INTERRELATIONSHIPS BETWEEN PYRIDOXINE AND METHIONINE IN CHICK DIETS

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PYRIDOXINE AND METHIONINE

KAZEMI

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Five experiments, using a total of 420 day-old male broiler type chicks, were conducted to investigate possible interrelationships between vitamin B_6 and methionine. In the first four experiments, purified type chick starter diets limiting in both vitamin B_6 and methionine were used while in the fifth experiment, a practical diet known to be marginal in methionine was used.

High vitamin B_6 supplementation (30 mg per kg) of a diet severely deficient in methionine resulted in performance inferior to that of a control diet adequate in both vitamin B_6 and methionine. However, high vitamin B_6 in the presence or absence of methionine was not significantly different from the control groups with adequate vitamin B_6. Vitamin levels in excess of 6 mg per kg (9, 6, 11, 16, 21, and 26 mg per kg) did not improve growth and feed efficiency in an otherwise complete diet. The vitamin B_6 deficient group was significantly inferior to all other vitamin B_6 supplemented groups. The data on body weight gain and feed consumption indicate that vitamin B_6 (6 mg per kg) could correct a marginal but not a severe methionine deficiency.

Chicks receiving 6 mg per kg of vitamin B_6 in a glycine deficient diet attained comparable growth to those receiving the same diet but adequate in glycine.

Plasma amino acids of B_6-deficient birds were lower than those receiving 6 mg per kg of vitamin B_6 with the exception of glycine, histidine, and threonine which were much higher. Liver vitamin B_6 was not significantly different at 6 mg per kg of vitamin B_6 with or without methionine when compared to a 3 mg of vitamin B_6 per kg with methionine and glycine. Moreover, carcass protein and SGO-T activities were significantly higher and carcass fat was significantly lower in methionine supplemented groups

compared to those not supplemented with this amino acid.

The results on a practical type ration showed that neither vitamin B_6 nor methionine supplementation significantly improved growth and feed utilization. Carcass protein was significantly lower in the methionine supplemented group than in all other treatments. Carcass fat was significantly different from one treatment to the other; the non-supplemented having the lowest carcass fat and the methionine supplemented the highest.

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I. INTRODUCTION

The field of nutrient interrelationships is one of the most dynamic phases of nutrition research at the present time. One group of these interrelationships involves vitamin B_6 and proteins. Research in the field of vitamin B_6 and protein interrelationships is becoming more oriented towards interactions of vitamin B_6 with each essential amino acid. The present study deals with the interrelationship of vitamin B_6 with one of these amino acids, namely methionine.

The involvement of the coenzyme form of vitamin B_6 in amino acid metabolism can be attributed to its known functions in transamination, deamination, reamination, decarboxylation, and racemization. There are many hypotheses, however, to explain the significance of each one of the above functions and also the mode of action of the vitamin in amino acid absorption.

Methionine is frequently listed as the principal limiting amino acid in most modern day poultry rations. Since the quality of the protein depends on the adequacy of each one of the essential amino acids, many research workers have attempted to improve protein quality by different methods such as using a mixture of proteins or adding the

synthetic forms of the deficient amino acids. Because protein rich feedstuffs are considered the most expensive ingredients in a ration, any attempt at inproving their utilization at a low cost should be taken into consideration. Vitamin B_6 can be purchased in crystalline form and the amount needed for supplementation in excess of the minimum requirement established (3 mg/kg of diet) is very minute. The supplementation, therefore, could be practiced with little additional cost per kg of diet. The practical implications of vitamin B_6 supplementation are more noticeable in today's modern broiler rations which are characterized by having high energy and high protein levels. Efforts directed towards faster growth and better feed utilization of broiler rations have ignored the importance of vitamin B_6 especially in a ration which is marginal in either vitamin B_6 or methionine.

In the first two preliminary experiments, an attempt was made to establish the vitamin B_6 level under the conditions of this study beyond which no extra growth or improvement in feed efficiency could be obtained; thus excluding one of the variable factors in subsequent experiments. The third experiment was designed to determine whether vitamin B_6 could completely alleviate a severe methionine deficiency, while in the fourth experiment, the effect of vitamin B_6 supplementation to a partially methionine deficient diet was investigated. The last experiment was

set up to study the effects of vitamin B_6 supplementation of a methionine-limiting practical chick starter ration.

II. REVIEW OF LITERATURE

Several reports have appeared in the literature concerning different aspects of vitamin B_6 and protein metabolism. Each study was carried out with the intention of elucidating a small part of the whole problem, a combination of these has rendered a better understanding of vitamin B_6 and protein interactions. Most of these studies were centered around the effects of dietary vitamin B_6 on the utilization of dietary proteins as measured by either vitamin B_6 or amino acid concentration in tissues. There are also a few reports on the influence of vitamin B_6 on carcass composition of rats and cockerels. Practical aspects of vitamin B_6 has gained some attention during the past decade. The review that follows covers selected aspects of the above mentioned areas.

Vitamin B_6 and Protein Metabolism

Vitamin B_6 is involved in oxidative deamination of the D-amino acids with subsequent reamination of the keto acids to the L-isomers (Berg, 1959, pp 57-96; Braunstein and Azarh, 1957). In transamination, it presumably acts as a carrier for the anino group through the formation of a shiff's base between pyridoxal phosphate and the amino acid

(Schlenk and Snell, 1945; Lichstein et al., 1945). In the absence of pyridoxine, reamination of the D-isomers is reduced.

There is some evidence that vitamin B_6 deficiency may affect D-amino acid oxidase activity as well. Homogenized kidney from rats deficient in pyridoxine exhibited only one third as much D-amino acid oxidase activity as did kidney homogenates from pyridoxine adequate rats (Armstrong et al., 1950). There are also differences in utilization of different D-amino acids as affected by the rates of reaction with D-amino acid oxidases. The D-isomers of valine, leucine and isoleucine are very poor substitutes for their L-isomers in comparison with D-methionine and D-tryptophan and their L-isomers (Berg, 1959, pp 57-96). Utilization of D-tryptophan is decreased in vitamin B_6 deficient rats due to an impairment of the formation of indolepyruvic acid from tryptophan (Braunstein and Azarh, 1957; Harding-Charconnet, 1959).

Although various functions of vitamin B_6 in protein metabolism are well established, several others are not yet clearly defined. Sauberlich (1961) proposed that when an amino acid is limiting, added amounts of vitamin B_6 in the diet may have a "sparing effect" by allowing more incorporation of the limiting amino acid into protein and growth instead of being channeled into oxidative pathways. Moreover, high levels of pyridoxine in the diet could enhance conversion

of the D-isomers of certain amino acids to the natural L-form and as such improve growth and feed efficiency as was confirmed by the work of Braunstein and Azarh (1957).

Christensen (1962, 1963) presented evidence for the participation of vitamin B_6 in the cellular uptake of the amino acids. He proposed that vitamin B_6 deficiency reduces cellular uptake of amino acids but the reactions involved are unknown. In a high or adequate vitamin B_6 ration, cellular uptake, including intestinal absorption was improved. The possibility that very high intake of the vitamin should be more effective than an adequate level was questioned. It was pointed out, however, that excess intakes might result in greater absorption of one amino acid at the expense of another, thus causing an amino acid imbalance with adverse effects.

Aketo et al. (1960) reported that the rate of disappearance of L-isomers of amino acids from the intestinal lumen was increased, while that of D-isomers was unaffected after injecting vitamin B_6 in rats. This suggests that vitamin B_6 is involved in the absorption of L-amino acids from the small intestine in this species.

Both quality and quantity of proteins in a ration seem to play an important role in determining the need of the animal for vitamin B_6. Cercedo and Foy (1944) observed that the onset of the characteristic dermatitis of vitamin B_6 deficiency in rats was accelerated on a high protein

diet. Furthermore, Cercedo et al. (1948) noticed better resistance to vitamin B_6 deficiency symptoms when the rats were fed a low-protein diet. In a 15% purified casein diet with no pyridoxine, survival was more than 100 days and the skin lesions appeared after about 90 days. When 0.31% DL-methionine was added to the above diet, skin lesions appeared after 17 days and average survival time was 41 days.

Kirchgessner and Frienecke (1963) concluded that as in the rat, the vitamin B_6 requirement in the growing cockerel is related to protein intake, and that, similar to the pig, there is a range of sub-optimal intake in which the deficiency signs do not appear but weight gain and nitrogen deposition are affected. In preliminary trials with day-old White Leghorns fed diets containing 18.7% protein and 2 mg of vitamin B_6 per kg, signs of deficiency were observed but no death occurred. When 22 or 28% protein was used, mortality was over 60% at the 2 mg per kg basal but not with the 2.6 mg of vitamin B_6 per kg.

Similar findings have been reported with mice (Miller and Baumann, 1945; Hawkins et al., 1959). Results of Tupule and Williams (1955) with rats, however, indicated that raising the protein level does not aggreviate vitamin B_6 deficiency as measured by both growth and tissue concentration of the vitamin.

Studies with lactic acid bacteria have shown that the omission of preformed amino acids from the medium increases the vitamin B_6 requirement (Snell and Keevil, 1954, pp 276-280; Holden et al., 1951). Under such conditions, amino acids must be synthesized from keto acids or hydroxy acid precursors by transamination or by other vitamin B_6-dependent reactions. Therefore, provision of non-essential amino acids in the diet should lower the demand for the vitamin, or conversely, increasing the pyridoxine intake should improve growth with diets lacking in non-essential amino acids.

Anderson et al. (1949) studied the effect of high levels of individual amino acids on growth of chicks fed low vitamin B_6 diets and diets containing adequate amounts of the vitamin. Growth was depressed by the addition of certain amino acids to a low vitamin B_6 diet and increased by the addition of the amino acid to a vitamin B_6-adequate diet.

Armstrong et al. (1950) reported that the supplementation with D-amino acids (leucine, isoleucine, phenylalanine, and valine) to a vitamin B_6-deficient diet greatly depressed nitrogen utilization in rats. An injection or an adequate dietary supply of vitamin B_6 completely overcame the effect. Supplementation of the L-isomers of the same amino acid or an equivalent amount of inorganic nitrogen caused a smaller depression of dietary nitrogen utilization.

Sauberlich (1961) worked on the influence of dietary supplements of vitamin B_6 on the activities of L-methionine, L-tryptophan, and L-valine in weanling rats. The diets were designed to be low in both vitamin B_6 and the amino acid under investigation. Growth and feed efficiency were improved by increased levels in the diet of either pyridoxine, the amino acid under study or both. The growth promoting activities of both the L- and D-isomers of the amino acid studied were enhanced with increased amounts of pyridoxine in the diet. With an optimum amount of vitamin B_6 in the diet, D-methionine and the hydroxy analogue of methionine were equal to L-methionine in promoting growth.

