine. These levels of methionine supplementation were selected from data on Tables 9 and 10 to coincide with having or not having an effect on feed intake and weight gain.

The free amino acid concentrations in plasma and brain of chicks fed the diets added with none, 0.2 or 0.3% levels of DL-methionine are shown in Tables 11 and 12, and depicted in Figure 4. Levels of individual amino acids of chicks fed the diets with 0 and 0.2% levels of methionine added were compared with those of control group chicks fed the diet with 0.3% level of methionine added to investigate the changes of amino acid patterns resulting from dietary treatments.

Threonine, serine, lysine and histidine in plasma were significantly increased, and methionine significantly decreased by the diet (MD diet) with no added methionine (Table 11). Cysteine showed a tendency for a slight increase as the levels of dietary methionine were increased. The differences in amino acid concentrations of the plasma between the diets of two different levels of methionine (0.2 and 0.3%) were not marked. Only threonine and serine were significantly higher in the chicks fed the diet with 0.2% added methionine than in the control. Alanine was

Table 11. Concentrations[1] of free amino acids (FAA) in plasma of chicks fed ad libitum the diets with different levels of DL-methionine. (Experiment II B)

| Amino acids | FAA in plasma, µmole/100 ml. % of DL-methionine added 0 | 0.2 | 0.3 | Trend for direction of change |
|---|---|---|---|---|
| ASP + ASN | 22.56± 0.92[2]a[3] | 28.22± 1.45[2]a[3] | 23.69± 2.48[2]a[3] | - |
| THR | 157.05±10.41 a | 71.80± 6.71 b | 45.13± 1.38 c | ↓ |
| SER | 199.84± 4.06 a | 146.56± 6.94 b | 98.02± 9.58 c | ↓ |
| GLU + GLN | 112.60± 6.93 a | 147.69±11.93 a | 114.93± 8.60 a | - |
| PRO | 26.49± 0.80 a | 23.39± 2.13 a | 21.07± 0.99 a | - |
| GLY | 73.47±12.86 a | 75.95± 3.95 a | 57.87± 6.91 a | - |
| ALA | 57.47± 3.05 a | 122.02± 4.49 b | 87.32±16.86 ab | ↑ |
| VAL | 45.87± 4.43 a | 57.00± 6.61 a | 43.00± 2.86 a | - |
| CYS | 5.96± 0.96 a | 6.68± 0.16 a | 7.88± 1.47 a | - |
| MET | 9.44± 0.65 a | 11.78± 0.09 ab | 16.32± 2.64 b | ↑ |
| ILE | 27.23± 3.85 a | 36.83± 5.42 a | 33.08± 4.95 a | - |
| LEU | 40.45± 4.23 a | 50.05± 6.23 a | 41.69± 2.80 a | - |
| TYR | 24.60± 1.50 a | 29.19± 1.36 a | 28.01± 2.20 a | - |
| PHE | 16.67± 1.32 a | 20.14± 1.16 a | 19.47± 1.95 a | - |
| LYS | 113.30±12.97 a | 76.31± 0.64 b | 66.60± 5.63 b | ↓ |
| HIS | 16.16± 0.88 a | 13.47± 1.26 ab | 11.95± 0.57 b | ↓ |
| ARG | 17.13± 1.10 a | 20.16± 1.85 a | 23.02± 1.00 a | - |
| TFAA[4] | 966.3 ±31.0 a | 937.2 ±11.5 a | 739.1 ±56.9 b | ↓ |
| NEAA[4] | 523.0 ±21.3 ab | 579.7 ±24.3 a | 438.8 ±40.4 b | ↓ |
| EAA[4] | 443.3 ±21.9 a | 357.5 ±14.4 b | 300.3 ±20.3 b | ↓ |
| NEAA/EAA | 1.18± 0.08 a | 1.62± 0.13 b | 1.46± 0.08 ab | ↑ |
| EAA/TFAA | 0.46± 0.02 a | 0.38± 0.02 b | 0.41± 0.02 ab | - |

[1] Means of 3 birds/rep. x 3 replications/treatment.
[2] Mean ± S.E.
[3] Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4] Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

Table 12. Concentrations[1] of free amino acids (FAA) in brain of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment II B)

| Amino acids | FAA in brain, µmole/g. tissue % of DL-methionine added | | | Trend for direction of change |
|---|---|---|---|---|
| | 0 | 0.2 | 0.3 | |
| ASP + ASN | 3.19±0.05[2]a[3] | 2.91±0.39[2]a[3] | 2.60±0.49[2]a[3] | - |
| THR | 1.30±0.20 a | 0.77±0.01 b | 0.44±0.01 c | ↓ |
| SER | 1.58±0.16 a | 1.94±0.15 a | 1.45±0.12 a | - |
| GLU + GLN | 10.97±0.62 a | 10.01±0.44 a | 11.13±2.00 a | - |
| PRO | 0.51±0.08 a | 0.53±0.06 a | 0.58±0.07 a | - |
| GLY | 1.62±0.40 a | 2.79±0.42 a | 2.21±0.41 a | - |
| ALA | 2.01±0.17 a | 2.12±0.29 a | 1.84±0.12 a | - |
| VAL | 0.41±0.03 a | 0.52±0.09 a | 0.48±0.04 a | - |
| CYS | 0.14±0.02 a | 0.13±0.01 a | 0.29±0.09 a | - |
| MET | 0.15±0.01 a | 0.15±0.02 a | 0.14±0.01 a | - |
| ILE | 0.35±0.02 a | 0.43±0.05 a | 0.32±0.03 a | - |
| LEU | 0.45±0.03 a | 0.52±0.06 a | 0.48±0.01 a | - |
| TYR | 0.29±0.01 a | 0.35±0.06 a | 0.32±0.01 a | - |
| PHE | 0.30±0.02 a | 0.34±0.05 a | 0.29±0.01 a | - |
| LYS | 1.29±0.15 a | 0.80±0.05 b | 0.68±0.05 b | ↓ |
| HIS | 0.35±0.02 a | 0.28±0.05 a | 0.21±0.01 a | - |
| ARG | 0.27±0.05 a | 0.41±0.04 a | 0.40±0.04 a | - |
| TFAA[4] | 25.2 ±1.6 a | 25.0 ±1.5 a | 23.9 ±1.9 a | - |
| NEAA[4] | 20.3 ±1.6 a | 20.8 ±1.3 a | 20.4 ±2.0 a | - |
| EAA[4] | 4.9 ±0.4 a | 4.2 ±0.2 ab | 3.4 ±0.1 b | ↓ |
| NEAA/EAA | 4.14±0.5 a | 4.08±0.2 a | 6.00±0.7 a | - |
| EAA/TFAA | 0.20±0.02a | 0.17±0.2 a | 0.15±0.02 a | - |

[1] Means of 3 birds/rep. x 3 replications/treatment.
[2] Mean ± S.E.
[3] Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4] Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

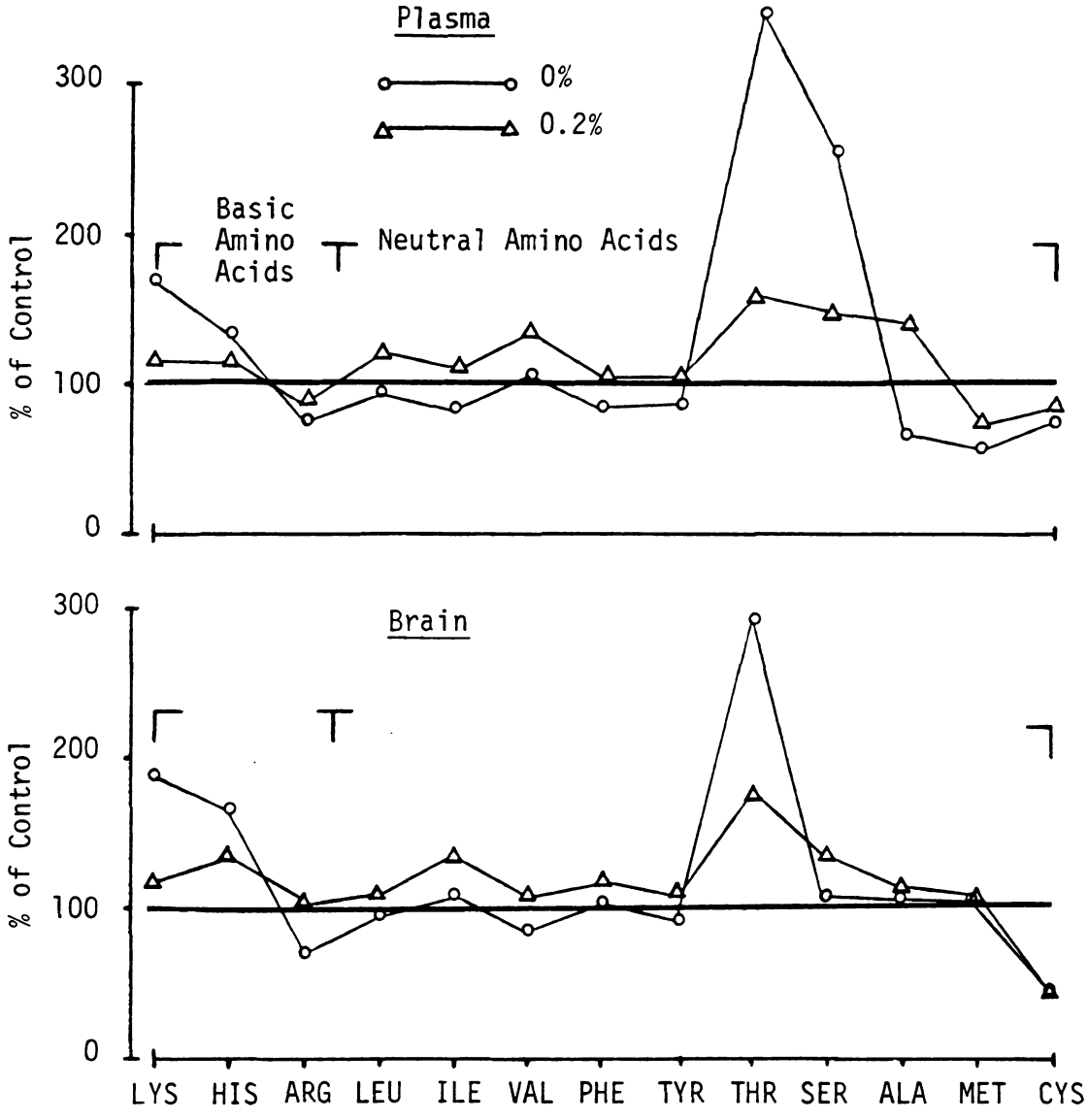


Figure 4.--Relative concentrations of plasma and brain free amino acids of chicks fed diets deficient (o——o), moderately deficient (Δ——Δ) or adequate in TSAA (methionine). The control group was fed the adequate diet (Experiment II B).

increased by the former diet to a level about 40% higher than that of the control without a statistical significance.

The concentrations of total free amino acids (TFAA), nonessential amino acids (NEAA) and essential amino acids (EAA) were all higher in the plasma of chicks fed the MD diet than in that of the control group (Table 11). The ratios of nonessential amino acids to essential amino acids, NEAA/EAA, and EAA/TFAA in plasma were significantly decreased and increased, respectively, by the diet deficient in methionine, compared to those of the control. Between the diets with 0.2 and 0.3% levels of added methionine, the former increased TFAA and NEAA with significance at $P < 0.01$. No differences were observed in levels of EAA, NEAA/EAA and EAA/TFAA in plasma between those two treatments (Table 11).

Of the changes of amino acids in brain (Table 12), the increased levels of threonine, lysine and histidine by the MD diet were of most significance. The only difference in the brain amino acids of chicks fed the diets of 0.2 and 0.3% levels of methionine was threonine which was higher on the former diet than on the control. The levels of brain cysteine obtained by the diets of the lower levels of methionine, i.e. 0 and 0.2%, were less than half of that of control, though there were no statistical differences. Meanwhile, the levels of brain methionine were not affected at all by the diet deficient in methionine.

No significant differences were observed in brain concentrations of TFAA, NEAA, NEAA/EAA and EAA/TFAA among

those chicks fed different levels of methionine diets. However, the level of EAA was higher ($P < 0.05$) in the group fed the MD diet than in the control, while the increased level of EAA in chicks fed the diet with 0.2% added methionine was significantly different from that of the control.

C. Experiment III

The requirement of TSAA (methionine + cystine), 0.665,for optimum growth was obtained in experiment II. Although the actual level of the amino acid required was 0.311%, the level of 0.32% DL-methionine was chosen to be added to the basal diet just to be certain of its adequacy. Thus, aside from the requirement level of TSAA at 0.665%, two decreasing levels of TSAA, i.e. 0.597% and 0.531%, were adopted to test the optimum ratio of methionine to cystine.

When chicks were fed the diet with the lowest level of TSAA (0.531%), feed intake, weight gain and gain/feed were always lower ($P < 0.01$) than for chicks receiving the higher levels of TSAA diets (Table 13). Between the treatments of two higher levels of TSAA (0.597% vs. 0.665%), no significant differences in those 3 measurements were observed at $P > 0.05$ . Meanwhile, when the percentage of methionine of TSAA in the diets was increased from 46% to 52% or 57%, there were no significant differences observed in feed intake, body weight gain or gain/feed among the treatments.

Table 13. Effects of feeding diets of various levels of TSAA and various proportions of methionine to cystine on feed intake, weight gain and gain/feed ratios of young chicks (Experiment III)

| | % TSAA of diet | % methionine of TSAA | | | Group Average |
|---|---|---|---|---|---|
| | | 46 | 52 | 57 | |
| Feed intake[1] g./bird/day | 0.531 | 32.0±0.7[2] | 32.3±0.4[2] | 31.4±1.5[2] | 31.9±0.3[2]a[3] |
| | 0.597 | 34.6±0.8 | 34.1±0.3 | 34.2±1.0 | 34.3±0.2 b |
| | 0.663 | 32.8±1.5 | 33.6±0.8 | 33.0±1.0 | 33.1±0.2 b |
| | Group-average | 33.1±0.8 | 33.3±0.5 | 32.9±0.8 | |
| | control[4] | | (31.7±0.7) | | |
| Weight gain[1] g./bird/day | 0.531 | 11.3±0.4 | 10.8±0.4 | 10.2±0.5 | 10.8±0.3 c |
| | 0.597 | 13.5±0.5 | 13.2±0.5 | 13.5±0.5 | 13.4±0.1 d |
| | 0.663 | 13.0±0.3 | 12.8±0.1 | 13.1±0.1 | 13.0±0.1 d |
| | Group-average | 12.6±0.7 | 12.3±0.7 | 12.3±1.0 | |
| | Control[4] | | (12.3±0.5) | | |
| Gain/Feed | 0.531 | 0.35±0.01 | 0.33±0.01 | 0.32±0.01 | 0.33±0.01 e |
| | 0.597 | 0.39±0.01 | 0.39±0.01 | 0.40±0.01 | 0.39±0.01 f |
| | 0.663 | 0.40±0.01 | 0.38±0.01 | 0.40±0.01 | 0.39±0.01 f |
| | Group-average | 0.38±0.02 | 0.36±0.02 | 0.37±0.03 | |
| | Control[4] | | (0.39±0.01) | | |

[1]Means of 6 bird/rep. x 3 replications/treatment.

[2]Mean ± S.E.

[3]Means not carrying the same subscript on each column for each parameter are significantly different at $P < 0.01$.

[4]Control diet with DL-methionine (0.32%) added to the basal diet as the only sulfur amino acid. The % methionine of TSAA is 73.0%.

The group fed the control diet containing 0.665% TSAA with 73% methionine of TSAA, was compared to the others by one way analysis. The feed intake of the control group was not significantly different from each datum of all the other groups ($P < 0.05$). Better performances in weight gain and feed efficiency were demonstrated by the control group than by the group fed the diet with 0.531% TSAA ($P < 0.05$), although no significant difference was observed between the control and the group with 0.531% TSAA and 46% of methionine of TSAA.

Thus, the chicks on the diets of 0.597% and 0.663% TSAA, including the control group, had the same amounts of feed intake, weight gain and feed efficiency. This observation suggests that the requirement for TSAA can be lowered, without any adverse effects, from 0.665% to 0.597% of diet if the proportion of methionine of TSAA is in the range of 46% to 57%. This experiment also provided an indication that as long as the proportions of dietary methionine of TSAA are within the range of 46 to 57%, the performances would not be affected by the proportion itself. However, when the level of TSAA was limiting (0.531%) in the diet, the higher ratio of methionine to cystine (57:43) could not maintain the normal weight gain or gain/feed ratio.

D. Experiment IV

Three experiments were conducted to determine the effects on feed intake, weight gain and feed efficiency by meal-feeding diets deficient or excessive in methionine.

Table 14. Feed intake, body weight gain and gain/feed of young chicks fed diets ad libitum or as meals, when such diets contain a deficiency, adequacy or excess of DL-methionine (Experiment IV A).

| Feeding Program | Feed intake[1] | | | Weight gain[1] | | | Gain/Feed | | |
|---|---|---|---|---|---|---|---|---|---|
| | Levels of DL-methionine supplemented, % | | | | | | | | |
| | 0 | 0.32 | 1.0 | 0 | 0.32 | 1.0 | 0 | 0.32 | 1.0 |
| Ad libitum feeding | 20.5±0.6[3,4] a | 28.3±1.3[3,4] b | 25.2±0.5[3,4] b | 3.5±0.5[3,4] h | 13.0±1.3[3,4] i | 11.1±0.3[3,4] i | 0.17±0.03[3,4] p | 0.47±0.02[3,4] q | 0.44±0.01[3,4] r |
| Meal feeding[2] | | | | | | | | | |
| 6 hr.interval | 14.6±0.4 c | 20.7±0.9 d | 22.0±0.7 d | 1.9±0.5 j | 10.3±1.0 k | 11.8±0.7 k | 0.13±0.03 s | 0.49±0.03 t | 0.54±0.02 t |
| 14 hr.interval | 11.4±0.4 e | 17.1±0.5 f | 19.0±0.4 f | -0.6±0.5 l | 8.1±0.4 m | 9.0±0.4 m | -- | 0.47±0.01 u | 0.47±0.02 u |
| 22 hr.interval | 6.8±0.8 g | 9.5±0.5 g | 9.3±0.9 g | -4.9±0.8 n | -1.3±0.2 o | -0.3±0.7 o | -- | -- | -- |

[1]Means of 6 birds/rep. x 3 replications/treatment. Data expressed as g./bird/day.

[2]Two hours of meal with indicated time-intervals during a period of 72 hours.

[3]Mean ± S.E.

[4]Means not carrying the same subscript in each row for feed intake, weight gain and gain/feed are significantly different at $P < 0.05$.

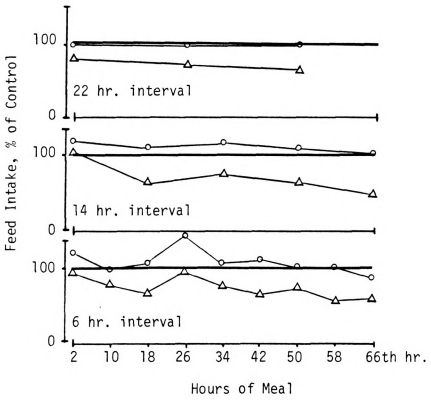


Figure 5.--Relative amount of each meal consumed by chicks fed diets deficient (Δ——Δ) or with excess (o——o) methionine presented with three different intervals of time between meals. The control group was fed the adequate diet (Experiment IV A).

