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Vitamin B6 metabolism in the chick

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VITAMIN B_6 METABOLISM IN THE CHICK

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INTRODUCTION

Slightly over a quarter century ago, vitamin B_6 was recognized as a distinct vitamin. Since the first report on vitamin B_6, researchers have investigated its biological functions quite extensively. However, this research has been performed mainly with mammalian species as the experimental animal. There have been reports on vitamin B_6 studies involving avian species, but these have been directed largely to the applied area of research rather than to the basic area.

Since literature contains indications of breed differences in vitamin B_6 requirements of chickens, the first trial was designed to study these relationships. Three breeds of chickens, which varied in type, body size, and growth rate, were used in attempting to determine the optimum vitamin B_6 requirement for each breed.

There is an apparent lack of information on effects of vitamin B_6 deficiency in chickens on hemoglobin level and packed cell volume. These relationships were studied in the second experiment. In this trial, a study was made on the effects of restricting feed intake on growth rate, feed conversion ratios, hemoglobin level, and packed cell volume. Chicks consuming diets containing adequate vitamin B_6 were restricted to the amount of feed consumed by chicks consuming diets deficient in vitamin B_6.

Since most practical poultry diets contain adequate vitamin B_6, studies on vitamin B_6 metabolism in chicks require feeding semi-purified diets. Since the semi-purified diets are more expensive than practical-type diets, the third trial was planned to study the possibility of using a practical diet containing a vitamin B_6 antagonist, desoxypyridoxine, in

producing a vitamin B_6 deficiency.

A vitamin B_6 deficiency in chickens produces apparent effects on growth rate; however, there is an apparent lack of information on the ability of chickens to recover from a vitamin B_6 deficiency. Experiment 4 was designed to study the effects of a vitamin B_6 deficiency on subsequent growth rate.

The overall objective of this research has been to attempt to fill in some voids in past vitamin B_6 research made with chicks and to extend our basic knowledge of vitamin B_6 metabolism in the chick.

REVIEW OF LITERATURE

The recognition of vitamin B_6 as a new member of the vitamin B-complex is credited to György (1935a, 1935b) who reported a "rat antipellagra factor" which he named vitamin B_6. These reports have been followed by extensive research and reports regarding the requirements, importance, and interrelationships of this vitamin. Most of the reports concern research with humans, or laboratory animals such as rats and mice, while reports on domestic fowl are more limited.

Requirements and Deficiency Symptoms in Domestic Fowl

Jukes (1939) and Hegsted et al. (1939) were two of the first to show that chicks needed a dietary source of vitamin B_6. These reports were followed shortly by those of Hegsted et al. (1940), Hogan et al. (1941), Lepkovsky and Kratzer (1942), and Briggs et al. (1942). These researchers generally reported vitamin B_6 deficiency symptoms in chicks as inefficient food utilization, reduced food consumption, reduced growth, and death. Some groups observed spasmodic convulsions preceding death. Daghir and Balloun (1963) also observed ruffled feathers and wing feather follicle hemorrhages in vitamin B_6-deficient chicks. Miller (1963) observed an abnormal incidence of pendulous crop in pyridoxine-deficient chicks.

Hogan et al. (1941) reported the chick's vitamin B_6 requirement to be between 300 and 500 mcg of vitamin B_6 per 100 g of ration. Briggs et al. (1942) reported the requirement of chicks as 275 to 300 mcg of vitamin B_6 per 100 g of ration. Research by Lucas et al. (1946) indicated that vitamin B_6 requirements may vary with the breed of chicken. Daghir and

Balloun (1963) found a statistically significant breed x vitamin B_6 interaction on 4-week chick weights. Rhode Island Red, Single Comb White Leghorn, and Vantress X Arbor Acres chicks were used in this research. For all three breeds, 2.2 to 2.6 mg of vitamin B_6 per kg of ration was adequate for best growth. The significant interaction was attributed to differences in response of Rhode Island Red chicks as compared to the response of the other two breeds. Weight gains of Rhode Island Red chicks consuming the 2.2 mg per kg ration was significantly different from the weight gains of Rhode Island Red chicks consuming the 2.6 mg per kg diet. This difference was not significant in the other two breeds.

Mills et al. (1947) observed no quantitative differences in vitamin B_6 requirements when chicks were maintained in either warm (90° Fahrenheit with 70 percent relative humidity) or moderate (70° Fahrenheit) environments.

Fuller and Kifer (1959) reported slight, but nonsignificant, weight gains in chicks when pyridoxine was added to a practical corn-soy type ration. When chicks were fed a semi-purified diet supplemented with 1, 2, and 4 mg of pyridoxine hydrochloride per pound, maximum growth was attained at the lowest level, indicating that 1.5 mg of vitamin B_6 per lb of ration met the chicks' requirement.

Kratzer et al. (1947) made comparative studies of the vitamin B_6 requirement of chicks and turkey poults. Research by this group indicated a requirement of 200 mcg of vitamin B_6 per 100 g of ration for chicks and 300 mcg of vitamin B_6 per 100 g of ration for turkey poults. Bird et al. (1943) had reported previously that deficiency symptoms in poults were

very similar to those of chicks: loss of appetite, poor growth, convulsions, hyperexcitability when disturbed, and death. The deficiency symptoms were prevented by adding 3.0 mg of pyridoxine per kg of deficient diet.

Hegsted and Rao (1945) studied vitamin B_6 metabolism in ducks. Symptoms of a deficiency in young ducklings were growth failure and severe microcytic anemia. In addition, older ducklings on deficient diets exhibited poor feather development, paralysis, and convulsions. The requirement for vitamin B_6 was reported as 250 mcg of vitamin B_6 per 100 g of ration.

Cravens et al. (1943, 1946) investigated the vitamin B_6 requirements of laying and breeding hens. This group observed a complete failure in hatchability in seven weeks and a cessation of production in eight weeks after hens started receiving deficient diets. The requirement for Single Comb White Leghorn hens was found to be approximately 2.0 mg of vitamin B_6 per kg of ratio. Fuller et al. (1961) found the vitamin B_6 requirement of S.C.W. Leghorn hens to be 2.3 mg per kg of diet for maximum production and 4.3 mg per kg for maximum hatchability.

Luckey et al. (1945) and Waibel et al. (1952) reported that pyridoxine, pyridoxal, and pyridoxamine are all active for chicks. However, pyridoxal and pyridoxamine are slightly less active than is pyridoxine.

Metabolic Relationships of Vitamin B_6

Coenzyme functions

Bellamy et al. (1945) confirmed the involvement of vitamin B_6 in decarboxylation reactions by demonstrating the need for vitamin B_6 by microorganisms in the formation of codecarboxylase.

Schlenk and Snell (1945) proposed a role for vitamin B_6 in transamination reactions. This role was subsequently confirmed by reports by Snell (1945) and Lichstein et al. (1945) which demonstrated the reversible interconversion of pyridoxal and pyridoxamine through transamination reactions involving glutamic acid and alpha-ketoglutaric acid.

Cammarata and Cohen (1950) reported on the importance of transamination reactions in the body, observing that 24 amino acids are able to transfer their amino group to alpha-ketoglutaric acid.

The involvement of vitamin B_6 in transamination reactions was further confirmed by Brin and Olson (1951). Using cardiac muscle from vitamin B_6-deficient ducks, these researchers observed greatly decreased transaminase activity compared with that of pair-fed controls receiving sufficient vitamin B_6. Treating deficient ducks with vitamin B_6 16 hours prior to making the determination of transaminase activity resulted in a restoration of the depressed activity to levels near that of control ducks.

Brin et al. (1954), in a similar study, observed a significant correlation between the transaminase activity in duck cardiac tissue and the degree of anemia in the vitamin B_6-deficient ducks.

Goswami et al. (1957) observed decreased liver cysteine desulfhydrase activity in chicks when a ration deficient in pyridoxine was fed. Goswami

and Robblee (1958) reported similar effects on aspartic-glutamic transaminase activity of chick heart, liver, and blood. Daghir and Balloun (1963) reported decreased serum glutamic-oxalacetic transaminase (SGO-T) levels in chicks fed a ration low in vitamin B_6. However, growth was observed to be a more sensitive criterion for determining vitamin B_6 adequacy than was SGO-T activity.

Snell (1958), in a review article, discussed the postulated mechanisms utilized by the vitamin B_6 complex in functioning as a coenzyme in metabolic reactions. Some of the enzymatic reactions which utilize vitamin B_6 as a cofactor are also outlined in the paper.

Amino acid metabolism in the avian species

Anderson et al. (1949, 1951) studied pyridoxine-amino acid relationships in the chick. Depressed growth was observed on a pyridoxine-low diet supplemented with selected amino acids.

Schulman and Richert (1957), in radiotracer studies with ducklings, observed decreased heme synthesis from labeled glycine or succinate when the ducklings were deficient in vitamin B_6. The incorporation of delta-aminolevulinic acid was essentially normal. Administering the delta-aminolevulinic acid to vitamin B_6-deficient ducklings did not prevent anemia.

Weissbach et al. (1957) observed lower tissue levels of serotonin in pyridoxine-deficient chicks. Deficient chicks were unable to convert administered 5-hydroxytryptophan to serotonin as efficiently as did control chicks.

Phillips et al. (1962) utilized germfree chicks to study effects of

a pyridoxine-deficient diet on liver levels of monoamine oxidase and serotonin. Determinations on germfree chicks gave higher monoamine oxidase levels with no difference in serotonin levels, when compared to normal flora chicks. Chicks with normal flora fed a vitamin B_6-deficient diet were observed to have more monoamine oxidase and less serotonin than the normal flora chicks consuming a diet containing sufficient vitamin B_6.

Lipid metabolism relationships with emphasis on poultry

Kratzer and Williams (1948) observed an apparent pyridoxine deficiency when a diet containing 30 percent linseed oil meal was fed to chicks. This diet was considered to be more than adequate in pyridoxine. The apparent deficiency was corrected by adding pyridoxine to the ration. The authors proposed several mechanisms which would explain this observation. First, the linseed oil meal could contain a factor which combined with the pyridoxine and formed a complex which rendered the pyridoxine unavailable. The second theory, proposed the presence of some antimetabolite in the linseed oil meal which substituted for pyridoxine in metabolic reactions, but lacked biological activity for normal functioning.

Prior to this, McHenry and Gavin (1941) had reported that rats required pyridoxine for conversion of a high protein, fat- and carbohydrate-free diet into body fat. No body fat was synthesized unless pyridoxine was present, regardless of other B-vitamin supplementation.