Williams (1962) carried out an experiment to study the effect of the non-essential amino acid composition of the diet on growth of rats fed a limited amount of vitamin B_6. It was postulated that growth of rats fed a diet supplemented with a single non-essential amino acid, together with a limited amount of vitamin B_6, would be less than that observed in rats fed an isonitrogenous diet containing a mixture of non-essential amino acids. Male rats previously depleted of vitamin B_6 were fed diets of purified L- and DL-amino acids with the vitamin B_6 intake of 3 ug per rat per day. The essential amino acid mixture based on whole egg protein contributed 0.95% of nitrogen per 100 grams of diet and was supplemented with 0.95% of nitrogen from different combinations of amino acids. During

the first week of vitamin B_6-supplementation, a mixture of seven non-essential amino acids (glutamic acid, aspartic acid, glycine, serine, cystine, proline, and tyrosine) permitted the greatest weight gain. After the first week of vitamin supplementation however, growth was affected much less by variation in the non-essential amino acid composition of the diet.

Wentworth et al. (1963) studied the vitamin B_6 requirement of the rat as affected by the presence or absence of preformed non-essential amino acids in the diet. The rats grew better when fed a diet containing ten essential amino acids plus 11 non-essential amino acids compared to an isonitrogenous ration with ten essential amino acids plus cystine and tyrosine. When the diets were supplemented with different levels of vitamin B_6, the growth and feed efficiency were better when more of the non-essential amino acids were present.

Genetic differences in vitamin B_6 metabolism have been observed in mice and chicks. Daghir and Balloun (1963) found that the vitamin B_6 requirement for growth was higher for Leghorns than for other breeds studied. Lyon et al. (1962) found that I strain mice, which were more rapidly depleted of vitamin B_6 had less pyridoxal phosphate and more pyridoxamine phosphate in brain and liver than that of C_{57} strain of mice.

Genetic differences in methionine metabolism have

been reported in chicks by McDonald (1957, 1958) who observed that supplements of methionine increased the growth of Leghorn chicks, but decreased the growth of Australorp chicks.

Vitamin B_6 and Tissue Amino Acids

Detailed studies of changes in the levels of plasma amino acids in vitamin B_6 deficiency have contributed to a better understanding of the importance of vitamin B_6 in amino acid metabolism.

Harding et al. (1964) measured the free amino acids in plasma and urine of human subjects fed vitamin B_6-deficient diets of low or high protein level. The deficiency period was preceded by a one-week control period when the subjects received 4.0 mg pyridoxine hydrochloride per day. The effect of vitamin B_6 deficiency on plasma amino acids depended upon the dietary protein. With the high protein diet, the level of leucine and valine increased significantly. With the low protein diet, there was no significant change in amino acid levels with the exception of phenylalanine which decreased significantly. With both diets, the levels of glycine, serine, and asparagine-glutamine increased. The low-protein diet produced higher levels of the non-essential amino acids, glycine, serine, and asparagine-glutamine when compared with the high-protein diet, either before or after the development of the deficiency. With both protein

levels, the glycine:serine ratio decreased significantly from approximately 0.9 to 0.6-0.7 because of the relatively greater increase in serine.

Richardson et al. (1953) carried out an experiment on the influence of the amount of a low-vitamin B_6 diet on the free amino acid content of the blood plasma of day-old chicks. Birds receiving the different basal diets grew at about the same rate, but the concentration of amino acids in the plasma as determined microbiologically was different. When the diet was low in vitamin B_6, the concentration of each of the amino acids in the plasma with the exception of lysine, was significantly higher than when the diet was adequate in that vitamin. Between the diets studied, the ones with lower amino acid content had lower plasma amino acid with the exception of arginine which was not reduced with a low dietary arginine.

Nadkarni and Sreenvassan (1958) determined total Liver amino acids in vitamin B_6-deficient and control rats. When amino acids were determined microbiologically, there was a decrease in the level of all amino acids in the deficient group as compared to the control.

Merrow et al. (1966) reported that in growing rats, glycine was the only amino acid which increased in plasma, Liver and muscle in vitamin B_6 deficiency. Men on diets poor in vitamin B_6 also showed increased plasma glycine, decreased taurine and glutamic acid as reported by the same workers. No drop in glutamic acid level occurred in rat

plasma, liver, muscle or spleen. After a protein meal, both rats and men showed an increase in most plasma amino acids.

Swendseid et al. (1964) analyzed the amino acid levels of plasma, muscle, and liver of rats which had been fed to appetite a purified diet with or without 2.5 mg of vitamin B_6 per kg for four to six weeks from weaning. The amino acid values, in general, were lower in deprived rats and the total essential amino acids decreased more than the total non-essential amino acids. Some of the changes were attributed to reduced metabolic interconversions. Glycine and aspartic acid were increased and serine and alanine decreased in plasma of deprived rats. Addition of 7.5% glycine to the deficient diet accentuated the changes in plasma amino acids.

In addition to its action on specific amino acids, dietary vitamin B_6 has been shown to affect tissue activity of several enzymes involved in transamination, deamination, and decarboxylation. Vitamin B_6 lowers the activity of serum glutamic oxaloacetic transaminase (SGO-T) in rats (Babcock, 1959; Yeh et al., 1960). Those fed a vitamin B_6-deficient diet were found to have 80% of the SGO-T activity as compared to the control group (Rosen and Milholland, 1960).

Tupule and Kschirsagor (1959) studied the effects of low protein, and adequate dietary protein levels with or

without vitamin B_6 supplementation on SGO-T in young rats. It was observed that SGO-T values of rats fed diets low in protein or vitamin B_6 were reduced. The effect was more pronounced when both nutrients were deficient.

Recent work with human SGO-T indicates that there is a fall in the enzyme activity but not consistently. This has led several workers to conclude that SGO-T values can not be taken as the sole criterion for determining the status of vitamin B_6 nutrition (Babcock et al., 1960; Raica and Sauberlich, 1964).

Goswami and Robblee (1958) reported that there was a considerable reduction in GO-T activity in blood, liver and heart of chickens on pyridoxine deficient diets. Daghir and Balloun (1963) confirmed the previous work and noted that the SGO-T activity varied with the breed of chickens, especially when the vitamin B_6 content of the diet was marginal.

Dietary Influence on Tissue Vitamin B_6

Certain variations in the diet have been shown to affect the vitamin B_6 content in liver and blood. Sheppard and McHenry (1946) reported that the concentration of vitamin B_6 in liver appeared to be independent of vitamin B_6 supply when the intake is greater than 25 ug per day, but is directly proportional to the percent of protein in the diet. The vitamin B_6 content of liver can be

significantly increased by supplying vitamin B_6 to rats maintained on a high protein diet. It was suggested that the higher storage was caused by an increased need for pyridoxine in the liver to handle the increase in amino acid metabolism.

Schweigert et al. (1946) indicated that the level of casein in the diet did not appear to affect either the storage or the depletion of vitamin B_6 from the tissue of rats. When a vitamin B_6-deficient diet was fed, there was less growth and more mortality at 50% casein levels compared to 10% levels. However, the tissue vitamin B_6 remained unchanged. When studies were continued on mice, the tissue reserve of the vitamin was found to diminish much more rapidly on 50% of casein than when the diet contained 10% of casein. The mice on the high protein diet lost more weight and had a higher mortality rate than those on the low protein diet.

Tupule and Williams (1955) stated that growth in rats does not necessarily reflect tissue pyridoxine level. On the other hand, Greenberg and Rainehart (1949) noted that the tissue levels of vitamin B_6 in rats and monkeys reflect vitamin B_6 intake.

Debey et al. (1952) indicated that dietary methionine has no significant effect on vitamin B_6 concentration in liver and blood of rats fed diets varying in vitamin B_6 and methionine content.

Beaton and McHenry (1953) noticed that liver tissue was more satisfactory than heart or kidney tissue for assessing the state of vitamin B_6 nutrition in the rat. Evidence was presented suggesting that vitamin B_6 is mobilized from extra hepatic tissue to liver during the deprivation of dietary vitamin B_6. Saturation of liver tissue with vitamin B_6 occurred with a lower level of vitamin B_6 intake than that which was sufficient to give maximum body weight.

Vitamin B_6 and Carcass Composition

McHenry and Gavin (1941) observed that in the absence of pyridoxine, there was a loss of weight in rats fed a high protein diet but devoid of fats and carbohydrates, regardless of the administration of any other known B vitamin. Rats fed a 96% protein diet and receiving supplements of thiamin, riboflavin, pantothenic acid, nicotinic acid, choline, and pyridoxine had double the carcass fat when compared to a group receiving the same treatment except for the omission of pyridoxine. When deficient animals were given vitamin B_6, there was a rapid increase in body weight and the amount of carcass fat tripled in three weeks thus contradicting the work of Halliday (1938) who found that vitamin B_6 deficient rats had a significantly heavier liver with a higher percentage of total fatty acids.

Beaton et al. (1953) reported that vitamin B_6 deficiency in the rat does not affect the synthesis of cellular protein or the activity of certain liver enzymes. They also stated that vitamin B_6 deficiency does not significantly alter the levels of carcass protein or of total serum protein in the rat.

Carter and Phizackerly (1951) reported that deficiency of vitamin B_6 in the rat lowers total lipid content of the body and neutral fat content of the liver. Inclusion of 20% fat in the diet restored the total body lipids to the level of that found in animals receiving an adequate intake of vitamin B_6. There seems to be no impairment in the absorption of fats, carbohydrates or the products of protein digestion in vitamin B_6 deficiency according to these workers.

Wentworth et al. (1963) noted that supplementation of 12 mg vitamin B_6 per kg of ration with ten essential amino acids and 11 non-essential amino acids resulted in better growth, but carcass protein was unaffected when compared to a control with ten essential amino acids plus cystine and tyrosine. However, high vitamin B_6 levels resulted in a higher percent of body moisture and a lower percent of body fat in comparison to low vitamin B_6 levels.

Kirchgessner and Frienecke (1963) added different levels of vitamin B_6 (2.2-5.0 mg per kg) to a purified diet and studied the effects on growth, feed efficiency, carcass

nitrogen and fat content of day-old cockerels. Weight gain and efficiency of feed utilization rose with the higher intake of vitamin B_6 but the nitrogen and fat content of the whole body were similar in all groups.

Vitamin B_6 Supplementation of Practical Poultry Rations

There are few reports on vitamin B_6 supplementation of practical poultry rations. Early workers in this field became interested in the problem after the appearance of some data indicating reduced activity of the vitamin in different feeds due to the presence of antimetabolites or low availability of some forms of the vitamin.

Luckey et al. (1945) reported that pyridoxal was more than half as active and pyridoxamine was about one fourth as active as pyridoxine. Waibel et al. (1952) noticed that pyridoxal and pyridoxamine are less active than pyridoxine in supporting chick growth on a complete ration containing large amounts of glucose and sucrose. However, the three forms of the vitamin are equally active when autoclaved starch is the only source of carbohydrate.

Many substances are known to be capable of inducing vitamin B_6 insufficiency state in chickens by virtue of their antagonistic effect on vitamin B_6 metabolism. The mode of action of the antagonist seems to be in inhibition of the active absorption of vitamin B_6 and by blocking the phosphorylation of the vitamin B_6-cofactors (Jacob et al.,

1959).

Kratzer et al. (1947, 1954) noticed that the vitamin B_6 requirement was increased in the presence of linseed oil meal because of the presence of a vitamin B_6 antagonist in the meal. Dillon (1962) confirmed the report of Kratzer and coworkers and stated that the inclusion of greater than 4% linseed oil meal in the diet resulted in a significant reduction in growth and feed consumption. Levels of linseed oil meal in excess of 12% depressed growth by 37.2%. When 2 mg of vitamin B_6 were added per kg of a diet containing 25% linseed oil meal and already adequate in vitamin B_6, growth increased significantly. However, the chick's response in growth and feed intake was best when 8 mg of vitamin B_6 were added per kg of this diet.

