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THE UTILIZATION OF NON-PROTEIN NITROGEN
BY THE DOMESTIC FOWL

by
Rolando Chavez

A Dissertation Submitted to the Faculty of the
COMMITTEE ON AGRICULTURAL BIOCHEMISTRY AND NUTRITION
In Partial Fulfillment of the Requirements
For the Degree of
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THE UNIVERSITY OF ARIZONA

1967
I hereby recommend that this dissertation prepared under my direction by Rolando Chavez entitled The Utilization of Non-Protein Nitrogen by the Domestic Fowl be accepted as fulfilling the dissertation requirement of the degree of Doctor of Philosophy.

Dissertation Director

After inspection of the dissertation, the following members of the Final Examination Committee concur in its approval and recommend its acceptance:*

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SIGNED: Rolando Chavez
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ABSTRACT

Ten experiments (five with laying hens and five with chicks) were carried out to determine if chicks and laying hens can utilize non-protein nitrogen (N.P.N.) as a partial protein substitute. In all but two of the experiments the level of protein in the diet was kept to a minimum so as to limit the protein intake. In the other two experiments protein intake was limited by feeding high energy diets. Non-protein nitrogen was supplemented to these diets and its effect was evaluated by comparing the performance of birds fed these diets to that of those fed intact protein.

The nitrogen from diammonium phosphate and diammonium citrate was utilized by laying hens to meet their protein requirements when supplemented to a low protein basal diet in which fish meal supplied a major portion of the intact protein. No protein-sparing effects were observed when N.P.N. was supplemented to low protein diets in which vegetable protein supplied a major portion of protein or to a low protein purified diet in which dried egg yolk supplied the protein. The availability of glycine in the diets with vegetable protein appeared to be low which could have been part of the problem in the failure of hens to utilize N.P.N.
Direct evidence of the utilization of non-protein nitrogen by chicks was obtained from only one of the experiments. Chicks fed a 15% protein diet supplemented with 3% protein equivalent supplied by a combination of diammonium phosphate and glutamic acid grew at a rate equal to that of chicks fed 18% intact protein. Indirect evidence, such as protein efficiency ratio (grams gain/grams protein consumed) when calculated under the assumption that the inorganic nitrogen was not available, as well as the amount of protein retained daily, indicated that the inorganic nitrogen was utilized by the chicks. Non-protein nitrogen did not have any effect on the absorption of the amino acids studied or on the activities of glutamic-oxalacetic and glutamic-pyruvic transaminase in liver tissue.

It would appear that both laying hens and chicks can utilize N.P.N. provided that their intact protein intake is limited and that the diets to which N.P.N. is supplemented contain the needed levels of the essential amino acids.
CHAPTER I
INTRODUCTION AND REVIEW OF LITERATURE

INTRODUCTION

The rapid increase in world population in the last century has caused great alarm, due primarily to the fact that the world's food supply does not appear to be keeping up with the needs of the population. Thus, the prediction of the English economist Robert T. Malthus, at the start of the 19th century, that the population of the world would increase faster than the food supply, appears to be approaching a reality.

At the present time, the most common nutritional deficiency observed throughout the world is that of protein (Shaefer 1962). This indicates that of the three principal nutrients (carbohydrates, protein and lipids), protein appears to be the most limiting at this time.

The name protein is derived from the Greek word "proteias", meaning primary or holding first place. This term was first used by the Dutch physiological chemist G. J. Mulder who recognized the complexity and understood the importance of protein in all forms of life. Nutritionally, the importance of proteins as sources of amino acids has been known for many years. As early as the 19th century the French physiologist Francois Magendie observed that gelatin was poorer in quality than meat. Later, the Americans
Osborn and Mendel showed that the quality of some protein sources could be improved by the supplementation of certain amino acids which apparently were not present in sufficient amounts to meet the needs of their experimental animals. Fortunately, not all of the amino acids found in proteins are required in the diets of most animals. The so-called non-essential amino acids can be synthesized within the organism from simple organic acids and nitrogen originating from other amino acids or perhaps from other nitrogen sources. The ability to synthesize non-essential amino acids varies between animal species, and this is why some amino acids may be essential to some organisms and not to others.

The efficiency of farm animals in converting feed protein into protein edible for humans in the form of meat, milk, eggs, etc., has increased significantly in the last 50 years. This increase in efficiency has resulted from better management, better breeds of animals and primarily through an increase in knowledge of the specific nutrient requirements of farm animals. However, even today the efficiency of farm animals as protein converters is not outstanding. This can be seen from the performance of laying hens, which are among the most efficient, but retain only approximately 35-40% of the protein which they consume and are able to convert to egg protein about 80% of the protein which they retain (Reid, 1966). The availability of egg protein on the
other hand approaches 100%; therefore, of the original pro-
tein ingested by the hen only approximately 25-35% is
indirectly available to humans. Unfortunately, not all
animal proteins are as available as egg protein. This has
caused nutritionists to fear the day in which feed normally
intended for animals will have to be processed for human
consumption. For this reason attempts are being made to
further improve the efficiency with which farm animals
utilize protein or to study the utilization of sources of
protein which are not edible for humans.

Non-protein nitrogen, the nitrogen from sources
other than protein, has been used extensively in ruminant
nutrition. The bacterial population in the rumen of the
animal is able to synthesize protein from simple carbon com-
ounds and inorganic nitrogen. This protein is then avail-
able to the animal in the lower portion of the digestive
tract. Non-ruminants do not have a complicated digestive
tract, and the bacterial population appears to be concen-
trated in the lower portion of the tract. Therefore, if
protein is synthesized by microorganisms present in the
digestive system of monogastric animals, it apparently is
not available to the animal. Nevertheless, numerous studies
have been carried out to determine whether monogastric
animals can utilize dietary inorganic nitrogen in lieu of
protein. To this date, however, most studies have failed to
show promising results.
REVIEW OF LITERATURE

The first studies with non-protein nitrogen (N.P.N.) in animal nutrition date back almost one hundred years. Weiske et al. studied the utilization of asparagines by animals in 1879 and Morgan in 1888 studied the utilization of amides. Zuntz in 1891 summarized the work on non-protein nitrogen which had appeared in the literature up to that date. He concluded from the work reported in the literature that N.P.N. had a protein sparing effect only in ruminants and proposed that this effect was primarily due to the assimilation of the inorganic nitrogen by the rumen bacteria. Since that time and especially in the last 20 years, N.P.N. has been widely used in ruminant nutrition. This has been due primarily to the rise in the synthetic nitrogen industry which has made the synthesis of compounds, such as urea, relatively inexpensive. In the United States alone 100,000 tons of urea are used in the feeding of ruminant animals annually.

Intensive studies with N.P.N. in the nutrition of monogastric animals were initiated in 1912. Abderhalden and Lampa (1912), however, failed to show any protein-sparing effect of ammonium salts in the nutrition of dogs. Later, Grafe (1913) and Grafe and Turbon (1913) reported no beneficial effects of N.P.N. in the nutrition of swine. Grafe
(1915) also reported that N.P.N. supplementation was not able to maintain nitrogen balance when fed to humans.

The first direct evidence that monogastric animals could use N.P.N. was shown by the work of Foster et al. (1938). Foster and co-workers fed rats diets supplemented with benzoic acid and $N^{15}$ ammonium salts and observed that more glycine was excreted as hippuric acid than was fed and furthermore that some of the nitrogen of the excreted glycine was $N^{15}$. Rose and co-workers (1949) demonstrated more directly that N.P.N. could be utilized by the rat. They supplemented diets, containing vitamins, minerals and the essential amino acids for the rat, with either ammonium salts, L-glutamic acid, glycine or urea and observed that these diets improved growth significantly. The nitrogen from L-glutamic acid, diammonium citrate (D.A.C.) and ammonium acetate appeared to be utilized effectively under the conditions in which they were fed. Lardy and Feldott (1949) and Frost and Sandy (1951) were able to confirm the findings of Rose et al. (1949). Rose and Dekker (1956) fed isotopically labeled urea to rats and observed that the label appeared in the carcass protein, and that of the amino acids present in the carcass protein, glutamic acid, aspartic acid, and cystine had the highest concentration of $N^{15}$ while the essential amino acids had a low concentration of the isotope. Birnbaum (1957) and Rechoigl et al. (1957) reported that excess L-amino acids could also be used as sources of
nitrogen for the synthesis of non-essential amino acids by rats. Liu et al. (1955) administered \( \text{N}^{15} \) urea orally to pigs and observed that there was a small but definite amount of the \( \text{N}^{15} \) incorporated into body protein, which provided evidence that at least a small portion of the ingested nitrogen could be utilized by swine.

Among the first workers to study the utilization of N.P.N. by poultry were Acherson et al. (1940), who fed growing chickens a diet in which 13% of the protein nitrogen was supplied by urea. They were unable to find any protein-sparing effect from the urea nitrogen. Bice and Dean (1942) confirmed the work of Acherson et al. (1940) with a similar study. In their study urea supplied one third of the total protein \((6.25 \times N)\) in diets fed to chicks. In addition some of the urea-supplemented diets were also given a soluble carbohydrate. These workers also failed to show any response to urea. Van der Meulen (1943) reported that urea had no protein-substituting effect in the diet of laying hens. Slinger et al. (1952), on the other hand, showed in a study with chicks that urea improved feed conversion, and that in the presence of penicillin, urea appeared to improve growth when fed at a level equivalent to 2% protein. Jones and Combs (1953), however, failed to show any response to urea, ammonium citrate or dibasic ammonium phosphate when fed to chicks in the presence or absence of aureomycin.
Nevertheless, the antibiotic appeared to have an effect in overcoming the toxicity of these compounds.

The utilization of methionine hydroxy analog as a source of methionine by poultry has been known for some time (Gordon and Sizer 1954, 1955 and Reid et al. 1954). Gordon and Sizer (1965) observed that this analog was rapidly converted to methionine by the chick and that it was incorporated into body tissue at a rate equivalent to that of methionine. However the exact origin of the nitrogen which replaces the hydroxy groups in the hydroxy analog is not known. Most practical-type diets for chicks normally have an excess of the non-essential amino acids which are eventually broken down and utilized for energy or converted to fat. This makes the amino groups available for the synthesis of methionine from its analog if it is supplied in the diet or for the synthesis of other amino acids. Sullivan and Bird (1957) showed for the first time that chicks could utilize the nitrogen from urea or diammonium citrate (D.A.C.) when fed a low protein diet in which the methionine and glycine were replaced by their hydroxy analogs. Apparently the basal diet did not have an excess of nitrogen, and therefore the nitrogen from urea or D.A.C. was used to synthesize glycine and methionine from the analogs. According to this work toxicity as a result of an excess of either of the N.P.N. sources could be prevented to a certain extent by raising the level of the supplemented hydroxy analogs.
In the last few years, primarily as a result of better knowledge of the nutrient requirements of chickens and laying hens, further evidence has appeared in the literature indicating that chicks and laying hens can utilize the nitrogen from inorganic sources. Featherston et al. (1962a) reported that chicks could utilize the D-isomers and excess L-amino acids as sources of nitrogen for the synthesis of non-essential amino acids. In additional studies, Featherston et al. (1962b) were able to show that chicks fed a diet containing all the essential amino acids and supplemented with several sources of nitrogen retained more nitrogen and had higher plasma levels of non-essential amino acids than those fed a control diet without the N.P.N. sources. In this study urea and D.A.C. were both effective sources of nitrogen for the chicks. Young et al. (1965) reported that laying hens fed a low protein diet were able to utilize N.P.N. to partially satisfy the total protein requirement. D.A.C. and glutamic acid both proved to be effective in a balanced diet, containing fish meal, for laying hens.

The exact mechanism by which monogastric animals, including poultry, are able to utilize the nitrogen from inorganic sources has not been proposed. Rose and Smith (1950) fed rats diets similar to those used in their earlier experiments (1949) with the addition of an antibiotic and observed that the nitrogen from urea was still utilized.
This, therefore, eliminated intestinal microorganisms as possible intermediates. The fact that the essential amino acids have to be present in order for the N.P.N. sources to be effective provides additional evidence that microorganisms are not involved. In poultry it has been observed that the nitrogen from D.A.C. is absorbed and later appears in the blood as glutamine and eventually is excreted as uric acid (Olsen et al. 1963). From this, therefore, it is logical to suppose that the ammonia molecule can be taken up by keto-acids to form non-essential amino acids.

To study the utilization of nitrogen from inorganic sources by poultry it is necessary to limit the amount of protein by limiting the non-essential amino acids and keeping the essential amino acids at a minimum. In the last few years linear-programming techniques have provided the nutritionist with a tool which he can use to formulate diets low in protein but which meet the essential amino acid requirements. These diets can then be supplemented with different sources of inorganic nitrogen and their effect evaluated by comparing the performance of birds fed these diets to those fed intact protein.

The studies reported herein were undertaken to determine if sources of inorganic nitrogen such as diammonium citrate, diammonium phosphate and urea have any protein-sparing effects when fed to the domestic fowl.
CHAPTER II
THE UTILIZATION OF NON-PROTEIN NITROGEN BY LAYING HENS
EXPERIMENTAL PROCEDURES

Experiment 1. This experiment involved 120 Single Comb White Leghorn pullets hatched May 29, 1964. The pullets were raised on straw litter until they were twelve weeks old at which time they were vaccinated against fowl pox and moved to range until four months of age. At four months of age, the pullets were moved into individual laying cages and randomly distributed into twenty-four groups of five birds each. Six experimental treatments were employed, and each treatment was replicated four times.

Two control diets were used in this study (Table 1). Both diets were formulated by digital computer to minimum essential amino acid restrictions according to the recommendations of the National Research Council (1960). Diet 1 was formulated to meet the essential amino acid requirements at a minimum protein level. The calculated percent protein of this diet was 12.75% and required that supplemental methionine be added at a level of 0.069% to meet the minimum requirement at a productive energy level of 890 Calories per pound. Diet 2 was formulated to meet both the essential amino acid requirements at least cost and a minimum protein
restriction of 15.75%, and at the same energy level as Diet 1.

Supplemented methionine required for Diet 1 was added as methionine hydroxy analog calcium and DL-methionine in order to compare these sources. Non-protein nitrogen utilization was studied with Diet 1 containing 0.060% added DL-methionine. The total nitrogen level of the diet was raised by replacing the cellulose pound for pound with diammonium citrate (D.A.C.) in one case and urea in another case at levels calculated to supply nitrogen equivalent to 3% protein (N x 6.25). In each case, the final calculated protein level was 15.75%, which was equivalent to that of Diet 2 (Table 1).