McFarland (1953) observed no decrease in atherosclerosis in chickens fed cholesterol when high pyridoxine levels were fed. In fact, there appeared to be an enhancement of the lesions.

Martens and Hoskins (1958) observed no effect due to parenterally

administered pyridoxine on the serum cholesterol levels of rabbits when they were fed a stock ration. When a cholesterol-rich diet was fed, the parenterally administered pyridoxine had no effect on the degree of hypercholesterolemia nor on the degree of atherosclerosis.

Sakuragi (1959) observed an apparent need by rats for vitamin B_6 in the utilization of linoleic acid. Johnson et al. (1961) reported that the effects of pyridoxine deficiency on fat metabolism in rats are of a secondary nature connected with changes in protein and carbohydrate metabolism.

Dam et al. (1958) observed significantly higher plasma cholesterol levels in chicks receiving a vitamin B_6-deficient diet as compared to the level in the control chicks. Daghir and Balloun (1962) also observed increased serum cholesterol levels in vitamin B_6-deficient chicks. Aortas from chicks fed vitamin B_6-deficient diets were observed to be significantly heavier than aortas from chicks receiving diets adequate in vitamin B_6.

Mineral metabolism

Gubler et al. (1949) observed increased total body iron and copper values in pyridoxine-deficient rats. This finding is not consistent with the "mucosal block" theory of iron absorption. In pyridoxine-deficient rats, the quantity of iron utilized was low, while the quantity of iron stored was high.

Hsu et al. (1958) have shown vitamin B_6 to have an effect on the electrolytic balance in rat muscle. A deficiency of vitamin B_6 resulted in increased potassium and decreased sodium concentrations in muscle

tissue. The sodium level in blood serum was also increased in vitamin B_6-deficiency. Serum sodium levels returned to normal values after pyridoxine was administered.

General metabolic effects on the body

Scudi et al. (1942) studied vitamin B_6 excretion in man, dogs, and rats. In these species, vitamin B_6 was recovered in unidentified conjugated forms in urine.

Krehl (1957), in a review article on vitamin B_6 metabolism, discusses the role of vitamin B_6 in metabolic functions within the body. These roles include neurological functions, a role in the development of dental caries in vitamin B_6-deficient monkeys maintained on deficient diets for periods of two years or longer, effects on tryptophan metabolism resulting in increased amounts of xanthurenic acid being excreted in vitamin B_6 deficiencies in mammals, and a possible role in the development of atherosclerosis in experimental animals and humans.

Fuller and Dunahoo (1959) observed drug additives caused vitamin B_6-deficiency symptoms to occur earlier in chicks receiving vitamin B_6-deficient diets when compared with chicks receiving vitamin B_6-deficient diets without drugs. Added pyridoxine hydrochloride overcame the growth depression when added to diets containing furazolidone and arsanilic acid. When nitrofurazone was fed with either furazolidone or arsanilic acid, or both, added pyridoxine did not overcome the growth depression. The statiscal analysis indicated that a highly significant interaction existed between drug treatment and requirement for vitamin B_6.

Whiteside et al. (1962) studied the hematology of turkey hens receiv-

ing diets deficient in B vitamins. Nonsignificant differences were observed in hemoglobin and hematocrit values when turkey hens consumed vitamin B_6-deficient diets. Decreased levels of the vitamin in the diet resulted in decreased levels of the vitamin in eggs produced by the turkey hens. Pyridoxine deficiency appeared to depress appetite.

Effects of vitamin B_6 antagonists on poultry

Ott (1946, 1947) observed the effects of vitamin B_6 antimetabolites on chicks. Four moles of methoxypyridoxine and two moles of desoxypyridoxine were capable of counteracting the vitamin activity of one mole of pyridoxine when the antimetabolite was given orally 15 to 30 minutes after the pyridoxine-deficient chicks received an oral dose of pyridoxine. Chicks on a commercial starting ration were able to tolerate an oral dose more than six times as great as the lethal dose to more than half of the pyridoxine-deficient chicks.

Mushett et al. (1947) observed the pathological effects of desoxypyridoxine and methoxypyridoxine on lymphoid organs and hemopoietic tissue in laboratory animals. In chicks maintained on vitamin B_6-deficient diets for three to four weeks, the spleen weight to body weight ratio decreased. During this period, the spleen weight to body weight ratio in control chicks receiving a complete ration was increasing. Values of 0.064 and 0.234 were obtained for chicks fed deficient and adequate diets, respectively. Adding 200 mcg of desoxypyridoxine per 100 g of diet slightly suboptimal in pyridoxine resulted in a spleen weight to body weight ratio very closely approaching that recorded for chicks fed a

pyridoxine-free diet during the same period of time. In both instances, hypoplasia, or a failure of the limphoid tissue to develop, was the most prominent pathological feature.

Cravens and Snell (1949) and Karnofsky et al. (1950) observed toxic effects on chick embryos when vitamin B_6 antimetabolites were injected into eggs prior to or during incubation. The effect of the injected antimetabolite on mortality during the incubation period varied, with the toxicity generally being greater as the period of incubation progressed.

INVESTIGATIONS

Experiment 1

Objective

This experiment was designed to further study the relationship of vitamin B_6 on poultry breeds differing widely in type and size in an attempt to determine if a quantitative vitamin B_6 requirement could be assigned to each breed studied. Daghir and Balloun (1963) in research into vitamin B_6 requirements of three breeds of chickens, have reported a range of 2.2 to 2.6 mg of vitamin B_6 per kg of ration adequate for best growth and feed conversion.

Response criteria selected for observation in this experiment were growth, feed conversion, and mortality.

Materials and methods

A replicated complete randomized block design with a factorial arrangement of treatments was selected for the experimental design. Three hundred sixty one-day-old vent-sexed cockerels were used, with each experimental unit consisting of a pen of 12 chicks. The three breeds used in this study were (1) Fayoumi, an Egyptian breed having a small body size; (2) Single Comb White Leghorn, obtained from the Iowa State University genetic research flock; and (3) Vantress X Arbor Acres broiler-type chicks, a broiler cross selected for fast growth. The vitamin B_6 levels selected were 0.7, 0.8, 0.9, 1.0, and 1.1 mg of vitamin B_6 per pound of complete ration. The experimental ration was the same as used previously for vitamin B_6 research (Daghir, 1962) and is shown in Table 1.

Table 1. Composition of ration used in Experiment 1

Ingredient	Percent
Dextrose	65.8
Isolated soybean protein[a]	22.0
Soybean oil	2.0
Non-nutritive fiber (Alphacel)	2.0
Vitamin premix[b]	1.0
Mineral premix[c]	5.3
DL-methionine	0.6
Glycine	0.3
Choline chloride (25%)	1.0
	100.0

Calculated analysis

Protein (%)	18.6
Fat (%)	2.0
Crude fiber (%)	2.0
Calcium (%)	1.2
Phosphorus (%)	0.6
Productive energy (calories per lb)	1110
Vitamin B_6, before supplementation (mg per lb)	0.44

[a]Isolated soybean protein obtained from Archer Daniels Midland Company, Cincinnati, Ohio, and Nutritional Biochemicals Company, Cleveland, Ohio.

[b]Vitamin premix contributed the following per lb of complete ration:

Vitamin A	4540 IU	Thiamine HCl	2 mg
Vitamin D	681 ICU	Inositol	60 mg
Vitamin E	10 IU	PABA	30 mg
Menadione	2 mg	Niacin	40 mg
Riboflavin	4 mg	Vitamin C	100 mg
Folic acid	1.5 mg	Vitamin B_{12}	10 mcg
Calcium pantothenate	10 mg	Biotin	100 mcg

[c]Mineral premix contributed the following per lb of complete ration:

Sodium chloride	2.27 g	Manganese	32 mg
Calcium	5.45 g	Iron	41.6 mg
Phosphorus	2.72 g	Cobalt	1.13 mg
Zinc	2.93 mg	Copper	3.15 mg
Potassium	5.14 g	Iodine	1.64 mg
Magnesium	400 mg		

Pyridoxine hydrochloride was added in amounts needed to obtain the desired vitamin B_6 levels.

All chicks were housed in standard electrically-heated starter batteries with each tier divided by a plywood-screen divider. Chicks, in the two pens on a given tier, shared a water pan, but had separate feed pans. The two sides of the battery were used as a replication effect.

A four-week experimental period was used. Feed and water were consumed ad libitum. Daily observations were recorded on any abnormalities as well as mortality. Individual weights were recorded initially and weekly thereafter. Feed weights were recorded initially and at the end of the experimental period.

Analyses of variance tests were made on weekly weight gains, experimental period feed conversion ratios, and experimental period mortality. Analyses of covariance tests were made on total weight gains adjusted for total feed consumption and on mean weight gains adjusted for mean initial weights of chicks.

All windows were covered with shades and artificial lights were used continuously throughout the experimental period. Water pans were cleaned daily by scrubbing with a brush prior to rinsing with water.

Results and discussion

The average weight gains, feed conversion ratios, and mortality are presented in Table 2. The experiment failed to be a sensitive indicator of optimum vitamin B_6 level since weight gains generally reached a peak at a vitamin B_6 level of 0.9 to 1.0 mg per lb and decreased thereafter, rather than leveling off as expected. Isolated soybean protein from two

Table 2. Average weight gains, feed conversion ratios, and mortality (Experiment 1)

Breed	Vitamin B_6 level	Weight gains (grams)			Feed/Gain			Mortality[a] (no. dead)	
	(mg/lb)	Rep 1	Rep 2	Mean	Rep 1	Rep 2	Mean	Rep 1	Rep 2
Fayoumi	0.7	128.4	125.1	126.8	2.55	2.31	2.43	3	2
	0.8	206.4	221.9	214.2	2.07	2.08	2.08	0	2
	0.9	213.2	218.4	215.8	2.08	2.04	2.06	0	0
	1.0	223.6	208.3	216.0	2.06	2.11	2.08	0	0
	1.1	199.6	184.1	191.8	2.44	2.18	2.31	1	0
Vantress X Arbor Acres	0.7	205.7	268.2	237.0	1.88	1.75	1.82	0	0
	0.8	359.5	336.3	347.9	1.80	1.81	1.80	0	0
	0.9	364.7	355.1	359.9	1.73	1.78	1.76	0	0
	1.0	324.0	362.8	343.4	1.81	1.70	1.76	0	0
	1.1	329.8	350.8	340.3	1.86	1.78	1.82	1	0
White Leghorn	0.7	132.9	118.0	125.4	2.11	1.83	1.97	1	1
	0.8	210.2	214.9	212.6	2.00	1.92	1.96	1	1
	0.9	211.5	221.6	216.6	2.06	1.94	2.00	3	0
	1.0	242.4	219.4	230.9	1.93	1.94	1.94	2	2
	1.1	207.7	183.2	200.4	2.07	2.08	2.08	1	2

[a]Each pen contained 12 cockerels initially.

different sources was used in mixing the rations. The rations with 1.0 mg per lb or more of vitamin B_6 contained a different source of protein than those rations containing less than 1.0 mg per lb. A two-week experiment with chicks, comparing these two sources of protein, did not show any differences in growth rate over the two-week period. However, mortality and feed conversion data indicate that some toxic factor may have been present in the rations containing the higher vitamin B_6 levels which contained soybean protein from the new source. Another possibility is that the new product had undergone more purification in processing and thus was lower in vitamin B_6 content than calculated.