Fuller and Dunahoo (1959) worked on the effect of various drug additives on the vitamin B_6 requirement of chicks. Nitrofurazone, furazolidone, and arsanilic acid separately and in all combinations, depressed growth in vitamin B_6-deficient chicks. Added vitamin B_6 overcame the growth depressing effect of furazolidone and arsanilic acid but not that of nitrofurazone.

The same workers investigated the vitamin B_6 supplementation of a complete corn-soybean meal broiler chick diet. When 4.4 mg of vitamin B_6 were added per kg of a ration already containing 5.7 mg of the vitamin per kg, no

additional growth was observed, although the feed efficiency was improved.

Sathe et al. (1964) studied the addition of 5 mg vitamin B_6 per kg of diet and 8.8 mg of folic acid per kg to low quality meat meal diets containing 30-35% protein. There was a significant improvement in both growth and survival but the improvement was mainly due to folic acid and in one case to pyridoxine and folic acid together.

Dillon (1962) investigated the growth of mixed White Leghorn chicks fed a methionine limiting ration containing wheat meal, meat meal and 12% soybean meal and observed the growth response and feed efficiency in a rapid assay and in a six-week experiment. A factorial type of experiment was designed with 5 mg vitamin B_6 per kg and 0.05% of DL-methionine. The addition of 5 mg pyridoxine per kg increased weight gain by 8.5% in the rapid assay and 7.1% in the six-week growth assay. The six-week growth comparison showed a response to methionine supplementation in the absence of vitamin B_6 but not in its presence.

III. MATERIALS AND METHODS

Experimental Animals and Management

Day-old male broiler type chicks (Cornish male x Plymouth Rock female) used in these studies were obtained from a commercial hatchery. All the experiments were carried out in a six-deck battery brooder equipped with wire floors and thermostatically-controlled electric heating units. The heat of the battery brooder was adjusted, so that the chick's thermal requirement was met.

In all the experiments, feed and water were provided ad libitum. Waterers were scrubbed and washed daily to keep microbial synthesis of vitamin B_6 at a minimum. Rations for experiments I, II, III, and IV were prepared from purified ingredients while in experiment V, natural ingredients were used.

Compounds and Ingredients

Vitamin B_6 was used as pure, crystalline pyridoxine hydrochloride in all the experiments. Methionine was added in the DL-form and glycine in the ammonia-free synthetic form.

Dextrose was obtained from the Corn Products Company

of New York, USA. For experiments I, II, and III, Promine-R, an isolated soybean protein containing 97% protein on a moisture free basis, was obtained from Central Soya, Chicago, Illinois. For experiment IV, Assay Protein C-1, an isolated soybean protein with 90% protein on moisture free basis was obtained from Skidmore Enterprises, 275 Mitchell Ave, Cincinnati, Ohio, USA. Alphacel, a non-nutritive fiber was obtained from Nutrition Biochemical Corporations, Cleveland, Ohio, USA and was used to provide bulkiness in the diet.

The vitamin mixture was a vitamin B_6-deficient mixture prepared at the AREC as presented in Table 1. Brigg's mineral mixture was added to all purified rations and its composition is shown in Table 1. The choline chloride used was a 70% aqueous solution obtained from Imperial Chemical Industries, Billingham, Durham, England.

Mazola, a pure corn oil product, was used as the fat supplement to the purified rations. In experiment V, however, when natural ingredients were used, beef tallow was added.

Records and Data

Chicks were randomized, wing-banded and weighed on a gram scale at the start of each experiment. They were weighed weekly and feed consumed recorded at the same time.

Records of mortality were kept daily.

All the data were analyzed statistically according to Snedecor (1957) and subjected to Duncan's multiple range test (Duncan, 1955) if found significant.

Collection of Samples

For SGO-T analysis, each bird was bled by heart puncture, using a 5 cc sterilized glass syringe and 20 gauge sterilized needle. The method used in obtaining blood was a modification of the method described by Hofstad (1950). Approximately 5 ml of blood were drawn from each bird, transferred directly to sterilized labeled centrifuge tubes, already wet with physiological saline solution, and covered with parafilms. Samples were allowed to clot at room temperature and then were centrifuged at 2000 rpm for 15 minutes. Each serum sample was placed in a sterile tube and SGO-T values were measured.

For plasma amino acid analysis, approximately 50 ml of blood were collected from each treatment as described previously in a heparinized centrifuge tube, so that 10 ml of plasma could be recovered.

Intact livers were collected from the representative birds from each pen and kept frozen at -20°C for vitamin B_6 determinations.

For carcass nitrogen and fat, the representative birds of each pen were killed by dislocating the spinal

cord. The birds were opened completely and dried in an oven at 100°C for 48 hours. Later, the dried birds including feathers were ground and a representative sample was taken for nitrogen and fat analysis.

Analytical Methods

SGO-T Determination

One ml of standardized asparatate glutarate reagent (Sigma substrate No. 505-1) with PH 7.5 was placed in labeled test tubes. The test tubes were kept in a water bath at 37°C. Exactly 0.2 ml of every serum sample was added to the respective tube and the contents were mixed by gentle shaking. Right after 60 minutes, 1 ml of standardized 2,4-dinitrophenyl hydrazine and HCl (Sigma color reagent No. 505-2) was added to each tube to stop enzymatic activity and start color reaction. Twenty minutes after adding the color reagent, 10 ml of 0.4 N sodium hydroxide (Sigma reagent No. 505-8) was added for color development, followed by gentle shaking. At least 5 minutes after adding sodium hydroxide, the optical density (O.D.) was read at 505 mu in a Beckman model B spectrophotometer using distilled water as a blank. Sigma-Frankel units of SGO-T of the serum was determined from a standard curve where O.D. was plotted against units of SGO-T per ml.

The standard curve was prepared as follows: Increasing levels of calibration standard solution (Sigma

No. 505-10) were added to six test tubes, 0.0, 0.1, 0.2, 0.3, 0.4, and 0.5 ml. One ml of Sigma color reagent No. 505-2 was added to each test tube and mixed by gentle shaking. The tubes were allowed to stand for 20 minutes at room temperature. Subsequently, 10 ml of 0.4 N sodium hydroxide were added to the tubes and mixed by inversion using a parafilm. Five minutes after adding the sodium hydroxide, the O.D. of all samples was read at a wave length of 505 mu in a Beckman model B spectrophotometer, using distilled water as a blank. The standard curve was plotted using the optical densities obtained versus the corresponding Sigma-Frankel units of SGO-T at 37°C as given in Sigma Bulletin No. 505 (Anonymous, 1960).

Vitamin B_6 Determination

Vitamin B_6 was determined by the method of Campling and Nixon (1954) as modified by Harley (1960). The lypholyzed organism, Saccharomyces Carlsbergensis 4228 (= ATCC 9080) was obtained from the American Type Culture Collection (ATCC). All the media were obtained from Difco Company and rehydrated as described by the Difco manual (Anonymous, 1966).

a. Preparation of sample for assay. Two grams of homogenized liver sample were suspended in about 90 ml of 0.05 N HCl. The suspension was heated in the autoclave at 120°C for 5 hours, cooled and adjusted to PH 5.0.

Subsequently, the volumes were raised to 100 ml with distilled water and filtered. The extracts were kept frozen for the appropriate time.

b. Assay procedure. To revive the organism, 0.4 ml of Bacto pyridoxine-y medium was taken with a serological pipette and mixed well with the lypholyzed organism. Then, the mixture was added to a test tube of Bacto pyridoxine-y medium and kept incubated at 28°C for 24 hours. Subsequently, the organism was cultured on slants of the lactobacilli Agar A.O.A.C. which were kept at 28°C for 24 hours. Immediately after, the organisms were stored at 10°C in the dark and subcultured every week.

The organisms were suspended in 10 ml of Bacto pyridoxine-y medium containing 30 mu pyridoxine hydrochloride and incubated at 28°C for 24 hours. The cells were centrifuged in an International Centrifuge (I.E.C.) at about 8000 rpm under aseptic conditions. The supernatant was decanted and the organisms were resuspended in 10 ml of sterile Bacto pyridoxine-y medium.

Liver extracts were added to 5 ml Bacto pyridoxine-y medium in screw capped tubes and volumes were raised to 10 ml with distilled water. Sterilization was done in the autoclave at 100°C for 10 minutes. After cooling, the tubes were inoculated with a drop of suspended organism and incubated at room temperature for 22 hours in an automatic shaker with 100 strokes per minute. After incubation, the

tubes were steamed in the autoclave for 5 minutes and the turbidity was measured at 600 mu in the spectrophotometer (Spectronic-20).

c. Preparation of the standard curve. Fifty mg of dried pyridoxine hydrochloride powder were dissolved in about 100 ml of 25% ethyl alcohol and subsequently diluted to 500 ml with additional 25% ethyl alcohol. This solution contained 100 mg pyridoxine hydrochloride per ml. Two ml of this solution were added to 998 ml of 25% ethyl alcohol to make a stock solution of 200 ug pyridoxine hydrochloride per ml. The stock solution was stored in a dark bottle in the refrigerator.

One ml of the stock solution was added to 9 ml of distilled water to make 20 ug of pyridoxine hydrochloride per ml.

For the standard curve, increasing volume of the above solution, namely, 0.01, 0.05, 0.10, 0.20, 0.30, 0.40, 0.50, and 0.60 ml were added to screw capped tubes containing 5 ml of Bacto pyridoxine-y medium and distilled water. The tubes were autoclaved at 100°C for 10 minutes. After cooling, the tubes were inoculated with the organism and incubated at room temperature for 22 hours in an automatic shaker with 100 strokes per minute. After the incubation, the tubes were steamed in the autoclave for 5 minutes and the turbidity was measured at 600 mu in the spectrophotometer (Spectronic-20).

Amino Acid Analysis

a. Preparation of samples. The blood plasma was deproteinized by the method of Stein et al. (1954). Ten ml of plasma were added to a glass-stoppered flask containing 50 ml of one percent picric acid solution. After a few seconds of shaking, it was centrifuged to obtain a clear centrifugate. Exactly 40 ml of the centrifugate were passed through a Dowex 2-10 (200 to 400 mesh), a strongly basic anion exchange resin in chloride form which is known to permit the amino acids to pass through quantitatively. The walls of the chromatograph tube and the resin bed were washed with five 3-ml volumes of 0.02 N HCl. The clear, colorless effluent and washings were concentrated in a Craig rotary evaporator to a volume of about 1 ml. To remove the suspended matter, about 4 ml of distilled water and few mg of celite were added to the suspension which was then filtered by gravity through a paper previously washed with 1 N HCl and distilled water. The filtrate and washings were concentrated to a volume of about 1 ml. The concentrate was washed into a small vial, the final volume being about 5 ml. At this point, the sample was kept frozen.