The levels of DL-methionine added to the methionine excess (ME) diets were 1.0% for experiment IV A and IV C and 1.32% for experiment IV B. The results of each experiment are given in Tables 14, 15 and 16 and Figures 5, 6 and 7 for experiments IV A, IV B and IV C, respectively. The figures present the data in terms of the relative amount of feed consumed by the chicks. The groups fed the diet to which 0.32% DL-methionine (methionine-adequate diet) was added, served as controls for all experiments.

The data in Tables 14 and 15 showed essentially the same results, in terms of the effects of the ME diets on performance of chicks. The observations uniformly obtained from the data in each table were that, when the diets were fed ad libitum, the chicks on the methionine deficient (MD) diet always consumed the least diet and grew the worst with the lowest feed efficiency among the treatments ($P < 0.01$). The MA diet always produced in chicks significantly higher feed intake, weight gain or feed efficiency than the ME diet, except for the feed efficiency in experiment IV A (Table 14).

The allowance of three 2-hour meals daily (6 hour interval) was not enough to allow the chicks to consume as much feed as their corresponding dietary groups fed ad libitum the diets with different levels of methionine (Table 14). Groups meal-fed the MD diet showed significantly lower ($P < 0.01$) feed intake, weight gain and gain/feed in comparison to those chicks on the MA or ME diets in each time-interval of meal-feeding (Table 14). However, a slightly

higher or equal amount of feed intake, and slightly improved weight gain were obtained by meal-feeding the ME diets as compared to meal-feeding the MA diets, however these differences were not significant at $P > 0.05$. A considerable improvement in feed efficiency was observed in chicks fed the ME diet as meals with 6 hours of time-interval over that of the group fed the same diet ad libitum.

To substantiate the effects of meal-feeding the ME diet on feed intake and feed efficiency, the experiments IV B and IV C were conducted.

The improved feed efficiency from the ME diet fed as meals was speculated to be due to the extra α-amino N coming from the excess methionine. The reason for the speculation was that the basal diet was low in protein (13.1%), and the levels of dispensable amino acids might not be sufficient in the diet for optimum performance of chicks. Thus, a postulation was made as follows. Chicks meal-fed a diet containing excessive amounts of α-amino N should show a better feed efficiency than the others on a diet with no excess amount of α-amino N. If not, then the effects of the meal-feeding of the ME diet previously observed should be methionine-specific.

The supplementation of L-glutamate, as a source of α-amino N, at levels of 1.0 or 1.32% to the MA or MD diet, respectively, tended to decrease feed intake and weight gain of chicks fed those diets ad libitum, compared to those of chicks fed ad libitum the same diets without added-glutamate (Table 15). However, these differences were not significant

Table 15. Feed intake, body weight gain and gain/feed of young chicks fed diets containing a deficiency or excess of methionine, or an excess of L-glutamate *ad libitum* or as meals[1] (Experiment IV B)

| Diets | Feed intake[2] | | Weight gain[2] | | Gain/Feed | |
|---|---|---|---|---|---|---|
| | *Ad libitum* | Meal-feeding | *Ad libitum* | Meal-feeding | *Ad libitum* | Meal-feeding |
| MD diet | 20.6±1.9[3]a[4] | 17.1±0.6[3]c[4] | -0.3±1.3[3]e[4] | 1.5±0.3[3]h[4] | - | 0.09±0.02[3]m[4] |
| MD + 0.32% MET | 34.8±1.3 b | 27.3±0.8 d | 14.5±1.1 g | 15.1±0.7 j | 0.42±0.02[3]k[4] | 0.55±0.01 n |
| MD + 1.32% MET | 21.7±0.1 a | 18.7±0.5 c | 6.5±0.6 f | 5.9±0.4 i | 0.30±0.02 l | 0.32±0.02 o |
| MD + 0.32% MET + 1.0% GLU | 29.3±2.6 b | 28.2±0.3 d | 10.8±2.1 g | 15.6±1.1 j | 0.36±0.03 kl | 0.55±0.04 n |
| MD + 1.32% GLU | 15.7±0.5 a | 16.9±0.1 c | -2.3±0.7 e | 1.9±0.5 h | - | 0.11±0.03 m |

[1]Two hours of meal with 6 hours of time-interval.

[2]Means of 5 birds/rep. x 3 replications/treatment. Data expressed as g./bird/day.

[3]Mean ± S.E.

[4]Means not carrying the same subscript in each column for feed intake, weight gain and gain/feed are significantly different at $P < 0.05$.

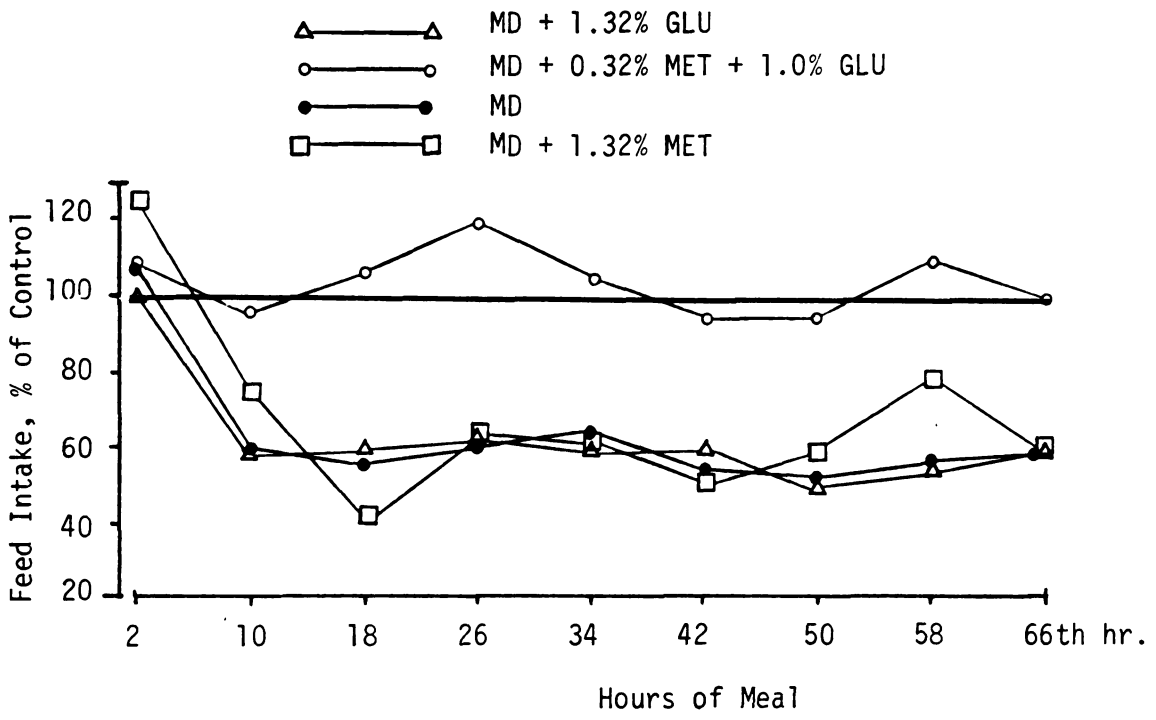


Figure 6.--Relative amount of each diet consumed by chicks given diets with added methionine and/or glutamic acid. The control group was meal-fed the MA diet (Experiment IV B).

at $P < 0.05$. The lowest levels of feed intake and weight gain ($P < 0.01$) of all treatments were produced by meal-feeding the MD diet or the MD diet + 1.32% L-glutamate. The birds meal-fed the ME diet (MD + 1.32% methionine) consumed less diet and, consequently, showed less weight gain ($P < 0.01$) than the groups meal-fed the MA diet (Table 15). This observation is different from those in the previous experiment IV A (Table 14), in which the chicks meal-fed the ME diet (MD diet + 1.0% methionine) grew better with a higher feed efficiency than those fed the MA diet as meals. The same levels of feed intake and weight gain were obtained with or without supplementing L-glutamate to the MD diet or to the MA diet in the meal-feeding program. Such supplementation decreased feed intake and weight gain when diets were provided ad libitum. Therefore, meal-feeding appears to have an effect of alleviating the detrimental effects on feed intake which occurred when excessive amounts of methionine or glutamate were added to the diet.

The gain/feed ratio was significantly improved ($P < 0.01$) by the MA diets meal-fed with or without 1.0% L-glutamate in comparison to data from the groups fed these diets ad libitum. Contrary to results of experiment IV A, no improvement in feed efficiency was observed by meal-feeding the ME diet (Table 15).

Because experiment IV B failed to substantiate the effects of meal-feeding on ME diet on feed intake and feed efficiency another experiment, IV C, was conducted.

The data in Table 16 showed the same result as in previous experiment, IV A, that meal-feeding the ME diet (1.0% added methionine) prevented the decline in feed intake, weight gain and feed efficiency observed by chicks meal-fed the MA diet. Although there was no significant difference, the former group grew about 10% faster than the latter on the MA diet. A significantly higher feed efficiency ($P < 0.05$) was observed for the group meal-fed the ME diet over those chicks fed the same diet ad libitum.

Figure 7 shows that chicks fed ad libitum consumed equal amounts of the MA and ME diets during the first 18 hours of feeding. At around 34 hours of feeding, the feed intake of the group fed the ME diet fell to approximately 70% of the control value, and thereafter remained relatively constant. The ME diet, when fed as meals, led to the same level of feed intake as that of the control diet through the entire experiment (66 hours). However, feeding the MD diet caused an immediate reduction of feed intake to about 80% of that of the control diet after the first 2 hours of feeding. Feeding the MD diet ad libitum reduced feed intake to a level of 70 to 50% of control, while meal-feeding it caused a more dramatic decrease to 41% of control by 66 hours of feeding. The feed intake of the MD diet fed as meals was continuously declining as the meal-feeding went on with 14 hours of time-interval.

Table 16. Feed intake, body weight gain, gain/feed of young chicks fed diets of methionine-deficiency, - adequacy, or -excess in ad libitum feeding or meal-feeding (Experiment IV C)

| Diets | Feed intake[1] | | Weight gain[1] | | Gain/Feed | |
|---|---|---|---|---|---|---|
| | Ad libitum | Meal-feeding[2] | Ad libitum | Meal-feeding[2] | Ad libitum | Meal-feeding[2] |
| MD diet | $18.4 \pm 0.2^{3}a^{4}$ | $11.0 \pm 1.3^{3}c^{4}$ | $-2.6 \pm 0.3^{3}e^{4}$ | $-5.2 \pm 1.0^{3}h^{4}$ | - | - |
| MD+0.32% MET | $29.2 \pm 2.4$ b | $19.4 \pm 0.6$ d | $8.6 \pm 0.9$ f | $5.0 \pm 0.7$ i | $0.30 \pm 0.01^{3}j^{4}$ | $0.26 \pm 0.03^{3}1^{4}$ |
| MD+1.0% MET | $22.7 \pm 0.6$ ab | $19.3 \pm 0.7$ d | $4.2 \pm 0.3$ g | $5.5 \pm 0.8$ i | $0.18 \pm 0.01$ k | $0.28 \pm 0.03$ 1 |

[1]Means of 6 birds/rep. x 3 replications/treatment. Data expressed as g./bird/day.

[2]Two-hours of meal with 14 hours of time-interval.

[3]Means $\pm$ S.E.

[4]Means not carrying the same subscript in each column for feed intake, weight gain and gain/feed are significantly different at $P < 0.05$.

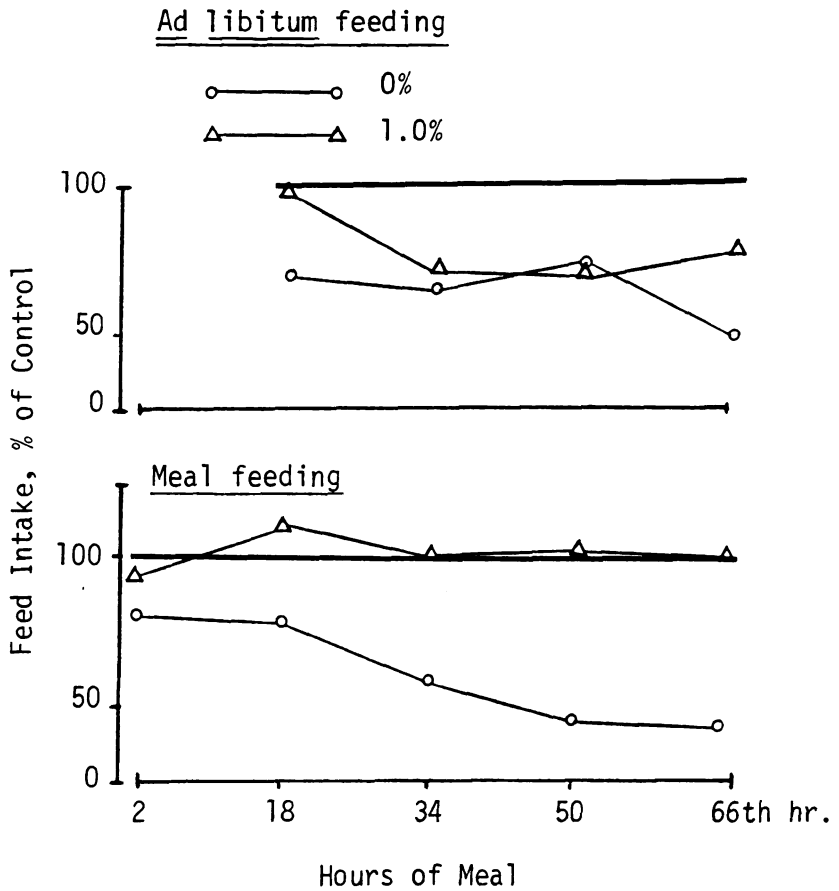


Figure 7.--Relative amount of feed consumed according to type of feeding program or level of methionine. The MD, MA or ME diets were added with levels of 0, 0.32 or 1.0% DL-methionine, respectively. The control was fed the MA diet (Experiment IV C).

Concentrations of free amino acids in plasma and brain of chicks fed *ad libitum* diets with a deficiency, adequacy or excess of methionine.

The data in Tables 17 and 19 are for amino acid levels in plasma, and the data in Tables 18 and 20 for amino acid levels in brain, all obtained by *ad libitum* feeding of the diets. The changes of relative concentrations of those amino acids listed in Tables 17 and 19 are depicted in Figures 8 and 9, respectively. The concentrations of each amino acid for chicks fed the MD (no methionine added), or ME (1.0 or 1.32% methionine added) diets were compared to those of the group fed the methionine adequate (MA) diet (0.32% methionine added) as a control.

The MD diet induced significant increases of threonine, serine, lysine or valine at $P < 0.05$, and decreased methionine and/or arginine in plasma with a significance at $P < 0.05$. Plasma cysteine was only slightly reduced by the diet (Tables 17 and 19).

The diet with excess levels of methionine (1.0 or 1.32%) significantly increased the levels of methionine, arginine and/or serine at $P < 0.05$ (Tables 17 and 19), and reduced the level of glycine in plasma (Table 17). Plasma cysteine was not significantly different from that of control, though it was significantly higher than the level of cysteine obtained by the MD diet (Table 17).

No significant differences in plasma TFAA, NEAA and EAA were observed among all the dietary treatments. However, the ratios of NEAA/EAA and EAA/TFAA for chicks fed the MD or

ME (1.0%) diets were significantly higher or lower ($P < 0.05$), respectively, than those of the control (Table 18). The increased ratio of NEAA/EAA and the decreased EAA/TFAA by the MD diet were the same as observed in experiment II (Table 11), while the NEAA/EAA or the EAA/TFAA in plasma of chicks fed the ME diet (1.32%) in Table 19 was higher or lower ($P < 0.05$), respectively, than those of chicks fed the MD or MA diets.

The level of plasma methionine was increased 12 times that of control by the diet with 1.32% added DL-methionine (Table 17). However, the ME diet with 1.0% added DL-methionine increased the level 2.9 times that of the control (Table 19).

The concentrations of amino acids in brain (Tables 18 and 20) were relatively less affected by dietary treatments than those in plasma. The relative changes of brain amino acids in chicks fed ad libitum such diets as MD, MA or ME diets are depicted in Figures 8 and 10, respectively, for the data in Tables 18 and 20.

Of the amino acids in brain, cysteine, tyrosine and phenylalanine were decreased at $P < 0.05$ (Table 18), and threonine was the only amino acid whose concentration was increased with a significance at $P < 0.01$ (Table 18) by the diet deficient in methionine. The diet with 1.32% added methionine increased the levels of valine and methionine, with the latter 6.8 times higher than that of control (Table 18), while the diet added with 1.0% added methionine reduced

Table 17. Concentrations[1] of free amino acids (FAA) in plasma of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment IV B)

| Amino acids | FAA in plasma, µmole/100 ml. % of DL-methionine added | | | Trend for direction of change |
|---|---|---|---|---|
| | 0 | 0.32 | 1.32 | |
| ASP + ASN | 11.75± 1.32[2] a[3] | 16.54± 1.39[2] a[3] | 13.42± 1.58[2] a[3] | - |
| THR | 67.35±16.41 a | 29.54± 2.33 b | 28.15± 3.03 b | ↓ |
| SER | 72.64± 7.74 a | 45.53± 5.51 b | 78.62± 5.14 a | - |
| GLU + GLN | 65.23±21.03 a | 61.44± 4.60 a | 68.84± 6.37 a | - |
| PRO | 17.98± 6.61 a | 14.77± 2.73 a | 20.09± 2.86 a | - |
| GLY | 45.38± 3.67 a | 41.15± 2.15 a | 29.39± 2.96 b | ↓ |
| ALA | 57.19± 2.60 a | 68.04± 8.21 a | 74.18±13.78 a | - |
| VAL | 24.38± 1.50 a | 16.62± 1.46 b | 13.52± 3.55 b | ↓ |
| CYS | 3.06± 0.26 a | 4.16± 0.35 ab | 5.11± 0.48 b | ↑ |
| MET | 4.17± 0.14 a | 8.54± 0.40 b | 104.27±10.45 c | ↑ |
| ILE | 14.90± 2.34 a | 13.27± 1.05 a | 10.48± 1.86 a | - |
| LEU | 17.10± 0.75 a | 17.35± 0.80 a | 13.79± 2.71 a | - |
| TYR | 12.69± 1.10 a | 16.68± 0.87 a | 13.27± 2.22 a | - |
| PHE | 9.27± 0.78 a | 9.97± 0.75 a | 8.95± 1.78 a | - |
| LYS | 109.23±19.12 a | 43.23± 5.50 b | 29.12± 2.80 b | ↓ |
| HIS | 17.16± 1.18 a | 13.04± 0.32 a | 17.29± 1.05 a | - |
| ARG | 16.26± 1.80 a | 23.27± 1.33 b | 33.86± 1.63 c | ↑ |
| TFAA[4] | 565.8 ±131.2 a | 443.7 ±18.9 a | 552.4 ±33.1 a | - |
| NEAA[4] | 285.9 ±59.4 a | 268.3 ±17.3 a | 293.0 ±22.3 a | - |
| EAA[4] | 279.9 ±71.8 a | 175.4 ± 3.2 a | 259.4 ±16.6 a | - |
| NEAA/EAA | 1.04± 0.04a | 1.53± 0.09 b | 1.13± 0.09 a | - |
| EAA/TFAA | 0.49± 0.01a | 0.40± 0.01 b | 0.47± 0.02 a | - |

[1] Means of 3 birds/rep. x 3 replications/treatment.
[2] Mean ± S.E.
[3] Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4] Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