A summary of the analyses of variance tests of weekly weight gains, feed/gain ratios, and mortality is presented in Table 3. The variance in weight gains associated with breed differences was significant each week. The differences in gains contributed by vitamin B_6 levels were significant after the first week. No statistical significance was observed in breed x vitamin B_6-level interaction. Daghir and Balloun (1963) reported a significant difference due to this effect in their analysis of four-week weight data. All sources of variation determined in the feed conversion ratio analysis of variance were significant. Since the replication effect was significant in this analysis, while not significant in the other tests, this significance probably is due to chance variation associated with a very small error mean square.

The analysis of mortality data showed a significant breed effect. This is shown by the moderate number of deaths in Fayoumi and White Leghorn breeds while the broiler-cross death loss of one out of 120 chicks

Table 3. Summary of the statistical analyses of weekly weight gains, feed/gain ratios, and mortality (Experiment 1)

Source of variation	Degrees of freedom	Mean squares					
		Weekly weight gains				Feed/Gain	Mortality
		First	Second	Third	Fourth		
Replications	1	0.08	16.14	1.37	35.21	0.0480*	0.300
Breeds (B)	2	699.74**	1187.83**	4614.74**	12245.68**	0.4041**	4.235*
Vit B_6 levels (V)	4	34.07	504.53**	1572.65**	1716.77**	0.0324**	0.385
B x V	8	13.86	39.77	115.08	27.70	0.0262**	1.108
Error	14	19.99	61.56	72.96	164.38	0.0061	0.586

*Significant at $P < .05$.

**Significant at $P < .01$.

is negligible. No consideration is given to the time within the experimental period when death occurred and since some deaths with Leghorn breed chicks occurred in the first week these deaths could have been due to starvation. Some hyperexciteability was noted in some pens, as well as apparent wing feather follicle hemorrhages.

Since large differences existed in total feed consumption, an analysis of covariance test was made of total experimental unit weight gains adjusting for experimental unit feed consumption. However, since loss of appetite is usually observed in vitamin B_6-deficient chicks, differences were expected to occur. A second analysis of covariance test was made, adjusting mean experimental unit weight gains for mean experimental unit initial weights. Summaries of these two analyses of covariance tests are presented in Table 4.

When total weight gains are adjusted for the effect of amount of feed consumed, breed differences were significant. The nonsignificance of the vitamin B_6-level mean square substantiates the reports on vitamin B_6 deficiency effects on loss of appetite.

The lack of a relationship between initial chick weights and subsequent weight gains is shown by the analysis of covariance test which adjusted mean experimental unit weight gains for mean experimental unit initial weights. In this test, both breed and vitamin B_6 level were significant as they were in the analyses of variance tests completed on second, third, and fourth week weight gains (Table 3).

Table 4. Summary of analyses of covariance tests of total weight gains and mean experimental unit weight gains (Experiment 1)

Source of variation	Degrees of freedom	Total weight gains adjusted for total feed consumption		Mean experimental unit weight gains adjusted for initial wt	
		Adjusted sums of squares	Adjusted mean squares	Adjusted sums of squares	Adjusted mean squares
Error	13	0.088	0.0068	4,399.57	338.43
Breed + error	15	0.622		8,562.37	
Difference for testing	2	0.534	0.267**	4,162.80	2,081.40*
Vit levels + error	17	0.172		47,206.42	
Difference for testing	4	0.084	0.021	42,806.85	10,701.71**
VxB + error	21	0.152		5,830.43	
Difference for testing	8	0.064	0.008	1,430.86	178.86

*Significant at $P < .05$.

**Significant at $P < .01$.

Experiment 2

Objective

There is an apparent lack of information about the effects of a vitamin B_6 deficiency on hemoglobin level and packed cell volume of deficient chicks. This experiment was designed to study these relationships and to determine if one or both of these values could be used in future vitamin B_6 research as additional criteria of response.

In addition, data on weight gains, feed consumption, and mortality were recorded. In order to determine what effect reduced feed consumption, due to loss of appetite in vitamin B_6 deficiency, has on response, one group of chicks on an adequate vitamin B_6 ration was pair-fed with the vitamin B_6-deficient group.

Materials and methods

Broiler-type vent-sexed cockerels were used in the four-week experiment. The cockerels were placed on a standard starter diet for a one-week pre-trial period, in order to eliminate any deaths due to starvation. Three replications, twelve chicks per replication, were used for each of the three treatments: (1) adequate B_6, consumption ad libitum (referred to as ad. B_6, ad lib.), (2) deficient B_6, consumption ad libitum (referred to as def. B_6, ad lib.), and (3) adequate B_6, consumption restricted within a replication to that consumed by the deficient B_6 group (referred to as ad. B_6, rest.). A completely random design was used for the experiment.

Chicks were housed in batteries similar to those used in Experiment

1, with other management techniques similar to those described previously. In an attempt to obtain better growth of chicks on these diets, the protein level was increased with a corresponding decrease in carbohydrate. Machlin and Gordon (1958) obtained excellent chick weight gains by feeding a semi-purified diet containing a higher protein level than that used in Experiment 1. The composition and calculated analysis of the diet used in this experiment are shown in Table 5. While the calculated tryptophan content was considered to be adequate, a small amount was added since tryptophan-vitamin B_6 relationship studies were planned for future research, in which this diet would be used as the experimental ration. The adequate B_6 ration contained added pyridoxine HCl giving a level of 1.3 mg of vitamin B_6 per lb of complete ration. No pyridoxine HCl was added to the deficient B_6 ration, thus providing approximately 0.6 mg of vitamin B_6 per lb. After mixing, all diets were stored in a walk-in cooler, being removed only during feeding periods.

The amount of feed consumed by the vitamin B_6-deficient groups, during the previous 24 hours, was determined each morning. This amount was then fed to the pair-fed group which received the adequate B_6, restricted ration. When mortality occurred in an experimental pen, mean feed consumption was determined for the deficient B_6 group and an adjustment made in the amount fed to the pair-fed group by converting from a mean intake basis to a total basis depending on the number of living chicks in the pen.

Weekly blood samples were obtained from six randomly selected chicks in each pen. A wing vein was punctured with a lancet and free flowing

Table 5. Composition and calculated analysis of experimental ration used in Experiment 2

Ingredient	Percent
Dextrose	58.85
Isolated soybean protein[a]	28.00
Soybean oil	2.00
Non-nutritive fiber (Alphacel)	3.00
Vitamin premix[b]	1.00
Mineral premix[b]	5.30
Choline chloride (25%)	1.00
DL-methionine	0.70
Glycine	0.10
DL-tryptophan	0.05
	100.00
Calculated analysis	
Protein (%)	22.96
Fat (%)	2.00
Crude fiber (%)	3.00
Calcium (%)	1.2
Phosphorus (%)	0.6
Productive energy	1075 calories per lb
Vitamin B_6, before supplementation	0.56 mg per lb

[a]Obtained from Nutritional Biochemicals Co., Cleveland, Ohio.

[b]Composition given at bottom of Table 1, page 14.

samples of blood were obtained for hemoglobin (Hb) and packed cell volume (PCV) determinations. The same six chicks in a given pen were used throughout the trial unless a death occurred, in which case a replacement was randomly selected from the remaining chicks in the pen.

Hemoglobin determinations were made using the technique of Bankowski (1942) as modified by Denington and Lucas (1955). Packed cell volumes were determined by a microcapillary method similar to that described by Natelson (1961). A clinical centrifuge with a head designed for micro-

capillary tubes was used. Centrifugation was at a speed of 5000 rpm for eight minutes. Packed cell volumes were read directly by utilizing a hematocrit reader.

The statistical analyses performed on these data included determination of the correlation between PCV and hemoglobin values and analyses of variance tests on weekly hemoglobin and PCV values. Planned comparisons were made comparing PCV and hemoglobin values of the chicks consuming the adequate B_6, restricted diet treatment with those of the deficient B_6, ad libitum group and PCV and hemoglobin values of the adequate B_6, ad libitum with the combined values of the adequate B_6, restricted diet group and the deficient B_6, ad libitum groups. An analysis of variance test was also made on experimental period feed conversion ratios, breaking the treatment sum of squares into the same planned comparisons made on PCV and hemoglobin values.

Results and discussion

The results of the analyses of variance tests on hemoglobin values are presented in Table 6 and Figures 1 and 2. The chicks on the vitamin B_6-deficient diet (0.6 mg of vit B_6 per lb) had depressed hemoglobin values (Figure 1). The lack of sufficient vitamin B_6 reduced hemoglobin to a significant degree compared with the pair-fed group values in the second, third, and fourth weeks. When these two groups were compared to the group receiving adequate vitamin B_6 on an ad libitum feeding regime (Figure 2), the mean hemoglobin value was significantly higher at the end of the first and third weeks. The F value at the end of the second week approached significance at the five percent level.

Table 6. Summary of mean squares from analyses of variance tests on weekly hemoglobin and packed cell volume values (Experiment 2)

Determination and Source of variation	Degrees of freedom	Week of experiment			
		First	Second	Third	Fourth
Hemoglobin					
Comparison 1[a]	1	0.0541	4.9504*	4.7171**	14.2913**
Comparison 2[b]	1	0.6087*	4.6309	4.7432**	0.0242
Error	6	0.0952	0.8176	0.2673	1.0388
Packed cell volume					
Comparison 1	1	3.53	37.50*	32.67	150.00*
Comparison 2	1	13.52*	44.18**	50.67	6.97
Error	6	1.368	2.957	10.845	15.390

[a]Comparison made between groups fed 0.6 mg vit B_6/lb, ad libitum intake and groups fed 1.3 mg vit B_6/lb, restricted intake.

[b]Comparison between group receiving 1.3 mg vit B_6/lb, ad libitum intake and combined value of groups receiving 0.6 mg vit B_6/lb, ad libitum intake and 1.3 mg vit B_6/lb, restricted intake.