Collection of data started December 2, 1964, and continued for 10 twenty-eight day periods ending September 7, 1965. Percent production, feed conversion, egg weight and body weight were summarized at the end of each period. Egg weight was determined by taking the average weight of the eggs collected over a three day period from each replicate of each treatment. The feed was analyzed every twenty-eight day period for protein, and fecal samples were collected for 3 days in every period for the determination of nitrogen. The nitrogen determinations were carried out by the Kjeldahl method as outlined by the A.O.A.C. (1960). Chromium oxide was used as a tracer and was determined by the method of Czarnocki et al. (1961). Percent nitrogen retention was
calculated every period by determining the content of chromium oxide in the feed and feces. The amino acid composition of the two control diets was determined one time during the study with a Beckman amino acid analyzer. The feed samples were prepared for the amino acid analyzer by hydrolyzing one gram of sample with 25 mls of 6N HCl at 100°C for twelve hours under 15 lbs. pressure and then removing the acid and dissolving the residue in a 2.2 pH buffer. Production, feed conversion and egg weight were treated statistically by analyses of variance (Snedecor, 1956), and the means separated by Duncan's multiple range test (1955).

Experiment 2. The pullets in this study were from the same flock as those used in Experiment 1. They were housed in twelve range pens in groups of 40 birds each, and all were treated alike for the first 24 weeks of egg production. The birds were all fed a diet which had a calculated protein level of 15% and 1,000 Calories of productive energy per pound.

At the start of the 25th week of lay, the experimental diets replaced the original diet. The experiment was blocked according to production level. There were two blocks of six pens each, and three treatments: each treatment diet was fed to four pens of birds, two of which were in each block.
In this experiment the utilization of the nitrogen from dianmonium phosphate was studied. It was used to replace cellulose pound for pound at a level calculated to supply 2% additional protein equivalent to a diet identical to control Diet 1 of Experiment 1, with 0.069% added methionine hydroxy analog calcium. The performance of birds fed this diet was compared to the performance of those fed the same diet without any additional protein and the same diet with 2% added protein supplied by fish meal. The fish meal replaced cellulose pound for pound at a level calculated to supply 2% protein. The addition of fish meal increased the productive energy from 890 to 917 Calories per pound of diet.

Collection of data was started May 20, 1965, and continued for four twenty-eight day periods which ended September 7, 1965. Data included percent production, feed conversion, and egg weight. All data were treated statistically as in Experiment 1.

**Experiment 3.** Four hundred Single Comb White Leghorn pullets hatched May 17, 1965 were used in this study. The pullets were reared in the same manner as those used in the first two experiments. At four months of age they were housed in floor pens and randomly distributed into ten pens each containing forty pullets. In addition, two White Leghorn males were housed in each pen so as to study fertility and hatchability. Five experimental treatments were
employed and each treatment was replicated twice. Each replicate consisted of a pen of forty females and two males.

Three control diets were used in this study (Table 2). These diets, as those used in the two previous experiments, were formulated by digital computer and differed from those of the two first experiments in the protein level and the ingredient composition. The protein levels used were 12%, 14% and 16% protein. The 12% protein diet was formulated to meet the essential amino acids as in the first two experiments; however, the ingredient composition was changed so as to make a combination of soybean meal and cottonseed meal the principal sources of protein other than the grain. Diammonium phosphate (D.A.P.) was tested in this study as a source of non-protein nitrogen, and it was supplemented to a basal diet identical to the 12% protein control diet at levels calculated to supply 2% and 4% protein (6.25 x N). The effects of the D.A.P. were evaluated by comparing the performance of the hens fed the diets supplemented with D.A.P. to those fed the control diets. All the diets had 950 Calories of productive energy per pound, and the calcium and phosphorus were the same for all the diets at 3.75% and 0.838% respectively.

Collection of data started December 1, 1965, and continued for 10 twenty-eight day periods ending September 6, 1966. The hens were weighed at the start and at the end of the study and in addition to the criteria used in the first
two experiments, percent shell, fertility and hatchability were also measured. Protein retention was determined three times throughout the study in the same manner as in Experiment 1, and the absorption of the amino acids was determined once by measuring the amino acids in the feed and feces with a Beckman amino acid analyzer. Absorption of the amino acids was calculated in the same manner as protein retention assuming that the amino acids were not excreted in the urine. Percent production, consumption and egg weight were treated statistically in the same manner as in the previous experiments.

Experiment 4. One hundred Single Comb White Leghorn hens from the same flock used in Experiment 3 and reared in the same manner were used in this experiment. The hens were placed in individual laying cages and randomly distributed into twenty groups. Five experimental treatments were used; each treatment was replicated four times.

Treatments 1 and 5 were fed diets identical to the 12% and 14% control diets used in Experiment 3 and were also used as controls in the study. Treatment 2 was fed a basal diet identical to the 12% control diet supplemented with D.A.P. at a 2% protein equivalent level. Treatment 3 was fed the same basal diet supplemented with glycine at a level of 0.25% and treatment 4 was fed the basal diet supplemented with both D.A.P. and glycine at the same levels as in treatments 2 and 3.
The study was carried out for four twenty-eight day periods beginning June 9, 1966, and ending September 29, 1966. Criteria used were egg production, egg weight and feed conversion, which were also treated statistically as in the previous experiments. Protein retention was determined twice during the study and glycine absorption was also determined twice by measuring the glycine in the feed and feces and calculating absorption in the same manner as protein retention. Blood samples were taken by cardiac puncture on the last day of the study from two birds from each replicate after fasting the birds for twelve hours and then feeding the experimental diets for two hours. The two blood samples from each replicate were heparinized, centrifuged and the plasma withdrawn and frozen immediately. Pooled plasma samples were later analyzed for free glycine. All the glycine determinations were carried out by the standard microbiological assay methods as outlined in the Difco Manual (1953) using the organism *Leuconostoc mesenteroides*.

**Experiment 5.** Twenty-one Single Comb White Leghorn hens from the same flock as those used in Experiments 3 and 4 were used in this study. The hens were randomly distributed into individual cages in a laying hen battery and fed for fourteen days, prior to the start of the experiment, a 9% protein purified diet calculated to meet the essential amino acid requirements. The experimental diets used are shown in Table 3. Diets 1 and 3 had a protein level of 7%
and 9% respectively and diet 2 had 9% of which 2% was supplied with D.A.P. The energy level was 1,000 Calories of productive energy per pound for the three diets.

Collection of data started June 21, 1966, and lasted 28 days which ended July 19. Criteria used were egg production, feed consumption, body weight, protein retention and the absorption of some of the essential amino acids. All these data were treated statistically as described earlier, and the amino acid determinations were carried out microbiologically using the same organism as in Experiment 4. In addition blood samples were taken by cardiac puncture at the end of the study from two birds from each treatment after a 3, 6 and 12 hour fast. Plasma was obtained as described earlier and analyzed for blood uric acid by the method of Brown (1945) and for total protein with a T.S. Meter (American Optical Company). The plasma samples from each treatment taken at each given time were pooled and deproteinized with trichloroacetic acid. The sample was then diluted with a pH 2.2 buffer and the free amino acids determined with a Beckman amino acid analyzer.
RESULTS AND DISCUSSION

Experiment 1. Determined protein values were slightly higher than the calculated values for the experimental diets. Analytical values were the average of 10 determinations made throughout the study (Table 4). A comparison of the calculated and determined amino acid levels of these diets agreed well with the calculated levels, although the determined values were based on one determination. The only exception was tyrosine, which was lower than the calculated value in each case, possibly due to its decomposition during hydrolysis of the feed samples for the determination.

The addition of 0.069% methionine hydroxy analog calcium or DL-methionine to the 12.75% protein diet, which was calculated to be deficient in methionine by that amount, did not significantly improve production rate (Table 5). The average production of the hens fed the diets supplemented with methionine hydroxy analog calcium or DL-methionine was only slightly higher than that of birds fed control Diet 1.

The average egg production of the birds fed the 15.75% protein diet (control 2) was significantly better than for birds fed the unsupplemented 12.75% protein diet (control 1), thus indicating that the 12.75% protein control
diet was deficient in protein (Table 5). However, when the 12.75\% protein diet was supplemented with 3\% protein equivalent from diammonium citrate in addition to 0.069\% DL-methionine, a significant improvement in production was obtained which was equal to that of the 15.75\% protein diet (control 2). The feeding of 3\% protein equivalent from urea in the 12.75\% protein basal diet did not significantly improve egg production. The average percent production for birds fed this diet was the same as the treatment fed the 12.75\% protein control diet, indicating that unlike the nitrogen from D.A.C., the nitrogen from urea was not utilized by the hen (Table 5). These results on egg production are in agreement with the work of Young \textit{et al}. (1965) who showed that laying hens fed low protein diets could utilize nitrogen from D.A.C. and glutamic acid for egg production.

Feed conversions, based on the kilograms of feed required to produce a dozen eggs, are given in Table 5. The feed conversion for the birds fed the low protein basal diet supplemented with D.A.C. and DL-methionine was significantly better than the other treatments except the one fed the 15.75\% protein diet.

Egg weights were significantly improved by feeding the low protein basal diet supplemented with 0.069\% DL-methionine or methionine hydroxy analog calcium (Table 6). The egg weights for the birds fed the 15.75\% protein diet and the low protein basal diet supplemented with DL-methionine
and D.A.C. were also significantly heavier than those from the treatment fed the unsupplemented basal diet; however, they were the same as those from the treatments fed the low protein basal diet supplemented with either methionine hydroxy analog calcium or DL-methionine. This can possibly be explained by the fact that the rate of lay for the birds in the treatments fed the higher protein levels was significantly higher than for those in the treatments fed the low protein basal diet supplemented with either methionine hydroxy analog calcium or DL-methionine, and generally it is agreed that egg production and egg weight are negatively correlated. The egg weight for the group fed the low protein basal diet supplemented with 0.069% DL-methionine and 3% protein equivalent from urea was depressed when compared to that for the treatment fed the basal diet supplemented only with DL-methionine.

No differences in feed consumption between the treatments were observed since the productive energy level of all diets had been equalized (Table 6). Body weights at the end of the experiment were the same for all treatments. The average nitrogen retention values agreed with the work reported by Reid et al. (1965) in that the nitrogen retained is inversely related to the level of protein in the diet (Table 6). The average protein consumed and retained was directly related to the protein in the diet and feed consumption (Table 6).
The values for the percent protein retained converted to egg protein (Table 6) were obtained by multiplying the average grams of eggs produced per hen per day (percent production \times average egg weight) by 12\%, which is the average amount of protein per egg, and then calculating the percent this factor was of the grams protein retained daily. These values ranged from 83.6\% to 92.5\%. For the treatment fed the diet which contained 3\% protein equivalent from D.A.C., this value was 91.9\%, employing a total dietary protein value of 15.75\%. However, when this was calculated, assuming that the nitrogen from D.A.C. was not utilized, a value of 113.5\% was obtained indicating that the nitrogen from D.A.C. was effectively utilized under the conditions of this study. Calculations in the same manner for the treatment fed the diet which contained 3\% protein equivalent from urea gave a value of 103.2\%, indicating that only a limited amount of nitrogen from urea was utilized.

**Experiment 2.** As in the previous experiment, the primary objective of this study was to determine whether laying hens could utilize non-protein nitrogen as a partial protein substitute. Previous studies (Reid et al. 1965) have indicated a lower protein requirement for laying hens for egg production during the last 5-6 months of the laying year. Egg size, however, was depressed during hot weather on the low-protein diets.
The average hen-day production for the last sixteen weeks of production is given in Table 7. Increasing the protein level from 12.75% to 14.75% with either fish meal or diammonium phosphate improved egg production significantly. Both fish meal and D.A.P. seemed to be equally effective. In both cases production was 4% higher than for the birds fed the 12% protein control diet. Feed conversions were improved slightly when the protein level was increased, but only in the case where fish meal was fed was conversion significantly better than for the group fed the 12.75% protein control diet. Egg weight, as shown in Table 7, was significantly improved by increasing the protein level to 14.75%. Diammonium phosphate was as effective as fish meal, since both supplements produced weights approximately one gram heavier than eggs from birds fed the 12.75% protein diet.

Birds fed the low-protein control diet were not able to lay at a rate equal to that of birds fed the higher nitrogen levels. Thus the protein requirements at this stage of production during hot weather were greater than 12.75%. It is also evident that the nitrogen from D.A.P. was effectively utilized, since both egg production and egg size were improved when D.A.P. was used to supply an equivalent of 2% additional protein to the control diet. Furthermore, the efficiency of the 2% additional protein from D.A.P. was as good as that from fish meal indicating that the control diet met all the essential amino acid requirements.
Experiment 3. In the work reported by Young et al. (1965) they mentioned that some of the non-protein nitrogen sources which they studied were effective as partial protein substitutes for laying hens when supplemented to diets in which fish meal supplied a major portion of the protein. In the 12% protein basal diet used in Experiments 1 and 2 fish meal supplied a major portion of the protein and the nitrogen from both diammonium citrate and diammonium phosphate was effectively utilized. The purpose of this experiment was to evaluate the utilization of nitrogen from diammonium phosphate by hens fed a diet in which a combination of soybean and cottonseed meals supplied a major portion of the protein.

The determined values for both protein and most of the amino acids are given in Table 8. The values for protein are an average of four determinations of samples taken throughout the course of the experiment, and the values for the amino acids are based on one determination. The measured values obtained for protein were slightly higher than the calculated, and with the exception of glycine and the aromatic amino acids most of the determined values for the amino acids were close to the calculated values.

Production, feed conversion, average egg weight and percent shell for the entire 280 days of the study are summarized in Table 9. Production, feed conversion, and average egg weight were higher for the hens fed 14% and 16% protein
control diets compared to the hens fed the 12% control diet. This indicated that the 12% protein control diet was inadequate in protein. An increase in the protein level of the 12% protein diet with D.A.P. appeared to be totally ineffective (Table 9). Diammonium phosphate at the level tested actually depressed production although not significantly. Feed conversion was affected only slightly by the D.A.P. and egg weight was not affected (Table 9). Percent shell was decreased by the higher level of protein possibly as a direct result of the higher rate of production. It was also decreased by the D.A.P. at both levels tested, even though the rate of production for these treatments was the same as that for the hens fed the 12% protein control diet. Diammonium phosphate supplied a major portion of the phosphorus to the diets in which it was supplemented; therefore, it may have altered the metabolism of other mineral elements important in the formation of the shell.

Diammonium phosphate did not appear to be toxic in that feed intake was not depressed (Table 10) when compared to the feed intake of the hens fed the 12% protein control diet. However, the average body weights, at the end of the study, for the hens fed the D.A.P. were approximately 150 grams lower than those of the hens fed the 12% protein control diet indicating that perhaps D.A.P. interfered with the utilization of certain nutrients (Table 10). Fertility and percent hatchability of the fertile egg did not appear to be
affected by any of the treatments, although the lowest average fertility was observed for the hens fed the 12% protein control diet. However, there was also a slight decrease in hatchability with an increase in D.A.P. In general percent fertility and hatchability were high for all of the treatments (Table 10).