Later, the sample was thawed out and the PH was adjusted to 7.2-7.5 with Hydrion paper by the drop-wise addition of 1 N NaOH. The solution was allowed to stand at room temperature for 4 hours for complete conversion of cysteine to cystine. Later, the sample was adjusted to

PH 2 by the addition of 1 N HCl. The volume was raised to 10 ml by PH 2.2 buffer and kept frozen for putting on the column.

b. Chromatography. Aliquots of 0.5 ml of the deproteinized frozen sample were analyzed on an Automatic Amino Acid Analyzer, Phoenix Model K-8000 based on the method devised by Spackman et al. (1958, pp 1190-1206). Two types of water jacketed columns packed with resin were utilized for the separation of the amino acids. One short column was used to separate the basic amino acids (lysine, histidine, arginine, and ammonia), the long column was used for the acidic and neutral amino acids.

c. Integration and interpretation of chromatograms. The amino acids present in the sample were chromatographed as a series of peaks, which were proportional to the quantities of the amino acids analyzed. A simplified method was used for calculating the peak areas (Phoneix Instruction Manual). This involved counting the number of dots, recorded at fixed time intervals around the upper half of the curve and multiplying by the net height of the peak. The volumes so obtained were compared with those for similar peaks for a standard solution containing one micromole quantities of each amino acid chromatographed under the same conditions. The ratio of the peak's area calculated for the unknown quantity of an amino acid to that of the standard was the amount in Umole present in the

aliquot analyzed. Using a dilution factor and the molecular weight of each specific amino acid, the amount in mg per 100 ml of plasma was calculated.

IV. RESULTS

Experiment I

Objective

Methionine has been reported frequently as the first limiting amino acid in chick starter rations. The present study was therefore initiated to investigate whether high dietary vitamin B_6 could partially overcome methionine insufficiency because of earlier reports of possible interactions between vitamin B_6 and methionine.

Procedure

A purified ration was formulated limiting in both vitamin B_6 and methionine. The experiment consisted of three levels of added vitamin B_6 (0, 3 and 30 mg/kg) and two levels of added methionine (0 and 0.5%) in a factorial arrangement. Since glycine was also deficient, synthetic glycine was added to all treatments. The composition of the pre-experimental and experimental rations are shown in Table 1. The pre-experimental differed from the experimental ration in having both DL-methionine and vitamin B_6 added at the rate of 0.5% and 3 mg per kg respectively. The different treatments consisted of the experimental rations with or without 0.5% DL-methionine and 3 or 30 mg

Table 1. Composition of the pre-experimental and experimental rations - Experiment I.

Ingredients	Pre-experimental (%)	Experimental-basal (%)
Dextrose	64.75	64.75
Isolated soybean protein	22.00	22.00
Corn oil	3.00	3.00
Alphacel	3.00	3.00
Vitamin mixture[1]	1.00	1.00
Mineral mixture[2]	5.30	5.30
Choline chloride	0.25	0.25
Glycine	0.20	0.20
DL-methionine[3]	0.50	-
Vitamin B_6 (mg per kg)	3.00	-

1 Vitamin mixture contributed the following to the kg of ration: 10,000 I.U. of vitamin A; 1936 I.C.U. of vitamin D_3; 22 I.U. of vitamin E; 132 mg inositol; 3.3 mg folic acid; 66 mg P.A.B.A.; 88 mg niacin; 22 mg calcium pantothenate; 8.8 mg riboflavin; 4.4 mg thiamin; 4.4 mg menadione; 220 mg ascorbic acid; 22 ug B_{12}; 220 ug biotin.

2 The mineral mixture supplied the following per kg of diet: Calcium carbonate 8.8 g; calcium phosphate 25.07 g; copper sulphate 2.13 g; feric citrate 176.5 mg; magnesium sulphate 2.65 mg; manganese sulphate 221 mg; potassium chloride 6.15 g; potassium iodide 9.0 mg; sodium chloride 3.5 g; sodium phosphatedibasic 6.15 g; zinc carbonate 115 mg.

3 Methionine replaced alphacel on equal weight basis.

vitamin B_6 per kg of diet.

A total of 108 birds were used in six treatments. Each treatment consisted of three replicates with six chicks per replicate making a total of 18 birds per treatment. The birds were fed the pre-experimental ration for one week; at the end of which, they were randomized and distributed so that all treatments had the same initial average body weight. The birds were kept on the experimental diets for three weeks and then were starved for 12 hours after which they were weighed.

Results

Data on body weight gain, feed consumption and feed efficiency are shown in Table 2. In general, growth was best when both vitamin B_6 and methionine were added and poorest when neither was added (basal). The data indicate that high vitamin B_6 (30 mg per kg) in the presence or absence of added methionine did not result in extra growth but gave poorer growth when compared to the treatments given 3 mg vitamin B_6 per kg of ration. The difference, however, was not statistically significant. The body weight gains of treatments receiving 3 mg and 30 mg of vitamin B_6 were not significantly different from those of the methionine supplemented treatments although there was a difference of 33 and 66 grams between means, respectively.

Feed consumption was significantly different among

Table 2. Body weight gain, feed consumption, and feed efficiency - Experiment I.

Dietary treatments	Body weight gain (g) (8-28 days)	Feed consumption (g/bird)	Feed consumption (g/bird/day)	Feed efficiency[2]
Basal	119±24[2]	365	18.2	3.12
Basal + 3 mg B_6/kg + 0.5% methionine	450±11	763	38.2	1.70
Basal + 30 mg B_6/kg + 0.5% methionine	405±14	679	34.0	1.68
Basal + 3 mg B_6/kg	250±17	605	30.2	2.53
Basal + 30 mg B_6/kg	222±21	526	26.3	2.53
Basal + 0.5% methionine	184±26	396	19.8	2.40

[1] Feed efficiency = $\frac{\text{Feed consumed}}{\text{Body weight gain}}$.

[2] Mean ± S.E.

the treatments with the exception of the basal and methionine-supplemented groups which were not significantly different from each other. This is shown in Table 4.

Feed efficiency was also best when both methionine and vitamin B_6 were added and poorest when neither was supplemented. The data also indicate that feed efficiency of the basal diet can be equally improved by supplementation of either vitamin B_6 (3 or 30 mg per kg) or methionine. The addition of vitamin B_6 or methionine singly gave significantly poorer feed efficiency than those supplemented with both of these nutrients.

Table 3. Analysis of variance of body weight gain, feed consumption, and feed efficiency - Experiment I.

Source of variation	d.f.	M.S.		
		Body weight gain (g)	Feed consumption (g/bird)	Feed efficiency
Replication	5	638	1964	0.121
Treatments	2	50243*	51820*	0.917*
Error	10	2276	15362	0.058

* Significant at 5% level of probability.

Table 4. Separation of means by Duncan's multiple range test - Experiment I.

	Body weight gain (g)					
Treatments[1]	B	B + M	B + 30 P	B + 3 P	B + 30 P + M	B + 3 P + M
Means[2]	119	184	222	250	405	450
	Feed consumption (g/bird)					
Treatments	B	B + M	B + 30 P	B + 3 P	B + 30 P + M	B + 3 P + M
Means	365	396	526	605	679	763
	Feed efficiency					
Treatments	B + 30 P + M	B + 3 P + M	B + M	B + 3 P	B + 30 P	B
Means	1.68	1.70	2.40	2.53	2.53	3.12

[1] For abbreviations see Appendix.

[2] Means not underlined by the same continuous line are significantly different ($P<0.05$).

Experiment II

Objective

The relative adverse effects of high vitamin B_6 supplementation observed in the previous experiment, necessitated the present study to investigate the effects of different levels of vitamin B_6 on growth and feed efficiency of day-old chicks. Moreover, it was felt that a fixed vitamin B_6 level giving maximum growth and feed efficiency should be established for use in the following experiments.

Procedure

One hundred and fifty chicks were placed on the pre-experimental ration for one week. At the end of the week, they were categorized according to body weight and were randomly distributed to the six treatments. Each treatment consisted of two replicates with six birds per replicate making a total of 12 birds per treatment. The birds were placed on the experimental ration for three weeks. At the end of the experiment, chicks on the deficient and 6 mg vitamin B_6 per kg rations were starved for 12 hours before collection of blood for amino acid analysis. The composition of the pre-experimental and experimental ration is shown in Table 5.

Table 5. Composition of pre-experimental and experimental rations - Experiment II.

Ingredients	%
Dextrose	64.75
Isolated soybean protein	22.00
Corn oil	3.00
Alphacel	3.20
Vitamin mixture[1]	1.00
Mineral mixture[2]	5.30
Choline chloride	0.25
Glycine	0.10
DL-methionine	0.40

[1] Same composition as shown in Table 1, pp 32 except that pyridoxine hydrochloride was added at 0, 6, 11, 16, 21, and 26 mg per kg levels in the experimental and at 3 mg per kg level in the pre-experimental.

[2] Same composition as shown in Table 1, pp 32.

Results

Data on body weight gain, feed consumption, and feed efficiency as presented in Table 6 indicated that there were no significant differences in growth and feed efficiency among the vitamin B_6-supplemented groups. However, there were significant differences ($P < 0.01$) between the non-supplemented group and each one of those supplemented. This showed that increasing the vitamin B_6 level up to 26 mg per kg had no detrimental effect on growth, feed consumption or feed efficiency. Although there was no significant difference in growth between the B_6-supplemented groups, the 6 mg vitamin B_6 per kg level gave the highest body gain and increasing the level of vitamin B_6 above 6 mg per kg caused a gradual but non-significant decrease in weight gain.

Amino acid levels were in general lower in the deficient group when compared to the adequate treatment (6 mg per kg). There was a severe drop in plasma levels of phenylalanine, methionine, valine, and cystine, and hydroxy proline, and a relatively moderate drop in lysine, tryptophan, alanine, and proline in the deficient birds. However, deficient chicks had about 50% increase in the plasma levels of three essential amino acids, namely, glycine, histidine, and threonine. Other amino acids like aspartic acid, glutamic acid, and tyrosine remained nearly unchanged. There was also a severe drop in both taurine and gamma amino butyric acid and a rise in cysteic acid,

Table 6. Body weight gain, feed consumption, and feed efficiency - Experiment II.

Dietary treatments	Body weight gain (g) (8-28 days)	Feed consumption (g/bird)	Feed consumption (g/bird/day)	Feed efficiency
Basal	95±22[1]	266	13.3	4.18
Basal + 6 mg B_6/kg	411±10	755	37.8	1.85
Basal + 11 mg B_6/kg	406±14	759	38.0	1.90
Basal + 16 mg B_6/kg	401±12	733	36.7	1.86
Basal + 21 mg B_6/kg	398±12	734	36.7	1.84
Basal + 26 mg B_6/kg	394± 3	727	36.4	1.82

[1] Mean + S.E.

ornithine and ammonia.

Table 7. Analysis of variance of body weight gain, feed consumption, and feed efficiency - Experiment II.

Source of variation	d.f.	M.S.		
		Body weight gain (g)	Feed consumption (g/bird)	Feed efficiency
Replication	1	21	1519	0.0070
Treatments	5	34674**	379167**	1.7318**
Error	5	487	4487	0.0017

** Significant at 1% level of probability.

Table 8. Separation of means by Duncan's multiple range test - Experiment II.