Table 18. Concentrations[1] of free amino acids (FAA) in brain of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment IV B)

| Amino acids | FAA in brain, µmole/g. tissue % of DL-methionine added 0 | 0.32 | 1.32 | Trend for direction of change |
|---|---|---|---|---|
| ASP + ASN | 5.60±1.14[2]a[3] | 7.26±0.46[2]a[3] | 4.92±1.21[2]a[3] | - |
| THR | 1.05±0.11 a | 0.77±0.01 a | 1.02±0.13 a | - |
| SER | 2.00±0.46 a | 2.25±0.15 a | 1.86±0.29 a | - |
| GLU + GLN | 10.65±0.49 a | 12.86±1.81 a | 9.27±0.90 a | - |
| PRO | 0.78±0.28 a | 1.26±0.07 a | 1.02±0.29 a | - |
| GLY | 2.40±0.46 a | 3.93±0.18 a | 3.03±0.45 a | - |
| ALA | 1.76±0.22 a | 2.41±0.17 ab | 2.81±0.25 b | ↑ |
| VAL | 0.48±0.08 a | 0.69±0.04 a | 0.93±0.02 b | ↑ |
| CYS | 0.12±0.02 a | 0.28±0.03 b | 0.23±0.02 b | ↑ |
| MET | 0.23±0.03 a | 0.32±0.04 a | 2.17±0.06 b | ↑ |
| ILE | 0.47±0.08 a | 0.53±0.01 a | 0.65±0.04 a | - |
| LEU | 0.72±0.21 a | 1.00±0.05 a | 0.88±0.27 a | - |
| TYR | 0.33±0.02 a | 0.53±0.01 b | 0.59±0.05 b | ↑ |
| PHE | 0.40± 0.06 a | 0.61±0.02 b | 0.74±0.01 b | ↑ |
| LYS | 1.10± 0.12 a | 1.08±0.05 a | 0.93±0.02 a | - |
| HIS | 0.46±0.08 a | 0.53±0.02 a | 0.58±0.03 a | - |
| ARG | 0.71±0.16 a | 0.84±0.02 a | 0.92±0.03 a | - |
| TFAA[4] | 29.4 ±3.5 a | 37.2 ±2.1 a | 32.6 ±2.9 a | - |
| NEAA[4] | 23.7 ±2.5 a | 30.8 ±2.2 a | 23.7 ±3.0 a | - |
| EAA[4] | 5.61±1.1 a | 6.45±0.1 a | 8.82±0.1 b | ↑ |
| NEAA/EAA | 4.41±0.50 a | 4.79±0.46 a | 2.69±0.36 b | ↓ |
| EAA/TFAA | 0.19±0.02 a | 0.18±0.02 a | 0.28±0.03 b | ↑ |

[1]Means of 3 birds/rep. x 3 replications/treatment.
[2]Mean ± S.E.
[3]Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4]Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

Table 19. Concentrations[1] of free amino acids (FAA) in plasma of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment IV C)

| Amino acids | FAA in plasma, µmole/100 ml. % of DL-methionine added | | | Trend for direction of change |
|---|---|---|---|---|
| | 0 | 0.32 | 1.0 | |
| ASP + ASN | 15.79± 4.89[2] a[3] | 10.49± 1.53[2] a[3] | 15.75± 3.66[2] a[3] | - |
| THR | 198.03±32.56 a | 95.40± 7.96 b | 84.56± 8.37 b | ↓ |
| SER | 222.76±23.28 a | 153.94± 5.15 b | 168.88± 2.16 b | ↓ |
| GLU + GLN | 82.23±10.68 a | 86.66± 2.6 a | 104.29±15.15 a | - |
| PRO | - | - | - | |
| GLY | 79.40± 9.25 a | 76.74± 6.34 a | 62.84± 4.93 a | - |
| ALA | 88.86± 7.53 a | 81.88± 1.73 a | 121.82± 4.49 b | ↑ |
| VAL | 38.23± 8.86 a | 49.79± 1.16 a | 43.03± 5.61 a | - |
| CYS | 5.01± 0.72 a | 5.10± 0.75 a | 6.49± 0.71 a | - |
| MET | 7.37± 0.14 a | 9.95± 0.27 b | 28.81± 2.46 c | ↑ |
| ILE | 21.09± 2.05 a | 26.89± 0.17 a | 24.57± 1.10 a | - |
| LEU | 33.37± 3.27 a | 36.51± 1.39 a | 35.45± 1.83 a | - |
| TYR | 20.93± 1.66 a | 24.36± 2.53 a | 30.42± 3.83 a | - |
| PHE | 16.73± 3.56 a | 18.15± 1.52 a | 19.90± 2.07 a | - |
| LYS | 129.89± 3.46 a | 120.16±15.12 a | 84.85± 7.13 a | - |
| HIS | 21.98± 1.04 a | 14.88± 1.72 a | 17.58± 2.75 a | - |
| ARG | 20.51± 0.95 a | 20.88± 0.18 a | 27.71± 0.48 b | ↑ |
| TFAA[4] | 1002.2 ±122.2 a | 831.8 ±22.6 a | 877.0 ±24.6 a | - |
| NEAA[4] | 515.0 ±57.7 a | 439.2 ±15.9 a | 510.5 ±30.2 a | - |
| EAA[4] | 487.2 ±64.7 a | 392.6 ±12.3 a | 366.5 ±12.4 a | - |
| NEAA/EAA | 1.06± 0.02 a | 1.12± 0.05 a | 1.40± 0.12 b | ↑ |
| EAA/TFAA | 0.49± 0.01 a | 0.47± 0.01 a | 0.42± 0.02 b | ↓ |

[1] Means of 3 birds/rep. x 3 replications/treatment.
[2] Mean ± S.E.
[3] Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4] Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

Table 20. Concentrations[1] of free amino acids (FAA) in brain of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment IV C)

| Amino acids | FAA in brain, μmole/g.tissue % of DL-methionine added | | | Trend for direction of change |
|---|---|---|---|---|
| | 0 | 0.32 | 1.0 | |
| ASP + ASN | 2.05±0.16[2] a[3] | 1.96±0.16[2] a[3] | 1.62±0.02[2] a[3] | - |
| THR | 0.88±0.07 a | 0.47±0.05 b | 0.28±0.02 c | ↓ |
| SER | 1.30±0.12 a | 1.02±0.08 a | 0.91±0.06 a | ↓ |
| GLU + GLN | 5.88±0.28 a | 6.14±0.43 a | 5.32±0.04 a | - |
| PRO | - | - | - | |
| GLY | 1.48±0.14 a | 1.41±0.12 a | 1.29±0.10 a | - |
| ALA | 0.99±0.10 a | 1.00±0.05 a | 0.86±0.01 a | - |
| VAL | 0.24±0.02 a | 0.26±0.01 a | 0.17±0.01 b | ↓ |
| CYS | 0.11±0.01 a | 0.10±0.02 a | 0.09±0.01 a | - |
| MET | 0.11±0.01 a | 0.11±0.01 a | 0.12±0.01 a | - |
| ILE | 0.17±0.01 a | 0.18±0.02 a | 0.13±0.01 a | - |
| LEU | 0.24±0.02 a | 0.25±0.02 a | 0.18±0.01 a | - |
| TYR | 0.17± 0.01 a | 0.15±0.01 a | 0.15±0.01 a | - |
| PHE | 0.15±0.02 a | 0.16±0.01 a | 0.14±0.01 a | - |
| LYS | 0.71±0.15 a | 0.55±0.07 a | 0.43±0.04 a | - |
| HIS | 0.20±0.03 a | 0.18±0.02 a | 0.18±0.01 a | - |
| ARG | 0.29±0.05 a | 0.23±0.01 a | 0.23±0.01 a | - |
| TFAA[4] | 15.7 ±0.6 a | 14.2 ±0.8 ab | 12.0 ±0.2 b | ↓ |
| NEAA[4] | 12.7 ±0.3 a | 11.8 ±0.8 a | 10.2 ±0.3 a | - |
| EAA[4] | 3.0 ±0.3 a | 2.4 ±0.1 ab | 1.8 ±0.1 b | ↓ |
| NEAA/EAA | 4.32±0.39 a | 4.93±0.34 a | 5.59±0.36 a | - |
| EAA/TFAA | 0.19±0.01 a | 0.17±0.01 a | 0.15±0.01 a | - |

[1] Means of 3 birds/rep. x 3 replications/treatment.
[2] Mean ± S.E.
[3] Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4] Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

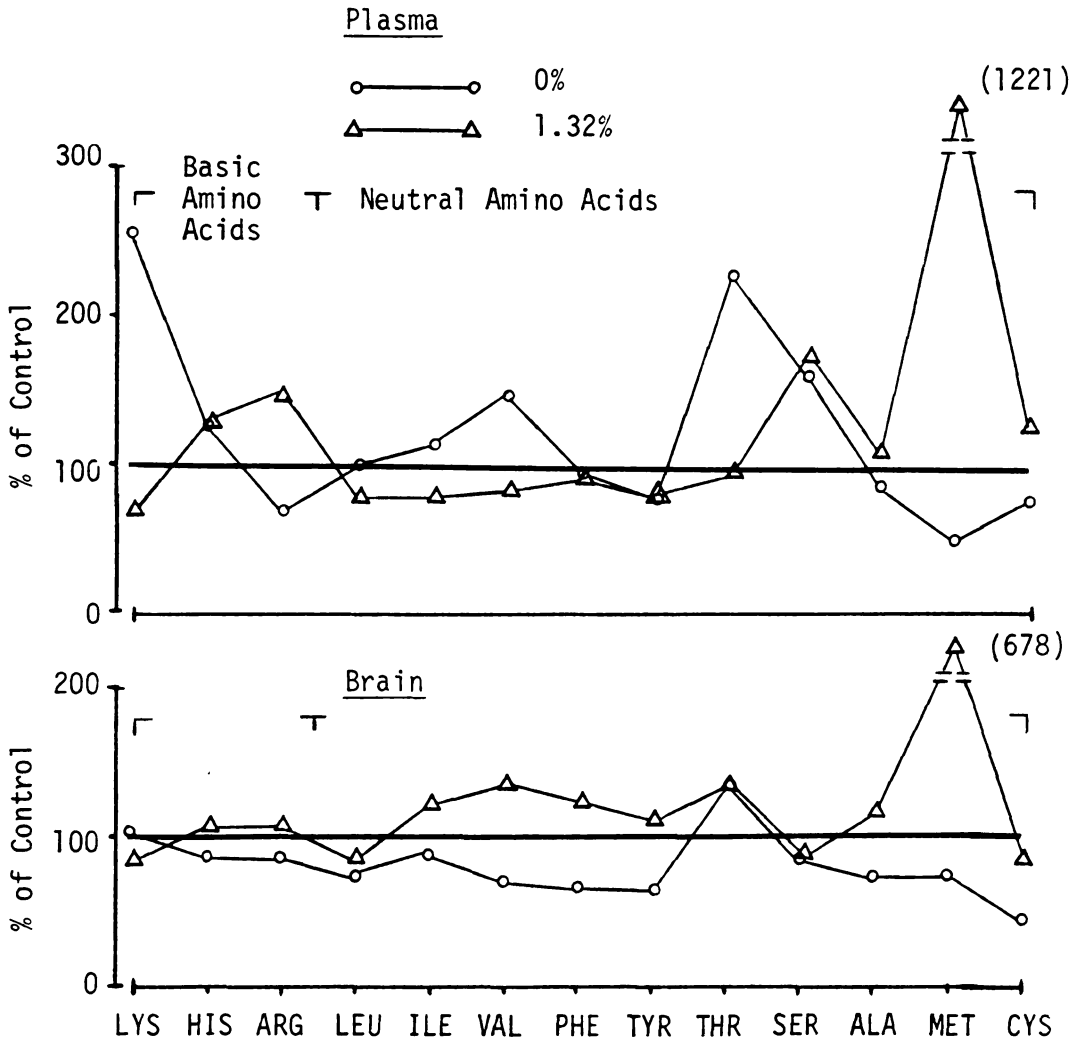


Figure 8.--Relative concentrations of plasma and brain free amino acids of chicks fed ad libitum diets of different levels of DL-methionine supplemented to basal diet. The control was fed the methionine adequate diet (Experiment IV B).

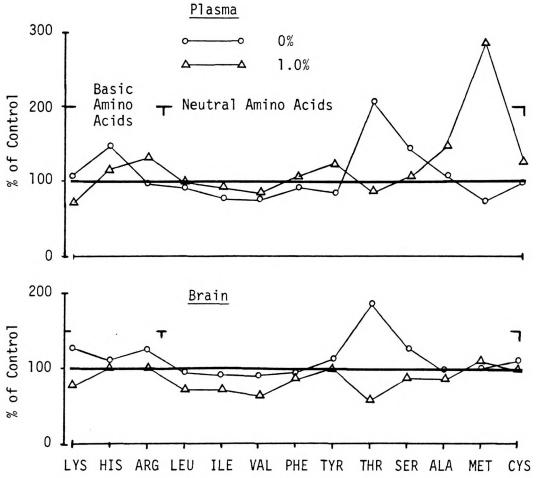


Figure 9.--Relative concentrations of plasma and brain free amino acids of chicks fed ad libitum diets of different levels of DL-methionine supplemented to basal diet. The control was fed the methionine adequate diet (Experiment IV C).

the levels of threonine and valine in brain. The levels of cysteine and methionine in brain were not affected at all by this excess methionine diet (1.0%).

No significant differences were observed in the levels of TFAA, NEAA, EAA, NEAA/EAA and EAA/TFAA in brain of chicks fed the MD or MA diets (Tables 18 and 20). This observation was the same as noticed in experiment II, except that the EAA was lowered by the MD diet in the previous experiment (Table 12). TFAA, NEAA and EAA were lowered by the ME diet (1.0%), compared to those of the MD diet (Table 20). The EAA and EAA/TFAA were higher and the ratio of NEAA/EAA was lower ( $P < 0.05$) for chicks on the ME diet (1.32%), respectively, than for those fed the MD or MA diets.

Concentrations of free amino acids in plasma and brain of chicks meal-fed the diets deficient, adequate or with excess methionine.

With the methionine adequate diet serving as a control, the diet deficient in methionine increased the levels of valine, threonine, lysine and glycine, and decreased those of methionine, tyrosine, glutamate + glutamine and arginine in plasma at $P < 0.05$ (Tables 21 and 23, Figures 10 and 11). The diet with 1.0 or 1.32% added methionine increased the levels of cysteine and, particularly, methionine, the latter increasing by 9.4 times that of the control, and decreased the levels of tyrosine, glutamate + glutamine or glycine in plasma (Tables 21 and 23).

The MD or ME diets increased, respectively, the levels of TFAA and EAA or that of EAA in plasma at $P < 0.05$ (Table 23).

These MD and ME diets tended to increase the level of NEAA/EAA and decrease that of EAA/TFAA as compared to those of the control (Tables 21 and 23).

Of the free amino acids in brain, the levels of threonine and lysine were increased (Table 24), and the levels of valine, isoleucine and alanine were reduced ($P < 0.05$) by the MD diet fed as meals (Table 22). Though a tendency for a decrease was observed for leucine, phenylalanine, tyrosine, histidine and arginine, the levels of methionine and cysteine in the brain of chicks meal-fed the MD diet were not different from those of the control (Tables 22 and 24). The ME diets (1.0 or 1.32% added) fed as meals increased only the levels of methionine in the brain 4.3 to 6.3 times higher than that of the control (Tables 22 and 24), and decreased the levels of serine, alanine and glutamate + glutamine (Table 24).

The levels of TFAA and NEAA in brain were not significantly altered by all the experiments. The alterations of the levels of brain EAA, NEAA/EAA and EAA/TFAA caused by the MD diet were, generally, not consistent. The ME diet tended to increase the level of EAA/TFAA and decrease that of NEAA/EAA as compared to those of the control (Tables 22 and 24).

The important aspects in understanding the effects of dietary treatments with different levels of methionine are to recognize the altered amino-acid patterns in plasma and brain as a direct effect of dietary levels of methionine or a result of protein deficiency due to a low feed consumption

Table 21. Concentrations[1] of free amino acids (FAA) in plasma of chicks meal-fed the diets of different levels of DL-methionine (Experiment IV B)

| Amino acids | FAA in plasma, μmole/100 ml % of DL-methionine added | | | Trend for direction of change |
|---|---|---|---|---|
| | 0 | 0.32 | 1.32 | |
| ASP + ASN | 15.62± 1.10[2]a[3] | 16.43± 0.75[2]a[3] | 13.74± 1.32[2]a[3] | - |
| THR | 92.55±20.39 a | 28.29± 2.21 b | 30.89± 7.52 b | ↓ |
| SER | 103.31±16.74 a | 49.39±13.88 a | 63.89± 3.63 a | ↓ |
| GLU + GLN | 54.37± 2.46 a | 74.28± 4.86 b | 57.30± 4.11 a | - |
| PRO | 25.35± 4.45 a | 17.66± 4.34 a | 19.08± 3.30 a | - |
| GLY | 53.11± 8.42 a | 38.26± 4.61 a | 29.73± 1.82 a | ↓ |
| ALA | 61.70± 8.77 a | 71.05± 4.14 a | 58.62±13.83 a | - |
| VAL | 29.36± 3.52 a | 17.72± 1.76 b | 13.09± 1.77 b | ↓ |
| CYS | 3.13± 0.36 a | 3.73± 0.36 a | 5.89± 0.74 b | ↑ |
| MET | 3.56± 0.36 a | 11.34± 0.53 b | 106.91± 9.00 c | ↑ |
| ILE | 15.22± 1.19 a | 15.54± 2.71 a | 9.10± 1.48 a | - |
| LEU | 20.92± 3.58 a | 19.54± 1.67 a | 12.39± 2.86 a | - |
| TYR | 12.95± 0.67 a | 22.57± 0.94 b | 11.79± 1.93 a | - |
| PHE | 11.06± 0.41 a | 12.37± 1.11 a | 8.98± 0.83 a | - |
| LYS | 73.05± 9.97 a | 32.34± 9.89 b | 28.30± 4.67 b | ↓ |
| HIS | 15.72± 1.56 a | 13.26± 1.07 a | 14.04± 1.40 a | - |
| ARG | 20.13± 2.32 a | 25.91± 2.16 a | 27.40± 2.59 a | - |
| TFAA[4] | 611.1 ± | 470.0 ±46.7 a | 511.1 ±42.2 a | - |
| NEAA[4] | 329.5 ± | 293.4 ±27.1 a | 260.0 ±26.9 a | - |
| EAA[4] | 281.6 ± | 176.6 ±20.0 a | 251.1 ±17.7 a | - |
| NEAA/EAA | 1.17± | 1.67± 0.06 b | 1.03± 0.06 a | - |
| EAA/TFAA | 0.45± | 0.38± 0.01 b | 0.49± 0.02 a | - |

[1] Means of 3 birds/rep. x 3 replications/treatment.
[2] Mean ± S.E.
[3] Means not carrying the same subscript in each row are significantly different at $P < 0.05$
[4] Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