*Significant at $P < .05$.

**Significant at $P < .01$.

Figure 1. Hemoglobin levels of chicks receiving a vitamin B_6-deficient diet, ad libitum intake (def. B_6, ad lib.) compared to the hemoglobin levels of chicks consuming a diet containing adequate vitamin B_6 with feed intake restricted (ad. B_6, rest.) to the amount consumed by the vitamin B_6-deficient group

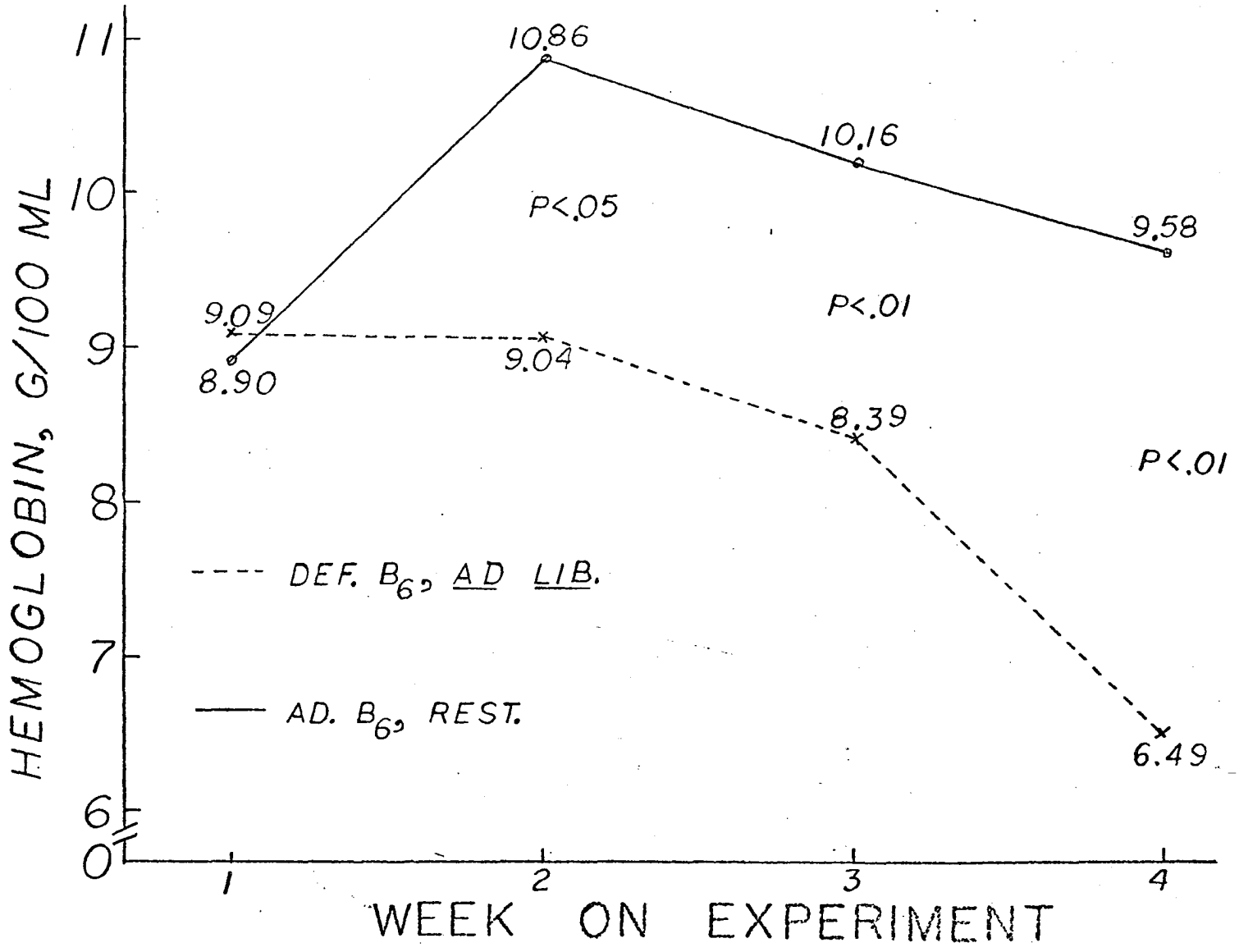
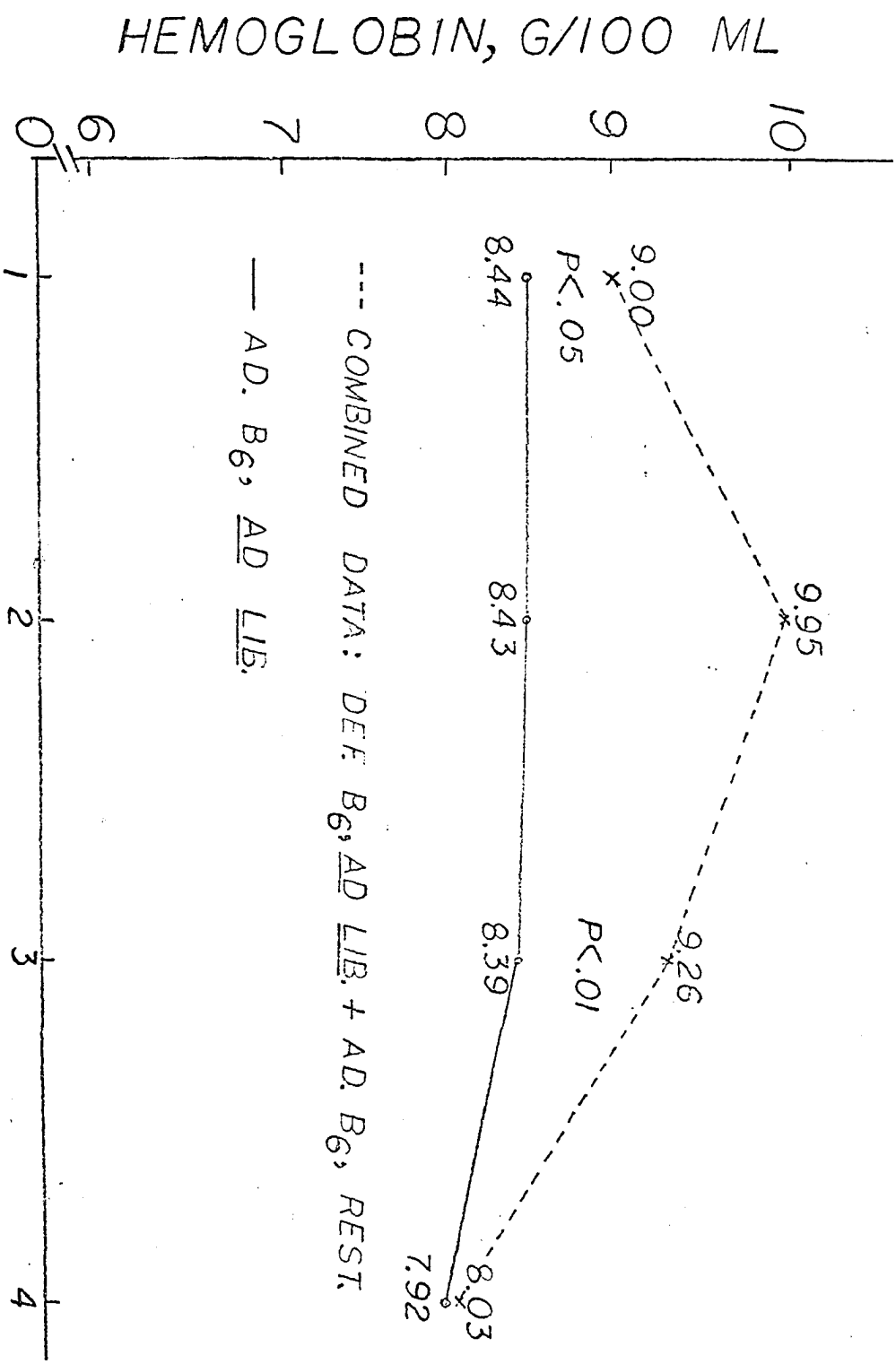

HEMOGLOBIN, G/100 ML
11
10
9
8
7
6
0
10.86
10.16
9.58
9.09
8.90
9.04
8.39
6.49
P<.05
P<.01
P<.01
---- DEF. B6, AD LIB.
—— AD. B6, REST.
1
2
3
4
WEEK ON EXPERIMENT

Figure 2. Hemoglobin levels of chicks consuming a diet containing adequate vitamin B_6, ad libitum intake (ad. B_6, ad lib.) compared to the combined data of chicks fed a vitamin B_6-deficient diet, ad libitum intake and chicks fed an adequate vitamin B_6 diet with feed intake restricted to the amount consumed by the vitamin B_6-deficient chicks (def. B_6, ad lib. + ad. B_6, rest.)


HEMOGLOBIN, G/100 ML
6
7
8
9
10
0
1
2
3
4
WEEK ON EXPERIMENT
9.00
8.44
8.43
8.39
7.92
9.95
9.26
8.03
P<.05
P<.01
--- COMBINED DATA: DEF. B6, AD LIB. + AD. B6, REST.
— AD. B6, AD LIB.

The PCV values exhibited more variability than the hemoglobin values with the analyses of variance tests showing a generally similar pattern. The results of the analyses of variance tests of PCV values are presented in Table 6, with a graphical presentation of PCV values in Figures 3 and 4.

Feed conversion data, presented in Table 7, were similar in the two groups receiving the same amount of feed. While some differences existed, these were not significant. The group receiving adequate vitamin B_6 with ad libitum intake had a significantly better feed conversion ratio than the combined value of the other two groups.

When chicks received essentially the same amount of feed, whether on the deficient B_6, ad libitum or adequate B_6, restricted rations, weight gains of surviving chicks were similar (Table 7). This indicates that a major cause of the greatly reduced rate of gain in vitamin B_6-deficient chicks is the loss of appetite and consequent reduced feed intake.