			Body weight gain (g)			
Treatments[1]	B	B + 6 P	B + 11 P	B + 16 P	B + 21 P	B + 26 P
Means[2]	95	394	398	401	406	411
			Feed consumption (g/bird)			
Treatments	B	B + 26 P	B + 16 P	B + 21 P	B + 6 P	B + 11 P
Means	266	726	733	734	755	759
			Feed efficiency			
Treatments	B + 26 P	B + 21 P	B + 6 P	B + 16 P	B + 11 P	B
Means	1.82	1.84	1.85	1.88	1.90	4.18

1 For abbreviations see Appendix.

2 Means not underlined by the same continuous line are significantly different ($P<0.05$).

Table 9. Plasma amino acid levels of vitamin B_6 adequate and deficient chickens (u mole/ 100 ml of plasma).

Amino acid	Control (6 mg B_6/kg)	Deficient	Percent change
A. Essential			
Histidine	2.87	5.86	+51.0
Isoleucine	7.80	9.94	-11.0
Leucine	11.80	10.64	-9.8
Lysine	61.51	33.07	-46.2
Methionine	2.38	0.67	-71.8
Phenylalanine	4.38	0.39	-91.0
Tyrosine	5.98	5.56	-7.0
Threonine	30.84	54.92	+43.8
Tryptophan	8.54	4.85	-43.2
Valine and cystine	77.44	11.39	-85.3
Glycine	34.88	78.49	+55.6
B. Non-essential			
Glutamic acid	16.48	15.38	-6.7
Proline	16.35	11.58	-29.2
Hydroxy proline	11.05	3.59	-67.5
Aspartic acid	3.12	3.13	+0.3
Alanine	40.92	28.22	-31.0
Taurine	17.98	1.13	-93.7
γ -Amino butyric acid	5.67	0.67	-88.2
Cysteic acid	1.95	5.52	+64.7
Ornithin	2.80	14.37	+80.5
Ammonia	17.32	47.66	+63.6

Experiment III

Objective

This experiment was set up to evaluate the effect of vitamin B_6 supplementation of a ration that is limiting not only in methionine but also in glycine which is considered the second limiting amino acid in soybean protein for the chick. Diets with 6 mg of vitamin B_6 per kg were compared to a basal with 3 mg per kg but supplemented with both methionine and glycine. Methionine and glycine were separately added to the 6 mg vitamin B_6 per kg diet to determine whether vitamin B_6 can partially spare either one of these amino acids. Criteria measured were body weight gain, feed efficiency, carcass composition, SGO-T, and liver vitamin B_6 content.

Procedure

Chicks were fed a pre-experimental ration (Table 5, pp 38) for one week prior to the start of the experiment. Seventy two birds with uniform weight were selected from a total of 150 chicks and randomly placed on the experimental diets. Each treatment had three replicates with six birds per replicate, making a total of 18 birds per treatment.

The experimental ration was identical to that used in experiment II except that glycine and DL-methionine were excluded and alphacel level raised to 3.70%. At the end of the third week, the birds were starved for 24 hours

and blood and liver samples were collected for SGO-T and vitamin B_6 determination, respectively. Moreover, one bird from each pen was selected for carcass analysis.

Results

The data on body weight gain, feed consumption and feed efficiency are presented in Table 10. When methionine was added to the 6 mg per kg basal diet, there was a significant improvement in growth although this was significantly lower than the growth of groups supplemented with methionine plus glycine and receiving 3 mg B_6 per kg basal. However, glycine supplementation in the absence of methionine did not result in a significantly better growth than the 6 mg B_6 per kg basal treatment. Total feed consumption was highest in the methionine and glycine supplemented group and lowest in the glycine supplemented group. The total feed intake of the glycine supplemented treatment was not significantly different from the 6 mg B_6 per kg basal, while groups receiving methionine alone or with glycine in the 3 mg B_6 per kg diet ate significantly more than those receiving the 6 mg B_6 per kg glycine-supplemented diet. Significant differences were also observed in feed efficiency among all the treatments. The birds on the 3 mg vitamin B_6 per kg plus methionine and glycine had the best feed efficiency followed by the methionine supplemented group, the glycine supplemented and then the 6 mg B_6 per kg basal.

Results on carcass analyses, liver vitamin B_6 and

Table 10. Body weight gain, feed consumption, and feed efficiency - Experiment III.

Dietary treatments	Body weight gain (g) (8-28 days)	Feed consumption (g/bird)	Feed consumption (g/bird/day)	Feed efficiency
Basal + 6 mg B_6/kg	199±13[1]	532	26.6	2.67
Basal + 6 mg B_6/kg + 0.4% methionine	279±12	579	29.0	2.02
Basal + 6 mg B_6/kg + 0.1% glycine	183±11	508	25.4	2.43
Basal + 3 mg B_6/kg + 0.4% methionine + 0.1% glycine	330± 4	631	31.6	1.94

[1] Mean + S.E.

SGO-T activity are all shown in Table 13. Carcass protein was significantly higher and carcass fat was significantly lower in the methionine supplemented groups versus those unsupplemented with this amino acid. Liver vitamin B_6 values ranged from 4.4-5.0 ug per gram of fresh tissue with no significant difference among any of the treatments studied. SGO-T activities, however, showed similar trends as carcass analysis whereby the methionine supplemented groups had significantly lower SGO-T values than the unsupplemented groups.

Table 11. Analysis of variance of body weight gain, feed consumption, and feed efficiency - Experiment III.

Source of variation	d.f.	M.S.		
		Body weight gain (g)	Feed consumption (g/bird)	Feed efficiency
Replication	2	636	40	0.0041
Treatments	3	1340**	8901*	0.3915**
Error	6	303	1182	0.0106

* Significant at 5% level of probability.
** Significant at 1% level of probability.

Table 12. Separation of means by Duncan's multiple range test - Experiment III.

Body weight gain (g)				
Treatments[1]	B + 6 P + 0.1% G	B + 6 P	B + 6 P + 0.4% M	B + 3 P + 0.4% M + 0.1% G
Means[2]	183	199	279	330
Feed consumption (g/bird)				
Treatments	B + 6 P + 0.1% G	B + 6 P	B + 6 P + 0.4% M	B + 3 P + 0.4% M + 0.1% G
Means	508	532	579	631

Feed efficiency				
Treatments	B + 3 P + 0.4% M + 0.1% G	B + 6 P + 0.4% M	B + 6 P + 0.1% G	B + 6 P
Means	1.94	2.02	2.43	2.67

[1] For abbreviations see Appendix.

[2] Means not underlined by the same continuous line are significantly different ($P < 0.05$).

Table 13. Carcass composition, liver vitamin B_6, and SGO-T activity - Experiment III.

Dietary treatments	Carcass composition[1]		Vitamin B_6 (ug/g of fresh liver)	SGO-T (Frankel units)
	Protein (%)	Fat (%)		
Basal + 6 mg B_6/kg	49.5±1.6[2]	43.1±1.7	4.4	198±17.2
Basal + 6 mg B_6/kg + 0.4% methionine	56.7±1.0	35.8±1.0	4.7	145±18.2
Basal + 6 mg B_6/kg + 0.1% glycine	49.6±1.6	42.5±1.2	4.5	212±15.3
Basal + 3 mg B_6/kg + 0.4% methionine + 0.1% glycine	55.0±0.6	37.0±1.7	5.0	136±20.0

[1] On dry weight basis.

[2] Mean ± S.E.

Table 14. Analysis of variance of protein, fat, and SGO-T - Experiment III.

Source of variation	d.f.	M.S.		
		Carcass protein	Carcass fat	SGO-T
Replication	2	6.81	12.55	-
	5	-	-	1234
Treatments	3	81.54**	82.81**	8735*
Error	6	4.39	5.84	-
	15	-	-	2111

* Significant at 5% level of probability.
** Significant at 1% level of probability.

Table 15. Separation of means by Duncan's multiple range test - Experiment III.

Carcass protein

Treatments[1]	B + 6 P	B + 6 P + 0.1% G	B + 3 P + 0.4% M + 0.1% G	B + 6 P + 0.4% M
Means[2]	49.54	49.61	55.05	56.67

Carcass fat

Treatments	B + 6 P + 0.4% M	B + 3 P + 0.4% M + 0.1% G	B + 6 P + 0.1% G	B + 6 P
Means	35.82	36.97	42.47	43.06

SGO-T

Treatments	B + 3 P + 0.4% M + 0.1% G	B + 6 P + 0.4% M	B + 6 P	B + 6 P + 0.1% G
Means	135.8	145.0	198.3	212.5

1 For abbreviations see Appendix.

2 Means not underlined by the same continuous line are significantly different ($P<0.05$).

Experiment IV

Objective

The results of the previous experiments indicate that vitamin B_6 could not replace methionine completely in a methionine deficient ration. Therefore, the question that remained to be answered was whether vitamin B_6 could correct a marginal methionine deficiency in a purified chick starter diet.

Procedure

The basal diet used was identical to that of the first experiment (Table 1, pp 32) except that glycine was added at 0.1% rather than 0.2% of the total ration. It was deficient in both methionine and vitamin B_6. A factorial experiment was designed with two levels of vitamin B_6 (1 and 6 mg per kg) and three methionine levels (0, 0.2, and 0.4%).

A total of 108 chicks were used in six different treatments. Each treatment consisted of three replicates with six chicks per replicate making a total of 18 birds per treatment. The average body weights of birds in each group were relatively equal at the start of the experiment.

Chicks were placed on the experimental diets at one day of age rather than at one week as in the previous experiments in order to get maximum response in the shortest time possible. At the end of the experimental period, one

bird was selected from each pen for carcass analysis.

Results

The data on body weight gain, feed consumption, and feed efficiency are presented in Table 16. Chicks fed 0.4% methionine and 6 mg vitamin B_6 per kg attained the highest body weight gain whereas chicks on the vitamin B_6-methionine deficient ration had the lowest body weight gain.

Statistical analysis revealed that there was no significant difference among birds fed a high level of vitamin B_6 and adequate methionine (6 mg B_6 per kg and 0.4% methionine), a low vitamin B_6 and adequate methionine (1 mg B_6 per kg and 0.4% methionine), and adequate vitamin B_6 and low methionine (6 mg B_6 per kg and 0.2% methionine). Moreover, body weight gain of chicks receiving 0.2% methionine with 1 mg B_6 per kg or 6 mg B_6 per kg were not significantly different. The data show that when methionine is partially deficient in a ration (0.2%), high vitamin B_6 (6 mg per kg) can spare the need for a higher level of this amino acid. The low body weight caused by a severe deficiency of methionine in a marginal B_6 ration can not be overcome by excess vitamin B_6 supplementation as shown by comparing the first two treatments in Table 16.

The total feed consumption was significantly lower in the treatment receiving 1 mg B_6 per kg than all the others. When the vitamin B_6 level was raised to 6 mg per kg, feed

Table 16. Body weight gain, feed consumption, and feed efficiency - Experiment IV.