Table 22. Concentrations[1] of free amino acids (FAA) in brain of chicks meal-fed the diets with different levels of DL-methionine (Experiment IV B)

| Amino acids | FAA in brain, μmole/g. tissue | | | Trend for direction of change |
|---|---|---|---|---|
| | % of DL-Methionine added | | | |
| | 0 | 0.32 | 1.32 | |
| ASP + ASN | 2.72±0.56[2]a[3] | 4.29±0.83[2]a[3] | 2.90±0.60[2]a[3] | - |
| THR | 0.84±0.11 a | 0.69±0.16 a | 0.70±0.15 a | - |
| SER | 1.48±0.09 a | 1.76±0.35 a | 1.97±0.56 a | - |
| GLU + GLN | 11.21±1.01 a | 8.69±0.41 a | 6.67±1.12 a | ↓ |
| PRO | 0.41±0.06 a | 0.48±0.03 a | 0.66±0.14 a | - |
| GLY | 1.34±0.11 a | 1.85±0.57 a | 1.85±0.30 a | - |
| ALA | 1.31±0.15 a | 2.36±0.23 b | 1.90±0.27 ab | ↑ |
| VAL | 0.17±0.03 a | 0.58±0.09 b | 0.40±0.15 ab | ↑ |
| CYS | 0.16±0.09 a | 0.17±0.02 a | 0.18±0.04 a | - |
| MET | 0.21±0.02 a | 0.29±0.05 a | 1.82±0.11 b | ↑ |
| ILE | 0.17±0.01 a | 0.44±0.09 b | 0.36±0.11 ab | ↑ |
| LEU | 0.18±0.02 a | 0.68±0.11 a | 0.45±0.18 a | ↑ |
| TYR | 0.17±0.01 a | 0.47±0.04 a | 0.32±0.12 a | ↑ |
| PHE | 0.13±0.01 a | 0.46±0.07 a | 0.40±0.16 a | ↑ |
| LYS | 0.82±0.15 a | 0.68±0.14 a | 0.55±0.11 a | - |
| HIS | 0.19±0.04 a | 0.38±0.03 a | 0.36±0.08 a | ↑ |
| ARG | 0.30±0.01 a | 0.59±0.05 a | 0.57±0.13 a | ↑ |
| TFAA[4] | 21.18±1.6 a | 24.9 ±1.4 a | 22.1 ±1.3 a | - |
| NEAA[4] | 18.8 ±1.5 a | 20.1 ±0.7 a | 16.4 ±0.4 a | - |
| EAA[4] | 3.01±0.10 a | 4.79±0.71 a | 5.61±1.17 a | - |
| NEAA/EAA | 6.62±0.39 a | 4.37±0.55 b | 3.21±0.65 b | ↓ |
| EAA/TFAA | 0.13±0.01 a | 0.19±0.02 ab | 0.25±0.04 b | ↑ |

[1]Means of 3 birds/rep. x 3 replications/treatment.
[2]Mean ± S.E.
[3]Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4]Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

Table 23. Concentrations[1] of free amino acids (FAA) in plasma of chicks meal-fed the diets with different levels of DL-methionine (Experiment IV C)

| Amino acids | FAA in plasma, μmole/100 ml. % of DL-methionine added | | | Trend for direction of change |
|---|---|---|---|---|
| | 0 | 0.32 | 1.0 | |
| ASP + ASN | 12.56± 0.03[2]a[3] | 14.52± 1.30[2]a[3] | 10.26± 1.18[2]a[3] | - |
| THR | 169.83± 9.47 a | 46.17± 2.61 b | 47.97± 3.62 b | ↓ |
| SER | 161.89± 3.81 a | 141.80± 5.11 a | 141.02± 4.33 a | - |
| GLU + GLN | 67.92± 5.48 a | 92.05± 7.49 a | 72.41±10.43 a | - |
| PRO | - | - | - | |
| GLY | 76.00± 4.62 a | 60.08± 0.84 b | 41.81± 1.64 c | ↓ |
| ALA | 101.31± 7.19 a | 112.18± 1.83 a | 109.24±13.41 a | - |
| VAL | 38.60± 2.44 a | 24.78± 2.43 b | 21.33± 2.19 b | ↓ |
| CYS | 4.15± 0.14 a | 5.06± 0.62 ab | 8.38± 1.20 b | ↑ |
| MET | 8.25± 0.23 a | 11.23± 1.32 a | 109.28±11.09 b | ↑ |
| ILE | 20.93± 1.15 a | 18.40± 1.69 a | 14.49± 1.09 a | ↓ |
| LEU | 29.02± 3.17 a | 24.20± 0.90 a | 20.82± 0.68 a | ↓ |
| TYR | 19.66± 1.47 a | 20.70± 1.56 a | 16.83± 1.63 a | - |
| PHE | 13.85± 0.67 a | 15.19± 0.82 a | 13.61± 1.12 a | - |
| LYS | 102.23±10.46 a | 48.97± 4.88 b | 33.65± 2.71 b | ↓ |
| HIS | 19.62± 0.75 a | 17.37± 0.77 a | 17.85± 0.48 a | - |
| ARG | 19.96± 1.33 a | 31.66± 3.48 b | 34.88± 2.02 b | ↑ |
| TFAA[4] | 865.4 ±33.6 a | 684.4 ±11.9 b | 713.8 ±51.6 ab | ↓ |
| NEAA[4] | 443.5 ±13.6 a | 446.4 ±14.8 a | 400.0 ±40.3 a | - |
| EAA[4] | 422.0 ±25.3 a | 238.0 ±11.1 b | 313.9 ±11.4 c | ↓ |
| NEAA/EAA | 1.05± 0.06 a | 1.89± 0.13 b | 1.27± 0.08 a | - |
| EAA/TFAA | 0.49± 0.01 a | 0.35± 0.02 b | 0.44± 0.02 ab | ↓ |

[1]Means of 3 birds/rep. x 3 replications/treatment.
[2]Mean ± S.E.
[3]Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4]Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

Table 24. Concentrations[1] of free amino acids (FAA) in brain of chicks meal-fed the diets with different levels of DL-methionine (Experiment IV C)

| Amino acids | FAA in brain, μ mole/g.tissue % of DL-methionine added | | | Trend for direction of change |
|---|---|---|---|---|
| | 0 | 0.32 | 1.0 | |
| ASP + ASN | 1.98±0.13[2]a[3] | 1.94±0.07[2]a[3] | 1.78±0.14[2]a[3] | - |
| THR | 0.80±0.06 a | 0.30±0.02 b | 0.20±0.03 b | ↓ |
| SER | 1.16±0.09 a | 1.07±0.13 ab | 0.70±0.06 b | ↓ |
| GLU + GLN | 5.86±0.11 ab | 6.64±0.38 a | 5.24±0.04 b | - |
| PRO | - | - | - | |
| GLY | 1.57±0.02 a | 1.49±0.13 ab | 1.21±0.07 b | ↓ |
| ALA | 0.92±0.04 a | 0.93±0.05 a | 0.78±0.01 b | ↓ |
| VAL | 0.25±0.03 a | 0.19±0.02 a | 0.18±0.02 a | - |
| CYS | 0.10±0.01 a | 0.12±0.01 a | 0.11±0.01 a | - |
| MET | 0.13±0.03 a | 0.13±0.02 a | 0.56±0.05 b | ↑ |
| ILE | 0.17±0.01 a | 0.17±0.01 a | 0.15±0.01 a | - |
| LEU | 0.25±0.01 a | 0.25±0.02 a | 0.20±0.01 a | - |
| TYR | 0.16±0.01 a | 0.18±0.02 a | 0.16±0.01 a | - |
| PHE | 0.15±0.01 a | 0.18±0.03 a | 0.15±0.02 a | - |
| LYS | 0.51±0.04 a | 0.33±0.01 b | 0.24±0.01 b | ↓ |
| HIS | 0.18±0.01 a | 0.18±0.01 a | 0.19±0.01 a | - |
| ARG | 0.25±0.02 a | 0.28±0.02 a | 0.26±0.02 a | - |
| TFAA[4] | 14.5 ±0.4 a | 14.4 ±0.7 a | 12.1 ±0.3 a | ↓ |
| NEAA[4] | 11.8 ±0.4 a | 12.4 ±0.6 a | 10.0 ±0.2 a | ↓ |
| EAA[4] | 2.7 ±0.1 a | 2.0 ±0.1 b | 2.1 ±0.1 b | ↓ |
| NEAA/EAA | 4.41±0.26 a | 6.14±0.03 b | 4.71±0.24 a | - |
| EAA/TFAA | 0.19±0.01 a | 0.14±0.01 b | 0.18±0.01 a | - |

[1]Means of 3 birds/rep. x 3 replications/treatment.
[2]Mean ± S.E.
[3]Means not carrying the same subscript in each row are significantly different at $P < 0.05$.
[4]Abbreviated forms each representing total free amino acids, non-essential amino acids and essential amino acids, respectively.

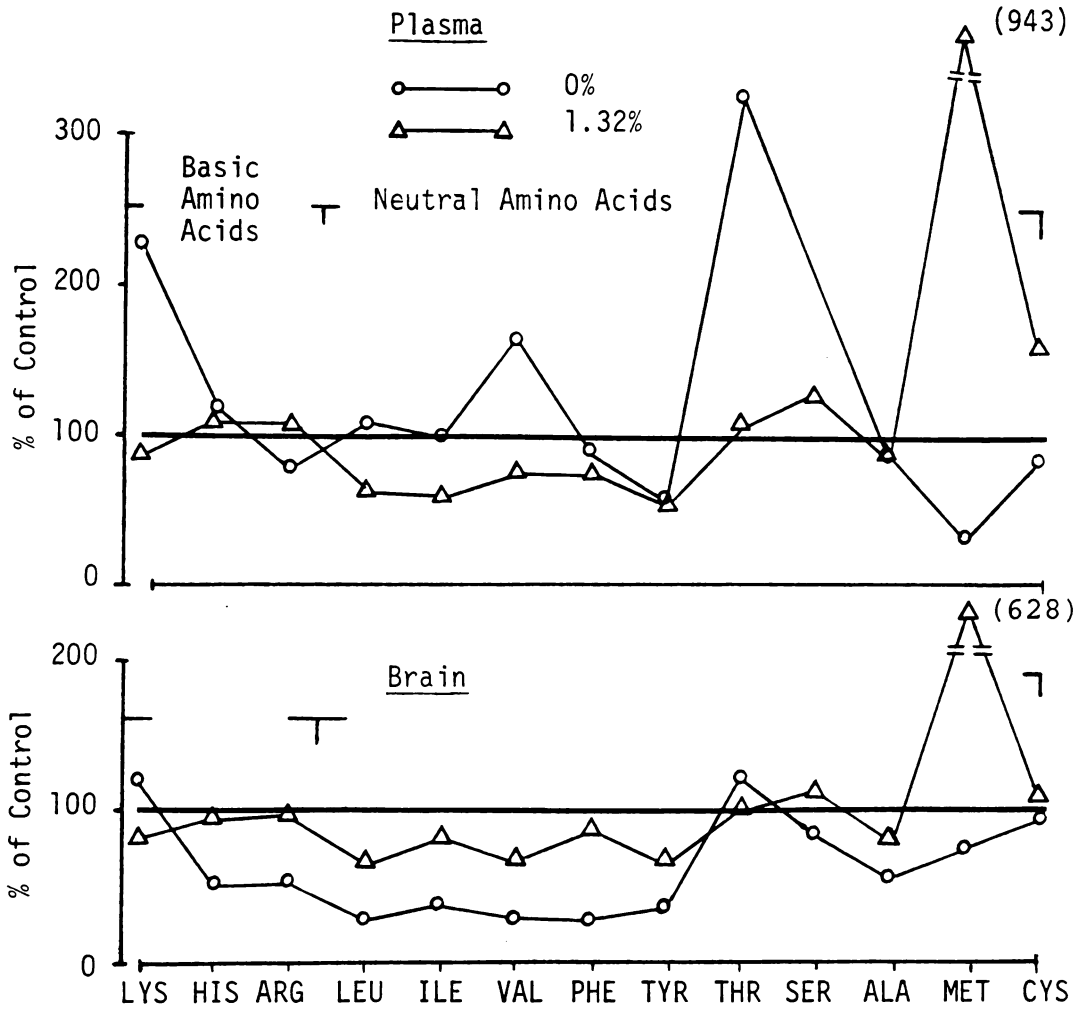


Figure 10.--Relative concentrations of plasma and brain free amino acids of chicks meal-fed the diets of different levels of DL-methionine supplemented to basal diet. The control was fed the methionine adequate diet (Experiment IV B).

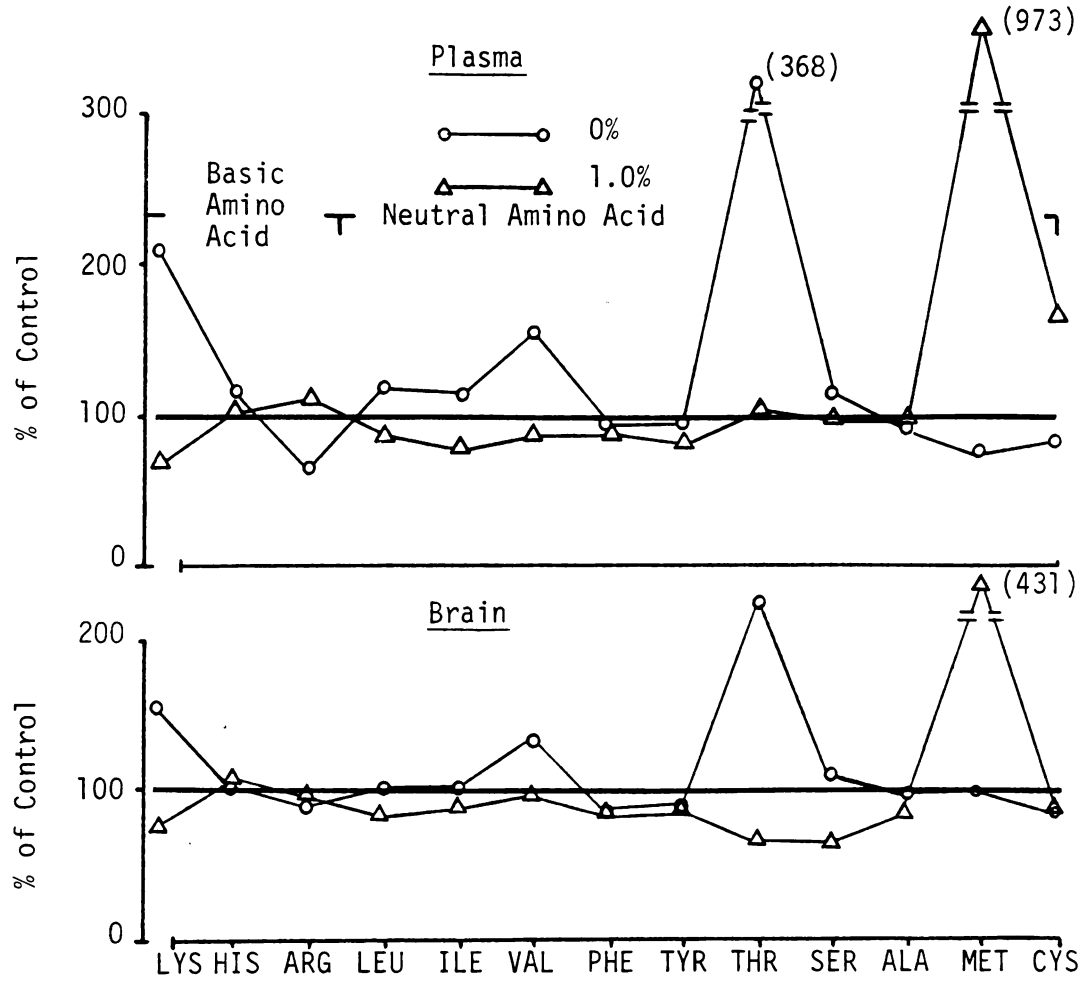


Figure 11.--Relative concentrations of plasma and brain free amino acids of chicks meal-fed the diets of different levels of DL-methionine supplemented to basal diet. The control was fed the methionine adequate diet (Experiment IV C).

Table 25. Free amino acids whose concentrations were changed in plasma and brain of chicks fed diets deficient (MD), or with excess methionine (ME) (Experiments II B, IV B and IV C)

| Direction of Change | Experiment Number | Free amino acids[1] in plasma: Ad libitum feeding — MD diet | Ad libitum feeding — ME diet | Meal-feeding — MD diet | Meal-feeding — ME diet |
|---|---|---|---|---|---|
| Increase | II B | THR LYS SER HIS | - | - | - |
| | IV B | THR LYS SER HIS VAL | SER ARG HIS - MET(12.2)[3] | THR, VAL SER[2] LYS | CYS MET(9.4)[3] |
| | IV C | THR HIS[2] SER | ALA ARG, MET(3.0)[3] | THR VAL GLY LYS | CYS[2] MET(9.4)[3] |
| Decrease | II B | CYS[2] ARG MET | - | - | - |
| | IV B | MET, ARG TYR[2] | GLY LYS[2] | MET GLU+ TYR GLN | ILE[2] GLU+ TYR, GLN, PHE[2] LEU[2] |
| | IV C | MET ILE | LYS[2] | ARG | GLY LYS[2] |

| | | Free amino acids[1] in brain: Ad libitum — MD diet | Ad libitum — ME diet | Meal-feeding — MD diet | Meal-feeding — ME diet |
|---|---|---|---|---|---|
| Increase | II B | THR HIS LYS, | - | - | - |
| | IV B | THR[2] | THR VAL MET (6.8)[3] | | MET(6.3)[3] |
| | IV C | THR | | THR LYS | MET(4.3)[3] |
| Decrease | II B | ARG, CYS[2] | - | - | - |
| | IV B | GLY[2] TYR CYS PHE | | ALA TYR VAL PHE ILE HIS LEU ARG | ASP+ TYR[2] ASN[2] LEU[2] |
| | IV C | | THR LEU[2] VAL | | SER GLU+ ALA GLN |

[1]Amino acids whose concentrations were significantly different from those of control at $P < 0.10$, except those indicated. The control was fed the diet adequate in methionine ad libitum or as meals.

[2]Amino acids whose concentrations were considerably different from control by more than 30% without statistical significance.

[3]The levels of methionine were increased by that number of times above control as indicated by the value in the parenthesis.

as well as a hormonal effect (glucocorticoids) created by dietary (metabolic) stress.

Table 25 was established by grouping the amino acids in plasma and brain according to their concentrations altered by dietary treatments. The directions of change, i.e. whether the levels of amino acids were increased or decreased, were determined by comparing the values to those of control groups which were fed the MA diets ad libitum or as meals. The table contains those amino acids whose concentrations were significantly different from those of the control at $P < 0.10$, and whose concentrations were considerably higher than those of the control by 30%, whether significant at $P < 0.10$, or not. The 30% alteration was chosen because it would show trends for a change.

The brain amino acids in Table 24, show that the levels of threonine and lysine were significantly ($P < 0.05$) increased by the MD diet. The level of methionine or cysteine in brain of the group meal-fed the MD diet was not significantly different from those of control. The ME diet induced the level of methionine to be 4.3 times higher ($P < 0.05$) than that of control, and significantly ($P < 0.05$) lowered the levels of such amino acids as glutamate + glutamine and alanine (Table 24).

The levels of TFAA and NEAA in brain were higher ($P < 0.05$) in the group fed the ME diet than those in the groups fed the MD or the MA diet. EAA were higher ($P < 0.05$) in the group fed the MD diet than those of the other groups.

The NEAA/EAA ratios of the groups meal-fed the MD or ME diets were lower ($P < 0.05$) than that of control, and the EAA/TFAA ratios of the former groups were higher ($P < 0.05$) than that of control group.

E. Experiment V

As shown in Table 26 (for Experiment V A), the feed intake of chicks fed ad libitum the MD diet was approximately 54% of that obtained by ad libitum feeding the MA diet (control group). Thus, weight gain and feed intake between these two treatments were significantly different at $P < 0.01$. However, force-feeding the MD diet significantly increased weight gain of chicks ($P < 0.05$) as comparable to that of the control group (17.7 g. vs. 14.2 g.) even with the same amount of feed ingested. Consequently the gain/feed ratio of the group force-fed the MD diet was better than that of the control ($P < 0.01$). The chicks force-fed the MA diet showed higher weight gain ($P < 0.05$) and feed efficiency ($P < 0.01$) than those on the other treatments.