Percent protein retained was determined three times during the course of the study, and the average values are given in Table 11. The values obtained for all the treatments were lower than those observed for Experiment 1, perhaps as a result of the higher feed intake for the hens in this experiment. There was little variation in the average protein retained between treatments. The absorption of the amino acids was determined once throughout the study and ranged from approximately 70% to 90% for most of the amino acids. The absorption of glycine, however, ranged from 43% to 58% indicating that the availability of this amino acid from these diets was low.

The results obtained from Experiment 2 clearly indicated that diammonium phosphate could be used in diets for laying hens as a partial protein substitute; however, the data from this experiment indicate the contrary. The only possible explanation is that the ingredient composition of the 12% protein basal diet used in this study did not contain as much fish meal as that used for Experiments 1 and 2. Fish meal has been considered for a long time as a source of
unidentified growth factors (Reid et al. 1958) which could play an important role in the metabolism of nitrogen. On the other hand, the fact that the availability of glycine was low could perhaps have also been a contributing factor in preventing the utilization of the inorganic nitrogen. Although the National Research Council does not list glycine as an essential amino acid for the laying hens, it could possibly be essential. The calculated level of glycine for the 12% protein basal diet was the same in all three experiments; nevertheless, the determined value was lower for the diet used in this study than for that used in the previous two.

Experiment 4. The availability of glycine from the diets used in the previous experiment appeared to be low in comparison to the availability of the other amino acids. This low availability of glycine was mentioned as a possible factor which could have prevented in some way the utilization of inorganic nitrogen as a source of protein. Therefore, the purpose of this experiment was to evaluate the effects of supplemented glycine and glycine + D.A.P. on the performance of hens fed the same 12% protein basal diet employed in the previous experiment.

Production and feed conversion were both improved by supplementing the 12% protein basal diet with 0.25% glycine (Table 12). Both production and feed conversion for the group fed the diet with the added glycine were equivalent to
that obtained for the group fed the 14% protein control diet. Egg weight, however, was not improved by the supplementation of glycine. Raising the protein level of the 12% protein basal diet to 14% with D.A.P. did not produce any beneficial effect, although it did not depress production or feed conversion and did increase egg weight slightly. Egg production and feed conversion were improved to some extent by supplementing the basal diet with both glycine and D.A.P. These results may be due only to the glycine since the supplementation of glycine alone produced a response. Feed consumption increased in direct relation with rate of lay (Table 13), and the supplementation of glycine or D.A.P. did not appear to affect consumption. Average body weight for the birds fed the glycine supplement was somewhat less than the average weight of the hens fed the 12% protein control diet, possibly due to the higher rate of production of the former (Table 13). However, the average body weight for the hens fed the 14% control diet was approximately 200 grams higher than any of the hens in the other treatments which was an indication that the level of protein in their diet was greater than their requirement.

The supplemented glycine improved the percent glycine which was absorbed (Table 13) indicating that the added glycine was utilized. A further indication of this was the level of glycine in the blood plasma after a two-hour fast (Table 14), which was directly related to the level of
glycine in the diet. The amount of glycine absorbed, expressed as grams absorbed per day, also was directly related to the level of glycine in the diet. The amount of protein retained increased in direct proportion to the level in the diet. Nevertheless, glycine supplementation improved the amount of protein retained by three-fourths of a gram per day over that retained by the hens fed an equivalent amount of whole protein (Table 14).

As in the previous experiment, the nitrogen from diammonium phosphate was not utilized by the hen to meet its protein requirements. However, it is evident from this study that glycine, which has normally not been considered to be an essential amino acid for the hen, is indeed essential. Furthermore, for efficient production under similar conditions it would appear that hens must retain at least 0.4 grams of glycine per day. There is also the probability that the supplemented glycine, which was equivalent to about 0.3% protein, may have contributed the amount of protein needed by laying hens at this stage of production.

Experiment 5. Dehydrated egg yolk was used as the source of protein in the diets used in this experiment. Prior to the start of the study the egg yolk was analyzed for protein and for amino acids and the control diets were formulated on the basis of the protein and amino acid content of the egg yolk which was the only source of protein in these diets.
The 9% protein control diet met all of the essential amino acids according to the recommendations of Lewis (1966) and the amino acids of the 7% protein diet were just slightly below the recommended allowances. The purpose of the study was to evaluate the effects of inorganic nitrogen supplementation to the low 7% protein diet on the performance of the hens. Inorganic nitrogen was supplied with D.A.P. at a level calculated to supply 2% protein equivalent.

The protein level in the diets employed here was well below the recommended level; nevertheless, production for the hens fed the 9% protein control was 43.0 grams of egg per day or approximately 80% and for those fed 7% protein it was 37.1 grams per day or approximately 67% (Table 16). The nitrogen from diammonium phosphate, however, did not appear to be of any nutritional value. Production and feed intake were both depressed by feeding the 7% protein basal diet supplemented with D.A.P. Moreover, the hens fed this diet lost an average of 82 grams over the 28 day period whereas the hens in the other two treatments gained weight (Table 15). Total plasma protein for the hens on the 9% protein diet was well within the range of hens fed adequate protein (Sturkie 1965) but was significantly lower for the hens fed 7% protein and 7% protein supplemented with D.A.P. (Table 15). Blood uric acid was higher for the hens fed D.A.P. supplement, even though the averages given in Table 15 are an average of the uric acid present in blood samples
taken at different times. This indicated that some of the inorganic nitrogen had been absorbed although it did not appear to be utilized.

Percent protein retained and the availability of a select number of the amino acids were relatively high for the hens in all the treatments primarily as a result of the high quality of egg yolk protein. Diammonium phosphate, however, depressed both the percent protein retained and the absorption of the amino acids studied (Table 16). The blood levels of the amino acids were determined on blood samples taken at three different times after fasting the hens. The non-essential amino acids, expressed as micromoles per 100 mls of blood plasma, are given in Table 17. The purpose of this was to compare the levels of the non-essential amino acids and to determine whether there was any evidence of synthesis of non-essential amino acids as a result of the inorganic nitrogen in the diet. Most of the non-essential amino acids were lower in the blood samples from hens fed D.A.P. than in hens from the other two treatments. Glutamic and aspartic acid, however, were higher in the blood samples from the hens fed the D.A.P. taken after a 3-hour fast compared to those fed the 7% protein diet alone. The same was observed for glutamic acid from blood samples taken after a 6-hour fast. The lower level of amino acids observed for the samples from the hens fed the D.A.P. could have been as a result of lower feed intake. On the other hand, the
slightly higher level of glutamic acid observed in the blood plasma of hens fed the D.A.P. could have been as a result of the inorganic nitrogen since Olsen et al. (1963) reported that glutamic acid and glutamine are intermediates in the metabolism of inorganic nitrogen by the avian species.

Under the conditions of the experiment it is evident that the nitrogen from diammonium phosphate was not utilized, and unlike the previous two experiments it appeared to be toxic. However, it was shown that laying hens can perform efficiently when fed low protein, provided that the protein is of high quality.
SUMMARY AND CONCLUSIONS

Five experiments were carried out to study the value of inorganic nitrogen sources in meeting the protein requirements of laying hens fed low levels of protein. Diammonium citrate and urea were tested in the first experiment and diammonium phosphate in Experiments 2 through 5. The control diets in all except Experiment 5 were formulated by digital computer to meet the essential amino acid requirements of the laying hen.

In the first experiment the nitrogen from diammonium citrate appeared to be utilized effectively by laying hens. Production and feed conversion of hens fed a 12.75% protein diet supplemented with 3% additional protein supplied by D.A.C. were equivalent to that of hens fed a 15.75% intact protein. Urea failed to improve the performance of hens when supplemented to the same diet at the same protein equivalent level as D.A.C. In the second experiment diammonium phosphate, when fed to hens in the same low protein diet as that used in the preceding experiment at a level of 2% protein equivalent, improved the performance of hens significantly over that of the hens fed the basal diet alone, and the performance was equal to that of hens fed an equivalent amount of protein from fish meal. The low protein basal diet used in these studies was characterized by
containing fish meal which supplied a major portion of the protein.

In Experiments 3 and 4 diammonium phosphate was tested as a source of protein. D.A.P. was supplemented to a low protein basal diet in Experiment 3 at levels calculated to supply 2% and 4% protein. Unlike the low protein basal diet used in Experiments 1 and 2, the amount of fish meal was restricted to 2.39% and a combination of soybean and cottonseed meal supplied a major portion of the protein. The performance of birds fed the D.A.P. supplemented diets was not improved over that of hens fed the basal diet alone. An evaluation of the absorption of the amino acids indicated that the availability of glycine was low, which could have contributed to the failure of hens to utilize the inorganic nitrogen. Experiment 4 was designed to test these possibilities. Glycine supplemented at a level of 0.25% to the low protein basal diet used in Experiment 3 improved the performance of hens but the D.A.P. did not have any effect. Nevertheless, the importance of glycine for laying hens was shown by this experiment.

In Experiment 5, D.A.P. was supplemented at a level of 2% protein equivalent to a 7% protein basal diet in which dried egg yolk was used as the only source of protein. Diammonium phosphate depressed production, body weight, protein retention and the absorption of the amino acids studied. An evaluation of the non-essential amino acids in the blood
failed to indicate if the inorganic nitrogen functioned in their synthesis. This experiment, however, showed that laying hens can perform efficiently with as low a protein level as 9%, providing that the protein is of high quality.

As a result of the five studies carried out the following general conclusions can be made:

1. The nitrogen from diammonium citrate and diammonium phosphate can be effectively utilized by laying hens fed a low protein diet which meets the essential amino acid requirements and in which fish meal supplies a major portion of the protein. Presumably, the nitrogen from the D.A.C. and D.A.P. is available for the synthesis of the non-essential amino acids.

2. Glycine which has generally not been considered to be an important amino acid for laying hens appears to be important. The availability of glycine from vegetable protein could be low and for maximum performance laying hens must retain at least 0.4 grams of glycine per day.

3. Laying hens can perform efficiently on low protein diets providing that the availability of the protein is high and that the essential amino acid requirements are met.
TABLE 1
COMPOSITION OF CONTROL DIETS
USED IN EXPERIMENT 1

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Diet 1</th>
<th>Diet 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground milo</td>
<td>72.34</td>
<td>67.60</td>
</tr>
<tr>
<td>Fish meal (65% protein)</td>
<td>5.21</td>
<td>7.74</td>
</tr>
<tr>
<td>Cottonseed meal (solvent)</td>
<td>-</td>
<td>2.50</td>
</tr>
<tr>
<td>Solka floe (cellulose)</td>
<td>3.15</td>
<td>3.10</td>
</tr>
<tr>
<td>Meat and bone scraps (50% protein)</td>
<td>-</td>
<td>.84</td>
</tr>
<tr>
<td>Dehydrated alfalfa meal (17% protein)</td>
<td>3.84</td>
<td>3.84</td>
</tr>
<tr>
<td>Wheat bran</td>
<td>4.42</td>
<td>4.03</td>
</tr>
<tr>
<td>Vitamin premix1</td>
<td>2.00</td>
<td>2.00</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>8.01</td>
<td>7.80</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>0.48</td>
<td>-</td>
</tr>
<tr>
<td>Sodium chloride</td>
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<td>0.25</td>
</tr>
<tr>
<td>Trace mineral mix2</td>
<td>0.10</td>
<td>0.10</td>
</tr>
<tr>
<td>Chromium oxide</td>
<td>0.20</td>
<td>0.20</td>
</tr>
</tbody>
</table>

1 Supplied the following per kg of diet: 9,900 I.U. vitamin A, 1,540 I.C.U. vitamin D₃, 4.4 mg riboflavin, 27.5 mg niacin, 11 mg calcium pentothenate, 880 mg choline chloride, 132 mcg vitamin B₁₂, 5.5 I.U. vitamin E, 2.2 mg vitamin K, 125 mg ethoxyquin (as a preservative) and 18.5 gms soybean meal as carrier.

2 Supplied the following per kg of diet: 20 mg iron, 60 mg zinc, 1 mg molybdenum, 60 mg manganese, 168 mg calcium, 4 mg copper, 1.5 mg iodine, and 1.5 mg cobalt.
**TABLE 2**

**COMPOSITION OF CONTROL DIETS USED IN EXPERIMENT 3**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Control I %</th>
<th>Control II %</th>
<th>Control III %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground milo</td>
<td>62.464</td>
<td>55.464</td>
<td>48.464</td>
</tr>
<tr>
<td>Soybean meal, solvent (44% protein)</td>
<td>4.403</td>
<td>10.253</td>
<td>16.103</td>
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<tr>
<td>Cottonseed meal, solvent (41% protein)</td>
<td>2.500</td>
<td>2.500</td>
<td>2.500</td>
</tr>
<tr>
<td>Fish meal, sardine (65% protein)</td>
<td>2.390</td>
<td>2.390</td>
<td>2.390</td>
</tr>
<tr>
<td>Dehydrated alfalfa meal</td>
<td>6.000</td>
<td>6.000</td>
<td>6.000</td>
</tr>
<tr>
<td>Animal fat(^1)</td>
<td>5.560</td>
<td>7.150</td>
<td>8.300</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.141</td>
<td>0.080</td>
<td>0.027</td>
</tr>
<tr>
<td>Vitamin premix(^2)</td>
<td>2.000</td>
<td>2.000</td>
<td>2.000</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>5.951</td>
<td>6.008</td>
<td>6.008</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>3.827</td>
<td>3.746</td>
<td>3.746</td>
</tr>
<tr>
<td>Salt</td>
<td>0.250</td>
<td>0.250</td>
<td>0.250</td>
</tr>
<tr>
<td>Trace minerals(^3)</td>
<td>0.100</td>
<td>0.100</td>
<td>0.100</td>
</tr>
<tr>
<td>Bentonite</td>
<td>4.414</td>
<td>4.059</td>
<td>0.027</td>
</tr>
</tbody>
</table>

\(^1\) Proctor and Gamble H.E.F.

\(^2\) Supplied the following per kgm of diet: 9,900 I.U. vitamin A, 1,540 I.C.U. vitamin D\(_3\), 4.4 mg riboflavine, 27.5 mg niacin, 11 mg calcium pantothenate, 880 mg choline chloride, 132 mcg vitamin B\(_{12}\), 5.5 I.U. vitamin E, 2.2 mg vitamin K, 125 mg ethoxyquin (as a preservative) and 18.5 gms soybean meal as carrier.