Dietary treatments	Body weight gain (g) (1-28 days)	Feed consumption (g/bird)	Feed consumption (g/bird/day)	Feed efficiency
Basal + 1 mg B_6/kg	146±14[1]	386	13.8	2.66
Basal + 6 mg B_6/kg	201±16	481	17.2	2.41
Basal + 1 mg B_6/kg + 0.2% methionine	266±13	572	20.4	2.10
Basal + 6 mg B_6/kg + 0.2% methionine	310±13	612	21.9	1.98
Basal + 1 mg B_6/kg + 0.4% methionine	317±15	603	21.5	1.93
Basal + 6 mg B_6/kg + 0.4% methionine	325±15	558	19.9	1.79

[1] Mean ± S.E.

consumption was not significantly different from that of a similar diet with 0.4% added methionine. No significant differences were observed in feed consumption among any of the methionine supplemented groups.

Feed efficiency, like body weight gain indicates that a high level of vitamin B_6 could replace methionine in a partially methionine deficient ration. Non-supplemented methionine groups resulted in a significantly poorer feed efficiency than all other supplemented treatments. Feed efficiency of chicks receiving 0.4% methionine and 6 mg B_6 per kg was significantly better than that of birds receiving 0.2% methionine at both levels of B_6 supplementation.

The data on carcass analysis are reported in Table 19. No significant differences were observed in carcass moisture and protein among any of the treatments. However, percent body fat showed significant differences among treatments. Statistical analysis indicates that only the birds receiving 1 mg per kg of vitamin B_6 had significantly ($P < 0.01$) lower fat content than the rest of the treatments. The high vitamin B_6 treatment, high vitamin B_6 and partially methionine supplemented treatment (0.2%) and low vitamin B_6 and methionine adequate (0.4%) were not significantly ($P < 0.05$) different from each other as shown in Table 20. The results indicate that a low level of B_6 (1 mg per kg) and marginal level of

methionine (0.2%) had similar effect on carcass fat as when both were high. Increasing one nutrient when the other is low tends to increase carcass fat.

Table 17. Analysis of variance of body weight gain, feed consumption, and feed efficiency - Experiment IV.

Source of variation	d.f.	M.S.		
		Body weight gain (g)	Feed consumption (g/bird)	Feed efficiency
Replication	2	691	727	0.0125
Treatments	5	1599*	22635*	0.3185*
Error	10	572	1689	0.0073

* Significant at 5% level of probability.

Table 18. Separation of means by Duncan's multiple range test - Experiment IV.

			Body weight gain (g)			
Treatments[1]	B + 1 P	B + 6 P	B + 1 P + 0.2% M	B + 6 P + 0.2% M	B + 1 P + 0.4% M	B + 6 P + 0.4% M
Means[2]	146	201	265	310	319	325

			Feed consumption (g/bird)			
Treatments	B + 1 P	B + 6 P	B + 6 P + 0.4% M	B + 1 P + 0.2% M	B + 1 P + 0.4% M	B + 6 P + 0.2% M
Means	386	481	558	572	603	612

			Feed efficiency			
Treatments	B + 6 P + 0.4% M	B + 1 P + 0.4% M	B + 6 P + 0.2% M	B + 1 P + 0.2% M	B + 6 P	B + 1 P
Means	1.79	1.93	1.98	2.10	2.41	2.66

1 For abbreviations see Appendix.

2 Means not underlined by the same continuous line are significantly different ($P<0.05$).

Table 19. Carcass composition - Experiment IV.

Dietary treatments	Moisture (%)	Protein[1] (%)	Fat[1]
Basal + 1 mg B_6/kg	75±3.5[2]	54.8±1.07	31.0±0.20
Basal + 6 mg B_6/kg	69±3.2	52.4±0.21	37.1±0.17
Basal + 1 mg B_6/kg + 0.2% methionine	67±5.1	51.9±0.16	32.8±0.02
Basal + 6 mg B_6/kg + 0.2% methionine	67±0.2	49.2±0.12	39.2±0.19
Basal + 1 mg B_6/kg + 0.4% methionine	58±4.4	55.2±1.24	34.3±0.03
Basal + 6 mg B_6/kg + 0.4% methionine	71±0.1	56.2±1.22	33.1±0.13

[1] On dry weight basis.

[2] Mean ± S.E.

Table 20. Analysis of variance of carcass composition - Experiment IV.

Source of variation	d.f.	M.S.		
		Carcass moisture	Carcass protein	Carcass fat
Replication	2	33.56	4.00	1.36
Treatments	5	100.36	41.18	554.29**
Error	10	32.56	59776.19	15.65

** Significant at 1% level of probability.

Table 21. Separation of means by Duncan's multiple range test - Experiment IV.

	Carcass fat					
Treatments[1]	B + 1 P	B + 1 P + 0.2% M	B + 6 P + 0.4% M	B + 1 P + 0.4% M	B + 6 P	B + 6 P + 0.2% M
Means[2]	30.97	32.83	33.12	34.34	37.11	39.21

1 For abbreviations see Appendix.

2 Means not underlined by the same continuous line are significantly different ($P < 0.05$).

Experiment V

Objective

This study was conducted to determine the effect of vitamin B_6 supplementation of a practical broiler ration limiting in methionine. It was stimulated in part by the results of experiment IV indicating increased growth and better feed efficiency upon vitamin B_6 supplementation of a purified basal ration partially limiting in both methionine and vitamin B_6.

Procedure

A practical broiler ration which is characterized by high protein (23.3%) and high energy (3130 Kcal per kg) was formulated (Table 22). The ration was made up of ingredients that are usually used in commercial present day broiler rations and as far as known was adequate in all nutrients except for a marginally adequate level of methionine. The calculated level of vitamin B_6 was 3.72 mg per kg which is above the minimum requirement of 3 mg per kg as established by NRC (1966).

The experiment consisted of four treatment groups, a basal, basal plus 0.1% methionine, basal plus 6 mg vitamin B_6 per kg, and basal plus 0.1% methionine and 6 mg vitamin B_6 per kg. A total of 106 birds were used with eight chicks per replicate and three replicates per treatment. The birds were categorized according to weight and distributed

Table 22. Composition of basal practical ration used in experiment V.

Ingredients	%
Yellow corn	55.15
Fish meal (65% protein)	5.00
Soybean meal (50% protein)	25.00
Peanut meal (50% protein)	5.00
Alfalfa meal (17% protein)	2.00
Bone meal	2.50
Salt	0.50
Limestone	0.50
Vitamin and trace mineral mixture*	0.35
Beef tallow	4.00

* Nopcosol M-5 furnished the following per kg of diet according to manufacturer's specifications: Vitamin A, 7700 U.S.P.; vitamin D_3, 2310 I.C.U.; vitamin E, 1.54 I.U.; riboflavin, 6.16 mg; niacin, 30.80 mg; choline chloride, 308 mg; d-pantothenic acid, 8.47 mg; vitamin B_{12}, 9.24 ug; menadione sodium bisulfate complex, 1.54 mg; manganese, 84 mg; zinc, 38.5 mg; iodine, 1.8 mg; iron, 28 mg; copper, 28 mg; cobalt, 2.8 mg.

Methionine and pyridoxine were added to the above ration replacing yellow corn on equal weight basis.

among the treatments at one day of age. Chicks were fed the experimental diets for four weeks at the end of which they were starved for 12 hours. One bird representing the mean weight of the group was selected from each pen for carcass analysis.

Results

The data on body weight gain, feed consumption, and feed efficiency are presented in Table 23. The results indicated that there were no significant differences in either growth, feed consumption or feed efficiency among the different treatments.

Data on carcass moisture, nitrogen, and fat are shown in Table 24. The results on carcass moisture were not found to be significantly different. There was some variation in carcass protein. The highest protein deposition occurred in the non-supplemented group. However, when the data were statistically analyzed, only the 0.1% methionine supplemented chicks had significantly lower carcass protein ($P < 0.01$) than all other treatments.

Data on carcass fat were significantly different from one treatment to another as shown in Table 26. This suggests that although the experimental treatments were all isocaloric, the percent fat laid down was different due to different treatments. The non-supplemented group had the lowest percent of carcass fat and the 0.1% methionine supplemented

group had the highest percent of carcass fat. Those receiving vitamin B_6 were intermediate.

Table 23. Body weight gain, feed consumption and feed efficiency - Experiment V.

Dietary treatments	Body weight gain 1-28 days	Feed consumption (g/bird)	Feed consumption (g/bird/day)	Feed efficiency
Basal	487±11[1]	795	28.4	1.63
Basal + 0.1% methionine	484±12	833	29.8	1.72
Basal + 6 mg B_6/kg	488±10	800	28.6	1.62
Basal + 0.1% methionine + 6 mg B_6/kg	487±29	832	29.7	1.72

[1] Mean ± S.E.

Table 24. Carcass composition (%) - Experiment V.

Dietary treatments	Moisture	Protein[1]	Fat[1]
Basal	69.2±2.20[2]	60.3±0.28	26.3±0.14
Basal + 0.1% methionine	67.0±1.51	52.7±0.26	42.7±0.11
Basal + 6 mg B_6/kg	68.8±0.23	58.1±0.03	36.3±0.88
Basal + 0.1% methionine + 6 mg B_6/kg	68.5±0.02	58.0±0.43	30.4±0.20

1 On dry weight basis

2 Mean ± S.E.

Table 25. Analysis of variance of body weight gain, feed consumption, feed efficiency, and carcass composition - Experiment V.

Source of variation	d.f.	M.S.					
		Body weight gain	Feed consumption	Feed efficiency	Carcass composition (%)		
					Moisture	Protein	Fat
Replication	2	274.02	11.80	0.013	1.44	-	-
	5	-	-	-	-	12.67	1.99
Treatments	3	12.66	3.70	0.008	2.95	63.50**	305.44**
Error	6	294.19	5.79	0.016	2.85	-	-
	15	-	-	-	-	2.50	2.29

** Significant at 1% level of probability.

Table 26. Separation of means by Duncan's multiple range test - Experiment V.

	Carcass protein			
Treatments[1]	B + 0.1% M	B + 6 P + 0.1% M	B + 6 P	B
Means[2]	52.66	57.95	58.13	60.13
	Carcass fat			
Treatments	B	B + 6 P + 0.1% M	B + 6 P	B + 0.1% M
Means	26.32	30.36	36.29	42.68

1 For abbreviations see Appendix.

2 Means not underlined by the same continuous line are significantly different ($P < 0.05$).

V. GENERAL DISCUSSION

Early in the history of vitamin B_6 studies, many research workers (Gyorgy, 1934; Carter and O'Brien, 1939) involved in determining the requirement for this vitamin, noticed that the suboptimal levels of vitamin B_6 caused growth retardation, appetite depression, and inefficiency in feed utilization. The present studies confirm those early findings and further illustrate the importance of both methionine and vitamin B_6 in growth, feed efficiency and feed consumption. In all experiments, when the above criteria were considered, groups adequate in both vitamin B_6 and methionine were always superior to those totally non-supplemented or not meeting the NRC requirements.