Mortality data presented in Table 7 indicate that some deaths occurring in a vitamin B_6 deficiency are due to starvation rather than due to direct metabolic effects, such as loss of coenzyme activity. Nearly one-third of the deaths of deficient chicks occurred before the second week while one-fifth of the deaths on the pair-fed adequate B_6, restricted group occurred during this period. The one death recorded in the adequate B_6, ad libitum group occurred during this period. Most reports indicate that vitamin B_6-deficiency symptoms occur at about 10-14 days, with deaths occurring at this time. The relative proportion of deaths occurring in the vitamin B_6-deficient group during this period would

Figure 3. Packed cell volume values of chicks consuming a vitamin B_6-deficient diet, ad libitum intake (def. B_6, ad lib.) compared with PCV of chicks consuming a diet containing adequate vitamin B_6 with feed intake restricted (ad. B_6, rest.) to the amount consumed by the vitamin B_6-deficient group

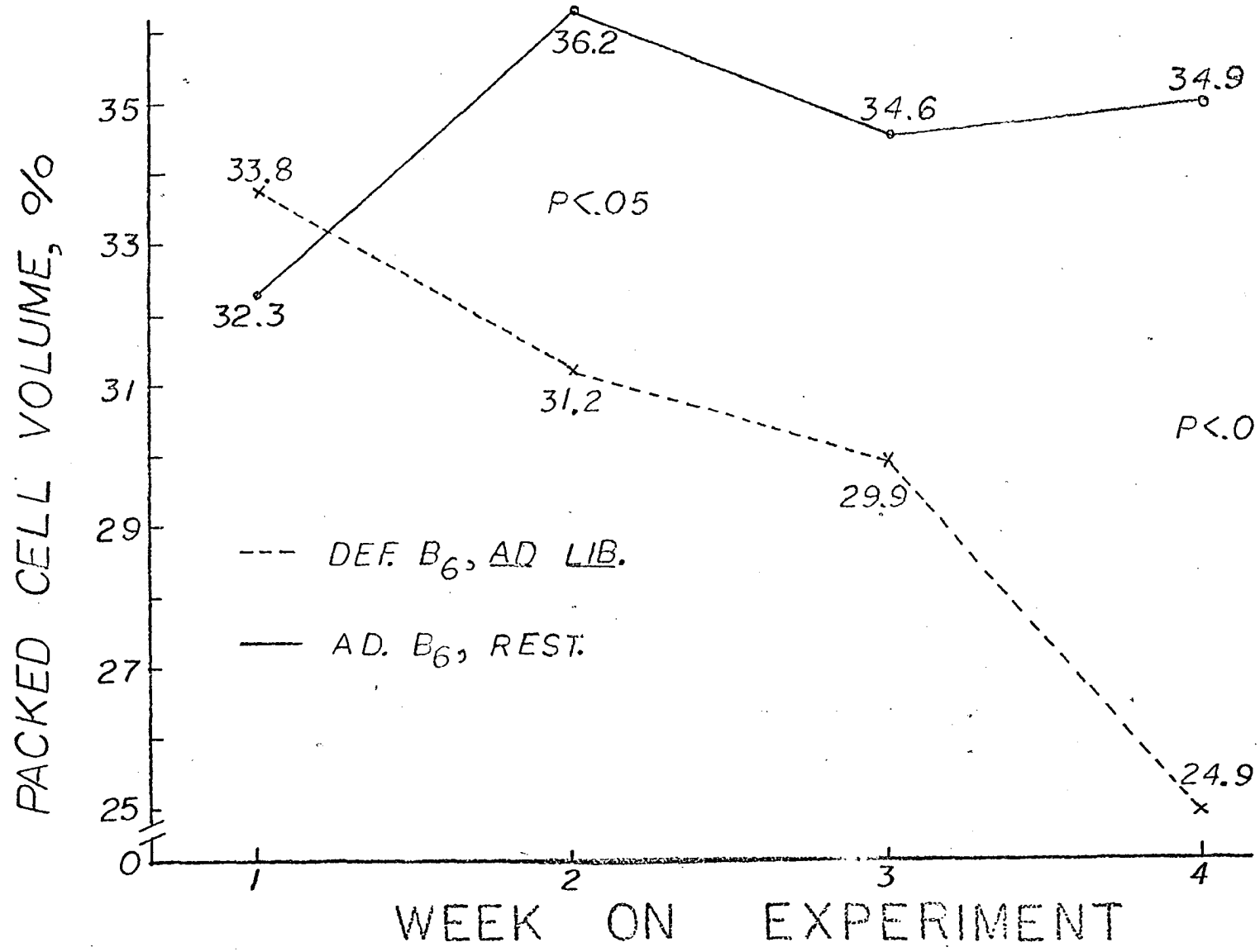

PACKED CELL VOLUME, %
35
33
31
29
27
25
0
1
2
3
4
WEEK ON EXPERIMENT
36.2
34.6
34.9
33.8
32.3
31.2
29.9
24.9
P<.05
P<.05
--- DEF. B6, AD LIB.
—— AD. B6, REST.

Figure 4. Packed cell volume of chicks consuming a diet containing adequate vitamin B_6, ad libitum intake (ad. B_6, ad lib.) compared to the combined data of chicks fed a vitamin B_6-deficient diet, ad libitum intake and chicks fed an adequate vitamin B_6 diet with feed intake restricted to the amount consumed by the vitamin B_6-deficient chicks (def. B_6, ad lib. + ad. B_6, rest.)

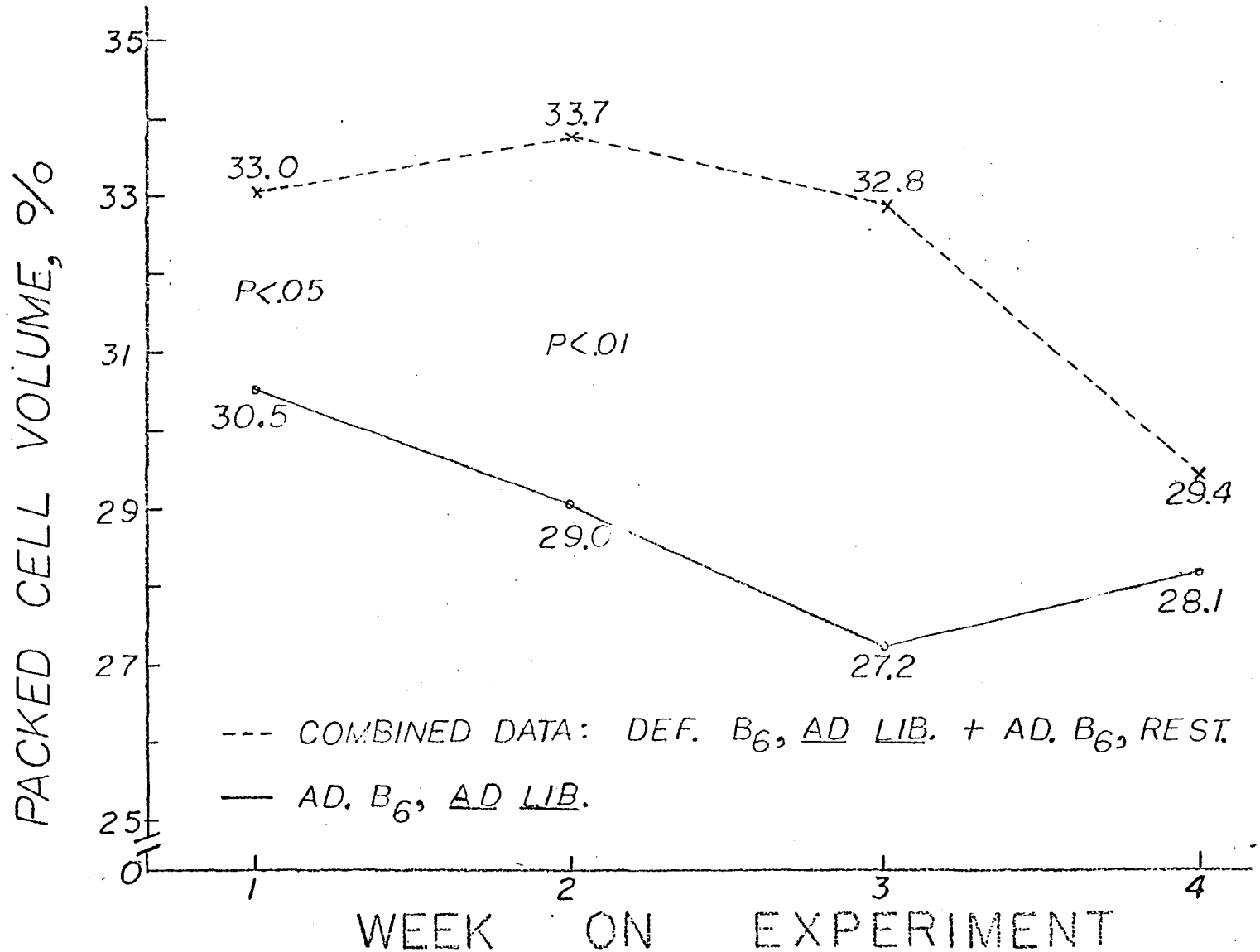

PACKED CELL VOLUME, %
35
33
31
29
27
25
0
33.0
33.7
32.8
29.4
30.5
29.0
27.2
28.1
P<.05
P<.01
--- COMBINED DATA: DEF. B6, AD LIB. + AD. B6, REST.
— AD. B6, AD LIB.
1
2
3
4
WEEK ON EXPERIMENT

Table 7. Average weight gains, feed conversion ratios, and mortality (Experiment 2)

Determination	Vitamin B_6 level and feeding regime[a]	Rep 1	Rep 2	Rep 3	Mean
Average 4-week weight gains of survivors (grams)	0.6 mg/lb, ad lib.	52.7	68.0	46.5	55.7
	1.3 mg/lb, rest.	55.9	58.1	73.9	62.6
	1.3 mg/lb, ad lib.	405.7	423.1	418.9	415.9
Feed conversion ratios	0.6 mg/lb, ad lib.	3.75	4.03	4.28	4.02
	1.3 mg/lb, rest.	4.68	4.64	4.04	4.45
	1.3 mg/lb, ad lib.	2.00	1.99	2.11	2.03
Mortality (number dead)[b]	0.6 mg/lb, ad lib.	9	10	10	
	1.3 mg/lb, rest.	4	2	4	
	1.3 mg/lb, ad lib.	0	1	0	

[a] Ad lib. is ad libitum, rest. is restricted.

[b] All pens contained 12 chicks initially.

indicate that these chicks were dying from a vitamin B_6 deficiency. This view was substantiated by post mortem findings of wing feather follicle hemorrhage, occurring in chicks from vitamin B_6-deficient groups. The degree of hemorrhage varied from slight in some chicks to severe in others. During periods when blood samples were being obtained for hemoglobin and PCV determinations, some chicks from deficient groups were observed to have increased blood clotting times, although no quantitative determinations

were made on this abnormality.

A correlation of 0.906 was obtained between hemoglobin and packed cell volume values. This correlation value was significantly different, at the one percent probability level, from a hypothesized correlation value of zero. The high correlation indicates that either determination could be used as a measure of chick hematological response to a vitamin B_6 deficiency.