\(^3\) Supplied the following per kg of diet: 20 mg iron, 60 mg zinc, 1 mg molybdenum, 60 mg manganese, 168 mg calcium, 4 mg copper, 1.5 mg iodine and 1.0 mg cobalt.
TABLE 3

COMPOSITION OF EXPERIMENTAL DIETS
USED IN EXPERIMENT 5

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percent</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose monohydrate</td>
<td>55.00</td>
<td>55.00</td>
<td>55.00</td>
<td></td>
</tr>
<tr>
<td>Solka floc (cellulose)</td>
<td>6.00</td>
<td>6.00</td>
<td>6.00</td>
<td></td>
</tr>
<tr>
<td>Egg yolk (45.60% protein)*</td>
<td>15.36</td>
<td>15.36</td>
<td>19.75</td>
<td></td>
</tr>
<tr>
<td>Purified vitamin mix</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
<td></td>
</tr>
<tr>
<td>Purified mineral mix</td>
<td>2.00</td>
<td>2.00</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>7.00</td>
<td>8.29</td>
<td>7.00</td>
<td></td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.16</td>
<td>0.16</td>
<td>0.16</td>
<td></td>
</tr>
<tr>
<td>Animal fat</td>
<td>2.52</td>
<td>2.52</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>3.33</td>
<td>1.44</td>
<td>3.33</td>
<td></td>
</tr>
<tr>
<td>Diammonium phosphate</td>
<td>-</td>
<td>1.51</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Bentonite</td>
<td>4.43</td>
<td>3.52</td>
<td>2.56</td>
<td></td>
</tr>
<tr>
<td>Chromium oxide</td>
<td>0.20</td>
<td>0.20</td>
<td>0.20</td>
<td></td>
</tr>
</tbody>
</table>

1 Determined protein content.

2 Supplied the following per kgm of diet: 10,000 I.U. vitamin A, 960 I.C.U. vitamin D₃, 8.8 I.U. vitamin E, 50 mg ethoxyquin (as a preservative), 8.8 mg thiamin HCl, riboflavin 12 mg, calcium pantothenate 15.2 mg, P-amino benzoic acid 20.0 mg, inositol 1,000 mg, niacin 88 mg, choline chloride (25%) 2206 mg, vitamin B₁₂ 30 mcg, biotin 0.2 mg, folic acid 2.0 mg, menadione 6.6 mg.

3 Supplied the following per kgm of diet: sodium 1969 mg, choline 5641 mg, manganese 104 mg, iron 289 mg, copper 41 mg, zinc 77 mg, potassium 3114 mg, cobalt 1.2 mg, magnesium 562 mg, molybdenum 3.2 mg, iodine 3.1 mg, sulfate (SO₄) 2907 mg.

4 Proctor and Gamble H.E.F.
**TABLE 4**

AMINO ACID AND PROTEIN COMPOSITION OF THE CONTROL DIETS USED IN EXPERIMENT 1

<table>
<thead>
<tr>
<th></th>
<th>Diet 1 Calculated %</th>
<th>Diet 1 Determined %</th>
<th>Diet 2 Calculated %</th>
<th>Diet 2 Determined %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>12.75</td>
<td>13.23</td>
<td>15.75</td>
<td>15.76</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.61</td>
<td>0.75</td>
<td>0.80</td>
<td>0.82</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.59</td>
<td>0.78</td>
<td>0.79</td>
<td>0.84</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.18</td>
<td>0.17</td>
<td>0.23</td>
<td>0.32</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.23</td>
<td>0.24</td>
<td>0.30</td>
<td>0.26</td>
</tr>
<tr>
<td>Methionine + cystine</td>
<td>0.41</td>
<td>0.41</td>
<td>0.53</td>
<td>0.58</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.63</td>
<td>0.64</td>
<td>0.83</td>
<td>0.71</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.61</td>
<td>0.72</td>
<td>0.72</td>
<td>0.75</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.47</td>
<td>0.35</td>
<td>0.56</td>
<td>0.32</td>
</tr>
<tr>
<td>Phenylalanine + Tyrosine</td>
<td>1.09</td>
<td>1.07</td>
<td>1.28</td>
<td>1.07</td>
</tr>
<tr>
<td>Valine</td>
<td>0.69</td>
<td>0.69</td>
<td>0.83</td>
<td>0.80</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.36</td>
<td>1.55</td>
<td>1.51</td>
<td>1.53</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.61</td>
<td>0.62</td>
<td>0.72</td>
<td>0.66</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.49</td>
<td>0.58</td>
<td>0.59</td>
<td>0.62</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.27</td>
<td>0.43</td>
<td>0.34</td>
<td>0.41</td>
</tr>
</tbody>
</table>

1 Average of ten determinations.
### TABLE 5

EFFECT OF NON-PROTEIN NITROGEN SOURCES ON EGG PRODUCTION AND FEED CONVERSION (EXPERIMENT 1)

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>% Protein</th>
<th>Protein equiv. NPN</th>
<th>Source NPN</th>
<th>% Methionine + cystine</th>
<th>% Production</th>
<th>Feed conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.75</td>
<td>0</td>
<td>-</td>
<td></td>
<td>0.41</td>
<td>64.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.89&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>12.75</td>
<td>0</td>
<td>-</td>
<td></td>
<td>0.48</td>
<td>65.2</td>
<td>1.92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(MHA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.75</td>
<td>0</td>
<td>-</td>
<td></td>
<td>0.48</td>
<td>65.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.91&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(DL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.75</td>
<td>3.0</td>
<td>DAC&lt;sup&gt;3&lt;/sup&gt;</td>
<td></td>
<td>0.48</td>
<td>68.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.78&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>15.75</td>
<td>3.0</td>
<td>Urea&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td>0.48</td>
<td>65.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.93&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>15.75</td>
<td>0</td>
<td>-</td>
<td></td>
<td>0.53</td>
<td>68.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.87&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Averages having different superscripts are statistically different at the 0.05 level of probability (Duncan, 1955).

<sup>2</sup> Kilograms of feed per dozen eggs.

<sup>3</sup> DAC = diammonium citrate (equivalent to 77.44% protein).

<sup>4</sup> Urea (equivalent to 262% protein).
<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>% Protein</th>
<th>% Protein equiv. NPN</th>
<th>Source NPN</th>
<th>% Meth. + cystine</th>
<th>Average egg weight (gms)</th>
<th>Avg. feed cons. gms/hen/day</th>
<th>Average gms prot/hen/day</th>
<th>% Nitrogen retained</th>
<th>Avg. prot/hen/day retained</th>
<th>Retained prot converted to egg protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.75 - -</td>
<td>0.41</td>
<td>54.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100</td>
<td>12.75</td>
<td>37.60</td>
<td>4.79</td>
<td>88.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.75 - -</td>
<td>0.48 (MHA)</td>
<td>55.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>103</td>
<td>13.13</td>
<td>36.24</td>
<td>4.75</td>
<td>91.4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.75 - -</td>
<td>0.48</td>
<td>55.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>102</td>
<td>13.01</td>
<td>36.21</td>
<td>4.71</td>
<td>92.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.75 3.0 DAC&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.48</td>
<td>55.55&lt;sup&gt;b&lt;/sup&gt;</td>
<td>101</td>
<td>15.91</td>
<td>31.37</td>
<td>4.99</td>
<td>91.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.75 3.0 Urea&lt;sup&gt;4&lt;/sup&gt;</td>
<td>0.48</td>
<td>54.52&lt;sup&gt;a&lt;/sup&gt;</td>
<td>103</td>
<td>16.22</td>
<td>31.38</td>
<td>5.09</td>
<td>83.5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15.75 - -</td>
<td>0.53</td>
<td>55.46&lt;sup&gt;b&lt;/sup&gt;</td>
<td>104</td>
<td>16.38</td>
<td>31.49</td>
<td>5.15</td>
<td>88.7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Averages having different superscripts are statistically different at the 0.05 level of probability (Duncan, 1955).

2 Averages for ten determinations.

3 DAC = diammonium citrate (equivalent to 77.44% protein)

4 Urea (equivalent to 262% protein).
TABLE 7

EFFECT OF DIAMMONIUM PHOSPHATE AS A SOURCE OF NON-PROTEIN NITROGEN FOR LAYING HENS FOR THE LAST 16 WEEKS OF PRODUCTION (EXPERIMENT 2)

<table>
<thead>
<tr>
<th>Dietary treatment</th>
<th>% Protein</th>
<th>Protein equiv. DAP</th>
<th>% Production (^2)</th>
<th>Feed Conversion (^3)</th>
<th>Average egg size (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.75</td>
<td>0</td>
<td>56.1(^a)</td>
<td>1.86(^b)</td>
<td>55.88(^a)</td>
<td></td>
</tr>
<tr>
<td>14.75</td>
<td>2.0</td>
<td>61.0(^b)</td>
<td>1.78(^{ab})</td>
<td>56.97(^b)</td>
<td></td>
</tr>
<tr>
<td>14.75(^4)</td>
<td>0</td>
<td>60.5(^b)</td>
<td>1.72(^a)</td>
<td>56.83(^b)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) DAP = diaminmonium phosphate (equivalent to 132.56% protein).

\(^2\) Averages having different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).

\(^3\) Kilogram of feed per dozen eggs.

\(^4\) Two percent of the protein supplied by fish meal.
<table>
<thead>
<tr>
<th></th>
<th>Control 1 Calculated</th>
<th>Determined</th>
<th>Control 2 Calculated</th>
<th>Determined</th>
<th>Control 3 Calculated</th>
<th>Determined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>12.00</td>
<td>12.60(^1)</td>
<td>14.00</td>
<td>14.61</td>
<td>16.00</td>
<td>16.09</td>
</tr>
<tr>
<td>Arginine</td>
<td>0.65</td>
<td>0.64</td>
<td>0.82</td>
<td>0.68</td>
<td>1.00</td>
<td>0.81</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.56</td>
<td>0.53</td>
<td>0.70</td>
<td>0.60</td>
<td>0.85</td>
<td>0.71</td>
</tr>
<tr>
<td>Methionine +</td>
<td>0.53</td>
<td>-</td>
<td>0.53</td>
<td>-</td>
<td>0.53</td>
<td>-</td>
</tr>
<tr>
<td>Cystine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glycine</td>
<td>0.60</td>
<td>0.47</td>
<td>0.71</td>
<td>0.48</td>
<td>0.83</td>
<td>0.58</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.62</td>
<td>0.54</td>
<td>0.71</td>
<td>0.60</td>
<td>0.80</td>
<td>0.67</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.49</td>
<td>0.30</td>
<td>0.57</td>
<td>0.32</td>
<td>0.65</td>
<td>0.41</td>
</tr>
<tr>
<td>Phenylalanine +</td>
<td>1.108</td>
<td>0.84</td>
<td>1.28</td>
<td>0.92</td>
<td>1.45</td>
<td>1.08</td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Valine</td>
<td>0.66</td>
<td>0.57</td>
<td>0.77</td>
<td>0.60</td>
<td>0.88</td>
<td>0.68</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.28</td>
<td>1.11</td>
<td>1.39</td>
<td>1.12</td>
<td>1.50</td>
<td>1.29</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.61</td>
<td>0.49</td>
<td>0.72</td>
<td>0.52</td>
<td>0.83</td>
<td>0.61</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.47</td>
<td>0.41</td>
<td>0.55</td>
<td>0.47</td>
<td>0.64</td>
<td>0.53</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.26</td>
<td>0.29</td>
<td>0.32</td>
<td>0.30</td>
<td>0.37</td>
<td>0.33</td>
</tr>
</tbody>
</table>

\(^1\) Protein values are an average of 4 determinations and the values for the amino acids are for one determination.
TABLE 9
EFFECT OF NON-PROTEIN NITROGEN ON PRODUCTION, FEED CONVERSION, EGG WEIGHT AND PERCENT SHELL (EXPERIMENT 3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Production</th>
<th>Feed Conversion</th>
<th>Average Egg Wt. (gms)</th>
<th>% Shell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I 12% Prot.</td>
<td>67.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.6&lt;sup&gt;bo&lt;/sup&gt;</td>
<td>9.29&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP&lt;sup&gt;3&lt;/sup&gt; 14% Prot.</td>
<td>65.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>57.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>9.13&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP 16% Prot.</td>
<td>65.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>57.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>9.22&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control II 14% Prot.</td>
<td>74.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.84&lt;sup&gt;c&lt;/sup&gt;</td>
<td>57.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>8.97&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control III 16% Prot.</td>
<td>74.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.90&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).

<sup>2</sup> Kilograms of feed consumed per dozen eggs.

DAP = diammonium phosphate (132.56% protein equivalent).
### TABLE 10

EFFECT OF NON-PROTEIN NITROGEN ON FEED CONSUMPTION, BODY WEIGHT, FERTILITY, AND HATCHABILITY (EXPERIMENT 3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Feed(^1) Consumption</th>
<th>Body Weight (gms.)</th>
<th>Fertility(^2)</th>
<th>Hatchability(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I (12% Prot.)</td>
<td>111.0(^{cd})</td>
<td>1712</td>
<td>77.69</td>
<td>92.52</td>
</tr>
<tr>
<td>Basal + DAP(^3) (14% Prot.)</td>
<td>113.8(^{bc})</td>
<td>1535</td>
<td>82.94</td>
<td>91.16</td>
</tr>
<tr>
<td>Basal + DAP (16% Prot.)</td>
<td>109.3(^d)</td>
<td>1578</td>
<td>80.87</td>
<td>88.43</td>
</tr>
<tr>
<td>Control II (12% Prot.)</td>
<td>114.7(^{ab})</td>
<td>1770</td>
<td>87.04</td>
<td>91.20</td>
</tr>
<tr>
<td>Control III (16% Prot.)</td>
<td>117.6(^a)</td>
<td>1818</td>
<td>84.68</td>
<td>93.75</td>
</tr>
</tbody>
</table>

\(^1\) Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).

\(^2\) Checked three times throughout the study.