The results of the first experiment indicated that supplementation of either methionine or vitamin B_6 equally and significantly improved growth and feed efficiency. This is in agreement with the work of Sauberlich (1961) indicating that when a diet is limiting in both vitamin B_6 and an essential amino acid, growth of rats can be improved by addition of either vitamin B_6 or the deficient amino acid. When methionine was added to the basal diet deficient in both vitamin B_6 and methionine, growth and feed

consumption were not significantly different from the basal, but feed efficiency was significantly improved. This contradicts the work of Anderson et al. (1949) who stated that growth was depressed when methionine was supplemented to diets already adequate in this amino acid and low or adequate in vitamin B_6. The high vitamin B_6 level (30 mg per kg) which is ten times the NRC requirement in the presence or absence of methionine caused slightly but not significantly poorer growth compared to the 3 mg per kg level. Christensen (1962) reported that excess of vitamin B_6 might favor absorption of one amino acid at the cost of the other and as such cause an amino acid imbalance. This is not supported by results of the second experiment in which vitamin B_6 levels up to 26 mg per kg had no detrimental effect on growth, feed consumption and feed efficiency when compared to the 6 mg per kg treatment. Kirchgessner and Frienecke (1963) reported that vitamin B_6 supplementation of a purified ration up to 10.8 mg per kg increased weight gain and feed efficiency in day-old cockerels.

The results of the third experiment indicated that 6 mg vitamin B_6 per kg of ration could not maintain growth in a severe methionine deficiency. The higher rate of growth in the methionine supplemented group was due to better feed efficiency rather than increased feed consumption. Glycine supplementation of a 6 mg vitamin B_6 per kg diet had

no significant effect on growth or feed consumption. On the other hand, the presence of this amino acid improved feed efficiency. When both methionine and vitamin B_6 were present, growth was significantly better than in the other two treatments. This growth improvement was due to better feed utilization rather than increased feed intake.

In the fourth experiment, an attempt was made at correcting a marginally deficient level of methionine by raising the vitamin B_6 level of the diet beyond the requirement. This increased vitamin B_6 level in a partially methionine-deficient ration completely restored growth to that of the high vitamin B_6 and adequate methionine treatment. This growth response was due to increased feed intake rather than improved feed efficiency.

In general, vitamin B_6 or methionine supplementation separately improved feed efficiency to some extent in all the present studies but it is not clear which one plays a more effective role. Considering the findings of the first two experiments, more credit should be given to vitamin B_6 whereas in experiment IV, more credit should be given to methionine.

There were no significant differences among the different treatments used in these studies in carcass moisture content. This indicated that this body constituent was not influenced by changes in dietary vitamin B_6 or methionine. Wentworth *et al.* (1963) on the other hand,

reported that high vitamin B_6 resulted in more carcass moisture in rats.

Results on carcass protein and fat showed some variation from one experiment to the other. The reports in the literature are also contradictory. McHenry and Gavin (1941) indicated that vitamin B_6 increased carcass fat deposition. On the other hand, Halliday (1938) reported that the deficiency of the vitamin resulted in higher carcass fat. Data of the third experiment indicate that better growth was accompanied by higher carcass protein while poorer growth was accompanied by more fat deposition. In the fourth experiment, high vitamin B_6 (6 mg per kg) in the presence or absence of methionine did not cause any change in carcass protein. Wentworth *et al.* (1963) also reported that high vitamin B_6 supplementation to a complete purified diet did not affect carcass protein. Carcass fat in our study, however, was highest when both methionine and vitamin B_6 were adequately supplied. When carcass fat of the birds on the practical ration was measured, there was a wide variation among treatments inspite of using isocaloric rations.

Amino acid data as reported in rats by Swendseid *et al.* (1964) indicated that in vitamin B_6 deficiency there was a lowering in most of the amino acids and the drop was more drastic in the essential amino acids. Richardson *et al.* (1953) determined the amino acids microbiologically

and found higher amino acid levels in vitamin B_6 deficiency. In view of newer and improved techniques in separation and measurement of the amino acids, one might doubt the latter findings. In experiment II, there was a significant drop in plasma levels of methionine, phenylalanine, valine and cystine, tryptophan, hydroxy proline and alanine. Gamma amino butyric acid (γ ABA) was drastically lower in the deficient group as compared to the control (0.67 versus 5.67 u mole per 100 ml respectively). Since γ ABA is mainly found in the central nervous system, many workers have attributed the nervous symptoms (hyperexcitability, convulsive movements, ... etc) of vitamin B_6 deficiency to changes in the level of γ ABA (Eugene et al., 1964, pp 504-556). Taurine was considerably decreased while cysteic acid increased. This is to be expected in vitamin B_6 deficiency since the conversion of cysteic acid to taurine is known to require the coenzyme pyridoxal phosphate. Glycine, histidine, and threonine increased significantly in plasma of vitamin B_6-deficient chicks. Increase of glycine in vitamin B_6 deficiency has been also reported in rats (Swendseid et al., 1964) and in man (Merrow et al., 1966; Harding et al. 1964).

SGO-T activity was reported to decrease in vitamin B_6 deficiency (Goswami and Robblee, 1958). However, in our studies, vitamin B_6 was adequate and the changes were probably due to methionine supplementation. Therefore, it

seems that lack of methionine increased the load on the coenzyme form of this vitamin in amino acid interconversions.

Data on liver vitamin B_6 indicated that the growth rate as influenced by vitamin B_6 and methionine levels of the ration did not reflect tissue vitamin B_6 level. This has previously been reported in rats by Tupule and Williams (1955) and Debey et al. (1952). Dietary protein level, however, has been shown to affect the tissue concentration of the vitamin (Schweigert et al., 1946; Sheppard and McHenry, 1946).

The fact that vitamin B_6 supplementation of a practical chick starter ration marginally adequate in methionine did not increase growth, feed efficiency or feed consumption might be due to an already high vitamin B_6 level in the ration (6 mg vitamin B_6 from natural ingredients as determined and 6 mg crystalline pyridoxine hydrochloride added). In view of the results of the second experiment, this lack of response is expected. The results also agree with the work of Fuller and Dunahoo (1959) but contradict the work of Dillon (1962), who found better growth and feed efficiency upon vitamin B_6 supplementation of a commercial ration. Dillon attributed the response to either to a natural factor like that of linseed oil meal having vitamin B_6 antagonistic action, or to an enhanced absorption of limiting essential amino acids from the digestive tract. Kratzer et. al. (1947, 1954) reported that linseed oil meal

contains a specific growth-inhibiting factor, the effect of which was greatly reduced by pyridoxine supplementation. It was also indicated that there were variations between samples in the level of the antagonist present. Considering the work of Fuller and Dunahoo (1959) and Dillon (1962), one might attribute the lack of growth response to additional vitamin B_6 and to the absence of vitamin B_6 antagonists in the ration used.

VI. SUMMARY AND CONCLUSIONS

Five experiments were carried out on day-old male broiler type (Cornish male x White Plymouth Rock female) chicks to elucidate some aspects of vitamin B_6-methionine interrelationships. The results of these studies indicated that the growth and feed efficiency were always superior when both of these nutrients were adequately present compared to control treatments. Vitamin B_6 could not spare methionine when this amino acid was severely deficient in a purified soybean-dextrose type ration. However, high vitamin B_6 could replace methionine for growth but not for feed utilization in a partially methionine deficient purified ration. Vitamin levels up to 26 mg per kg had no detrimental effect on chicks' response to growth, feed consumption or feed efficiency.

Carcass composition varied in different experiments. In the third study, carcass protein was significantly higher and carcass fat significantly lower in the methionine supplemented treatments. In the fourth study, carcass protein did not change significantly with changes in either vitamin B_6 or methionine level of the diet. However, carcass fat was highest in the high vitamin B_6 and partially methionine supplemented group. In the fifth study, the

non-supplemented group had the lowest carcass fat and highest carcass protein.

Liver vitamin B_6 of treatments receiving 6 mg vitamin B_6 per kg alone or with methionine or glycine were not significantly different nor were they different from a control of 3 mg vitamin B_6 per kg plus both amino acids. SGO-T activities, however, were significantly higher in the methionine supplemented groups as compared to the non-supplemented.

Deficient birds had in general lower plasma amino acid levels than adequate ones (6 mg vitamin B_6 per kg). Methionine, phenylalanine, valine and cystine, and hydroxy proline were drastically lower in the deficient birds. Some amino acids, namely aspartic acid and glutamic acid were unchanged, whereas glycine, histidine and threonine increased considerably in the deficient birds.

When a practical type chick starter ration was supplemented with vitamin B_6 or methionine, no response in growth, feed consumption or feed efficiency was observed.

From the results obtained in these studies it can be concluded that:

1. Vitamin B_6 can correct a partial methionine deficiency from the standpoint of growth and feed consumption in a purified type soybean-dextrose diet. However, it can only partially correct a severe deficiency of this amino acid.

2. Vitamin B_6 supplementation of a purified diet up to 26 mg per kg has no detrimental effect on chick growth, feed consumption or feed efficiency.

3. Carcass composition does not vary consistently with changes in dietary vitamin B_6 or methionine.

4. Plasma amino acid levels of vitamin B_6-deficient birds are in general lower than those adequate in this vitamin.

5. Liver vitamin B_6 does not seem to indicate dietary vitamin B_6 status.

6. Vitamin B_6 supplementation of a corn-soybean type chick starter ration known to be adequate in all nutrients does not improve chick growth or feed utilization.

LITERATURE CITED

Aketo, H., T. Sugawa, S. Yashikawa, and M. Suda. 1960. Intestinal absorption of amino acids. 1. The effect of vitamin B_6 on the absorption of L-amino acids through the intestine. J. Biol. Chem. 47: 289-297.

Anderson, J.O., G.E. Lamb, and G.M. Briggs. 1949. Importance of pyridoxine in amino acid metabolism. Poultry Sci. 28: 755.

Anonymous. May 1960. A simplified method for the colorimetric determination of glutamic-oxaloacetic and glutamic pyruvic transaminases. Sigma Technical Bulletin No. 505.

Anonymous. 1966. Difco Laboratory Manual. Difco Laboratories, Detroit, Michigan, 48201, USA.

Armstrong, K.L., G. Feldott, and A.A. Lardy. 1950. Relationship of vitamin B_6 to metabolism of D-amino acids. Proc. Soc. Exper. Biol. Med. 73: 159-163.

Babcock, M.J. 1959. Serum glutamic oxaloacetic transaminase activity of vitamin B_6 deficient rats. J. Nutrition. 67: 205-212.

Babcock, M.J., M. Brush, and E. Sostman. 1960. Evaluation of vitamin B_6 in nutrition. J. Nutrition. 70: 369-376.

Beaton, J.R., J.L. Bearse, G.H. Benton, and E.W. McHenry. 1953. Studies on vitamin B_6. The effect of vitamin B_6 on protein synthesis and maintenance in rats. J. Biol. Chem. 204: 715-719.

Beaton, B.H., and E.W. McHenry. 1953. Vitamin B_6 levels in rat tissues. J. Nutrition. 7: 357-363.

Berg, C.P. 1959. Protein and Amino Acid Nutrition. Academic Press, New York.

Braunstein, A.E., and R.M. Azarh. 1957. Influence of suppression of transamination on the synthesis of amino acids in liver slices and homogenates. Biohimija. 22: 430-438. Abstracted in Nutrition Abstracts and Reviews. 1957. pp 1163.

Campling, J.D., and D.A. Nixon. 1954. The inositol content of foetal blood and foetal fluids. J. Physiology. 126: 71-80.

Carter, C.W., and J.R. O'Brein. 1939. Vitamin B complex in relation to the nutrition of chick and pigeon. World's Poultry Congress Proc. 7: 126-128.