Experiment 3

Objective

Semi-purified diets are more expensive than practical diets and require special handling in order to prevent destruction or inactivation of vitamins by heat, light, or minerals present in the diets. Since Ott (1946) had shown desoxypyridoxine to be a potent inhibitor of vitamin B_6 in chicks, it was desired to investigate the use of this compound in producing a vitamin B_6 deficiency in chicks receiving a practical diet.

Materials and methods

A completely randomized design was used in this experiment with three replications of each of the six treatments. Broiler type, vent-sexed males were used with ten cockerels randomly allotted to each pen. Chicks were housed in starter batteries similar to those used in Experiments 1 and 2. During the 24-day experiment, chicks consumed feed and water *ad libitum*.

Response was measured by mortality, weight gains, PCV values, and feed conversion ratios. Weights were recorded initially, on the ninth

day, and at the end of the experiment. Observations on mortality were made daily. Packed cell volume determinations were made on four chicks in each pen on the ninth and on the last day of the experiment. Feed conversion ratios were determined at the end of the trial.

The composition of the practical ration used in this experiment is shown in Table 8. Levels of desoxypyridoxine used were 0.0, 2.3, 5.3, 12.2, 28.1, and 64.6 mg per lb of ration, selection of the levels being based on publications of the National Academy of Sciences - National Research Council (1956, 1958). These publications list the pyridoxine content of corn as 3.8 mg per lb, while no report is given on vitamin B_6 content of soybean oil meal. Using this analysis gave a calculated vitamin B_6 content in the ration of 2.3 mg per lb of complete ration.

During the trial, corn and soybean oil meal were assayed for vitamin B_6 content. This was done in order to determine more precisely the level of antimetabolite which effectively inhibited the "natural" vitamin B_6 content of the ration. Procedures and results of this assay are presented in Appendix A.

Analyses of variance tests were made on PCV values, feed conversion ratios, and mean weight gains. Dunnett's test (1955) was used to compare weight gains of groups receiving the antimetabolite to the weight gains of the control group which received no supplemental antimetabolite. A median lethal dose (LD 50) was determined from mortality data using the technique of probit analysis (Finney, 1952).

Table 8. Composition and calculated analysis of experimental ration (Experiment 3)

Ingredient	Percent
Ground yellow corn	61.5
Soybean oil meal (50% solvent)	32.0
Soybean oil	2.0
Mineral mixture[a]	0.5
Dicalcium phosphate	2.5
Ground oyster shell	1.0
Vitamin mixture[b]	0.5
	100.0

Calculated analysis

Protein (%)	22.6
Fat (%)	5.0
Crude fiber (%)	2.4
Calcium (%)	1.2
Phosphorus (%)	0.9
Productive energy (calories per lb)	1015
Vitamin B_6 (mg/lb)	2.3

[a]Mineral mixture provided per lb:

Sodium chloride	1.93 g	Iron	23 mg
Manganese	57 mg	Copper	2.27 mg
Zinc	23 mg		

[b]Vitamin mixture provided following levels per lb:

Vitamin A	2500 IU	Ca pantothenate	2 mg
Vitamin D	500 ICU	Niacin	12 mg
Vitamin E	5 IU	Choline	220 mg
Menadione	1 mg	Vitamin B_{12}	6 mcg
Riboflavin	2 mg		

Results and discussion

Data on PCV values were combined over the two sampling periods and a random day effect and a day x treatment interaction determined in the analysis of variance. These data and the analysis of variance are presented in Tables 9 and 10, respectively. The main effect due to days

Table 9. Effect of desoxypyridoxine on packed cell volume values (Experiment 3)

Level of anti-metabolite (mg per lb)	Packed cell volume, %							
	9th day				24th day			
	Rep 1	Rep 2	Rep 3	Mean	Rep 1	Rep 2	Rep 3	Mean
0.0	33.2	36.8	32.2	34.1	29.8	31.2	33.2	31.4
2.3	32.8	33.0	31.0	32.3	30.5	32.5	31.0	31.3
5.3	33.2	34.8	30.8	32.9	31.8	32.2	30.8	31.6
12.2	29.5	29.5	32.5	30.5	29.5	32.2	31.2	31.0
28.1	30.3	32.0	31.8	31.4	31.5	29.8	30.5	30.6
64.6	29.0	28.0	25.0	27.3	25.8	29.5	23.0	26.1

and the day x treatment interaction were nonsignificant. The treatment effect was significant at the one percent probability level. The most noticeable effect of treatments on PCV was a depression of the PCV values by the highest level of desoxypyridoxine (64.6 mg per lb).

Experimental period mean weight gains and the analysis of variance of these data are presented in Tables 11 and 12, respectively. Dunnett's test indicated that the addition of the two highest levels of

Table 10. Analysis of variance of packed cell volume values (Experiment 3)

Source of variation	Degrees of freedom	Sum of squares	Mean square	Expected mean square[a]
Days (D)	1	10.454	10.454	$\sigma^2 + 12\ \sigma^2_D$
Treatment (T)	5	141.415	28.283**	$\sigma^2 + 3\ \sigma^2_{DT} + 6\ \sigma^2_T$
D x T	5	7.676	1.535	$\sigma^2 + 3\ \sigma^2_{DT}$
Error	24	74.127	3.089	σ^2

[a]Treatment effect fixed, day effect random.

**Significant at $P < .01$.

desoxypyridoxine depressed growth to a significant degree ($P < .01$) over that attained by control chicks. The calculated vitamin B_6 content in the diet, using assay results (Appendix A), was 1.93 mg per lb of complete ration. The results of this experiment confirm the findings of Ott (1946) that chicks are able to tolerate the antimetabolite when receiving a practical ration containing sufficient vitamin B_6.

Rabinowitz and Snell (1951) discuss some shortcomings in the microbiological assay utilizing Saccharomyces carlsbergensis. However, assuming the vitamin B_6 assay to be accurate, statistical analysis of weight gains indicated that a level of desoxypyridoxine 14.6 or greater (levels of 28.1 and 64.6 mg per lb) than the assayed vitamin B_6 content significantly depressed growth over that attained by the control ($P<.01$).

Table 11. Effect of desoxypyridoxine on weight gains (Experiment 3)

Level of antimetabolite (mg per lb)	Weight gains, grams			
	Rep 1	Rep 2	Rep 3	Mean
0.0	347.8	344.4	360.7	351.0
2.3	357.2	369.9	344.5	357.2
5.3	339.8	345.5	310.5	331.9
12.2	317.0	280.1	335.4	310.8
28.1	241.7	268.2	183.0	231.0[a]
64.6	174.5	192.5	184.0	183.7[a]

[a]Significantly different from control ($P < .01$).

Table 12. Analysis of variance of weight gains (Experiment 3)

Source of variation	Degrees of freedom	Sum of squares	Mean square
Treatments	5	75,323.93	15,064.79**
Linear	1	65,504.47	65,504.47**
Quadratic	1	8,437.89	8,437.89**
Remainder	3	1,381.57	460.52
Error	12	6,726.38	560.53

**Significant at $P < .01$.

The median dose level (12.2 mg per lb) resulted in a growth depression which approached significance at the five percent level of probability (difference of 48.3 grams needed for significance). The median level was 6.3 times greater than the assayed vitamin B_6 content of the diet. These results indicate, that in this experiment, a level of antimetabolite approximately ten times the vitamin B_6 level would result in a significant depression of weight gains. Linear and quadratic effects of weight gains on log dose were highly significant, indicating that a linear depression of weight gains occurred with a log increase in dose. The statistically significant quadratic effect probably is due to the apparent growth stimulation which occurred when chicks received the lowest level of antimetabolite (2.3 mg per lb).

Results of the probit analysis of mortality data are presented in Appendix B. The median lethal dose was 31.6 mg of desoxypyridoxine HCl per lb of complete ration with five percent fiducial limits of 24.3 and 41.8 mg per lb for lower and upper limits, respectively. A comparison of the statistical analyses of growth and mortality data indicates that a level of desoxypyridoxine 14 times greater than the assayed vitamin B_6 content of the ration produced apparent vitamin B_6 deficiency in chicks. This level is higher than that reported by Ott (1946), however, his evaluation was based on fewer chicks observed over a shorter period of time. Oral dosing was used in his research rather than feeding the antimetabolite as part of the complete ration.

Experiment 4

Objective

The previous trials have not indicated to what extent, if any, the chick is able to recover from a vitamin B_6 deficiency when placed on a diet containing sufficient vitamin B_6. No reports on this aspect of vitamin B_6 metabolism were found in the literature search.

This experiment was designed to study the recovery rate of vitamin B_6-deficient chicks when placed on a diet suboptimal or optimal in vitamin B_6. The rate of development of a vitamin B_6 deficiency was also studied.

Response criteria were weight gains (expressed as average daily gains), feed conversion ratios, and change in PCV values.

Materials and methods

Twelve vent-sexed male broiler-type chicks were used in this experiment. All chicks were housed in standard starter batteries during a two-week pre-trial period. During this pre-trial period, a standard starter ration was fed for the first ten days followed by a semi-purified diet containing 0.6 mg of vitamin B_6 per lb of complete ration. At the end of this pre-trial period, chicks were randomly assigned to individual cages and housed in these for the eight-week experimental period. The individual wire cages measured 18 x 18 x 14 inches and contained individual waterers and feeders. Chicks consumed feed and water *ad libitum* throughout the experiment.

A balanced extra-period change-over design was used (Patterson and Lucas, 1962) with the length of each period arbitrarily selected as two

weeks. The experimental unit consisted of the individual chick. During the trial, one chick was inadvertently killed thereby necessitating calculation of missing data. These calculations were based on the method of Lucas (1957).

Details of the semi-purified, low vitamin B_6 basal diet used in this trial are given in Table 13. Pyridoxine HCl was added to the basal ration

Table 13. Composition and calculated analysis of basal diet used in Experiment 4

Ingredient		Percent
Dextrose		59.10
Isolated soybean protein[a]		28.00
Soybean oil		2.00
Non-nutritive fiber (Alphacel)		3.00
Vitamin premix[b]		1.00
Mineral premix[b]		5.30
Choline chloride (25%)		1.00
DL-methionine		0.40
Glycine		0.10
DL-tryptophan		0.10
Calculated analysis		
Protein (%)		22.96
Fat (%)		2.00
Crude fiber (%)		3.00
Calcium (%)		1.2
Phosphorus (%)		0.6
Productive energy (calories per lb)	1075	
Vitamin B_6, before supplementation (mg per lb)[c]	0.19	

[a]Obtained from Nutritional Biochemicals Company, Cleveland, Ohio.