Liver weight and its appearance were severely affected by dietary treatments (Table 26). Force-feeding the MD or MA diets produced significantly heavier livers than ad libitum feeding of these same diets ($P < 0.05$). The birds on the MD diet fed ad libitum had the smallest ($P < 0.05$). livers and of normal color, whereas the livers of chicks force-fed the MD diet were the heaviest and largest but of pale color and fragile structure. The chicks fed the MA diet, whether on ad libitum feeding or force-feeding programs,

had livers which were of normal color. Those force-fed the MA diet had livers 1.34 times heavier than their controls.

Table 27 shows the effects of ad libitum feeding or force-feeding the MD or ME diet (1.0% level of added methionine) on weight gain, feed efficiency, and liver size (Experiment V B).

Feed intake of chicks fed ad libitum the MD diet or ME diet was 42% or 78%, respectively, of that of control, substantiating the data obtained in Experiment IV. The control group was fed ad libitum the MA diet. Force-feeding the MD diet produced a 25% higher weight gain than that of the control chicks but with no significance at $P > 0.05$. Chicks force-fed the MA or ME diets grew heavier ($P < 0.05$) than controls. As a whole, the chicks force-fed the diets with different levels of methionine grew faster than those fed the MA diet ad libitum. Gain/feed ratio was highest for chicks force-fed the ME diet ($P < 0.10$), and next to highest for chicks force-fed the MA diet. This improved feed efficiency from force-feeding the ME diet seems comparable to the observation made on the effect of feed efficiency from meal-feeding the ME diet (Tables 14 and 16).

Liver sizes in the ad libitum feeding group were of the same weight despite different levels of dietary methionine. However, those in chicks force-fed the same diets were larger ($P < 0.05$), with the largest in the group force-fed the MD diet. Their livers were also the lightest in color. Force-feeding the ME and MA diets produced the same size of liver.

Table 26. Effects of force-feeding and ad libitum feeding of diets deficient or adequate in methionine on feed intake, weight gain, gain/feed and liver size (Experiment V A)

| | Levels of DL-methionine added, % | | | |
|---|---|---|---|---|
| | 0 | | 0.32 | |
| | Ad libitum | Force-feeding | Ad libitum | Force-feeding |
| Feed intake[2] g./bird/day | 30.9±1.0[3]a[4] | 57.2[1] | 57.2±0.1[3]b[4] | 57.2[1] |
| Weight gain[2] g./bird/day | -3±0.9 c | 17.2±0.3[3]d[4] | 14.2±0.3 e | 21.6±0.6[3]f[4] |
| Gain/Feed | - | 0.31±0.01 g | 0.25±0.01 h | 0.38±0.01 i |
| Liver size[2] % body wt. | 3.0±0.1 j | 6.2 ±0.1 k | 3.6±0.1 l | 4.8 ±0.2 m |

[1]Chicks were force-fed the same amount of feed consumed by chicks fed ad libitum the diet with 0.32% DL-methionine, at 5 times a day with a 3-hour time-interval.

[2]Means of 5 birds/rep. x 2 replications/treatment.

[3]Mean ± S.E.

[4]Means not carrying the same subscript in each row are significantly different at $P < 0.05$.

Analysis of variance for feed intake, weight gain, gain/feed and liver size

| Source of variation | Feed intake | | Weight gain | | Gain/Feed | | Liver size | |
|---|---|---|---|---|---|---|---|---|
| | d.f. | Mean Square | d.f. | Mean Square | d.f. | Mean Square | d.f. | Mean Square |
| Total | 3 | | 7 | | 5 | | 39 | |
| Treatment | 1 | 694.3** | 3 | 235.2** | 2 | 0.0085** | 3 | 19.89** |
| Error | 2 | 1.0 | 4 | 0.96 | 3 | 0.0001 | 36 | 0.16 |

** $P < 0.01$.

Table 27. Effects of force-feeding and ad libitum feeding of diets containing different levels of DL-methionine on feed intake, weight gain, gain/feed and liver size (Experiment V B)

| | Levels of DL-methionine added, % | | | | | |
|---|---|---|---|---|---|---|
| | 0 | | 0.32 | | 1.0 | |
| | Ad libitum | Force-feeding | Ad libitum | Force-feeding | Ad libitum | Force-feeding |
| Feed intake[2]-g./bird/day | 19.9±0.8[3]a[4] | 47.0[1] | 46.9±0.2[3]b[4] | 47.0[1] | 36.5±0.5[3]c[4] | 47.0[1] |
| Weight gain[2]-g./bird/day | -10.4±0.2 d | 8.6±2.0[3]fg[4] | 6.9±0.1 f | 13.1±0.1[3]gh[4] | 0.6±0.4 e | 15.8±1.0[3]h[4] |
| Gain/Feed | - | 0.18±0.05 j | 0.15±0.01 j | 0.28±0.01jk | 0.02±0.01 i | 0.34±0.02k |
| Liver size[2]-% | 3.2±0.2 l | 5.4±0.2 n | 3.4±0.1 l | 4.5±0.2 m | 3.4±0.1 l | 4.6±0.1 m |

[1]Chicks were force-fed the same amount of feed consumed by chicks fed ad libitum the diet with 0.32% DL-methionine added, at 4 times a day with a 4-hour time-interval.

[2]Means of 5 birds/rep. x 2 replications/treatment.

[3]Mean ± S.E.

[4]Means not carrying the same subscript in each row are significantly different at $P < 0.05$.

F. Experiment VI

The dried crop-contents of chicks administered purified-type diets which were either deficient, adequate or with excess methionine are shown in Table 28 and Figure 12 (Experiment VI A).

Two hours after the administration of either of 3 diets, approximately 40% of the diet had been discharged from the crop. However, 4 hours after the administration a significantly ($P < 0.01$) lesser amount of MA diet was recovered than MD diet, with the amount of ME diet being intermediate.

The same trend of results as in the first experiment was observed with a practical-type diet in the second experiment as shown in Table 29 and Figure 13. Three hours after the administration of the practical-type diet, approximately 60% of the diet had passed from the crop. At this time, no dietary effect on emptying rate was observed. Significantly more feed was collected from the crops of chicks given the MD diet 6 hours previously than from chicks given the MA or ME diets. No effect of excess methionine on the emptying rate was observed.

G. Experiment VII

The levels of dietary methionine as % of protein for pullets during the period from 8 to 13 weeks of age were 1.46, 1.74 and 2.0% for light breed, and 1.43, 1.71 and 2.0% for heavy breed, respectively, for the diets deficient (MD),

Table 28. Dried weight of crop contents remaining at 2 and 4 hours after administration of purified-type liquid diets containing different levels of methionine (Experiment VI A)

| Collection time after administration of diets | Levels of DL-methionine added. % | | | | | |
|---|---|---|---|---|---|---|
| | 0 | | 0.32 | | 1.0 | |
| | Weight[1] | % | Weight[1] | % | Weight[1] | % |
| 0 hour[2] | 16.8±0.08[3] | 100 | 16.8±0.08[3] | 100 | 16.8±0.08[3] | 100 |
| 2 hour | 10.4±0.43 | 62 | 10.0±0.51 | 60 | 10.8±0.46 | 64 |
| 4 hour | 9.1±0.25 a[4] | 54 | 6.9±0.60 b[4] | 41 | 8.0±0.43 ab[4] | 48 |

[1]Means of individually collected crop contents from 9 chicks per treatment. Dried weight of the crop content expressed as g./bird.

[2]Not actually administered into crop, but collected on a balance.

[3]Mean ± S.E.

[4]Means not carrying the same subscript in each row are significantly different at $P \leq 0.05$.

Analysis of variance of dried weight of crop contents

| Source of variation | d.f. | Mean Square | |
|---|---|---|---|
| | | Collection time after administration of diets | |
| | | 2 hour | 4 hour |
| Total | 26 | | |
| Treatment | 2 | 1.470 | 11.504** |
| Error | 24 | 1.976 | 1.866 |

**P <0.01

Table 29. Dried weight of crop contents remaining at 3 and 6 hours after administration of practical-type liquid diets containing different levels of methionine (Experiment VI B)

| Collection time after administration of diets | Levels of DL-methionine added, % | | | | | |
|---|---|---|---|---|---|---|
| | 0 | | 0.094 | | 1.0 | |
| | Weight[1] | % | Weight[1] | % | Weight[1] | % |
| 0 hour[2] | 13.9±0.04[3] | 100 | 13.9±0.04[3] | 100 | 13.9±0.04[4] | 100 |
| 3 hour | 5.7±0.18 a | 41 | 5.8±0.37 a | 42 | 5.6±0.36 a | 40 |
| 6 hour | 4.2±0.28 a | 30 | 1.2±0.40 b | 9 | 2.1±0.31 b | 14 |

[1]Means of individually collected crop contents from 9 chicks per treatment.

[2]Not actually administered into crop, but collected on a balance.

[3]Mean ± S.E.

[4]Means not carrying the same subscript in each row are significantly different at $P \leq 0.05$.

Analysis of variance of dried weight of crop contents

| Source of variation | d.f. | Mean Square | |
|---|---|---|---|
| | | Collection time after administration of diets | |
| | | 3 hour | 6 hour |
| Total | 26 | | |
| Treatment | 2 | 0.089 | 21.603** |
| Error | 24 | 0.910 | 1.019 |

**P <0.01

Figure 12. Crop emptying rate of chicks measured with 2 and 4 hours of time-interval after administration of purified-type diets deficient (0% added), adequate (0.32% added) or with excess (1.0% added) methionine (Experiment VI A)

Figure 13. Crop emptying rate of chicks measured with 3 and 6 hours of time-interval after administration of practical-type diets deficient (0% added), adequate (0.094% added), or with excess (1.0% added) methionine (Experiment VI B)

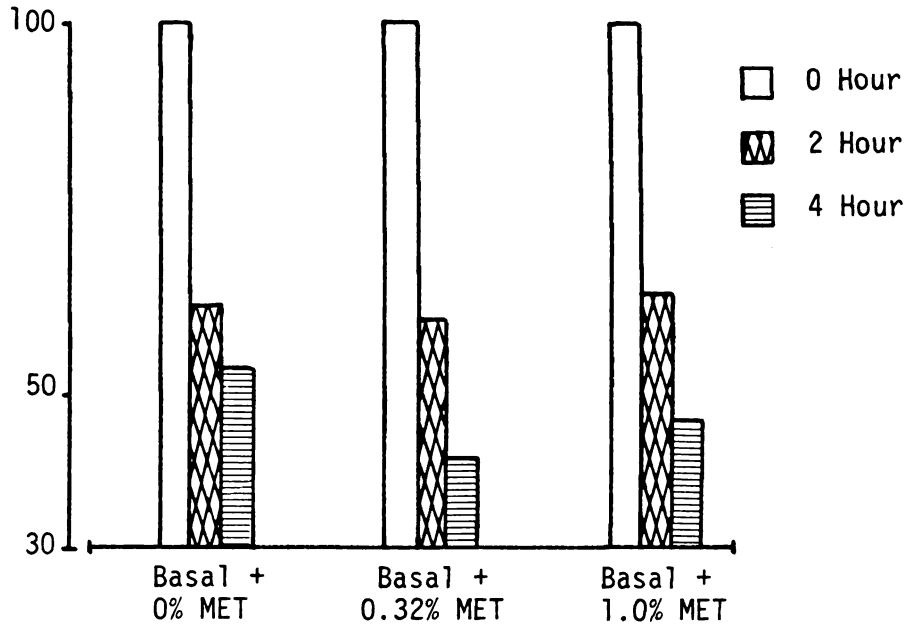


Figure 12.

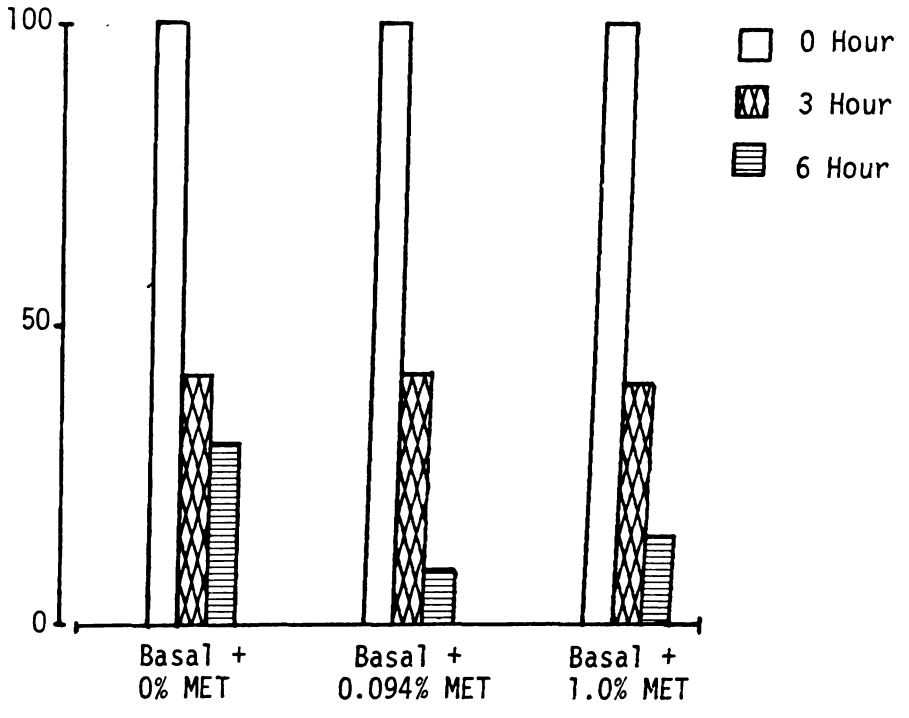


Figure 13.

moderate deficient (MMD) or adequate (MA) in methionine (Table 30). The diets for chickens during the period from 14 to 19 weeks of age contained levels of 1.26, 1.73 and 2.0% methionine for light breed, and 1.43, 1.71 and 2.0% for heavy breed, respectively, for the MD, MMD or MA diet (Table 31). For protein levels of each diet, see Table 6.

The data for responses in feed intake, weight gain, and feed efficiency of each breed to the diet of various levels of methionine are shown in Tables 30 and 31, respectively, for two different stages of the experiment.

During the period when the chickens were 8 to 13 weeks of age, the highest amount of feed ($P < 0.05$) was consumed by the light breed chickens fed the diet with a level of 1.46% methionine. For heavy breed chickens, though no significant differences among the treatments were found at $P < 0.05$, there was a trend that chickens fed the diet with the lowest level of methionine (1.43%) consumed more diet than those fed the diets with higher levels of methionine (1.71 and 2.0% methionine). No differences in weight gain were observed among groups on all treatments. The birds on the MD diets for light and heavy breeds demonstrated the poorest feed efficiency ($P < 0.05$). However, no difference in food efficiency was found between the light breed chickens fed the MMD or MA diets. A better efficiency in feed utilization ($P < 0.05$) was observed in heavy breed chickens fed the diet with 2.0% methionine than in those on the diet with 1.71% methionine.

Table 30. Effects of various levels of methionine on feed intake, weight gain and gain/feed in light and heavy breed pullets during the 8th to 13th week of age (Experiment VII)

| | % methionine of protein[1] | 8-13 weeks of age | | |
|---|---|---|---|---|
| | | Feed intake[2] g./bird/day | Weight gain[2] g./bird/day | Gain/Feed |
| Light breed | 1.46 | 71.7±1.3[3]a[4] | 12.3±0.2[3]c[4] | 0.17±0.01[3]d[4] |
| | 1.74 | 66.4±0.8 b | 12.7±0.2 c | 0.19±0.01 e |
| | 2.00 | 68.8±0.7 b | 12.8±0.3 c | 0.19±0.01 e |
| Heavy breed | 1.43 | 170.9±2.6 | 33.9±0.8 | 0.20±0.01 f |
| | 1.71 | 165.4±3.1 | 34.4±0.7 | 0.20±0.01 f |
| | 2.00 | 162.2±0.9 | 34.4±0.6 | 0.21±0.01 g |

[1] For protein levels of each diet, see Table 6.

[2] Means of 6 birds/rep. x 6 replications/treatment.

[3] Mean ± S.E.

[4] Means not carrying the same subscript in each column of each breed are significantly different at $P < 0.05$.

Table 31. Effects of various levels of methionine on feed intake, weight gain and gain/feed in light and heavy breed pullets during the 14th to 19th week of age (Experiment VII)

| | % methionine of protein[1] | 14-19 weeks of age | | |
|---|---|---|---|---|
| | | Feed intake[2] g./bird/day | Weight gain[2] g./bird/day | Gain/Feed |
| Light breed | 1.46 | 83.8±1.8[3] | 10.9±0.4[3] | 0.13±0.01[3] |
| | 1.74 | 80.0±1.3 | 10.1±0.3 | 0.13±0.01 |
| | 2.00 | 82.5±0.9 | 10.5±0.3 | 0.13±0.01 |
| Heavy breed | 1.43 | 167.8±4.0 | 23.1±1.2 | 0.14±0.01 |
| | 1.71 | 178.8±4.9 | 25.9±1.2 | 0.15±0.01 |
| | 2.00 | 170.2±4.1 | 26.1±0.8 | 0.15±0.01 |

[1]For protein levels of each diet, see Table 6.

[2]Means of 6 birds/rep. x 6 replications/treatment.

[3]Mean ± S.E.

Table 32. Effects of various levels of methionine on liver weight, % liver lipid and amount of liver lipid in light and heavy breed pullets (Experiment VII)

| | % methionine of protein | Liver weight[2] g./100 g.body wt. | % liver[2] lipid | Liver lipid[2] g. |
|---|---|---|---|---|
| Light breed | 1.46 | 2.57±0.05[3] | 3.96±0.08[3] | 1.04±0.03[3] |
| | 1.74 | 2.44±0.03 | 3.94±0.15 | 1.02±0.06 |
| | 2.00 | 2.51±0.13 | 4.05±0.17 | 1.06±0.05 |
| Heavy breed | 1.43 | 1.83±0.08 | 5.89±0.96 | 2.99±0.55 |
| | 1.71 | 1.94±0.19 | 5.83±1.44 | 3.58±1.44 |
| | 2.00 | 1.98±0.07 | 5.03±0.96 | 2.87±0.63 |

[1]For protein levels of each diet, see Table 6.
[2]Each mean represents the average value of 6 birds.
[3]Mean ± S.E.

Analysis of variance of liver weight, % liver lipid and amount of liver lipid

| | Source of Variation | d.f. | Mean Square: Liver weight | % lipid | Amount of lipid |
|---|---|---|---|---|---|
| Light breed | Total | 17 | | | |
| | Treatment | 2 | 0.026 | 0.020 | 0.003 |
| | Error | 15 | 0.040 | 0.112 | 0.014 |
| Heavy breed | Total | 17 | | | |
| | Treatment | 2 | 0.036 | 1.304 | 0.871 |
| | Error | 15 | 0.097 | 7.799 | 5.562 |

The performances of each breed during the 14th week through 19th week of age (Table 31) were not affected by dietary treatment. No significant differences in feed intake, weight gain, and feed efficiency were found among the groups on different levels of methionine for each breed.

Table 32 shows the effects of feeding the diets of various levels of methionine on liver weight, % of total lipid of liver, and amount of total liver lipid. No statistical differences in the measurements of the three parameters were found ($P < 0.05$) among the treatments of different levels of dietary methionine for each breed.