Carter, C.W., and P.J.R. Phizackerly. 1951. The influence of pyridoxine on fat metabolism in the rat. Biol. J. 49: 227-231.

Cercedo, L.R., and J.R. Foy. 1944. Protein intake and pyridoxine deficiency in the rat. Arch. Biochem. 5: 207-210.

Cercedo, L.R., J.R. Foy, and E.C. Derenzo. 1948. Protein intake and vitamin B_6 deficiency in the rat. The effect of supplementation of a low-protein diet with cystine or methionine. Arch. Biochem. 17: 397-401.

Christensen, H.N. 1962. Intestinal absorption with special reference to amino acids. Federation Proc. 21, Suppl. 1: 37-42.

Christensen, H.N. 1963. Chemical and biological aspects of pyridoxal catalysis. Vitamins and Hormones. Vol. 21. Pergamon Press, New York.

Daghir, N.J., and S.L. Balloun. 1963. Evaluation of the effects of breed on vitamin B_6 requirement of chicks. J. Nutrition. 79: 279-287.

Debey, H.J., E. Snell, and C.A. Baumann. 1952. Studies of the interrelationship between methionine and vitamin B_6. J. Nutrition. 46: 203-213.

Dillon, J.E. 1962. An investigation of the status of vitamin B_6 in chick diets. World's Poultry Congress Proc. 157-168.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics. 11: 1.

Eugene, R., J. Wein, and O.G. Simonsen. 1964. Aminobutyric acid (γ ABA), vitamin B_6, and neural functions. Vitamins and Hormones. Vol. 22. Academic Press, New York.

Fuller, H.J., and W.S. Dunahoo. 1959. The effect of various drug additives on the vitamin B_6 requirement of chickens. Poultry Sci. 38: 1150-1154.

Goswami, M.N.D., and A.R. Robblee. 1958. Aspartic-glutamic transaminase activity in chick liver. J. Poultry Sci. 37: 96-99.

Greenberg, L.D., and J.F. Reinehart. 1949. Blood pyridoxine of vitamin B_6-deficient monkeys. Proc. Soc. Exper. Biol. Med. 70: 20-25.

Gyorgy, P. 1934. Vitamin B_2 and the pellagra-like dermatitis in rats. Nature. 133: 498-499.

Halliday, N. 1938. Fatty livers in vitamin B_6 deficient rats. J. Nutrition. 16: 285-290.

Harding-Charconnet, F. 1959. Role of pyridoxine in metabolism of L- and D-tryptophan. Comparison of the effects of vitamin B_6 deficiency, direct or produced by isonicotinic hydrazide (INH). Arch. Sci. Physiology. 13: 227-270. Abstracted in Nutrition Abstracts and Reviews. Vol. 30. 1960. pp 863.

Harding, R.S., J.E. Canham, and H.E. Sauberlich. 1964. Unpublished results. Reviewed in Vitamins and Hormones. 1958. 16: 562. Academic Press, New York.

Hawkins, W.W., V.C. Leonard, and C.M. Coles. 1959. The excretion of nitrogen by the rat in vitamin B_6 deficiency. J. Nutrition. 69: 383-386.

Hofstad, M.S. 1950. A method of bleeding chickens from the heart. J. Amer. Vet. Med. Assoc. 116: 353-354.

Holden, J.T., R.B. Willman, and E.E. Snell. 1951. Growth promotion by keto and hydroxy acids and its relation to vitamin B_6. J. Biol. Chem. 191: 559-576.

Hurley, N.A. 1960. Note on the modification of the Atkin method for vitamin B_6 assay. J. Association of Official Agricultural Chemists. 43: 43-45.

Jacob, F.A., J.L. Coon, and R.S.L. Hamilton. 1959. Influence of pyridoxine, pyridoxal phosphate, deoxy pyridoxine and 2,4-dinitrophenol on methionine absorption. J. Biol. Chem. 235: 1372-1375.

Kirchgessner, M., and H. Frienecke. 1963. Growth and body composition of chicken given different amounts of vitamin B_6. Arch. Geflugelk. 27: 412-418. Abstracted in Nutrition Abstracts and Reviews. Vol. 22. 1964. pp 411.

Kratzer, F.H., H. Bird, V.S. Asmundson, and S. Lepkovsky. 1947. The comparative pyridoxine requirements of chickens and turkey poults. Poultry Sci. 26: 453-456.

Kratzer, F.H., D.E. Williams, B. Marshall, and P.N. Davis. 1954. Some properties of the chick growth inhibitor in linseed oil meal. J. Nutrition. 52: 555-563.

Lichestein, H.C., I.C. Gunsalus, and W.W. Umberit. 1945. Functions of vitamin B_6 group: Pyridoxal phosphate in transamination. J. Biol. Chem. 161: 311-320.

Luckey, T.D., G.M. Briggs, Jr., C.A. Elvehjem, and E.B. Hart. 1945. Activity of pyridoxine derivatives in chick nutrition. Proc. Soc. Exper. Biol. Med. 58: 340-344.

Lyon, J.B., Jr., H.L. Williams, and A. Arnold. 1962. The pyridoxine deficient state in two strains of inbred mice. J. Nutrition. 66: 261-275.

McDonald, M.W. 1957. Methionine supplements in chicken diets. I. Effect of supplementary methionine and choline on growth and folic acid requirements of White Leghorn chickens. Australian J. Agric. Res. 8: 218-324.

McDonald, M.W. 1958. Methionine supplements in chicken diets. III. The biochemical difference in sulphur amino acid metabolism between White Leghorn and Australorp. Australian J. Agric. Res. 9: 161-169.

McHenry, E.W., and J. Gavin. 1941. The B vitamins and fat metabolism. The synthesis of fat from proteins. J. Biol. Chem. 138: 471-475.

Merrow, S.B., M.J. Babcock, F.H. Radke, and A.I. Rasmussen. 1966. Effect of vitamin B_6 on the amino acids and their metabolism in the rat and man. Vermont Agric. Exper.St. Bulletin 647.

Miller, E.C., and C.A. Baumann. 1945. Relative effects of casein and tryptophane on the health and xanthuranic acid excretion of pyridoxine-deficient mice. J. Biol. Chem. 157: 555-562.

Nadkarni, G.B., and A. Sreenvassan. 1958. A study of variation in total amino acids of rat liver as influenced by dietary deficiencies of pyridoxine, biotin and folic acid. Proc. Indian Acad. Sci. 47: 193-201.

Raica, N., and H.E. Sauberlich. 1964. Blood cell transaminase activity in human vitamin B_6 deficiency. Amer. J. Clin. Nutrition. 15: 67-75.

Richardson, L.R., L.G. Blaylock, and C.M. Lyman. 1953. Influence of the level of vitamin in the diet on the concentration of free amino acids in the plasma of chicks. J. Nutrition. 49: 21-27.

Rasen, F., and R. Milholland. 1960. Effect of pyridoxine depletion on three enzymes requiring pyridoxal phosphate as a cofactor. Fed. Proc. 19: 414-416.

Sathe, B.S., R.B. Cumming, and G.L. Meclymont. 1964. Nutritional evaluation of meat meals for poultry. Australian J. Agric. Res. 15: 698-718.

Sauberlich, H.E. 1961. Effects of vitamin B_6 on the growth of rats fed diets limiting in an essential amino acid and on the utilization of isomers of tryptophan, methionine and valine. J. Nutrition. 74: 289-297.

Schlenk, F., and E.E. Snell. 1945. Vitamin B_6 transamination. J. Biol. Chem. 157: 425-426.

Schweigert, B.S., H.E. Sauberlich, C.A. Elvehjem, and C.A. Baumann. 1946. Dietary protein and the vitamin B_6 content of mouse tissue. J. Biol. Chem. 165: 187-196.

Sheppard, E.C., and E.W. McHenry. 1946. The pyridoxine content of tissues of rats fed various diets. J. Biol. Chem. 165: 649-655.

Snedecor, G.W. 1957. Statistical Methods Applied to Experiments in Agriculture and Biology. The Iowa State College Press. Ames, Iowa, 5th ed.

Snell, E.E., and C.S. Keevil. 1954. The Vitamins III. Sebrell, W.H., and W. Harris (Editors). Academic Press, New York.

Spakman, D.H., W.H. Stein, and S.Moore. 1958. Automatic recording apparatus for use in the chromatography of amino acids. Anal. Chem. 30: 1190-1192.

Stein, W.H., A.G. Bearn, and S. Moore. 1954. The amino acid content of the blood and urine in Wilson's disease. J. Clin. Invest. 33: 410-419.

Swendseid, M.E., J. Vilalobes, and B. Friedrich. 1964. Free amino acids in plasma and tissue of rats fed a vitamin B_6-deficient diet. J. Nutrition. 82: 206-208.

Tupule, P.G., and S.G. Kshirsagor. 1959. Effect of dietary protein, calories and pyridoxine on glutamic-oxaloacetic transaminase (GOT) activity of blood. Indian J. Med. Res. 46: 452-460. Abstracted in Biological Abstracts (12749). Vol. 34, September 1959.

Tupule, P.G., and J.N. Williams, Jr. 1955. Relationship of dietary protein food intake to pyridoxine nutrition in the rat. J. Nutrition. 57: 529-539.

Waibel, P.E., W.W. Cravens, and E.E. Snell. 1952. The effect of diet on the comparative activities of pyridoxal, pyridoxamine and pyridoxine for chicks. J. Nutrition. 48: 531-538.

Wentworth, J., C. Kies, and H. Linkswiler. 1963. Amino acid and vitamin B_6 requirement. Fed. Proc. 22: 322-324.

Williams, N.A. 1962. Effects of non-essential amino acids on the growth of vitamin B_6-deficient rats. J. Nutrition. 76: 35-40.

Yeh, S.D.J., E. Duran, and B.F. Chow. 1960. Growth response and plasma GOT levels in rats with chronic and intermittent B_6 deficiencies. Fed. Proc. 19: 322-328.

A P P E N D I X

Abbreviations

Agricultural Research and Education Center	AREC
American Type Culture Collection	ATCC
and others	et al.
Association of Official Agricultural Chemists	A.O.A.C.
avenue	Ave
basal	B
centigrade	°C
cubic centimeter(s)	cc
degrees of freedom	d.f.
dextrorotatory	D
dextrorotatory and levorotatory	DL
gamma amino butyric acid	γ ABA
glycine	G
glutamic oxaloacetic transaminase	GOT
gram(s)	g
International Centrifuge	IEC
International Chick Unit(s)	I.C.U.
International Unit(s)	I.U.
kilocalorie(s)	Kcal
kilogram(s)	kg
levorotatory	L
mean sum of squares	M.S.
methionine	M
microgram(s)	ug
milligram(s)	mg
milligram pyridoxine hydrochloride per kilogram of ration	P
milliliter(s)	ml
millimicron(s)	mu
normal	N
number	No.
Nutrition Research Council	NRC
optical density	O.D.
page(s)	pp
para amino benzoic acid	PABA
percent	%
revolution per minute	rpm
serum glutamic oxaloacetic transaminase	SGO-T
standard error	S.E.
United States Pharmacopoeia	U.S.P.