\(^3\) DAP = Diammonium phosphate (132.56\% protein equivalent).
TABLE 11

EFFECT OF NON-PROTEIN NITROGEN ON PERCENT PROTEIN RETENTION AND AMINO ACID ABSORPTION (EXPERIMENT 3)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>27.881</td>
<td>31.44</td>
<td>27.36</td>
<td>26.80</td>
<td>27.67</td>
</tr>
<tr>
<td>Lysine</td>
<td>71.312</td>
<td>82.07</td>
<td>78.65</td>
<td>76.60</td>
<td>83.09</td>
</tr>
<tr>
<td>Histidine</td>
<td>73.82</td>
<td>86.27</td>
<td>83.07</td>
<td>82.67</td>
<td>86.43</td>
</tr>
<tr>
<td>Anginine</td>
<td>87.03</td>
<td>89.57</td>
<td>89.30</td>
<td>90.07</td>
<td>92.45</td>
</tr>
<tr>
<td>Threonine</td>
<td>76.70</td>
<td>74.95</td>
<td>73.18</td>
<td>68.72</td>
<td>82.44</td>
</tr>
<tr>
<td>Glycine</td>
<td>54.32</td>
<td>54.00</td>
<td>47.52</td>
<td>43.62</td>
<td>58.25</td>
</tr>
<tr>
<td>Alanine</td>
<td>86.42</td>
<td>85.66</td>
<td>84.69</td>
<td>80.50</td>
<td>86.82</td>
</tr>
<tr>
<td>Valine</td>
<td>81.22</td>
<td>81.34</td>
<td>78.55</td>
<td>80.51</td>
<td>82.91</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>81.58</td>
<td>84.66</td>
<td>81.86</td>
<td>77.03</td>
<td>87.32</td>
</tr>
<tr>
<td>Leucine</td>
<td>89.48</td>
<td>89.34</td>
<td>87.90</td>
<td>86.53</td>
<td>90.43</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>83.60</td>
<td>86.13</td>
<td>78.19</td>
<td>31.92</td>
<td>87.80</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>89.04</td>
<td>83.94</td>
<td>90.77</td>
<td>79.91</td>
<td>88.73</td>
</tr>
</tbody>
</table>

1 Protein retentions are based on three determinations throughout the study.

2 Amino acid retentions are based on one determination.

3 DAP = diammonium phosphate (132.56% protein equivalent).
### TABLE 12

**EFFECT OF NON-PROTEIN NITROGEN AND GLYCINE SUPPLEMENTATION ON PRODUCTION, FEED CONVERSION AND EGG WEIGHT (EXPERIMENT 4)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glycine</th>
<th>% Production</th>
<th>Feed Conversion</th>
<th>Egg wt. (gms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I (12% prot.)</td>
<td>0.600</td>
<td>48.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.23&lt;sup&gt;c&lt;/sup&gt;</td>
<td>58.9&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP&lt;sup&gt;1&lt;/sup&gt; (14% prot.)</td>
<td>0.600</td>
<td>47.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.39&lt;sup&gt;c&lt;/sup&gt;</td>
<td>59.7&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + 0.25% Gly. (12% prot.)</td>
<td>0.850</td>
<td>58.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.92&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>57.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP + 0.25% Gly. (14% prot.)</td>
<td>0.850</td>
<td>54.6&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.17&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>58.8&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control II (14% prot.)</td>
<td>0.650</td>
<td>62.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>60.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 DAP = Diammonium phosphate (132.56% protein equivalent).

2 Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).

3 Kilograms of feed consumed per dozen eggs.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Glycine</th>
<th>Body weight (gms)</th>
<th>% Protein Ret.</th>
<th>% Glycine absorbed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I (12% prot.)</td>
<td>0.600</td>
<td>1773</td>
<td>42.38</td>
<td>57.65</td>
</tr>
<tr>
<td>Basal + DAP(^1) (14% prot.)</td>
<td>0.600</td>
<td>1802</td>
<td>40.63</td>
<td>55.76</td>
</tr>
<tr>
<td>Basal + 0.25% Gly. (12% prot.)</td>
<td>0.850</td>
<td>1689</td>
<td>48.08</td>
<td>68.48</td>
</tr>
<tr>
<td>Basal + DAP + 0.25% Gly. (14% prot.)</td>
<td>0.850</td>
<td>1746</td>
<td>41.15</td>
<td>63.13</td>
</tr>
<tr>
<td>Control II (14% prot.)</td>
<td>0.650</td>
<td>2039</td>
<td>51.24</td>
<td>66.95</td>
</tr>
</tbody>
</table>

\(^1\) DAP = Diammonium phosphate (132.56% protein equivalent).


**TABLE 14**

EFFECT OF NON-PROTEIN NITROGEN AND GLYCINE SUPPLEMENTATION ON FEED CONSUMPTION, GRAMS PROTEIN RETAINED, GRAMS GLYCINE ABSORBED AND PLASMA LEVELS OF GLYCINE (EXPERIMENT 4)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Glycine Gms/day</th>
<th>Gms. Protein retained per day</th>
<th>Gms. Glycine absorbed per day</th>
<th>Micromoles Gly. per 100 ml of Plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I (12% prot.)</td>
<td>0.600</td>
<td>89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.44</td>
<td>0.295</td>
</tr>
<tr>
<td>Basal + DAP&lt;sup&gt;2&lt;/sup&gt; (14% prot.)</td>
<td>0.600</td>
<td>90&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.13</td>
<td>0.289</td>
</tr>
<tr>
<td>Basal + 0.25% Gly. (12% prot.)</td>
<td>0.850</td>
<td>92&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.25</td>
<td>0.508</td>
</tr>
<tr>
<td>Basal + DAP + 0.25% Gly. (14% prot.)</td>
<td>0.850</td>
<td>93&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>5.36</td>
<td>0.463</td>
</tr>
<tr>
<td>Control II (14% prot.)</td>
<td>0.650</td>
<td>96&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.19</td>
<td>0.435</td>
</tr>
</tbody>
</table>

<sup>1</sup> These calculations are based on the determined values for protein.

<sup>2</sup> DAP = Diammonium phosphate (132.56% protein equivalent).

<sup>3</sup> Blood samples for glycine determinations were taken after fasting birds for twelve hours and then feeding the experimental diets for two hours.
TABLE 15

EFFECT OF NON-PROTEIN NITROGEN SUPPLEMENTATION TO A LOW PROTEIN DIET ON PRODUCTION, CONSUMPTION, TOTAL PLASMA PROTEIN AND BLOOD URIC ACID (EXPERIMENT 5)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Protein</th>
<th>Eggs/gm</th>
<th>Feed cons./gms/day</th>
<th>Body weight change (gms)</th>
<th>% Plasma prot.</th>
<th>Blood uric acid mg/100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control I</td>
<td>7</td>
<td>37.1ab</td>
<td>124</td>
<td>+27</td>
<td>5.18b</td>
<td>1.26a</td>
</tr>
<tr>
<td>Basal + DAP¹</td>
<td>9</td>
<td>31.3a</td>
<td>101</td>
<td>-82</td>
<td>4.65c</td>
<td>1.96b</td>
</tr>
<tr>
<td>Control II</td>
<td>9</td>
<td>43.0b</td>
<td>118</td>
<td>+11</td>
<td>7.02a</td>
<td>1.45ab</td>
</tr>
</tbody>
</table>

¹ DAP = Diammonium phosphate (equivalent to 132.56% protein).

² Averages having different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
TABLE 16
EFFECT OF NON-PROTEIN NITROGEN SUPPLEMENTATION TO A LOW PROTEIN DIET ON THE RETENTION OF PROTEIN AND ABSORPTION OF SOME OF THE AMINO ACIDS (EXPERIMENT 5)

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>7% Protein control</th>
<th>7% Protein + 2% Prot. (DAP)</th>
<th>9% Protein control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>73.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Methionine</td>
<td>96.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>95.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>97.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lysine</td>
<td>92.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>90.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.8&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>92.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>89.2&lt;sup&gt;c&lt;/sup&gt;</td>
<td>94.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Leucine</td>
<td>93.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>90.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>85.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Histidine</td>
<td>91.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>91.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Arginine</td>
<td>93.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycine</td>
<td>79.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>65.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>82.0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Serine</td>
<td>81.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>81.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Averages with different superscripts are statistically different at the 0.05 level of probability.
<table>
<thead>
<tr>
<th>Amino acid</th>
<th>3 Hours</th>
<th>6 Hours</th>
<th>12 Hours</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treatment</td>
<td></td>
<td>Treatment</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>12.0</td>
<td>8.3</td>
<td>10.2</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>9.3</td>
<td>11.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Serine</td>
<td>163.8</td>
<td>163.0</td>
<td>213.0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>17.3</td>
<td>25.7</td>
<td>26.3</td>
</tr>
<tr>
<td>Proline</td>
<td>15.0</td>
<td>12.5</td>
<td>11.2</td>
</tr>
<tr>
<td>Glycine</td>
<td>39.8</td>
<td>37.7</td>
<td>32.2</td>
</tr>
<tr>
<td>Alanine</td>
<td>54.5</td>
<td>44.8</td>
<td>65.7</td>
</tr>
</tbody>
</table>

1 Amino acids are expressed as micro moles per 100 ml of blood plasma.

2 Treatment 1 = 7% protein control.
   Treatment 2 = 7% protein basal + 2% protein from DAP.
   Treatment 3 = 9% protein control.
CHAPTER III
THE UTILIZATION OF NON-PROTEIN NITROGEN BY YOUNG CHICKS
EXPERIMENTAL PROCEDURES

Experiment 1. One hundred and twenty-eight day-old sexed, Hubbard broiler chicks were randomly distributed into sixteen pens in a battery brooder with raised screen floors. There were eight treatments, and each treatment was replicated twice. Each replicate consisted of eight chicks (four males and four females).

The study was carried out for a period of twenty-eight days and the basal diet used is shown in Table 18. This semi-purified diet was formulated by a digital computer to meet the essential amino acid requirements according to the recommendations of the National Research Council (1960). The energy level of this diet was 900 kcal productive energy per pound and the protein level was restricted to 15%. The chicks in treatment 1 were fed the basal diet and those in treatments 2, 3 and 4 were fed the basal diet supplemented with D.A.C., D.A.P. and glutamic acid (G.A.), respectively. The levels of these N.P.N. sources fed were calculated to supply 3% protein equivalent (N x 6.25). The chicks in treatments 5, 6 and 7 were fed the basal diet supplemented with either D.A.C., D.A.P. or isolated soybean protein each in combination with glutamic acid. In each case glutamic
acid supplied one-half of the additional 3% protein, and the rest was supplied by the inorganic sources or by the isolated soybean protein. The chicks in treatment 8 were fed the basal diet supplemented with isolated soybean meal protein at a level calculated to supply 3% protein and therefore were the positive control. In each case the supplemented test ingredient replaced bentonite pound for pound. The total phosphorus level was constant in all of the test diets at 0.89%.

The male and the female chicks in each replicate were weighed separately, as a group at one day of age and individually at the end of the study. The individual body weights were subjected to an analysis of variance and the means separated by Duncan's multiple range test. Fecal samples were collected for three days during the third week of the study. Both feed and feces were analyzed for nitrogen, and protein retention was calculated from the values of the chromium oxide in the feed and the feces as described earlier. At the end of the study two birds from each replicate (one male and one female) were bled and the plasma separated as described earlier. The plasma was analyzed for uric acid as described by Brown (1945) and for total protein with a Total Solids Meter (American Optical Company). An accurate record of the feed intake was kept and the protein intake per bird was calculated in order to calculate protein efficiency ratio (P.E.R.) as described by Osborn and Mendel.
(1919). P.E.R. was calculated both with and without inclusion of the non-protein nitrogen.

**Experiment 2.** One hundred and twenty male chicks of the same strain as those used in the previous study were distributed throughout a battery brooder and fed a 21% protein, chick-starter diet for fourteen days. At the end of this period they were individually weighed and randomly distributed into 30 pens. Six experimental treatments were used; each was replicated five times and each replicate contained four chicks. The birds in treatment 1 were fed the 15% basal diet used in the previous experiment and those in treatments 2, 3, 4 and 5 were fed the basal diet supplemented with D.A.C., D.A.P., D.A.C. + G.A., and D.A.P. + G.A. respectively, at levels calculated to supply 3% protein equivalent. The chicks in treatment 6 were fed the basal diet supplemented with 3% protein from isolated soybean protein. As in experiment 1 the level of total phosphorus was constant in all diets at 0.89%.

The study was carried out for fourteen days and the chicks were weighed individually at the end of the study and group weighed every third day. Feed consumption was determined every third day and from this the protein intake was calculated. Protein efficiency ratios were calculated as in the previous experiment. Fecal samples were collected and the nitrogen determined and from this the amount of protein retained was calculated.
Experiment 3. Seven treatments were used in this study, each involving a total of twenty-four, sexed, day-old chicks of the same strain as those in the previous studies. The basal diet is given in Table 23 and was identical in essential amino acid composition to the one used in the previous two studies. The calculated protein of this diet was 16%, and methionine was added at a level of 0.323% to meet the minimum requirement of the chick.

The chicks in treatment 1 were fed the basal diet without any added methionine. Those in treatments 2 and 3 were fed the basal diet supplemented with 0.323% DL-methionine and methionine hydroxy analog calcium respectively. Treatment 2 diet was used in treatments 4 and 5, and treatment 3 diet was used in treatments 6 and 7; however, diammonium phosphate was added to the diet in treatments 4 and 5 at a level calculated to supply 2% protein. Isolated soybean meal protein was added to the diet for treatments 6 and 7 at a level calculated to supply 2% protein.

The study was carried out for twenty-eight days and all data were treated as in the previous experiments. In addition to weight, feed conversion and P.E.R., the absorption of lysine and methionine were also determined. These amino acids were determined in feed and feces by a standard microbiological assay as described in the Difco Manual (1953), and their absorption was calculated as described in Experiment 4 of the hen studies (page 15).
Experiment 4. Unlike the three previous experiments a purified diet (20.5% protein) with a productive energy level of 1,200 kcal per pound was employed (Table 26). This diet in essence was also deficient in protein due to the high energy level. The vitamin supplement used in this study did not have any vitamin B₆ and the calculated vitamin B₆ content of the diet itself was 1.1 mg. per pound.

Eight treatments were used in this study, each involving a total of 24, sexed, day-old chicks of the same strain as those used in the previous experiments. In treatment 1 the basal diet without any added vitamin B₆ was employed and in treatments 2 and 3 the basal diet was supplemented with vitamin B₆ at levels of 0.6 and 1.2 mg. per pound respectively. Diammonium phosphate was added to the basal diet at a level of 4% protein equivalent in the diets used in treatments 4, 5 and 6; and vitamin B₆ was added to these diets at a level of 0.0, 0.6 and 1.2 mg. per pound respectively. Treatments 7 and 8 were fed the basal diet supplemented with isolated soybean meal protein at a level calculated to supply 4% protein, and vitamin B₆ was added at levels of 0.0 and 1.2 mg. per pound, respectively.

At the end of the study, which lasted twenty-eight days, four chicks (two males and two females) were sacrificed and liver samples were taken and frozen immediately. Glutamic-oxalacetic and glutamic-pyruvic transaminase activities were measured in the liver homogenates by the method
of Sigma Chemical Co. (1961). The percent protein retained and the absorption of methionine, lysine, glycine and phenylalanine were calculated as described earlier. The chicks and feed were weighed as in the previous experiments and all data were treated statistically as before.

**Experiment 5.** The same basal diet used in the previous experiment was employed in this study, with the exception of the glycine supplement. Analysis of the diet indicated it was adequate in this amino acid, and the N.P.N. sources tested were supplemented at a level calculated to supply 2% protein. This diet also had 50 calories of productive energy more per pound supplied as glucose monohydrate. A total of nine treatments were used, each with the same number of sexed chicks of the same strain as those used in the previous study. The chicks in treatment 1 were fed the basal diet without any supplemental vitamin B₆ and those in treatments 2 and 3 were fed the basal diet supplemented with 0.6 and 1.2 mg. of vitamin B₆ respectively. The chicks in treatments 4-9 were fed the basal diet supplemented with D.A.P., glycine and isolated soybean meal protein at a level calculated to supply 2% protein and at two levels of supplemental vitamin B₆ (0.6 and 1.2 mg. per pound).