# V. DISCUSSION

## The most limiting amino acid in the basal diet and the requirement of sulfur amino acids

Grau and Kamei (1950) reported that, for young chicks, TSAA were the most deficient of indispensable amino acids in a diet with 10% protein whose source was only isolated-soy-protein. Warnick and Anderson (1968) proved that TSAA were the most limiting in raw or heat-treated type of soybean meal when fed to chicks in a diet with 14% protein. Threonine and valine were calculated to be the next limiting amino acids in soybean meal prepared for commercial use, but were not limiting in the experimental diet with 13% protein that was used in these studies. Only methionine of the four amino acids tested (threonine, tryptophan, methionine and lysine) was found limiting in the isolated-soy-protein.

Supplementation of methionine at 0.18 and 0.31% to the diet containing 13% protein, all supplied by isolated-soy-protein, improved feed intake and weight gain maximally at those respective levels. Thus, the requirement of methionine for appetite control was found to be much less than the requirement for maximum weight gain. The contribution of methionine and cystine from the soy protein added

to the supplemented amount of methionine yielded a requirement of TSAA for maximum growth and maximum feed intake to be 4.99 and 4.06%,respectively, of dietary protein, assuming 100% biological availability. However, this value of 4.99% appears to be higher than the requirement of 3.75% at 20% protein in the diet, calculated for chicks from data by NAS-NRC (1971). This suggests that the TSAA requirement is not a constant proportion of the level of dietary protein, and increases as the level of protein declines. The observation agrees with that of Grau and Kamei (1950), who noted that lysine and TSAA accounted for a higher percentage of protein as its level declined. However, the value of 4.99% could be lowered to 4.55% by additions of cystine as well as methionine, as long as methionine was in the range of 46 to 57% of TSAA. So, the experiment revealed that methionine conversion to cystine is not 100% efficient, because the lower requirement of TSAA for maximum weight gain was obtained when the cystine requirement was furnished directly rather than through supplementation with methionine. The reverse reaction is known not to occur (Baker, 1976). Nevertheless, the higher requirement for methionine by chicks to achieve maximum growth without any additional feed intake was an evidence for the known role of methionine in anabolism for tissue growth. At the TSAA level for maximum feed intake, growth was 82% of maximum, achieved by supplementation to soy protein with only methionine. Efficiency of feed utilization was optimum at TSAA levels at

or below, but not greater than, that amount of TSAA required for maximum growth rate. In fact, excess methionine at 1.0 to 1.32% levels of supplementation were less efficient in promoting growth or had an adverse effect on feed intake. The daily requirements for TSAA for maximum feed intake, gain/feed ratio, and weight gain were calculated to be, respectively, 149, 174, and 180 mg. per bird per day.

Determination of optimum levels of dietary TSAA or ratios of methionine to cystine for feed intake and weight gain

The requirement of TSAA for optimum growth was determined to be lowered from 0.665% to 0.597% of diet when the proportion of methionine of TSAA was in range of 46 to 57%. This is in agreement with that of Graber and Baker (1971). These latter investigators had demonstrated that on a weight basis the requirement for TSAA was less when supplied to chicks as methionine alone. The diet with 0.665% level of TSAA and 73% methionine of TSAA did not produce significantly different values in feed intake, weight gain and gain/feed from those of chicks fed the diets with 0.597% level of TSAA at the proportions of methionine to TSAA of 46, 52 and 57%. This result also supports the observation made by Graber and Baker (1971).

The observation, in the present experiment, that no significant differences were observed on feed intake, and weight gain from the diets with different ratios of methionine and cystine suggests that cystine can be used up to 54%

of TSAA in diets for growing chicks without any adverse effects when the protein level is 13.1%. Graber et al. (1971) found, in young chicks fed a diet in which methionine was the only source of SAA, that, when cystine replaced methionine, the maximum amount that could be added was 56 and 60% of SAA using gain and gain/feed, respectively. Sasse and Baker (1974) have found in young chicks, the cystine sparing values of 48.4% using gain and 55.7% using gain/feed in the presence of added $K_2SO_4$. The National Research Council (1971) recommends 0.4% methionine and 0.35% cystine at 20% protein diet. On that basis, calculation shows that cystine is 46.7% of TSAA. Sasse and Baker (1974) also pointed out that the response of chicks to dietary TSAA was dependent upon the presence of inorganic sulfur. Although the inorganic sulfur was not separately considered in the present experiment, 0.01% of inorganic sulfur was calculated to be present in the diet from the added salt mixture. Byington et al. (1972) observed that the addition of inorganic sulfur did not significantly influence chick's performances of feed intake and weight gain.

Various ratios of methionine to cystine in the diets deficient in TSAA (0.531%) did show the same trend in feed intake, weight gain and gain/feed as those on the diets of higher levels of TSAA, though the former diet reduced the performances of chicks in those 3 parameters. Sasse and Baker (1974) have made the same observation on chicks fed a deficient level of TSAA diet, although they expected a

considerably different response at a deficient level of TSAA from that occurring at an adequate level of TSAA.

Effect of methionine deficiency on feed intake and weight gain

Methionine is shown to play a major role in appetite control of chickens. The role is complicated and its mode of action not clearly defined. Nevertheless, the data definitely indicate that its role involves alteration of crop emptying time, plasma EAA levels and a role in energy metabolism.

Severe methionine deficiency produces a marked depression in food intake, in confirmation of data reported by Shoji et al. (1966) and Baldini (1961). In these studies, TSAA was only about 50% of requirements for maximum weight gain. On the other level, a moderate deficiency in methionine had no effect on feed intake but prevented maximal growth, as observed in young chicks used in this study, or resulted in an enhanced feed intake at maximal growth, as observed in the older chickens of this study, and in chickens of various ages in studies by Carew and Hill (1961), Slinger et al. (1953), Nelson et al. (1960), Hill (1965), and Solberg et al. (1971). In all of these studies TSAA was 70-80% of requirements, regardless of whether the diets were composed of purified or practical-type ingredients. Clearly, feed efficiency at any level of methionine deficiency marginal or severe, declines proportionally to the extent of the deficiency. Harms and Waldroup (1963) showed that the response to amino acid content in the chick's diet was dependent

upon the level of protein, the season, and apparently the strain of the chick. However, the data reviewed above indicate that the most dominant factor governing the methionine response for appetite control is its relationship as part of TSAA to the level of protein.

Shoji et al. (1966) and Baldini (1961) showed that severe methionine deficiency decreased energy deposition in the chick carcass and lowered the efficiency of utilization for metabolizable energy. Carew and Hill (1961) pointed out that although the increased intake of dietary energy was metabolized with normal efficiency as a result of (marginal) methionine deficiency, it did not appear as additional weight gain because of changes in body composition. They could find no evidence of significantly increased heat production. The chicks force-fed methionine deficient diets in this study had fatter livers of greater fragility than force-fed chicks given a methionine-adequate diet. All of those data indicate that a methionine (marginal) deficiency alters the energy metabolism of the chick. Solberg et al. (1971) showed that birds receiving a MD diet excrete a greater proportion of their dietary nitrogen as uric acid, indicative of a more active state of uric acid synthesis in these birds. They related the higher feed intake associated with the (marginally) deficient methionine diet to the increased heat production for uric acid synthesis. According to Sekiz et al. (1975), reduced growth was a stimulus for chicks to overcome a MD diet to meet an inner need for the energy

required for growth of lean body tissue. In doing so, the total methionine intake was increased, thus allowing normal weight gain but at the reduced efficiency of feed utilization. The data from the studies reported in this thesis support that hypothesis, and also indicate that the methionine deficiency results in preferential formation of lipid in the chick's liver and as shown by Carew and Hill (1961) in the carcass. Presumably, this lipid is formed from deaminated residues of amino acids which are low molecular weight fatty acids and which accumulate because of the reduced synthesis for protein and increased tissue breakdown caused by the methionine deficiency. Therefore, the hypothesis is presented that the craving for energy is the more dominant factor for appetite control than is marginal methionine deficiency, and not until methionine deficiency is severe does it become the dominant force to reduce feed intake. Further postulated is that removal of the factors which allow the dominant factor (energy) governing feed intake to express itself will unmask the lesser factor (methionine deficiency) governing feed intake. This, then, is why the slower crop emptying time, which could not ordinarily be detected in chicks fed ad libitum diets marginally deficient in methionine, can be detected when the chicks are force-fed the marginally MD diet without access to feed ad libitum.

Velu et al. (1972) showed that chicks respond to L-lysine addition in a crystalline amino acid diet devoid of

lysine by attaining maximum feed consumption at a lower level (0.71%) than that required for maximum weight gain (0.95%); however, in their studies with L-leucine feed intake and weight gain were maximized at the same level. Thus lysine, but not L-leucine, belongs in the same category as methionine in the ability to separate the points at which feed intake and weight gain are maximized upon additions of these amino acids to diets specifically deficient in them.

Meal-feeding and force-feeding were used in an attempt to overcome the depressing effect on feed intake caused by a diet severely deficient in methionine. The data show that hunger induced by extending the time between meals was not a dominant enough factor to overcome the effect of methionine deficiency on feed intake control. The MD chicks consistently ate about 30% less feed than their counterpart controls given MA diets as meals, an amount comparable to the effect obtained by comparing the data on ad libitum feeding of the MD and MA diets. For example, chicks fed MD diets ad libitum consumed 72.4% of the amount by chicks fed the MA diet ad libitum; whereas, those meal-fed the MD diet with 6, 14 or 22 hourly intervals consumed 70.5, 66.7 and 71.6% of the amount by those fed the MA diet at those respective hourly intervals. Nevertheless, one should note that when feed intake values are calculated on the basis of hours actually allowed to eat, chicks on meal schedules ate at a more rapid rate than their counterparts

fed ad libitum. Thus, rate of fill is apparently not influenced to as marked a degree by methionine deficiency within the two-hour span for eating. The slower crop-emptying time would appear to account for a lesser capacity to fill during the two-hour meal or the receptors for capacity to fill are accounting for that 30% reduction in feed intake. The data agree with the observations made with rats by Leung et al. (1968), Harper (1964) and Harper and Rogers (1966), that there exists a point at which the deficiency of the most limiting amino acid could not be overcome by forcing an increase in feed intake; in the above case by inducing greater hunger, in the other case, by force-feeding. There is a question regarding the word "limiting" when applied to the studies by Harper and his co-workers. Fisher et al. (1960) and Fisher and Shapiro (1961) consider an "imbalanced" diet which was used by Harper and co-workers, as an exaggeration of a specific amino acid deficiency. On that basis, the study with chicks on diets limiting in methionine are considered comparable to the study on threonine or histidine "imbalanced" or "limiting" in diets employed by Harper et al. (1964) and Leung et al. (1968). Also, supporting the work on crop-emptying time with chicks were the observations on rats that a diet high in methionine (3% of the diet) caused a delay in stomach emptying (Benevenga and Harper, 1970). The same effect on crop-emptying time by ME diets was found on chicks. In contrast, Leung and Rogers (1971), and Peng et al. (1972)

did not detect a consistent effect from a threonine deficient (imbalanced) diet on stomach emptying of rats.

These observations support the postulation made by Polin and Wolford (1973) on regulation of feed intake in chicks, that the crop plays a major role in governing feed intake by regulating activities of rate of fill, capacity and rate of discharge. Methionine deficiency is apparently operating within the context of this hypothesis.

Harper (1974b) stated that when the intake of protein exceeds the capacity of the animal for degradation of amino acids, then the amino acids will accumulate in body fluids. As a result, entry of the amino acids into the body may be slowed by a reduction in the rate of stomach emptying. The same statement can be adopted to explain the slower rate of crop emptying of chicks on the MD diet, an explanation discussed in greater detail in a subsequent section of this discussion. The chicks on the MD diet always showed a high concentration of plasma amino acids (PAA), probably due to a rapid tissue breakdown (Hill and Olsen, 1963). This high PAA or high plasma EAA concentration may provide a signal to reduce the emptying rate of the crop and thereby result in a decreased feed intake. Cooke and Ward (1976) indicated that excess tryptophan slowed gastric emptying of dogs by exciting a receptor in the gut and not by a direct effect on the stomach or brain or via its major metabolites.

Some investigators have assumed that emptying is independent of intragastric volume (Hildes and Dunlop, 1951).

However, according to Dubois et al. (1977), this assumption is in contradiction with experimental evidence which indicates that emptying rate of water is proportional to the intragastric volume. Thus, the emptying rate obtained by force-feeding a 30 ml. volume of liquid diet, which is larger than the normal volume of crop contents, may not be similar under a different volume of diet.

Effect of methionine excess on feed intake and weight gain

Diets with excessive levels of methionine generally cause depressions in feed intake and growth rate in rats (Cohen et al., 1958; Benevenga and Harper, 1967). The same observations were made for chicks fed ad libitum the diets with 13% protein and excessive levels of methionine, i.e. 1.0 or 1.32%, supplemented to the basal diet. However, the process of meal-feeding the ME diets overcame the depressing effect on feed intake control, and the adverse effect on weight gain. Thus, the mode of action on regulation of feed intake by methionine excess is not the same as that caused by a methionine deficiency. The latter's effect could not be overcome. The ME diet will support equal or greater growth in chicks if its adverse effect on the regulating system for feed intake can be overcome as shown by the studies on meal-feeding or force-feeding. Leung et al. (1968), Kumta and Harper (1961), and Harper and Rogers (1966) provided the same results with rats fed amino acid imbalanced diets. Benton (1964) produced similar results by force-feeding rats a diet supplemented with 3% L-leucine.

Some proposed mechanisms by which excess methionine in diets fed ad libitum were known to exhibit adverse effects on growth rate are 1) excessive labile methyl group (Cohen and Berg, 1951; Benevenga, 1974), 2) homocysteine accumulation (Katz and Baker, 1975), and 3) competitive transport of amino acids (Peng et al. 1973). The reversal of these mechanisms would be applicable to explain the fact that meal-feeding the diet with excess methionine led to the same feed intake as that of the MA diet fed as meals. However, no indications were observed that meal-feeding increased the oxidation of excess methionine.

The role played by excess methionine in reducing feed intake was shown not to be from its contribution of excess α-amino N per se. Replacing excess methionine with the dispensable glutamate reversed to a major extent the adverse effect on feed intake under ad libitum or meal-feeding regimens. The fact that the addition of 1% glutamate to the MA diet did not improve feed intake or weight gain observed with the MA diet indicated that the MA diet was not deficient in dispensable amino acids or α-amino N.

Force-feeding diets at time-intervals, another form of meal-feeding, provided the observation that the ME diet fed as meals improved feed efficiency. Chicks force-fed the same amount of an MA diet showed a tendency for slower growth and poorer feed efficiency than those on the ME diet. The observation is in agreement with that of Benton (1964) who produced equal body weight gain in rats by force-feeding

diets with or without supplemental 3.0% L-leucine. The improved feed efficiency resulting from the meal-feeding of a diet with an excess indispensable amino acid may be due to an improved intestinal digestion and absorption of nutrients, an increased body fat synthesis, or an increased body protein synthesis by an unknown reason. Leveille et al. (1975) observed that, in meal-fed chicks, the hepatic lipogenesis was increased more than twice that observed for liver of chicks fed ad libitum. Meanwhile, Nir et al. (1974) reported that, during a maximal growth stage in young chicks, deposition of fat was negligible, and most of the body weight-gain was due to protein synthesis. Thus, a further study is needed to determine the effect of meal-feeding a diet with excess amino acid on body composition.

Apparently, the strength of the signal to prevent feed intake when methionine is in excess is weaker than that produced when it is severely deficient. In both experiments on crop emptying time a trend, though not significant, was observed for a slower rate. This is interpreted to indicate that hunger induced by meal-feeding programs was the stronger signal. Meal-feeding programs overcame the moderate refusal to eat.

Effects of force-feeding the diets of methionine deficiency or excess on weight gain and liver size

Though force-feeding was employed to maintain a constant feed intake, birds responded with different weight gains and liver sizes to the levels of methionine in the diets they were fed.

On only one half the amount of TSAA but the same amount of dietary energy intake, the weight gain of chicks force-fed the MD diet was higher than that of chicks fed the MA diet ad libitum. This observation may suggest that the increased weight gain of chicks force-fed the MD diet may not be due to an increase of nitrogen retention, but to an increase of body fat accumulation. This effect can be attributed solely to the force-feeding itself since birds on both groups were offered the same amount of energy. Nir et al. (1974) demonstrated that force-feeding resulted in a more efficient energy utilization. Shoji et al. (1966) found that methionine deficiency decreased energy deposition in the carcass and decreased the efficiency of metabolizable energy utilization presumably as a result of increased heat production. However, birds receiving a MD diet excreted a greater proportion of their dietary nitrogen as uric acid and showed a more active state of uric acid synthesis (Solberg et al., 1971). Thus the nitrogen is removed leaving the carbon skeleton of the excess amino acids to be deposited as fat.

The better weight gain and gain/feed obtained by force-feeding than by ad libitum feeding of the MA diet definitely provided the evidence of a more efficient energy utilization and better nitrogen retention by force-feeding. Nir et al. (1974) reported that the increased growth in very young chicks by force-feeding an excessive amount of feed is mainly from lean body substance and partially from fat deposition.

The increased liver size by force-feeding may be due to fat accumulation. Nir et al. (1974) showed no changes in protein and glycogen concentrations in the liver by force-feeding but about a 20% increase of fat content. Meanwhile, force-feeding a diet containing a threonine-deficient amino acid mixture to young rats for several days resulted in an increase of liver size, uptake of amino acids into protein and RNA content and a shift of polysomes toward heavier aggregates (Staehelin et al., 1967). A more extensive incorporation of labeled amino acids into microsomes prepared from the livers of these animals was confirmed by Sidransky et al. (1964). The general mechanisms responsible for lipid accumulation in the liver may be an increased synthesis of triglycerides, a decreased oxidation of triglyceride fatty acids, a decreased mobilization of triglyceride from the liver, or a combination of these factors (Alfin-Slater and Aftergood, 1973). However, the involvement of SAA in the production of fatty liver is, still, not quite well understood, although it may be related to choline metabolism (Sidransky and Verney, 1969). Actually, the activity of choline oxidase in chicken liver was reduced by low methionine or low lysine diets (Garanca and Cielens, 1971) and also by dietary ethionine (Sidransky and Verney, 1969). The latter group of workers suggested that the reduced choline-oxidase activity may conserve some choline and play a role in the ethionine inhibition of choline-deficient fatty liver. This suggestion can explain the

normal size of liver obtained by ad libitum feeding of the MD diet. However, it can not properly explain the enlarged liver size induced by force-feeding of the MD diet. One postulate to consider is that methionine deficiency results in tissue breakdown and an increase in carbon skeletons of deaminated amino acids. These are not burned fast enough for energy. Instead the liver responds to their excess and converts them to lipid.

Patterns of free amino acids in plasma

The present observation of an increased threonine and serine in chick plasma by a MD diet is in agreement with other reports by Nakagawa and Masana (1967) in men, and by Girard-Globa et al. (1972) in rats. Sanchez and Swendweid (1969), and Girard-Globa et al. (1972) reported that hepatic enzyme activity of threonine dehydrase (or serine dehydrase) was decreased in rats fed a diet devoid of sulfur amino acids and increased as dietary methionine level was increased up to a toxic level. Ohno et al. (1972) also reported an increased level of serine in plasma of adult cockerels fed a MD diet. Evidence indicates that a low protein diet which was deficient in methionine induced an increase in the activity of enzyme, 3-phosphoglycerate dehydrogenase, needed for serine biosynthesis and maintained a higher plasma serine concentration by reducing the breakdown of serine (Schepartz, 1973). The increase of the enzyme activity was prevented by an inclusion of extra cysteine or methionine in

the diet (Schepartz, 1973). The above mentioned studies support the results of the present experiment. In addition to that, the fact that threonine and serine belong to the same transport groups as methionine for intestinal absorption (Wiseman, 1968), also provides a plausible support for the present observation on threonine and serine.