[b]Composition given at bottom of Table 1, page 14.

[c]Based on assay; results of assay given in Appendix A.

in amounts needed to give the desired levels of vitamin B_6 in the three treatments, 0.6, 0.9, and 1.2 mg of vitamin B_6 per lb of complete ration. The supplemental amount of pyridoxine HCl was determined from calculations based on assayed vitamin B_6 content of isolated soybean protein (Appendix A). The assayed content (0.67 mg per lb) was considerably less than the content listed for the similar product used in Experiment 1 (2.01 mg per lb). After mixing, the semi-purified diet was kept in a walk-in cooler until fed.

Chick weights, PCV value determinations, and feed weights were recorded at the beginning and end of each two-week period. After these data were recorded, chicks were fed the diet that they were to consume in the succeeding period.

Results and discussion

Average daily gains, feed conversion ratios, and change in packed cell volume values over the two-week experimental period are presented in Appendix C. A summary of the analyses of variance tests of these response criteria is presented in Table 14.

A large portion of the total sum of squares was attributed to differences associated with periods within squares. One would expect differences in average daily gain as chicks become older, provided the diet was nutritionally adequate. Poorer feed efficiency values in the older chicks (Periods 3 and 4) can be attributed in part to feed wastage. Packed cell volume values generally decreased throughout the trial. However, in Period 3, these values generally increased. This change in the trend probably was sufficient to account for the large sum of squares associated

Table 14. Summary of analyses of variance of average daily gains, feed conversion ratios, and change in packed cell volume (Experiment 4)

Source of variation	Degrees of freedom	Average daily gains		Feed conversion ratios		Change in packed cell volume	
		Sums of squares	Mean squares	Sums of squares	Mean squares	Sums of squares	Mean squares
Squares	3	97.83		0.0220		51.90	
Periods within squares	12	790.63		5.9935		422.94	
Chicks within squares	8	191.25		0.6436		47.31	
Direct effects	2	11.77	5.885	0.0059	0.0029	7.91	3.955
Residual effects	2	4.26	2.130	0.0165	0.0082	40.39	20.195
Error	18[a]	87.99	4.888	0.7455	0.0414	389.02	22.884

[a]Error degrees of freedom for change in packed cell volume is 17.

with this effect.

No statistical differences due to residual or direct effects of treatments were detected in any of the three response criteria. The lack of significance seems to indicate that an error had been made in the vitamin B_6 assay of isolated soybean protein, or that the two-week periods were of too short duration for differences to become apparent. Since severe vitamin B_6 deficiency usually develops in two weeks, it would seem likely that no diets were actually vitamin B_6 deficient.

GENERAL DISCUSSION AND CONCLUSIONS

Weight gains attained by chicks in Experiment 1 did not plateau as one would expect at the optimum vitamin B_6 level, instead, the weight gains exhibited a peak at the median vitamin B_6 level and thereafter decreased somewhat with higher levels of vitamin B_6. There are several possible explanations for this phenomenon. Since a different isolated soybean protein source was used in the rations containing the higher levels of vitamin B_6, it is possible that the vitamin B_6 content in this product was lower than expected. Another possibility is the presence of a toxic factor in this isolated soybean protein product. This phenomenon could be related to an error in mixing, such as omitting some ingredients. Using a vitamin premix which had lost some vitamin activities could also result in a growth depression, however, the same vitamin premix was used throughout, thus apparently eliminating this possibility. The two most likely reasons appear to be an error in mixing or deficiencies in the isolated soybean protein source such as lower vitamin B_6 content than expected or the presence of some toxic factor.

In this trial, adjusting weight gains for initial weights, by an analysis of covariance, resulted in significant breed and vitamin B_6 level effects. As expected, broiler males, selected for fast growth, had larger weight gains than the White Leghorn or Fayoumi males. Weight gains of White Leghorns and Fayoumi males were quite similar.

The lack of sensitivity encountered in this trial did not allow a quantitative vitamin B_6 requirement to be assigned to each breed utilized in the trial. A level of 0.9 mg of vitamin B_6 per lb provided optimum

growth in this trial, however, this level probably is slightly suboptimal for the fast growing chicks which would appear to have a more critical need for this coenzyme than chicks growing at a slower rate. In order to quantitatively determine if breed differences exist in vitamin B_6 requirements of chicks, it will be necessary to repeat this trial using a protein source of known vitamin B_6 content and supplementing to obtain desired dietary levels of vitamin B_6. The basal ration probably should contain a higher protein level since the ration used in this trial may be suboptimal in protein.

Feed conversion values were quite variable in Experiment 1 without any clear trends exhibited. Mortality data generally were quite variable as well, with more deaths occurring in the White Leghorn chicks. Essentially no mortality was observed in the broiler cross, with a moderate number of deaths occurring with Fayoumi chicks.

In Experiment 2, when daily feed consumption of chicks receiving a ration containing sufficient vitamin B_6 was reduced to that of vitamin B_6-deficient chicks, weight gains were essentially the same. Feed conversion ratios also were similar. Mortality in the group receiving sufficient vitamin B_6 indicated that some of the deaths observed in deficient chicks can be related to starvation due to a loss of appetite in vitamin B_6 deficiency. Loss of appetite has been reported by most researchers as one of the symptoms of a vitamin B_6 deficiency in chicks.

The extent to which weight gains were depressed and the number of deaths encountered in pair-fed chicks receiving vitamin B_6-adequate rations was unexpected. These results indicate that vitamin B_6 is very

important in stimulating chicks to eat. The method whereby vitamin B_6 influences appetite is unknown, but possibly is due to indirect effects such as a decreased rate of metabolism resulting in increased serum glucose levels. The possible role of vitamin B_6 in loss of appetite in chicks seems to be an area for future vitamin B_6 research.

Changes in hemoglobin and packed cell volume throughout the experiment generally showed similar patterns. During the last week of the trial, vitamin B_6-deficient chicks exhibited depressed hemoglobin and packed cell values. The degree of depression generally was statistically significant. The high correlation (0.906) between hemoglobin and packed cell volume determinations is associated with a close relationship between level of hemoglobin and packed cell volume in chicks. This high correlation indicates that a vitamin B_6 deficiency results in a reduced rate of formation of erythrocytes in chicks rather than in a change in the size of cell produced. Hegsted and Rao (1945) observed microcytic anemia in vitamin B_6-deficient ducklings. Whiteside et al. (1962) observed nonsignificant differences in hemoglobin and hematocrit values in turkey hens consuming vitamin B_6-deficient diets as compared to turkey hens consuming diets containing adequate vitamin B_6. A comparison of these results would seem to indicate that a species difference between turkeys and chickens exists in vitamin B_6 metabolism.

Results of Experiment 3 indicate that a vitamin B_6 deficiency can be produced on a practical ration by supplementing the ration with a vitamin B_6 antimetabolite. Since results of the vitamin B_6 assay on ration ingredients seem to be too low, the effective level of desoxypyridoxine

was not determined quantitatively. The results did indicate that a level of desoxypyridoxine 14.6 times greater than the vitamin B_6 content produced a significant depression in weight gains.

Level of desoxypyridoxine had a significant effect on packed cell volume values, with a distinct depression of these values in chicks receiving the highest level of antimetabolite. Ott (1946) conducted somewhat similar research, but used oral dosing on three alternate days rather than constant feeding of the antimetabolite in the ration as was done in this experiment. The effective level determined in this assay is higher than that determined by Ott (1946). However, it is difficult to make a direct comparison between the trials because of differences in techniques and procedures. The quantitative determination of level of antimetabolite needed to produce a vitamin B_6 deficiency will need to be based upon an accurate assay of vitamin B_6 content in the practical ration.

The last experiment did not attain the desired objectives. There are several readily apparent, possible explanations for this failure. Since the results of the vitamin B_6 assay on corn and soybean meal appear questionable, the same is true for the vitamin B_6 assay of isolated soybean protein. The results of this assay appear to be too low when the assayed vitamin B_6 content of this protein source is compared to that given for a similar product (0.67 mg per lb versus 2.01 mg per lb). A second possible explanation for the lack of treatment differences, either direct or residual, is that the two-week experimental periods were of too short duration to produce vitamin B_6-deficiency symptoms. Although the chicks were two weeks old when placed on the experiment, it appears

doubtful that they would have had sufficiently high vitamin B_6 stores or reserves in the body to last for the entire eight-week experiment. A final evaluation of the results of this experiment depends on knowing the actual vitamin B_6 content of the protein source. Since difficulties were encountered in the microbiological assay with Saccharomyces carlsbergensis, it is recommended that this assay be based on a different microorganism or on a colorimetric method or on both methods.

SUMMARY

Studies were made on different aspects of vitamin B_6 metabolism in male chicks. An attempt to quantitatively determine if differences existed in vitamin B_6 requirements of three breeds of chickens did not attain the desired goal due to a lack of sensitivity. In this experiment, weight gains of all breeds studied peaked when chicks consumed the median vitamin B_6 level (0.9 mg of vitamin B_6 per lb) and decreased thereafter.

Results of Experiment 2 indicate that vitamin B_6 plays an important role in the rate of formation of hemoglobin. Hemoglobin and packed cell volume were significantly correlated, indicating that a vitamin B_6 deficiency results in lowered levels of both criteria due to a decreased rate of formation of hemoglobin. When chicks receiving a diet containing sufficient vitamin B_6 were pair-fed with vitamin B_6-deficient chicks, significant differences existed in second, third, and fourth week hemoglobin values and second and fourth week packed cell volume values. When the deficient and the pair-fed group values were combined and compared to the values of chicks receiving sufficient vitamin B_6 and consuming the diet ad libitum, differences in hemoglobin level were significant at the end of the first and third weeks. Packed cell volume values were significantly different at the end of the first and second weeks.

Deaths were observed in pair-fed chicks indicating that a vitamin B_6 deficiency is capable of depressing appetite sufficiently so that starvation is a factor in deaths of some deficient chicks. Feed conversion ratios were similar in deficient and pair-fed chicks. Chicks on adequate

vitamin B_6 diets with consumption ad libitum required about one-half the feed per unit gain of the other two groups.

Results of Experiment 3 indicate that a vitamin B_6 antimetabolite, desoxypyridoxine, was capable of producing a vitamin B_6 deficiency when added to a practical ration at sufficiently high levels. Based on assay results of vitamin B_6 content in corn and soybean meal, a level of antimetabolite 14.6 times greater than the assayed vitamin B_6 content resulted in a significant depression of growth. The median lethal dose (LD 50) determined in this experiment was 31.6 mg of desoxypyridoxine per lb of complete ration, with five percent fiducial limits of 24.32 and 41.84 mg per lb for lower and upper limits, respectively.