The duration of the study was twenty-eight days, and the same criteria as in the previous study were used in this experiment with the exception of glutamic-pyruvic transaminase which was not determined in this study. All data were treated as before.
RESULTS AND DISCUSSION

Experiment 1. Although the protein level of the basal diet was low in comparison to the recommended allowances, the chicks fed this diet alone grew very well illustrating the adequacy of the essential amino acids (Table 19). Nevertheless, the fact that this diet was inadequate in protein was shown by the significantly better growth obtained with the chicks fed this same basal diet supplemented with 3% protein from isolated soybean meal protein. The addition of 3% protein equivalent to the basal diet from diammonium citrate reduced growth slightly, but on the other hand D.A.P., G.A. and D.A.P. + G.A. appeared to have growth-promoting effects. However, the average weight of the birds fed the D.A.P. + G.A. was statistically greater than those fed the basal diet alone and equivalent to that of those fed the basal diet supplemented with the isolated soybean meal protein. Feed conversion, expressed as grams gain per gram of feed consumed, was improved by all the supplements, which indicated that feed was better utilized as a result of decreased consumption and the better growth for the birds in these treatments.

Percent protein retained decreased with the increase in protein except for the chicks fed the basal diet supplemented with the glutamic acid plus the isolated soybean meal
protein (Table 19). This apparently was due to the lower feed intake which was compensated by the retention of more protein. In general the amount of protein retained per bird per day did not appear to vary significantly between the treatments. However, approximately 17% of the protein in the diets supplemented with the N.P.N. sources was supplied by these sources which would mean that some of the retained protein originated from the inorganic nitrogen.

Protein efficiency ratio as proposed by Osborn and co-workers (1919) is defined as the grams gained in body weight per gram of nitrogen or protein consumed. Theoretically this technique is an excellent tool for evaluating the adequacy of a protein for growth provided that the diets tested contain the same level of protein. For this reason the protein efficiency ratios for the birds fed the basal diet supplemented with N.P.N. were calculated on the assumption that the inorganic nitrogen supplied part of the protein, and also on the assumption that this nitrogen was not available to the bird. If the calculated P.E.R. by the latter method was higher than that obtained for the birds fed the low control diet, it would in essence provide indirect evidence of the utilization of the inorganic nitrogen by the chicks. In Table 20 are given the P.E.R.'s for all the treatments calculated both ways. The protein efficiency ratios were higher for the birds fed N.P.N. than for those fed the control diets when the former were calculated under
the assumption that the inorganic nitrogen was not available. This provided evidence that at least a portion of the inorganic nitrogen was utilized.

From work reviewed by Albanese (1959) in his book entitled *Protein and Amino Acid Nutrition* it is evident that blood or plasma proteins are an indication of protein nutrition. Total plasma protein values (Table 20) were significantly higher for the birds fed the basal diet supplemented with 3% intact protein in comparison with that of chicks fed the basal diet alone. Both of the values were higher than the average value reported for the fowl (Sturkie 1965); nevertheless, this indicated that the birds fed the higher level of protein were more adequately nourished than those fed the lower level of protein. The values obtained for the birds fed the N.P.N. sources, except those fed the D.A.C. alone, were also significantly higher than those fed the low protein control diet and were equal to those fed the 18% intact protein diet. Uric acid determined on the same plasma samples as those used for the determination of total protein followed the same trend as the plasma protein and increased with an increase in the nitrogen content of the diet (Table 20). Although uric acid is the end product of protein metabolism in birds, it did not serve as a criterion of the utilization of inorganic nitrogen; but it provided evidence indicating the absorption of inorganic nitrogen at a rate apparently equal to that of birds fed intact protein.
Experiment 2. The purpose of feeding a standard chick-starter diet prior to the start of the study was to test the utilization of non-protein nitrogen with more mature, growing birds. The design of this experiment was very similar to the preceding.

Average body weight, feed conversion and protein retention for the fourteen day period are summarized in Table 21. There were no differences in body weight between the treatments, and there was no response to the supplementation of 3% whole protein to the 15% protein basal diet. All the N.P.N. sources tested appeared to depress body weight slightly, although this was not significant. Feed conversions on the other hand, for the chicks fed N.P.N. (excepting D.A.P.), were statistically better than for birds fed the basal diet and equal to those fed the 18% whole protein diet. Percent protein retained decreased with the increase of protein in the diet; but the amount of protein retained per day was practically the same for the chicks in all of the treatments, except those fed the basal supplemented with D.A.C. which was statistically lower than the rest. As in the previous experiment approximately 17% percent of the protein in the diets with N.P.N. was supplied by the inorganic nitrogen sources, which would mean that some of retained nitrogen was inorganic nitrogen.

Protein efficiency ratios, calculated in the same manner as in the previous experiment, appeared to follow the
same pattern. However, only in the case of the chicks fed the basal diet with D.A.C. was the P.E.R. greater than that obtained for those fed the basal diet, when the former were calculated without the N.P.N. The P.E.R.'s for the chicks fed the other N.P.N. sources and calculated without the inorganic nitrogen were equal to those obtained for the chicks fed the basal diet (Table 22).

The purpose for weighing the chicks every third day and determining the feed consumed over the same period was to plot the growth of the chicks versus protein consumption. It is generally agreed that the relationship between protein intake and body weight is essentially linear (Munro and Allison 1964). The slope of the line obtained by plotting growth versus protein intake can therefore be considered a qualitative measure of nutritive growth. This linear relationship for the chicks fed N.P.N. was plotted both with and without the N.P.N. and the slope determined in both of the curves (Table 22). The values obtained for the curves of the chicks fed intact protein were both 0.99 and lower for that of the chicks fed N.P.N. However, the values for the latter, obtained from the curves plotted without considering the N.P.N., were unity or greater than unity in the case of the chicks fed the D.A.C. or the D.A.P. + G.A. This provided evidence that some of the inorganic nitrogen was utilized.
Experiment 3. This experiment was designed primarily to test the work of Sullivan and Bird (1957). These workers were the first to show that the avian species could utilize non-protein nitrogen when fed in diets containing the hydroxy analogs of either methionine, glycine or both.

Although the essential amino acid composition of this diet was identical to that of the one used in the previous two experiments the total protein content was higher by 1% indicating that this diet had a higher content of non-essential amino acids.

The basal diet contained only 42% of the required methionine, and therefore the addition of methionine either as the DL or the methionine hydroxy analog produced a significant growth response (Table 24). Both forms of methionine used were equally effective. However, the addition of D.A.P. (equivalent to 2% protein) to the basal diet containing DL-methionine or the hydroxy analog did not produce any additional effects; nevertheless, body weight was slightly depressed for the chicks fed the D.A.P. supplemented to the diet containing DL-methionine. There appeared to be a response to the supplementation of intact protein to the basal diet containing either the DL-methionine or the hydroxy analog of methionine, although the response appeared to be greater when the intact protein was added to the basal diet containing the DL-methionine. Due to an error in the preparation of the latter two diets, which resulted in a total
intact protein level of 20%, a true comparison could not be made between the growth of the birds fed these diets to those fed the N.P.N. Feed conversion was improved by the addition of methionine to the basal diet, but the inorganic nitrogen did not improve it further. No differences in feed consumption were noted (Table 24).

The absorption of both methionine and lysine was improved by the supplementation of methionine to the basal diet (Table 25). However, the absorption of methionine by the birds fed the diets supplemented with the analog of methionine was not improved. This was perhaps due to an inaccuracy in the determination of the amino acid in these diets, since the organism used for the assay does not utilize the analog of methionine and therefore an accurate determination was not possible. The supplementation of non-protein nitrogen to the basal diet with the added methionine did not produce any additional effects on the absorption of these amino acids.

Protein efficiency ratios calculated as before are given in Table 25. The supplementation of methionine to the basal diet improved protein efficiency ratios significantly. Unlike the previous two experiments, the values obtained for the chicks fed the N.P.N., but calculated without it, were equal only to those of the chicks fed the diet supplemented with methionine only.
There was no evidence in this study that the chicks utilized the inorganic nitrogen. Perhaps this was a result of higher protein level used in the diet in comparison to that used in the previous two experiments since the energy level was the same for both diets.

Experiment 4. Unlike the three previous experiments in which the protein in the diet was restricted, the protein in the diet used in this experiment was not restricted. However, the energy level was 1200 Calories of productive energy per pound equivalent to a calorie: protein ratio of 59:1 instead of the recommended 42:1. This therefore restricted the protein intake of the chicks by limiting the amount of feed consumed. The purpose of feeding the different levels of vitamin B₆ was to test the effects of this vitamin on the utilization of inorganic nitrogen since vitamin B₆ is part of the enzyme systems involved in the transamination reactions. The activities of glutamic-oxalacetic (G.O.T.) and glutamic pyruvic (G.P.T.) transaminases in liver tissue were measured in an attempt to evaluate the effects of vitamin B₆ and N.P.N. on these enzyme systems.

Although the basal diet contained a calculated level of 1.1 mgs. of vitamin B₆ per pound, all the chicks fed the diets without supplemental B₆ died during the course of the study. Growth was not improved by feeding a level of supplemental vitamin B₆ higher than 0.6 mgs. per pound or by increasing the protein content of the basal diet by 4\% with
N.P.N. or intact protein (Table 27). No differences in feed consumption were observed, but as predicted, the feed intake was lower than that observed for the chicks in the previous experiments. Feed conversions appeared to be improved slightly but not significantly when higher levels of protein were fed either as N.P.N. or as intact protein (Table 27).

Vitamin B₆ did not have any effect on protein efficiency ratio, but the higher level of vitamin B₆ significantly improved both the percent and the amount of protein retained (Table 28), as well as the absorption of the amino acids studied (Table 29). On the other hand the P.E.R.'s for the chicks fed N.P.N. were significantly higher than those observed for the chicks fed the basal diet supplemented only with vitamin B₆ when the former were calculated without the inorganic nitrogen. In these treatments the higher level of vitamin B₆ also improved the percent and the amount of protein retained, but only the absorption of lysine was improved by feeding the higher level of B₆ with the N.P.N. The activity of glutamic-oxalacetic transaminase appeared to be depressed by the higher levels of vitamin B₆, but this was not significant. Inorganic nitrogen did not influence the activity of G.O.T. or G.P.T.

Protein efficiency ratios and protein retentions provided the only evidence of the utilization of non-protein nitrogen by the chicks in this study. Increasing levels of
vitamin B₆ influenced the retention of the protein and the absorption of the amino acids studied.

**Experiment 5.** As a result of a higher level of energy in the basal diet employed, feed consumption was lower than that observed for the chicks in the previous experiment. This apparently led to an excellent utilization of feed by the chicks in all the treatments, as is evident by the feed conversions shown in Table 31. However, no significant differences in feed conversions were observed.

Growth appeared to be improved slightly by increasing the level of protein in the basal diet with either source of non-protein nitrogen or intact protein. The effects of vitamin B₆ on growth, however, were variable and inconclusive.

Protein efficiency ratios were better than those observed for the chicks in the previous experiment (Table 31) possibly due to the lower intake of protein. In general, the P.E.R.'s were inversely related to the level of protein in the diet with the exception of that obtained for the chicks fed glycine at a level equivalent to 2% protein in addition to 0.6 mgs. vitamin B₆ per pound. The P.E.R.'s obtained for the chicks fed the N.P.N. calculated without consideration of the N.P.N. addition were equal to those observed for the chicks fed the basal diet alone with the exception of the value for the chicks fed the glycine supplemented diet. This latter value was statistically greater
than that observed for the chicks fed the basal diet alone. The effects of vitamin B6 on P.E.R.'s were variable but seemed to have a slightly depressing effect. As in the previous experiment no conclusive evidence on the effects of B6 on N.P.N. was provided by the measurement of activity of liver glutamic-oxaloacetic transaminase (Table 32).

The retention of protein and the absorption of the amino acids were not increased by the higher level of B6 as in the previous experiment (Table 33). No differences in the retentions of protein were observed between the treatments, although the chicks fed more protein as N.P.N. or as intact protein retained more protein per day. There was no apparent effect on the absorption of the amino acids as a result of feeding non-protein nitrogen. The highest absorption of glycine, however, was observed for the chicks fed 2% protein equivalent from glycine. Both of the glycine supplemented diets contained approximately 3% glycine by analysis, and the comparatively high absorption indicated that the supplemented glycine was utilized. However, not enough evidence was obtained from this study to conclude that the retained glycine was utilized to meet the protein needs of the chicks.
SUMMARY AND CONCLUSIONS

Five experiments were carried out with young chicks to study the effectiveness of non-protein nitrogen in meeting their protein requirements. The diets employed in all of the experiments were semi-purified or purified diets formulated to meet the essential amino acid requirements. The protein intake in three of the experiments was limited by restricting the amount of protein in the diet and in the other two experiments by employing diets with a high energy level. Diammonium citrate, diammonium phosphate, and glutamic acid were tested as sources of protein in the low protein diets in three of the experiments. Diammonium phosphate and glycine were tested in the other two experiments and vitamin B₆ was fed at two levels (0.6 and 1.2 gms. per pound) with each of the supplements to test its effect on the utilization of non-protein nitrogen by chicks. In each of the experiments the effects of N.P.N. as a source of protein were evaluated by comparing the performance of chicks fed N.P.N. to those fed the basal diet alone and to those fed the basal diet supplemented with intact protein.

Direct evidence of the utilization of N.P.N. was obtained in only one of the experiments in which growth was significantly improved by supplementing N.P.N. at a level of 3% protein equivalent using a combination of diammonium...
phosphate and glutamic acid. In this same experiment the total plasma protein for all the chicks fed N.P.N. was higher than for those fed the basal diet alone and was equal to that for chicks fed the basal diet plus intact protein. Protein efficiency ratios and protein retentions provided indirect evidence in four of the five experiments which indicated that young chicks could utilize N.P.N. Vitamin B₆ did not affect the utilization of N.P.N., although it increased the protein retained and the absorption of the amino acids studied in one of the experiments.