The elevation of lysine was one of the most significant changes caused by methionine deficiency (MD). Other investigators reported the same effect of MD on plasma lysine in chicks (Richardson et al., 1953) and on tissue lysine in rats (Denton et al., 1950). Though Ohno et al. (1972) observed an increase of plasma lysine in adult cockerels with increasing levels of dietary methionine, that was not substantiated by the data from feeding the ME diet which consistently reduced the level of lysine. The relationship between plasma lysine level and the level of dietary methionine will be discussed in greater detail later on.

The increased histidine in plasma may be related to the depressed hepatic histidase activity resulting from a diet deficient in protein and methionine (Schepartz, 1973). The lowered enzyme activity can reduce the rate of degradation of histidine. The level of valine was increased when the MD diet was fed ad libitum or as meals, probably due to its metabolic character as a branched-chain amino acid (BCAA). The MD diet usually raised the levels of TFAA and EAA in plasma higher than those of the control possibly as a

result of increased tissue breakdown. Oxidation of BCAA occurs in extra hepatic tissue, i.e. muscle, whereas, that of the other amino acids occurs in liver (Harper et al., 1970). Thus, the amino acids other than BCAA are removed with higher efficiency from circulating blood by the liver than is BCAA by muscle tissue. As a result, the level of BCAA in plasma remains relatively high.

Methionine was expected to be reduced by MD and to be increased by the ME diet, because, generally, the pattern of plasma free amino acids reflects, in part, that of dietary amino acids (Longenecker and Hause, 1959). Also, there is evidence that incorporation into tissue protein of the amino acid in short supply was increased by an imbalanced amino acid mixture resulting in its depletion from a plasma pool (Harper and Rogers, 1965).

The lowering of arginine by the MD diet seemed to be related to lysine-arginine antagonism, because excess lysine decreases the level of arginine through the elevation of arginase activity (Jones, 1964). This antagonism, however, may not be the main reason for the lowered arginine, since the arginase activity, also, was shown to be depressed in chicks by an excess level of dietary threonine (Austic and Nesheim, 1970), and threonine level in plasma was elevated. Tyrosine, which was reduced by the MD diet, may be responsive to the secretion of the adrenal gland, because the catabolic enzyme for tyrosine, tyrosine transaminase, is induced by glucocorticoids (Schepartz, 1973). A diet deficient

in protein and methionine might provide a stress to stimulate the secretion of the adrenal gland. Also, the hepatic enzyme, phenylalanine hydroxylase, is reduced by a low protein diet thereby limiting the breakdown of phenylalanine to tyrosine, and accounting for the unchanged level of phenylalanine.

Plasma amino acids altered by ad libitum feeding of the ME diet to chicks were those previously reported to be changed in rats (Sanchez and Swendseid, 1969; Klavins, 1965). Arginine, histidine and alanine were increased and glycine decreased. The level of arginine may be related to lysine-arginine antagonism, because the ME diet reduced the level of lysine. The high level of plasma histidine was related to some alterations in the metabolism of the single carton atom (Sanchez and Swendweid, 1969). However, histidase activity, which is probably the major catabolic enzyme, has been increased by glucocorticoids or high protein diets (Schepartz, 1973 ; Lee and Harper, 1977). Thus, the high level of histidine could be explained by an inhibited histidase activity by the same reason described for the effect of MD as follows. Chicks fed ad libitum the diet added with excess levels of methionine consumed an insufficient amount of diet and, consequently, dietary protein. This low consumption of dietary protein can lower the histidase activity to keep a high level of histidine (Schepartz, 1973).

Glycine and serine are the amino acids that are readily interconvertible. This would account for a decrease of glycine and an increase of serine by the ME diet. The enzyme, serine transhydroxy methylase, that converts glycine to serine can be elevated by glucocorticoids (Schepartz, 1973), and methionine has been known to increase blood corticosterone levels in rats (Munro et al., 1963).

A slightly different response in plasma amino acids was obtained by meal-feeding of the ME diet from that of the same diet fed ad libitum. Methionine and cysteine were the only amino acids elevated by the former treatment. The reason that amino acids such as serine, arginine or histidine in plasma were not increased by the excess methionine diet fed as meals can not be explained at the present moment.

Patterns of free amino acids in brain

Generally, the relationship between circulating and brain tissue levels of amino acids does not follow any consistent pattern. This is partly due to a competitive interaction in membrane transport, variations in intracellular metabolism and the existence of blood brain barrier (Roberts, 1968).

A clue to methionine's effect on control of feed intake was not obvious from the direction of change in methionine or cystein levels in plasma and brain when diets were deficient or excess in methionine. The effect on feed intake was for a decline in the case of both diets, but the

shifts in methionine or cysteine concentrations in plasma and brain for both amino acids were not consistent. Methionine levels in brain tended to remain constant from feeding MD diets; whereas, they were definitely higher in brain and plasma of chicks fed diets with excess methionine. The latter is in agreement with the observation of Lajtha and Toth (1961) who noted in the cerebral tissues of rats elevated concentrations of the amino acid administered in large quantities. The former observation on methionine in brain agrees with that of Rubin et al. (1974) who found no changes in brain levels of methionine in rats fed a protein-free diet. Cysteine levels were usually unchanged in plasma or trended lower in brain under the influence of MD diets. These observations imply that cysteine is regulated within a narrow range at a high degree of sensitivity. In plasma, the level of cysteine could be regulated by liver cystathionase whose activity is depressed by a methionine deficiency (Daniel and Waisman, 1969b). Had this been so, the cysteine levels in chick plasma would have reflected this, which it did not. Brain level of cysteine would be depressed if the enzyme pathways favored methionine formation rather than degradation to cysteine. Ordonez and Wurtman (1973) have shown that rat brain contains all of the enzymes needed to regenerate methionine from homocysteine, using serine as a source of methyl groups and folic acid derivatives as co-factors. Therefore, the shifts in methionine and cysteine in plasma and brain appear to be more correlated to the

activity of the enzymes in the body than to changes brought forth to act as signals for directly regulating food intake.

The experiments with chicks showed that the brain appears to reflect some of its amino acid pattern according to the plasma amino acid pattern. Increased levels of threonine, lysine and histidine were common in plasma and brain of chicks fed MD diets ad libitum. The pattern of free amino acids in chick brain is in the same trend as that exhibited in rats by Denton et al. (1950). In addition these investigators observed a lower level of tryptophan caused by methionine deficiency, an amino acid not studied in these chick experiments. On the other hand, they had no reports on cysteine levels which were previously discussed. Denton et al. (1950) found that valine was lower in brains of rats fed MD diets, whereas chicks fed MD diets showed no consistent change in their brain levels of valine.

Daniel and Waisman (1969b) reported a tendency for serine and threonine to decrease in brain of rats fed diets with excess methionine. In 3 of 4 experiments with chicks fed ME diets ad libitum or as meals the tendency for serine to be lower in brain was also observed. These observations tend to fit the observation that in rats force-fed ME diets serine dehydratase activity was elevated (Sanchez and Swendseid, 1969) and that the influence of ME diets is to inhibit the enzyme 3-phosphoglycerate dehydrogenase (Schepartz, 1973), in the biosynthetic pathway to serine. Baker (1976) observed that threonine oxidation was enhanced markedly when

excess methionine was fed to chicks, and a significant portion of the threonine catabolized in the chick was converted to glycine via the threonine aldolase pathway. With the latter pathway enhanced, and the former inhibited, the changes in serine seem unlikely to show any consistent trends. Threonine was found lower in brain in 2 of 4, showed no change in one, and an increase in another experiment when ME diets were fed. These data are not consistent enough to allow any reasoning an enzymatic pathways involved. Threonine was definitely elevated in brain and plasma of chicks fed diets deficient in methionine, an effect also noted for serine levels in plasma, but not for brain.

Aspartate transaminase in liver cytosole was elevated in typical gluconeogenic conditions by administration of glucocorticoids, and by other circumstances leading to increased rates of protein catabolism, such as imbalanced amino acid diets or diets containing toxic levels of methionine (Schepartz, 1973). The enzyme, glutamate dehydrogenase, converting glutamate to α-ketoglutarate, has not been consistent in rise and fall with the requirement for gluconeogenesis in rats (Schepartz, 1973). Alanine is one of the amino acids most readily convertible to glucose through gluconeogenesis (Felig, 1973). These can probably explain the reduced levels of those amino acids by the MD diet fed ad libitum or as meals, because the chicks on this diet were under gluconeogenic conditions. However, those reduced levels of aspartate + asparagine, and glutamate + glutamine

by meal-feeding of the ME diet are probably a specific-effect of excess-methionine in the diet fed as meals, because the effects were not found in the groups fed the ME diet ad libitum even with a decreased feed intake.

Relationship between plasma lysine and dietary methionine levels

The inverse relationship between the levels of plasma lysine and dietary methionine were in agreement with previous reports in the literature. There are many reports showing considerable decreases of lysine in tissues (Daniel and Waisman, 1969a; Sanchez and Swendseid, 1969) and in blood (Klavins, 1965) obtained by feeding an excess methionine diet to rats. Furthermore, Dean and Scott (1966) observed an increase of plasma methionine from chicks fed a lysine deficient diet.

The decreasing trend of plasma lysine observed in chicks fed the ME diet ad libitum or as meals was not likely due to the dramatic increase of plasma methionine, and consequent limitation of the pool size for free lysine. Increased levels of some amino acids, particularly arginine, were observed under the same condition, and furthermore, these two amino acids, lysine and methionine, do not share a common transport system through the intestinal wall (Wiseman, 1968).

According to Wang and Nesheim (1972), and Grove and Roghair (1971), the formation of saccharopine rather than that of pipecolic acid is the major pathway for L-lysine

degradation in chicks. The enzyme, lysine-ketoglutarate reductase, involved in the conversion of lysine to saccharopine, requires NADPH as a coenzyme. Thus, the NADPH availability may become a limiting factor in regulation of lysine degradation *in vivo*. Since most of the NADPH is generated from carbohydrate metabolism, when chicks are fasted or when they are consuming low quantities of feed, the NADPH availability may be diminished (Wang and Nesheim, 1972). Therefore, the decreased enzyme activity for lysine degradation may lead to the increased plasma lysine. Another possible explanation was reported by Hill and Olsen (1963). They observed that plasma lysine was markedly increased when chicks were fasted, but not when they were fed nonprotein diets. They postulated that this was due to a rapid breakdown of tissue protein during fasting and a slow rate of lysine degradation as compared to other amino acids.

Nevertheless, the NADPH hypothesis (Wang and Nesheim, 1972) does not seem exclusively pertinent for the observation in this study. If one considers that the difference in feed intake between the chicks fed *ad libitum* the MD and ME diet (1.32%) was only 1.1 g./bird/day, then the possibility of less available NADPH in the MD diet group looks very small. However, the low methionine level in the MD diet may increase tissue protein breakdown, and eventually, this accounts for the higher plasma lysine levels due to a slow rate of lysine breakdown. Such explanations could partly explain the negative relationship observed between the two amino acids.

Feed intake and amino acid patterns in plasma and brain

The assumption was made that there should be no real difference between the amino acid patterns in plasma and brain of chicks fed diets moderately-deficient or adequate in methionine because their feed intakes are similar. If there were any differences, these would not account for methionine's role in the regulation of feed intake because there is no difference in food intake of chicks fed these diets. However, the earlier discussion considered the prospect that the demand for energy craving was a more dominant force than a marginal methionine deficiency. Therefore, the changes reflected in plasma and brain of chicks moderately-deficient in methionine should be in the same direction as those of severely deficient chicks, only of lesser magnitude. This would consider these data to be confirmatory of reports which indicate that the intake of feed is regulated by the plasma or brain amino acid pattern (Mellinkoff et al., 1956; Leung and Rogers, 1969; Harper et al., 1970; and Peng et al., 1972).

Gradient responses toward increasing levels were observed among three dietary treatments of methionine, of 0.3, 0.2 and no additions to basal, for threonine, serine, TFAA, and NEAA in plasma; and for threonine to be higher and cysteine lower in brains of MD chicks.

The observations discussed earlier that meal-feeding could not overcome the depressed food intake of methionine deficiency, but could overcome it when caused by methionine

excess would suggest two separate modes of action for these two methionine treatments. The most characteristic change observed in the meal-feeding experiments was that methionine was the only amino acid whose concentration was highly elevated in plasma and brain by the ME diet. This same amino acid is markedly lower in plasma and brain when MD diets are fed. Thus, methionine level *per se* appears to be ruled out as a signal for regulating food intake. Interestingly, threonine appears to fit the pattern. It is elevated in plasma and brain by MD diets fed *ad libitum* or as meals, and is no longer elevated when ME diets are meal-fed.

Although threonine appears to fit the pattern in these studies, it was considered not effective on the control of food intake for rats (Sauberlich, 1961; Peng and Harper, 1970; Harper *et al.*, 1970). According to Rogers and Leung (1973) the receptor in the anterior prepyriform cortex area in rat brain is sensitive to the concentration of the growth-limiting amino acids. Rats fed a threonine imbalanced diet show only minor and inconsistent effects on stomach emptying-time (Leung and Rogers, 1971; Peng *et al.*, 1972). However, tryptophan showed gastric emptying significantly in a dose-related response. Unfortunately, the plasma and brain samples were not analyzed for tryptophan.

These data, on both methionine and threonine seem contradictory to those hypotheses that excessive methyl group (Cohen and Berg, 1951; Benevenga, 1974), homocysteine accumulation (Katz and Baker, 1975) or competitive transport

through intestine (Peng et al., 1973) are factors causing a depression of food intake from diets with excess methionine. Actually this is not so, if the meal-feeding procedures could be shown to reduce these factors through metabolic pathways.

The observation that no differences in feed intake were observed even with such highly altered concentrations of individual amino acids including very high levels of methionine in plasma and brain allows an inference that the changes of individual amino acids in plasma or brain, whether they were increased or decreased, may not be responsible for the reduced feed intake. This suggestion is in contradiction to many other reports. Almquist (1954), Mellinkoff et al. (1956) and Peng et al. (1969) have suggested that an elevated concentration of plasma amino acids which cannot be channeled into protein synthesis may serve as a satiety signal for a food intake regulating mechanism and thereby result in depressed food intake. The increase of histidine in plasma and brain was more harmful than lysine or threonine (Sauberlich, 1961; Peng et al., 1973). However, the increase of histidine in plasma or brain of chicks was too minor to be effective on feed intake.

Anderson et al. (1969) and Peng and Harper (1970) reported that an elevation of total free amino acids in plasma had a correlation to the amount of feed intake. Peng and Harper (1970) obtained a coefficient of -0.68 between the feed intake and total plasma EAA concentrations from

rats fed a low protein diet with isonitrogenous amounts of amino acid mixtures differing greatly in amino acid composition. This suggestion was examined in the present study and the correlation between feed intake and EAA/TFAA in plasma was -0.69 and -0.65, for ad libitum and meal-feeding programs, respectively. The correlation for plasma NEAA/EAA and feed intake was -0.59 for both feeding programs. Thus, the increase in essential amino acids would appear to be the most closely correlated of three measurements, TFAA, EAA, and NEAA, to feed intake control. Again, the high rate of tissue breakdown, and lowered capacity for amino acid degradation may be the reasons for the increased EAA in plasma (Anderson et al., 1968). The observation that the MD diet lowered the NEAA/EAA ratio in plasma suggests that relatively more NEAA than EAA was converted to glucose via gluconeogenesis to compensate for that shortage of energy.

Recently, Anderson and Ashley (1976) found that plasma TYR/PHE ratio correlated consistently with energy intake. Generally, they reported, the correlation between the ratio and energy intake were between the ranges of 0.68 to 0.98. Based on the observation that changes in plasma tyrosine can change brain tyrosine and catecholamine concentrations (Wurtman et al., 1974; Fernstrom, 1976), Anderson and Ashley (1976) suggested that changes in the plasma TYR/PHE ratio reflect or stimulate, at least in part, a mechanism operating via the central nervous system to control energy intake. However, the TYR/PHE ratio has the

correlations of 0.60 or 0.62 with the amount of feed intake (energy intake), respectively, for the diets fed ad libitum or as meals. Furthermore, the correlation between the changes of TYR/PHE in plasma and those in brain of the chicks on the same dietary treatments was only 0.34. Thus, the present data do not provide support for a correlation between the TRY/PHE ratio and energy intake. This may probably be due to the different levels of methionine in the diets. Anderson (1977) indicated that the problem with the TYR/PHE ratio is that the relationship between the alterations in catecholamine concentrations in the brain and the energy intake was not determined yet.

Although, the ratio of EAA/TFAA showed a better correlation with amount of feed intake than NEAA/EAA ratio or TYR/PHE ratio, a further study is required to achieve a better conclusion.

# VI. SUMMARY AND CONCLUSION

Seven experiments were conducted to determine the effects of dietary methionine on feed intake and amino acids in plasma and brain. Growing S.C.W.L. male chicks were used for experiments I to VI, and pullets of light and heavy breeds for experiment VII. A purified-type diet deficient in methionine (or TSAA) and with 13.1% level of protein, whose only source was isolated-soy-protein, was adopted as basal diet for all experiments except experiment VI B and VII. For experiments VI B, a practical-type containing 16.6% protein was formulated to be deficient, adequate or excessive in methionine. Diets containing 16.6 and 21.2% levels of protein and composed of practical ingredients were formulated, respectively, for pullets of light and heavy breeds to be deficient or adequate in methionine for experiment VII.

In Experiment I, amino acids such as DL-methionine, L-lysine·HCl, L-threonine or L-tryptophan were added to the basal diet individually or as a mixture of different combinations lacking in one of the amino acids to determine the most limiting amino acids. Methionine was found to be the only limiting amino acid in the diet. It, and cysteine were calculated to be at levels of 0.165 and 0.180%, respectively.

The requirements of total sulfur amino acids (TSAA) for maximum weight gain, feed intake and feed efficiency were found to be 0.665, 0.532 and 0.641% of diets, respectively. These values were obtained in experiment II by plotting the data for growth, feed intake or gain/feed against the various levels of DL-methionine added to the basal diet. However, a lower requirement of TSAA for optimum growth was obtained in a study using a factorial design with 3 different proportions of methionine of TSAA, i.e. 46, 52 or 57%, and 3 levels of TSAA, i.e. 0.531, 0.597 or 0.663% of diets. Feed intake and weight gain were similar for chicks fed the diets with 0.597 or 0.663% levels of TSAA and with methionine accounting for 46 to 57% of TSAA. Chicks fed the diets with 0.531% TSAA ate and grew to a lesser degree.

The purified-type diet severely deficient in methionine and with 13.1% protein always produced very poor feed intake and weight gain in young chicks. However, pullets of light and heavy breeds, when they were fed practical-type diets deficient in methionine but with normal levels of protein, showed a higher feed intake but no greater weight gain, compared to those fed the methionine adequate diet in experiment VII. This discrepancy in response of birds to diets deficient in methionine was suggested as probably due to the different extent of methionine deficiency between the two types of diets.