Not enough evidence was provided by these experiments to definitely conclude that chicks do utilize N.P.N. However, as was shown in the studies with laying hens, the importance of restricting the amount of protein and of meeting the essential amino acid requirements before the N.P.N. can be shown to be utilized was also evident.
TABLE 18

COMPOSITION OF BASAL DIET USED
IN CHICK EXPERIMENTS 1 AND 2

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground milo</td>
<td>19.533</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>25.682</td>
</tr>
<tr>
<td>Soybean meal (44% Protein)</td>
<td>21.600</td>
</tr>
<tr>
<td>Animal fat</td>
<td>5.000</td>
</tr>
<tr>
<td>Dried skim milk</td>
<td>1.688</td>
</tr>
<tr>
<td>Blood fibrin</td>
<td>2.879</td>
</tr>
<tr>
<td>Purified vitamin mix</td>
<td>4.000</td>
</tr>
<tr>
<td>Purified mineral mix</td>
<td>2.000</td>
</tr>
<tr>
<td>Solka floc (cellulose)</td>
<td>3.000</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.325</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.079</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>0.177</td>
</tr>
<tr>
<td>L-Glycine</td>
<td>0.262</td>
</tr>
<tr>
<td>L-Valine</td>
<td>0.040</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.250</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>3.485</td>
</tr>
<tr>
<td>Chromium oxide</td>
<td>0.300</td>
</tr>
<tr>
<td>Slack</td>
<td>9.700</td>
</tr>
</tbody>
</table>

Total: 100.000

1 Proctor and Gamble H.E.P.

2 Supplied the following per kgm of diet: 10,000 I.U. vit. A, 960 I.C.U. vit. D₃, 8.8 I.U. vit. E, 50 mg ethoxyquin, 8.8 mg thiamin HCl, Riboflavin 12.00 mg, D-calcium pantothenate 15.2 mg, P-amino benzoic acid 20.0 mg, inositol 1000 mg, niacin 88 mg, choline chloride (25%) 2206 mg, vit. B₁₂ 30 mcg, biotin 0.2 mg, folic acid 2.0 mg, methionine 6.6 mg.

3 Supplied per kgm of feed: sodium 1969 mg, chlorine 5641 mg, manganese 104 mg, iron 289 mg, copper 41 mg, zinc 77 mg, potassium 3114 mg, cobalt 1.2 mg, magnesium 562 mg, molybdenum 3.2 mg, iodine 3.1 mg, sulfate 2907 mg.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Prot.</th>
<th>Four Week(^5) Average Body Wt. (gms)</th>
<th>Gms Gain</th>
<th>Gms Feed Cons.</th>
<th>% Prot. Ret.</th>
<th>Gms Protein ret./day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>15.00</td>
<td>537(^{bc})</td>
<td>2.04</td>
<td>36</td>
<td>71.44</td>
<td>4.0</td>
</tr>
<tr>
<td>Basal + DAC(^1)</td>
<td>18.00</td>
<td>505(^{c})</td>
<td>1.99</td>
<td>33</td>
<td>67.86</td>
<td>4.1</td>
</tr>
<tr>
<td>Basal + DAP(^2)</td>
<td>18.00</td>
<td>581(^{ab})</td>
<td>1.92</td>
<td>37</td>
<td>64.55</td>
<td>4.4</td>
</tr>
<tr>
<td>Basal + GA(^3)</td>
<td>18.00</td>
<td>568(^{ab})</td>
<td>1.77</td>
<td>33</td>
<td>66.93</td>
<td>4.0</td>
</tr>
<tr>
<td>Basal + DAC + GA</td>
<td>18.00</td>
<td>537(^{bc})</td>
<td>1.90</td>
<td>33</td>
<td>60.79</td>
<td>3.7</td>
</tr>
<tr>
<td>Basal + DAP + GA</td>
<td>18.00</td>
<td>598(^{a})</td>
<td>1.80</td>
<td>35</td>
<td>64.93</td>
<td>4.2</td>
</tr>
<tr>
<td>Basal + GA + ADM(^4) Prot.</td>
<td>18.00</td>
<td>533(^{bc})</td>
<td>1.75</td>
<td>32</td>
<td>73.30</td>
<td>4.3</td>
</tr>
<tr>
<td>Basal + ADM Prot.</td>
<td>18.00</td>
<td>613(^{a})</td>
<td>1.80</td>
<td>36</td>
<td>64.74</td>
<td>4.3</td>
</tr>
</tbody>
</table>

\(^1\) DAC = Diammonium citrate (77.44% protein equivalent).
\(^2\) DAP = Diammonium phosphate (132.56% protein equivalent).
\(^3\) GA = Glutamic acid (57.81% protein equivalent).
\(^4\) ADM = Archer Daniels Midland Company isolated soybean meal protein (82% protein).
\(^5\) Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
**TABLE 20**

EFFECT OF VARIOUS NPN SOURCES ON PROTEIN EFFICIENCY RATIO, TOTAL PLASMA PROTEIN AND BLOOD URIC ACID (EXPERIMENT 1)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Prot.</th>
<th>P.E.R.</th>
<th>P.E.R. w/o NPN</th>
<th>Total Plasma Prot. gms/100 ml.</th>
<th>Blood Uric Acid mg/100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>15.00</td>
<td>3.17</td>
<td>-</td>
<td>4.55&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>3.77&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAC&lt;sup&gt;1&lt;/sup&gt;</td>
<td>18.00</td>
<td>2.73</td>
<td>3.24&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.33&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.54&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>18.00</td>
<td>2.85</td>
<td>3.42</td>
<td>5.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.36&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + GA&lt;sup&gt;3&lt;/sup&gt;</td>
<td>18.00</td>
<td>3.14</td>
<td>3.78</td>
<td>4.93&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.56&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAC + GA</td>
<td>18.00</td>
<td>2.92</td>
<td>3.49</td>
<td>4.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.93&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP + GA</td>
<td>18.00</td>
<td>3.07</td>
<td>3.66</td>
<td>4.90&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.16&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + GA + ADM&lt;sup&gt;4&lt;/sup&gt; Prot.</td>
<td>18.00</td>
<td>3.03</td>
<td>3.29</td>
<td>4.85&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>6.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + ADM Prot.</td>
<td>18.00</td>
<td>3.04</td>
<td>-</td>
<td>4.95&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.51&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Diammonium citrate (77.44% protein equivalent).
2 Diammonium phosphate (132.56% protein equivalent).
3 Glutamic acid (57.81% protein equivalent).
4 Archer Daniels Midland Co. isolated soybean meal protein (82% protein).
5 Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
### TABLE 21

EFFECT OF VARIOUS NON-PROTEIN NITROGEN SOURCES ON CHICK GROWTH, FEED CONVERSION AND PROTEIN RETENTION (EXPERIMENT 2)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Prot.</th>
<th>Four week avg. body wt. (gms)</th>
<th>Gms. gain feed cons.</th>
<th>% Protein ret.</th>
<th>Gms. prot. ret per day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>15.00</td>
<td>552&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.98&lt;sup&gt;c&lt;/sup&gt;</td>
<td>77.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAC&lt;sup&gt;1&lt;/sup&gt;</td>
<td>18.00</td>
<td>524&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>69.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>18.00</td>
<td>539&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>67.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAC + GA&lt;sup&gt;3&lt;/sup&gt;</td>
<td>18.00</td>
<td>543&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.93&lt;sup&gt;b&lt;/sup&gt;</td>
<td>75.31&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP + GA</td>
<td>18.00</td>
<td>547&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.92&lt;sup&gt;b&lt;/sup&gt;</td>
<td>68.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + ADM&lt;sup&gt;4&lt;/sup&gt; Prot.</td>
<td>18.00</td>
<td>558&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.87&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>74.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> DAC = Diammonium citrate (77.44% protein equivalent).

<sup>2</sup> DAP = Diammonium phosphate (132.56% protein equivalent).

<sup>3</sup> Glutamic acid (57.81% protein equivalent).

<sup>4</sup> Archer Daniels Midland Company isolated soybean meal protein (82% protein).

<sup>5</sup> Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
## TABLE 22

**EFFECT OF VARIOUS NON-PROTEIN NITROGEN SOURCES ON PROTEIN EFFICIENCY RATIO AND THE SLOPE OF THE CURVE OBTAINED BY PLOTTING GROWTH VERSUS PROTEIN INTAKE (EXPERIMENT 2)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Protein</th>
<th>P.E.R. (^5)</th>
<th>P.E.R. w/o NPN</th>
<th>Slope</th>
<th>Slope w/o NPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>15.00</td>
<td>3.22(^a)</td>
<td>3.22(^b)</td>
<td>0.99</td>
<td>-</td>
</tr>
<tr>
<td>Basal + DAC(^1)</td>
<td>18.00</td>
<td>2.89(^b)</td>
<td>3.46(^a)</td>
<td>0.90</td>
<td>1.06</td>
</tr>
<tr>
<td>Basal + DAP(^2)</td>
<td>18.00</td>
<td>2.68(^c)</td>
<td>3.11(^b)</td>
<td>0.87</td>
<td>1.00</td>
</tr>
<tr>
<td>Basal + DAC + GA(^3)</td>
<td>18.00</td>
<td>2.97(^b)</td>
<td>3.23(^b)</td>
<td>0.87</td>
<td>1.00</td>
</tr>
<tr>
<td>Basal + DAP + GA</td>
<td>18.00</td>
<td>2.91(^b)</td>
<td>3.22(^b)</td>
<td>0.96</td>
<td>1.05</td>
</tr>
<tr>
<td>Basal + ADM(^4) Prot.</td>
<td>18.00</td>
<td>3.07(^ab)</td>
<td>3.07(^b)</td>
<td>0.99</td>
<td>-</td>
</tr>
</tbody>
</table>

1 DAC = Diammonium citrate (77.44\% protein equivalent).
2 DAP = Diammonium phosphate (132.56\% protein equivalent).
3 GA = Glutamic acid (57.81\% protein equivalent).
4 ADM = Archer Daniels Midland Company isolated soybean meal protein (82\% protein).
5 Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
# TABLE 23
## COMPOSITION OF BASAL DIET USED IN EXPERIMENT 3

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground milo</td>
<td>50.174</td>
</tr>
<tr>
<td>Animal fat&lt;sup&gt;1&lt;/sup&gt;</td>
<td>8.000</td>
</tr>
<tr>
<td>Dried whey</td>
<td></td>
</tr>
<tr>
<td>Blood fibrin</td>
<td>2.879</td>
</tr>
<tr>
<td>Purified vitamin mix&lt;sup&gt;2&lt;/sup&gt;</td>
<td>4.000</td>
</tr>
<tr>
<td>Purified mineral mix&lt;sup&gt;3&lt;/sup&gt;</td>
<td>2.000</td>
</tr>
<tr>
<td>Soybean meal</td>
<td>16.600</td>
</tr>
<tr>
<td>Glucose monohydrate</td>
<td>2.012</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.250</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>3.488</td>
</tr>
<tr>
<td>L-Lysine</td>
<td>0.086</td>
</tr>
<tr>
<td>L-Arginine</td>
<td>0.237</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.268</td>
</tr>
<tr>
<td>Solka floe (cellulose)</td>
<td>3.000</td>
</tr>
<tr>
<td>Chromium oxide</td>
<td>0.200</td>
</tr>
<tr>
<td>Slack</td>
<td>5.121</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>100.000</td>
</tr>
</tbody>
</table>

1 Proctor and Gamble H.E.F.

2 Supplied the following per kgm of diet: 10,000 I.U. vit. A, 960 I.C.U. vit. D₃, 8.8 I.U. vit. E, 50 mg ethoxyquin, 8.8 mg thiamin HCl, Riboflavin 12.00 mg, D-calcium pantothenate 15.2 mg, P-amino benzoic acid 20.0 mg, inositol 1000 mg, niacin 88 mg, choline chloride (25%) 2206 mg, vit. B₁₂ 30 mcg, biotin 0.2 mg, folic acid 2.0 mg, menadione 6.6 mg.

3 Supplied per kgm of feed: sodium 1969 mg, chlorine 5641 mg, manganese 104 mg, iron 289 mg, copper 41 mg, zinc 77 mg, potassium 3114 mg, cobalt 1.2 mg, magnesium 562 mg, molybdenum 3.2 mg, iodine 3.1 mg, sulfate 2907 mg.
### TABLE 24
EFFECT OF DL-METHIONINE, METHIONINE HYDROXY ANALOG AND NPN SUPPLEMENTATION ON CHICK GROWTH AND FEED CONVERSION (EXPERIMENT 3)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Prot.</th>
<th>% Added meth.</th>
<th>Avg. 4 wk. body wt.</th>
<th>Gms gain gms feed cons.</th>
<th>Gms feed cons/day per bird</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>16</td>
<td>-</td>
<td>487&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.14&lt;sup&gt;a&lt;/sup&gt;</td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DL-Meth.</td>
<td>16</td>
<td>0.324</td>
<td>594&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.77&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + MHA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>16</td>
<td>0.324</td>
<td>600&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.81&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + MHA + DAP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>18</td>
<td>0.324</td>
<td>597&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>1.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DL-Meth. + DAP</td>
<td>18</td>
<td>0.324</td>
<td>560&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + MHA + ADM</td>
<td>18</td>
<td>0.292</td>
<td>620&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.68&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DL-Meth. + ADM</td>
<td>18</td>
<td>0.292</td>
<td>642&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.65&lt;sup&gt;c&lt;/sup&gt;</td>
<td>35&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 Methionine hydroxy analog (Monsanto Chem. Corp.)
2 DAP = Diammonium phosphate (132.56% protein equivalent).
3 ADM = Archer Daniels Midland Company isolated soybean meal protein (82% protein).
4 Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
### TABLE 25

**EFFECT OF DL-METHIONINE, METHIONINE HYDROXY ANALOG AND NPN SUPPLEMENTATION ON THE ABSORPTION OF LYSINE AND METHIONINE AND PROTEIN EFFICIENCY RATIO (EXPERIMENT 3)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>% Prot</th>
<th>% Absorption</th>
<th>P.E.R.</th>
<th>P.E.R. w/o NPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>16</td>
<td>86.70</td>
<td>89.85</td>
<td>2.92&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DL-meth.</td>
<td>16</td>
<td>89.31</td>
<td>96.03</td>
<td>3.49&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + MHA&lt;sup&gt;1&lt;/sup&gt;</td>
<td>16</td>
<td>85.05</td>
<td>95.62</td>
<td>3.44&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + MHA + DAP&lt;sup&gt;2&lt;/sup&gt;</td>
<td>18</td>
<td>83.30</td>
<td>94.99</td>
<td>3.00&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DL-meth. + DAP</td>
<td>18</td>
<td>92.90</td>
<td>95.44</td>
<td>3.04&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + MHA + ADM&lt;sup&gt;3&lt;/sup&gt;</td>
<td>18</td>
<td>86.59</td>
<td>95.52</td>
<td>2.97&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DL-meth. + ADM</td>
<td>18</td>
<td>92.38</td>
<td>95.38</td>
<td>3.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup> Methionine hydroxy analog (Monsanto Chem. Corp.).

<sup>2</sup> DAP = Diammonium phosphate (132.56% protein equivalent).

<sup>3</sup> ADM = Archer Daniels Midland Company.

<sup>4</sup> Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
### TABLE 26

**COMPOSITION OF BASAL DIET USED IN EXPERIMENT 4**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose Monohydrate</td>
<td>48.92</td>
</tr>
<tr>
<td>ADM&lt;sup&gt;1&lt;/sup&gt; Protein (&lt;82% protein)</td>
<td>25.00</td>
</tr>
<tr>
<td>Animal Fat&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10.00</td>
</tr>
<tr>
<td>Purified Vitamin premix&lt;sup&gt;3&lt;/sup&gt;</td>
<td>4.00</td>
</tr>
<tr>
<td>Purified Mineral premix&lt;sup&gt;4&lt;/sup&gt;</td>
<td>2.00</td>
</tr>
<tr>
<td>Chromium oxide</td>
<td>0.20</td>
</tr>
<tr>
<td>Dicalcium phosphate</td>
<td>3.84</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>0.66</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.17</td>
</tr>
<tr>
<td>DL-Methionine</td>
<td>0.49</td>
</tr>
<tr>
<td>Bentonite</td>
<td>4.72</td>
</tr>
<tr>
<td>Total</td>
<td>100.00</td>
</tr>
</tbody>
</table>

1. Archer Daniels Midland Co. isolated soybean meal protein.
2. Proctor and Gamble H.E.P.
3. Supplied the following per kgm of diet: 10,000 I.U. vit. A, 960 I.C.U. vit. D<sub>3</sub>, 8.8 I.U. vit. E, 50 mg ethoxyquin, 8.8 mg thiamin HCl, Riboflavin 12.00 mg, D-calcium pantothenate 15.2 mg, P-amino benzoic acid 20.0 mg, inositol 1000 mg, niacin 88 mg, choline chloride (25%) 2206 mg, vit. B<sub>12</sub> 30 mcg, biotin 0.2 mg, folic acid 2.0 mg, menadione 6.6 mg.
4. Supplied per kgm of feed: sodium 1969 mg, chlorine 5641 mg, manganese 104 mg, iron 289 mg, copper 41 mg, zinc 77 mg, potassium 3114 mg, cobalt 1.2 mg, magnesium 562 mg, molybdenum 3.2 mg, iodine 3.1 mg, sulfate (SO<sub>4</sub>) 2907 mg.
**TABLE 27**

**EFFECT OF NON-PROTEIN NITROGEN AND VITAMIN B₆ ON CHICK GROWTH, FEED CONSUMPTION AND FEED CONVERSION (EXPERIMENT 4)**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Added Vit. B mg/lb. B₆</th>
<th>% Prot.</th>
<th>4 Week Avg. body wt. (gms)</th>
<th>Feed Consum. gm/bird/day</th>
<th>Gms Gain gms feed cons.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>-</td>
<td>20.50</td>
<td>-1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + B₆</td>
<td>0.6</td>
<td>20.50</td>
<td>552ᵃ</td>
<td>31ᵃ</td>
<td>1.80ᵃ</td>
</tr>
<tr>
<td>Basal + B₆</td>
<td>1.2</td>
<td>20.50</td>
<td>538ᵃ</td>
<td>31ᵃ</td>
<td>1.79ᵃ</td>
</tr>
<tr>
<td>Basal + DAP¹</td>
<td>-</td>
<td>24.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + B₆ + DAP</td>
<td>0.6</td>
<td>24.50</td>
<td>553ᵃ</td>
<td>30ᵃ</td>
<td>1.66ᵃ</td>
</tr>
<tr>
<td>Basal + B₆ + DAP</td>
<td>1.2</td>
<td>24.50</td>
<td>575ᵃ</td>
<td>31ᵃ</td>
<td>1.69ᵃ</td>
</tr>
<tr>
<td>Basal + ADM² Prot.</td>
<td>-</td>
<td>24.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + B₆ + ADM Prot.</td>
<td>1.2</td>
<td>24.50</td>
<td>564ᵃ</td>
<td>30ᵃ</td>
<td>1.66ᵃ</td>
</tr>
</tbody>
</table>

¹ DAP = Diammonium phosphate (132.56% protein equivalent).

² ADM = Archer Daniel Midland Co. isolated soybean meal protein.

³ Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).

⁴ All the birds in these treatments died prior to the end of the study.
**TABLE 28**

EFFECT OF NON-ProtEIN NITROGEN AND VITAMIN B<sub>6</sub> ON PROTEIN EFFICIENCY RATIO (PER) AND ON PERCENT PROTEIN RETAINED AND GRAMS RETAINED PER DAY (EXPERIMENT 4)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Added Vit. B&lt;sub&gt;6&lt;/sub&gt; mg/lb.</th>
<th>% Protein</th>
<th>PER&lt;sup&gt;3&lt;/sup&gt; w/o NPN</th>
<th>PER&lt;sup&gt;3&lt;/sup&gt; w/o NPN</th>
<th>PROTEIN Ret.</th>
<th>Gms Prot. Ret./day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td></td>
<td>20.50</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.6</td>
<td>20.50</td>
<td>2.56&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>2.56&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>68.49&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>4.8&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>1.2</td>
<td>20.50</td>
<td>2.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.61&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>78.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.2&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0</td>
<td>24.50</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal + DAP + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.6</td>
<td>24.50</td>
<td>2.38&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.74&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4.9&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>1.2</td>
<td>24.50</td>
<td>2.32&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.75&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>71.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + ADM&lt;sup&gt;2&lt;/sup&gt; prot.</td>
<td>0</td>
<td>24.50</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal + ADM + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>1.2</td>
<td>24.50</td>
<td>2.45&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>2.48&lt;sup&gt;c&lt;/sup&gt;</td>
<td>72.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 DAP = Diammonium phosphate (132.56% protein equivalent).
2 ADM = Archer Daniels Midland Co. isolated soybean meal protein.
3 Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
4 All birds in these treatments died before the termination of the experiment.
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Added B&lt;sub&gt;6&lt;/sub&gt; mg/lb.</th>
<th>% Protein</th>
<th>% Absorption&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Lysine</th>
<th>Glycine</th>
<th>Meth.</th>
<th>Phenylalanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0</td>
<td>20.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.6</td>
<td>20.50</td>
<td>93.62&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.54&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>94.87&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>1.2</td>
<td>20.50</td>
<td>95.25&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>89.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.39&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + DAP&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0</td>
<td>24.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + DAP + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.6</td>
<td>24.50</td>
<td>92.76&lt;sup&gt;c&lt;/sup&gt;</td>
<td>87.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>93.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>94.76&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + DAP + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>1.2</td>
<td>24.50</td>
<td>94.48&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.81&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>94.96&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + ADM&lt;sup&gt;2&lt;/sup&gt;-Prot.</td>
<td>0</td>
<td>24.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + ADM + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>1.2</td>
<td>24.50</td>
<td>96.00&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>92.62&lt;sup&gt;b&lt;/sup&gt;</td>
<td>95.38&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>1</sup> DAP = Diammonium phosphate (132.56% protein equivalent).

<sup>2</sup> ADM = Archer Daniels Midland Co. isolated soybean meal protein.

<sup>3</sup> Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).

<sup>4</sup> All birds in these treatments died before the termination of the experiment.
TABLE 30

EFFECT OF NON-PROTEIN NITROGEN AND VITAMIN B₆ ON
GLUTAMIC-OXALACETIC AND GLUTAMIC-PYRUVIC
TRANSAMINASE IN CHICK LIVER (EXPERIMENT 4)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Added Vit. B₆ mg/lb.</th>
<th>% Prot.</th>
<th>Glutamic-Oxalacetic Transaminase O.D. change/ min/gm tissue</th>
<th>Glutamic-Pyruvic Transaminase O.D. change/ min/gm tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>-</td>
<td>20.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + B₆</td>
<td>0.6</td>
<td>20.50</td>
<td>135.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12.7&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B₆</td>
<td>1.2</td>
<td>20.50</td>
<td>106.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP&lt;sub&gt;1&lt;/sub&gt;</td>
<td>0</td>
<td>24.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + DAP + B₆</td>
<td>0.6</td>
<td>24.50</td>
<td>123.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + DAP + B₆</td>
<td>1.2</td>
<td>24.50</td>
<td>93.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.5&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + ADM&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0</td>
<td>24.50</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + ADM + B₆</td>
<td>1.2</td>
<td>24.50</td>
<td>101.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

1 DAP = diammonium phosphate (132.56% protein equivalent).

2 ADM = Archer Daniels Midland Co. isolated soybean meal protein.

3 Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).

4 Birds in these treatments died before the termination of the experiment.
## TABLE 31

EFFECT OF NON-PROTEIN NITROGEN AND VITAMIN B6 ON CHICK GROWTH, FEED CONVERSION AND FEED CONSUMPTION (EXPERIMENT 5)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Added Vit. B6 mg/lb.</th>
<th>% Prot.</th>
<th>4 Week Avg. body wt. (gms)</th>
<th>Gms Gain</th>
<th>Gms feed cons.</th>
<th>Gms feed cons/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>-</td>
<td>20.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + B6</td>
<td>0.6</td>
<td>20.50</td>
<td>522&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + B6</td>
<td>1.2</td>
<td>20.50</td>
<td>538&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + B6 + DAP&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.6</td>
<td>22.50</td>
<td>553&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28&lt;sup&gt;ab&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + B6 + DAP</td>
<td>1.2</td>
<td>22.50</td>
<td>554&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.60&lt;sup&gt;a&lt;/sup&gt;</td>
<td>29&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + B6 + Gly.&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.6</td>
<td>22.50</td>
<td>546&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.53&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + B6 + Gly.</td>
<td>1.2</td>
<td>22.50</td>
<td>526&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + B6 + ADM Prot.&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.6</td>
<td>22.50</td>
<td>523&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>26&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Basal + B6 + ADM Prot.</td>
<td>1.2</td>
<td>22.50</td>
<td>559&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.58&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27&lt;sup&gt;bc&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

1 DAP = Diammonium phosphate (equivalent to 132.56% protein).
2 Glycine = equivalent to 116.62% protein.
3 ADM Protein = Archer Daniels Midland Co. isolated soybean meal protein (82% protein).
4 Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
5 All the birds in the treatment died.
TABLE 32

EFFECT OF NON-PROTEIN NITROGEN AND VITAMIN B6 ON PROTEIN EFFICIENCY RATIO AND ON GLUTAMIC OXALACETIC TRANSAMINASE (G.O.T.) IN LIVER TISSUE (EXPERIMENT 5)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Added Vit. B6 mg/lb.</th>
<th>% Prot.</th>
<th>P.E.R. w/o N.P.N.</th>
<th>G.O.T. change/min per gm tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>-</td>
<td>20.50</td>
<td>-</td>
<td>G.O.T. 87.76ab</td>
</tr>
<tr>
<td>Basal + B6</td>
<td>0.6</td>
<td>20.50</td>
<td>3.01ab</td>
<td>3.01b 91.82ab</td>
</tr>
<tr>
<td>Basal + B6</td>
<td>1.2</td>
<td>20.50</td>
<td>2.88abc</td>
<td>2.88b 91.82ab</td>
</tr>
<tr>
<td>Basal + B6 + DAP1</td>
<td>0.6</td>
<td>22.50</td>
<td>2.75cd</td>
<td>3.02b 83.20ab</td>
</tr>
<tr>
<td>Basal + B6 + DAP</td>
<td>1.2</td>
<td>22.50</td>
<td>2.73cd</td>
<td>2.99b 100.00a</td>
</tr>
<tr>
<td>Basal + B6 + Gly.2</td>
<td>0.6</td>
<td>22.50</td>
<td>3.06a</td>
<td>3.37a 81.88ab</td>
</tr>
<tr>
<td>Basal + B6 + Gly.</td>
<td>1.2</td>
<td>22.50</td>
<td>2.89d</td>
<td>2.78b 86.44ab</td>
</tr>
<tr>
<td>Basal + B6 + ADM</td>
<td>0.6</td>
<td>22.50</td>
<td>2.89abc</td>
<td>2.89b 95.20ab</td>
</tr>
<tr>
<td>Basal + B6 + ADM</td>
<td>1.2</td>
<td>22.50</td>
<td>2.81cd</td>
<td>2.89b 70.96b</td>
</tr>
</tbody>
</table>

1 DAP = Diammonium phosphate (equivalent to 132.56% protein).
2 Glycine = equivalent to 116.62% protein.
3 ADM Protein = Archer Daniels Midland Co. isolated soybean meal protein (82% protein).
4 Averages with different superscripts are statistically different at the 0.05 level of probability (Duncan 1955).
5 All the birds in the treatment died.
### TABLE 33

EFFECT OF NON-PROTEIN NITROGEN AND VITAMIN B<sub>6</sub> ON PROTEIN RETENTION AND THE ABSORPTION OF SOME OF THE ESSENTIAL AMINO ACIDS (EXPERIMENT 5)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Added Vit. B&lt;sub&gt;6&lt;/sub&gt; mg/lb.</th>
<th>% Prot.</th>
<th>% Prot. ret.</th>
<th>Gms Prot. Ret./bird/day</th>
<th>Absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal</td>
<td>0</td>
<td>20.50</td>
<td>-5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>0.6</td>
<td>20.50</td>
<td>61.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.3</td>
<td>93.56&lt;sup&gt;ab&lt;/sup&gt; 95.78&lt;sup&gt;ab&lt;/sup&gt; 78.81&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt;</td>
<td>1.2</td>
<td>20.50</td>
<td>61.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.6</td>
<td>92.63&lt;sup&gt;c&lt;/sup&gt; 95.33&lt;sup&gt;b&lt;/sup&gt; 74.57&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt; + DAP&lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.6</td>
<td>22.50</td>
<td>65.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4</td>
<td>94.33&lt;sup&gt;a&lt;/sup&gt; 96.19&lt;sup&gt;ab&lt;/sup&gt; 78.43&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt; + DAP&lt;sup&gt;1&lt;/sup&gt;</td>
<td>1.2</td>
<td>22.50</td>
<td>64.75&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.3</td>
<td>93.74&lt;sup&gt;ab&lt;/sup&gt; 95.80&lt;sup&gt;ab&lt;/sup&gt; 77.06&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt; + Gly.&lt;sup&gt;2&lt;/sup&gt;</td>
<td>0.6</td>
<td>22.50</td>
<td>59.41&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.4</td>
<td>93.15&lt;sup&gt;ab&lt;/sup&gt; 95.23&lt;sup&gt;b&lt;/sup&gt; 89.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt; + Gly.&lt;sup&gt;2&lt;/sup&gt;</td>
<td>1.2</td>
<td>22.50</td>
<td>62.82&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9</td>
<td>92.73&lt;sup&gt;c&lt;/sup&gt; 94.92&lt;sup&gt;b&lt;/sup&gt; 83.03&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt; + ADM Prot.&lt;sup&gt;3&lt;/sup&gt;</td>
<td>0.6</td>
<td>22.50</td>
<td>64.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8</td>
<td>92.78&lt;sup&gt;c&lt;/sup&gt; 95.80&lt;sup&gt;ab&lt;/sup&gt; 75.46&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Basal + B&lt;sub&gt;6&lt;/sub&gt; + ADM Prot.&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1.2</td>
<td>22.50</td>
<td>63.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.9</td>
<td>94.28&lt;sup&gt;c&lt;/sup&gt; 95.56&lt;sup&gt;ab&lt;/sup&gt; 77.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

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REFERENCES