The purified-type diets with different levels of methionine were fed to chicks as meals or by force to

determine the effects on feed intake and weight gain (experiments IV and V). Feeding the diet deficient in methionine as a 2-hour meal and with 6 or 14 hours between meals caused feed intake and weight gain to be less than when the same diet was fed ad libitum. That same diet, given by force in an amount equal to the intake of the chicks fed ad libitum, resulted in a marked improvement in weight gain but was not equal to the effect produced by force-feeding the diet with adequate methionine. Thus, the depressed growth rate produced by methionine deficiency was partly because of its influence on food intake and partly because of its deficiency per se. The diet with an excess of DL-methionine (1.0% added) and fed as meals did not produce an adverse effect on feed intake and weight gain, as had this same diet fed ad libitum. Also, the chicks meal-fed the diet with excess methionine showed an improvement in feed efficiency. Force-feeding the diet with excess methionine improved weight gain and feed efficiency as opposed to poorer responses by chicks fed this diet ad libitum.

L-glutamic acid added as a source of α-amino N to the methionine adequate or deficient diets improved the responses in weight gain or feed efficiency of chicks meal-fed the diets, compared to those fed their corresponding diets ad libitum. However, meal-feeding the diet adequate in methionine without added L-glutamic acid also improved feed efficiency, compared to that obtained by the same diet fed ad libitum. Thus, these observations suggest that the

improved weight gain and feed efficiency caused by the excess methionine diet (1.0% added) fed as meals was an effect of the meal-feeding itself and not due to the excess α-amino N coming from the diets with an added 1% of DL-methionine. There was a definite trend for more feed (about 6.5% more) to be collected from the crops of chicks fed the methionine excess diet than from those fed the adequate diet. This suggested that the depressed feed intake of chicks fed the deficient or excessive diets ad libitum could probably be related to the delayed crop-emptying rate.

Amino acids in plasma and brain were determined in experiments II and IV in association to their effects on feed intake.

An inverse relationship was found between plasma levels of lysine and the levels of dietary methionine. When the dietary level of methionine was low, the level of plasma lysine was increased and vice versa.

The methionine deficient diet fed ad libitum or as meals usually increased the levels of threonine, lysine, histidine, or serine in plasma and/or brain. However, the declines in levels of methionine, cystine, arginine, or tyrosine in plasma or brain by the deficient diet were not consistent. Particularly, the level of methionine in brain was unchanged throughout the various levels of dietary methionine from severe deficiency to adequacy. The methionine excess diet fed ad libitum or as meals consistently increased the level of methionine in plasma and brain, but did not

always increase cysteine. However, these shifts of concentrations of individual amino acids were not associated with decreased feed intake. One of the observations for this statement is that chicks meal-fed the diet with an added 1.0% of methionine did not show the adverse effects in feed intake and weight gain, although these chicks had a dramatic increase of methionine in plasma and brain. The other observation is that feed intake was comparable for chicks fed diets with 0.2% or 0.3% added methionine, while the plasma levels of methionine were markedly different. Also, threonine and serine were significantly higher in brain and plasma of chicks fed the diet with an added 0.2% methionine, yet food intake was not influenced.

The diets deficient or with excess methionine usually increased the levels of total free amino acids, essential amino acids or EAA/TFAA in plasma. A negative correlation with a coefficient of -0.67 was found between the amount of feed intake and the plasma EAA/TFAA ratio.

The data on plasma and brain amino acid levels are discussed in relationship to lower feed intake caused by methionine deficiency or excess in the diet. The hypothesis is presented that crop emptying time is the primary mode of action through which there is an effect by an upset in dietary balance of methionine.

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# APPENDIX

Appendix Table 1. Analysis of variance for compositions of free amino acids in plasma of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment II B)

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 26.9 | 10,251** | 7,781** | 1,155 | 22.1 | 288 | 3,130* | 163.9 | 2.8 | 49.1$^{+}$ | 70.2 |
| Error | 6 | 9.1 | 155 | 156 | 264 | 6.2 | 228 | 313 | 71.5 | 3.1 | 13.5 | 68.8 |

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LEU | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | NEAA/EAA | EAA/TFAA |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 81.9 | 17.1 | 10.2 | 1,822** | 13.7* | 26.0$^{+}$ | 45,878 | 15,080* | 15,548 | 0,179* | 0.458* |
| Error | 6 | 64.5 | 8.9 | 6.9 | 183 | 1.6 | 5.6 | 4,333 | 2,676 | 2,676 | 0.030 | 0.083 |

+ P< 0.10
* P< 0.05
**P< 0.01

Appendix Table 2. Analysis of variance for compositions of free amino acids in brain of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment II B)

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 0.261 | 0.566** | 0.196 | 1.105 | 0.004 | 1.033 | 0.057 | 0.010 | 0.024 | 0.0001 | 0.011 |
| Error | 6 | 0.637 | 0.040 | 0.063 | 4.562 | 0.015 | 0.511 | 0.128 | 0.009 | 0.008 | 0.0005 | 0.004 |

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LEU | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | NEAA / EAA | EAA / TFAA |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 0.004 | 0.002 | 0.002 | 0.319** | 0.014† | 0.012 | 1.529 | 0.176 | 1.557* | 2.627 | 0.178 |
| Error | 6 | 0.004 | 0.004 | 0.001 | 0.028 | 0.004 | 0.004 | 8.513 | 8.211 | 0.198 | 0.844 | 0.063 |

† P <0.10
* P <0.05
**P <0.01

Appendix Table 3. Analysis of variance for feed intake, weight gain and gain/feed of chicks fed the diets of different levels of TSAA and ratios of methionine to cystine (Experiment IV)

| Source of variation | d.f. | Mean Squares | | |
|---|---|---|---|---|
| | | Feed intake | Weight gain | Gain/Feed |
| Treatment | | | | |
| Ratio (A) | 2 | 0.448 | 0.319 | 0.022 |
| TSAA (B) | 2 | 13.445** | 18.715** | 0.535** |
| A x B | 4 | 0.392 | 0.422 | 0.023 |
| Error | 18 | 2.963 | 0.495 | 0.015 |

**P< 0.01

Appendix Table 4. Analysis of variance for feed intake, weight gain and gain/feed of chicks fed ad libitum or as meals with various time-intervals the diets of various levels of methionine (Experiment IV A)

| Source of variation | d.f. | Mean Square (Feed intake) | | | |
|---|---|---|---|---|---|
| | | Ad libitum | Meal-feeding, time-interval | | |
| | | | 6 hr. | 14 hr. | 22 hr. |
| Total | 8 | | | | |
| Treatment | 2 | 46.65** | 46.63** | 46.72** | 7.17† |
| Error | 6 | 2.21 | 1.31 | 0.59 | 1.80 |

| Source of variation | d.f. | Mean Square (Weight gain) | | | |
|---|---|---|---|---|---|
| | | Ad libitum | Meal-feeding, time-interval | | |
| | | | 6 hr. | 14 hr. | 22 hr. |
| Total | 8 | | | | |
| Treatment | 2 | 74.94** | 85.97** | 83.79** | 17.83** |
| Error | 6 | 1.99 | 1.79 | 0.65 | 1.17 |

(Gain/Feed

| Source of variation | Ad libitum | | Meal-feeding, time-interval 6 hr. | | 14 hr. | |
|---|---|---|---|---|---|---|
| | d.f. | Mean Square | d.f. | Mean Square | d.f. | Mean Square |
| Total | 8 | | 8 | | 5 | |
| Treatment | 2 | 0.0761** | 2 | 0.1612** | 1 | 0.00001 |
| Error | 6 | 0.0014 | 6 | 0.0017 | 4 | 0.00060 |

† P< 0.10
**P< 0.01

Appendix Table 5. Analysis of variance for feed intake, weight gain and gain/feed of chicks fed ad libitum or as a meal with 6 hour-interval the diets of various levels of methionine (Experiment IV B)

| Source of variation | d.f. | Mean Square | | | |
|---|---|---|---|---|---|
| | | Ad libitum | | Meal-feeding | |
| | | Feed intake | Weight gain | Feed intake | Weight gain |
| Total | 14 | | | | |
| Treatment | 4 | 172.4** | 153.0** | 95.5** | 143.9** |
| Error | 10 | 11.0 | 4.9 | 0.8 | 1.3 |

| Source of variation | Gain/Feed | | | |
|---|---|---|---|---|
| | Ad libitum | | Meal-feeding | |
| | d.f. | Mean Square | d.f. | Mean Square |
| Total | 8 | | 14 | |
| Treatment | 2 | 0.0100* | 4 | 0.1549** |
| Error | 6 | 0.0017 | 10 | 0.0022 |

* P< 0.05
**P< 0.01

Appendix Table 6. Analysis of variance for feed intake, weight gain and gain/feed of chicks fed ad libitum or as a meal with 14 hour-interval the diets of various levels of methionine (Experiment IV C)

| Source of variation | d.f. | Mean Square | | | |
|---|---|---|---|---|---|
| | | Feed intake | | Weight gain | |
| | | Ad libitum | Meal-feeding | Ad libitum | Meal-feeding |
| Total | 8 | | | | |
| Treatment | 2 | 88.48** | 68.53** | 95.43** | 108.48** |
| Error | 6 | 5.96 | 2.32 | 0.92 | 2.02 |

| Source of variation | d.f. | Mean Square (Gain/Feed) | |
|---|---|---|---|
| | | Ad libitum | Meal-feeding |
| Total | 5 | | |
| Treatment | 1 | 0.0183** | 0.0009 |
| Error | 4 | 0.0003 | 0.0030 |

** P< 0.01

Appendix Table 7. Analysis of variance for compositions of free amino acids in plasma of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment IV B)

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE | LEU |
| Total | 8 | | | | | | | | | | | | |
| Treatment | 2 | 17.7 | 1,483* | 933* | 41.1 | 21.5 | 206* | 222 | 94.0* | 3.17* | 9,601** | 15.02 | 11.83 |
| Error | 6 | 19.6 | 284 | 170 | 754 | 59.3 | 27 | 263 | 17.0 | 0.42 | 109 | 10.05 | 8.56 |

| Source of variation | d.f. | Mean Square | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | NEAA/EAA | EAA/TFAA |
| Total | 8 | | | | | | | | | | |
| Treatment | 2 | 13.95 | 0.81 | 5,486** | 13.24† | 235.5** | 13,451 | 485 | 9,196 | 0,199* | 72.4* |
| Error | 6 | 6.92 | 4.35 | 229 | 2.61 | 18.2 | 18,655 | 4,327 | 5,432 | 0.019 | 7.2 |

† P< 0.10
* P< 0.05
**P< 0.01

Appendix Table 8. Analysis of variance for compositions of free amino acids in brain of chicks fed ad libitum the diets with different levels of DL-methionine. (Experiment IV B)

| Source of variation | d.f. | Mean Square | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 4.20 | 0.065 | 0.116 | 9.9 | 0.17 | 1.77† | 0.85* | 0.15** | 0.022** | 3.484 | 0.027 |
| Error | 6 | 3.01 | 0.097 | 0.320 | 4.3 | 0.17 | 0.44 | 0.14 | 0.01 | 0.002 | 0.005 | 0.008 |

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LEU | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | $\frac{\text{NEAA}}{\text{EAA}}$ | $\frac{\text{EAA}}{\text{TFAA}}$ |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 0.059 | 0.055** | 0.089** | 0.028 | 0.011 | 0.035 | 47.0 | 49.5 | 8.33* | 3.74* | 90.27* |
| Error | 6 | 0.117 | 0.003 | 0.004 | 0.017 | 0.008 | 0.026 | 25.2 | 19.9 | 1.16 | 0.58 | 11.40 |

† P< 0.10
* P< 0.05
** P< 0.01

Appendix Table 9. Analysis of variance for compositions of free amino acids in plasma of chicks meal-fed the diets with different levels of DL-methionine. (Experiment IV B)

| Source of variation | d.f. | Mean Square | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 11.24 | 3,969* | 2,336† | 416.6* | 50.2 | 420† | 145 | 210.8** | 6.29** | 9,938** | 39.5† |
| Error | 6 | 8.19 | 477 | 486 | 66.7 | 49.5 | 95 | 252 | 18.6 | 0.89 | 81 | 11.0 |

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LEU | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | $\frac{\text{NEAA}}{\text{EAA}}$ | $\frac{\text{EAA}}{\text{TFAA}}$ |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 62.9 | 105.1** | 8.79 | 1,838* | 4.77 | 44.2 | 15,801 | 3,626 | 8,741 | 0.322** | 108.3* |
| Error | 6 | 23.8 | 5.1 | 3.08 | 220 | 5.55 | 16.8 | 8,653 | 1,790 | 3,371 | 0.028 | 12.6 |

†P< 0.10
*P< 0.05
**P<0.01

Appendix Table 10. Analysis of variance for compositions of free amino acids in brain of chicks meal-fed the diets with different levels of DL-methionine (Experiment IV B)

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE | LEU |
| Total | 8 | | | | | | | | | | | | |
| Treatment | 2 | 5.13 | 0.25 | 0.96 | 18.9† | 0.18 | 1.26 | 0.89* | 0.128* | 0.0001 | 2.77** | 0.061* | 0.188† |
| Error | 6 | 2.15 | 0.14 | 0.40 | 4.15 | 0.21 | 1.05 | 0.18 | 0.024 | 0.010 | 0.02 | 0.011 | 0.046 |



| Source of variation | d.f. | Mean Square | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | $\frac{\text{NEAA}}{\text{EAA}}$ | $\frac{\text{EAA}}{\text{TFAA}}$ |
| Total | 8 | | | | | | | | | | |
| Treatment | 2 | 0.068† | 0.098† | 0.055 | 0.032† | 0.078† | 9.11 | 10.11 | 6.00 | 8.99* | 103.5* |
| Error | 6 | 0.015 | 0.024 | 0.052 | 0.009 | 0.020 | 6.12 | 3.01 | 1.87 | 0.88 | 19.7 |

† P <0.10
* P <0.05
**P <0.01

Appendix Table 11. Analysis of variance for compositions of free amino acids in plasma of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment IV C)

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 27.9 | 11,763* | 3,930* | 408 | - | 237 | 1,365** | 101 | 2.1 | 411.1** | 25.6$^{+}$ |
| Error | 6 | 39.6 | 1,944 | 572 | 350 | - | 150 | 114 | 111 | 1.6 | 6.2 | 5.4 |

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LEU | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | NEAA/EAA | EAA/TFAA |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 7.7 | 69.3$^{+}$ | 7.6 | 1,685$^{+}$ | 38.6 | 49.4** | 23,374 | 5,426 | 12,101 | 0.175* | 36.94* |
| Error | 6 | 16.0 | 17.2 | 19.3 | 469 | 13.5 | 1.2 | 16,043 | 4,490 | 4,492 | 0.017 | 5.77 |

$^{+}$ $P < 0.10$
* $P < 0.05$
** $P < 0.01$

Appeneix Table 12. Analysis of variance for compositions of free amino acids in brain of chicks fed ad libitum the diets with different levels of DL-methionine (Experiment IV C)

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 0.12 | 0.24** | 0.11† | 0.41 | - | 0.02 | 0.015 | 0.006† | 0.0002 | 0.0001 | 0.0016 |
| Error | 6 | 0.06 | 0.01 | 0.03 | 0.32 | - | 0.05 | 0.015 | 0.001 | 0.0004 | 0.0002 | 0.0005 |

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LEU | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | NEAA/EAA | EAA/TFAA |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 0.0035 | 0.0005 | 0.0004 | 0.052 | 0.0005 | 0.003 | 7.97* | 3.71† | 0.829* | 0.974 | 8.72 |
| Error | 6 | 0.0011 | 0.0002 | 0.0006 | 0.034 | 0.0015 | 0.002 | 1.15 | 0.85 | 0.128 | 0.400 | 3.91 |

†P< 0.10
*P< 0.05
**P< 0.01

Appendix Table 13. Analysis of variance for compositions of free amino acids in plasma of chicks meal-fed the diets with different levels of DL-methionine (Experiment IV C)

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 13.6 | 15,073** | 419 | 494 | - | 878** | 95 | 251** | 14.9* | 9,915** | 31.5* |
| Error | 6 | 4.5 | 109 | 318 | 177 | - | 25 | 234 | 17 | 1.8 | 125 | 5.4 |

| Source of variation | d.f. | Mean Square | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | LEU | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | NEAA/EAA | EAA/TFAA |
| Total | 8 | | | | | | | | | | | |
| Treatment | 2 | 50.9† | 12.0 | 2.16 | 3,887** | 4.21 | 185* | 28,318* | 2,028 | 25,646** | 0.558** | 151.06** |
| Error | 6 | 11.3 | 7.3 | 2.40 | 140 | 1.39 | 18 | 3,929 | 2,029 | 893 | 0.027 | 7.03 |

† P< 0.10
* P< 0.05
** P< 0.01

Appendix Table 14. Analysis of variance for compositions of free amino acids in brain of chicks meal-fed the diets with different levels of DL-methionine (Experiment IV C)

| Source of variation | d.f. | Mean Square | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ASP+ ASN | THR | SER | GLU+ GLN | PRO | GLY | ALA | VAL | CYS | MET | ILE | LEU |
| Total | 8 | | | | | | | | | | | | |
| Treatment | 2 | 0.032 | 0.304** | 0.180* | 1.483 | - | 0.110* | 0.023* | 0.005 | 0.0035 | 0.189** | 0.0005 | 0.003 |
| Error | 6 | 0.042 | 0.005 | 0.029 | 0.158 | - | 0.021 | 0.004 | 0.002 | 0.0012 | 0.003 | 0.0003 | 0.001 |

| Source of variation | d.f. | Mean Square | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | TYR | PHE | LYS | HIS | ARG | TFAA | NEAA | EAA | NEAA / EAA | EAA / TFAA |
| Total | 8 | | | | | | | | | | |
| Treatment | 2 | 0.0007 | 0.001 | 0.058** | 0.0001 | 0.0007 | 5.60* | 4.80* | 0.396* | 2.56* | 17.32** |
| Error | 6 | 0.0004 | 0.001 | 0.002 | 0.0001 | 0.0007 | 0.79 | 0.58 | 0.047 | 0.13 | 1.43 |

* P<0.05
** P<0.01

Appendix Table 15. Analysis of variance for feed intake, weight gain, gain/feed and liver size of chicks force-fed the diets with various levels of DL-methionine (Experiment V B)

| Source of Variation | Feed intake | | Weight gain | | Gain/Feed | | Liver size | |
|---|---|---|---|---|---|---|---|---|
| | d.f. | Mean Square | d.f. | Mean Square | d.f. | Mean Square | d.f. | Mean Square |
| Total | 5 | | 11 | | 9 | | 59 | |
| Treatment | 2 | 370.9** | 5 | 288.3** | 4 | 0.0307* | 5 | 6.88** |
| Error | 3 | 0.93 | 6 | 6.54 | 5 | 0.0035 | 54 | 0.25 |

* P< 0.05
**P< 0.01

Appendix Table 16. Analysis of variance for feed intake, weight gain and gain/feed ratio in light breed and heavy breed pullets during the periods on grower diets and developer diets (Experiment VII)

| | Source of variation | d.f. | Mean Square | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Grower Period | | | Developer Period | | |
| | | | Feed intake | Weight gain | Gain/Feed | Feed intake | Weight gain | Gain/Feed |
| Light breed | Total | 17 | | | | | | |
| | Treatment | 2 | 42.359** | 0.417 | 0.0006** | 21.587 | 0.844 | 0.00001 |
| | Error | 15 | 5.482 | 0.405 | 0.00004 | 11.532 | 0.547 | 0.00006 |
| Heavy breed | Total | 17 | | | | | | |
| | Treatment | 2 | 117.828† | 0.558 | 0.00026** | 200.105 | 16.477 | 0.0003 |
| | Error | 15 | 33.977 | 2.826 | 0.00004 | 113.900 | 6.641 | 0.0001 |

† P< 0.10
* P< 0.05
**P< 0.01