Formulations used in Experiment 4 were based on assayed vitamin B_6 content in the isolated soybean protein source. Results of this assay appear questionable, which could explain, in part, the failure to produce a vitamin B_6 deficiency in this experiment. This experiment was designed to study the rate of development and the rate of recovery from varying degrees of a vitamin B_6 deficiency. In this trial, no statistically significant differences, due to residual or direct effects of vitamin B_6 level, were detected in average daily gains, feed conversion ratios, or change in packed cell volume. The vitamin B_6 levels used in this experiment included a level which had produced a vitamin B_6 deficiency in a previous experiment.

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APPENDIX A. VITAMIN B_6 ASSAY

Method

The design for the vitamin B_6 assays was based on principles discussed by Bliss (1952). The microbiological assay was made according to the method of Association of Vitamin Chemists, Inc. (1951) using Saccharomyces carlsbergensis 4228. The statistical analysis of data was made using the techniques of Clarke (1952). Hopkins and Pennington (1947) had demonstrated previously that assay results obtained with Saccharomyces carlsbergensis were similar to results obtained in rat assays.

Results and Analysis

Corn and soybean oil meal

Table 15. Response and computation table for 6-point assay for vitamin B_6 content of corn and soybean oil meal (SOM)

Dose (units)[a]	Standard	Corn	SOM	Total
1/2	347[b] 351 (698)	277 302 (579)	294 305 (599)	
1	482 530 (1012)	421 430 (851)	425 460 (885)	
Total	1710	1430	1484	4624
Q	2460	-186	342	2616
b	243.600000	255.400000	273.000000	
H	384	307	313	1004

[a]1 unit of standard = 0.024 mcg of vitamin B_6; 1 unit of corn = 4.0 mg of corn; 1 unit of SOM = 4.0 mg of SOM.

[b]Responses are given in 10^3 x optical density; treatment totals shown in parentheses.

Table 16. Analysis of variance for 6-point assay of vitamin B_6 content in corn and soybean oil meal

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Between treatments	5	74,626.67	
Regression	3	74,259.800	24,753.266**
Intersections	2	366.870	183.435
Within treatments	6	2,186.00	364.333
Total	11	76,812.67	

**Significant at $P < .01$.

$$R_1 = \frac{b_{corn}}{b_{std}} = (0.743306)\ \frac{0.024}{4.0}$$ = 0.004460 mcg of vitamin B_6 per mg of corn.

= 2.02 mg of vitamin B_6 per 1b of corn.

$$R_2 = \frac{b_{SOM}}{b_{std}} = (0.794528)\ \frac{0.024}{4.0}$$ = 0.004767 mcg vitamin B_6 per mg of SOM.

= 2.16 mg of vitamin B_6 per 1b of SOM.

Isolated soybean protein

Table 17. Response and computation table for 7-point assay of vitamin B_6 content of isolated soybean protein (ISP)

Dose (units)[a]	Blank	Standard	ISP	Total
0	331[b] 276 (607)			
1/3		355 426 (781)	409 408 (817)	
2/3		444 449 (893)	465 449 (914)	
1		562 505 (1067)	542 488 (1030)	
Total	607	2741	2761	6109
Q		3722	3491	7213
b		209.835165	206.299451	
H		1883	2122	4005

[a]1 unit of standard = 0.0225 mcg of vitamin B_6; 1 unit of ISP = 15.0 mg of ISP.

[b]Responses are given in 10^3 x optical density; treatment totals shown in parentheses.

Table 18. Analysis of variance for 7-point assay of vitamin B_6 content of isolated soybean protein

Source of variation	Degrees of freedom	Sums of squares	Mean squares
Between treatments	6	73,360.71	
Regression	2	71,485.62	35,742.81**
Blanks	1	844.67	844.67
Intersections	1	680.01	680.01
Deviations from individual regressions	2	350.41	175.21
Within treatments	7	7,256.50	1,036.64
Total	13	80,617.21	

**Significant at $P < .01$.

$$R = \frac{b_{ISP}}{b_{std}} = (0.98315)\ \frac{0.0225}{15.0} = 0.00147 \text{ mcg of vitamin } B_6 \text{ per mg.}$$

$$= 0.67 \text{ mg of vitamin } B_6 \text{ per lb.}$$

APPENDIX B. PROBIT REGRESSION EQUATION OF MORTALITY

Explanation of Symbols Used in Analysis

Computation tables

Z = concentration of antimetabolite in mg/lb of complete ration.

x = $\log_{10} Z$

n = number of chicks receiving respective treatment.

r = number of dead chicks in the respective treatment group.

p' = percentage dead = r/n.

p = percentage dead adjusted for deaths in control group.
= (p' - c)/(1 - c), where c = p' for the control group or an estimate of this value if c appears to be an overestimate.

Empirical probit = tabular value which changes the proportions (p) to probits, thus transforming the normal sigmoid curve of mortality to a straight line.

Y = expected probits which are read from a graph made by plotting empirical probits against x and connecting the points with a straight line.

nw = product of a weighting coefficient and the corresponding n. The weighting coefficients are tabular values which depend upon c and the respective Y value.

y = expected probit which depends upon the respective p and Y value.

nwx = product of the respective nw's and the corresponding x.

nwy = product of the respective nw's and the corresponding y.

Y' = estimates of Y obtained from the regression equation obtained in the first cycle.

Y'' = estimates of Y obtained from the regression equation obtained in the second cycle.

Sums

Snw = sum of the nw values.

Snwx = sum of the nwx values.

Snwy = sum of the nwy values.

Means

$\bar{x}$ = Snwx/Snw.

$\bar{y}$ = Snwy/Snw.

Sums of products

$Sxx = (Snwx)(x) - (Snwx)^2/Snw$

$Sxy = (Snwx)(y) - (Snwx)(Snwy)/Snw$

$Syy = (Snwy)(y) - (Snwy)^2/Snw$

Regression coefficient

$b = Sxy/Sxx$

Regression equation

$Y' = \bar{y} + b(x - \bar{x})$

Calculations

Tables 19 and 20 are the computation tables used in obtaining the probit regression equation. Table 21 contains the calculated values obtained in the analysis.

Mortality on control group gave c = .07, an apparent overestimate when one observes that the two lowest levels of antimetabolite caused no mortality. In the analysis, c was estimated as equal to .01.

LD 50 is determined by letting $Y'' = 5.000$ and solving for x. In which case x = 1.50, therefore Z = 31.6 mg of desoxypyridoxine per lb of complete ration. The five percent fiducial limits are 24.32 and 41.84 mg per lb for lower and upper limits, respectively.

Table 19. Computation table for fitting of the probit regression equation (1st cycle)[a]

Z	x	n	r	p'	p	Empirical probit	Y	nw	y	nwx	nwy
64.6	1.80	30	23	.77	.77	5.74	5.7	15.7	5.74	28.260	90.118
28.1	1.44	30	13	.43	.42	4.80	4.9	18.6	4.80	26.784	89.280
12.2	1.08	30	6	.20	.19	4.12	4.1	13.4	4.12	14.472	55.208
5.3	0.72	30	0	.00	.00	-	3.3	5.1	2.83	3.672	14.433
2.3	0.36	30	0	.00	.00	-	2.4	0.4	2.06	0.144	0.824
0.0		30	2	.07							

[a]Explanation of column headings given on page 66.

Table 20. Computation table for fitting of the probit regression equation (2nd cycle)[a]

x	n	p	Y'	nw	y	nwx	nwy	Y''
1.80	30	.77	5.75	15.7	5.74	28.260	90.118	5.76
1.44	30	.42	4.85	18.6	4.80	26.784	89.280	4.85
1.08	30	.19	3.95	12.4	4.13	13.392	51.212	3.94
0.72	30	.00	3.05	4.2	2.74	3.024	11.508	3.03
0.36	30	.00	2.15	0.1	1.88	0.036	0.188	2.13

[a]Explanation of column headings given on pages 66 and 67.

Table 21. Sums, sums of products, and regression equations from probit analysis of mortality

Cycle	Sums	Sums of products	Regression equations
First	Snw = 53.2	Sxx = 6.68003	$Y' = \bar{y} + b(x - \bar{x})$
	Snwx = 73.332	Sxy = 16.67222	$= 4.697 + 2.496(x - 1.378)$
	Snwy = 249.863	Syy = 42.29632	$= 1.256 + 2.496x$
Second	Snw = 51.0	Sxx = 5.86158	
	Snwx = 71.496	Sxy = 14.75350	$Y'' = 4.751 + 2.517(x - 1.402)$
	Snwy = 242.306	Syy = 37.99268	$= 1.222 + 2.517x$

APPENDIX C. DATA FROM EXPERIMENT 4

Average Daily Gains

Table 22. Individual data and sums (grams) used in the analysis of variance

Square	Period	Individual data[a]			Sum
		Chick 1	Chick 2	Chick 3	
1	1	24.6 (1)	25.4 (2)	23.0 (3)	73.0
	2	32.9 (3)	31.8 (1)	35.6 (2)	100.3
	3	33.6 (2)	37.6 (3)	39.0 (1)	110.2
	4	36.3 (2)	38.9 (3)	35.4 (1)	110.6
	Sum	127.4	133.7	133.0	394.1
		Chick 4	Chick 5	Chick 6	
2	1	20.7 (3)	25.9 (2)	21.6 (1)	68.2
	2	28.9 (1)	31.5 (3)	28.9 (2)	89.3
	3	28.6 (2)	33.9 (1)	31.5 (3)	94.0
	4	30.4 (2)	34.0 (1)	29.9 (3)	94.3
	Sum	108.6	125.3	111.9	345.8
		Chick 7	Chick 8	Chick 9	
3	1	27.0 (3)	25.5 (2)	22.8 (1)	75.3
	2	34.5 (1)	29.1 (3)	34.5 (2)	98.1
	3	37.5 (2)	27.9 (1)	34.9 (3)	100.3
	4	39.1 (2)	30.7 (1)	28.6 (3)	98.4
	Sum	138.1	113.2	120.8	372.1
		Chick 10[b]	Chick 11	Chick 12	
4	1	24.5 (1)	22.6 (3)	26.3 (2)	73.4
	2	33.1 (3)	29.4 (2)	33.4 (1)	95.9
	3	33.6 (2)	31.1 (1)	36.0 (3)	100.7
	4	35.5 (2)	28.9 (1)	38.7 (3)	103.1
	Sum	126.7	112.0	134.4	373.1

[a]Treatments are shown in parenthesis: (1) is 0.6 mg of vit B_6 per lb, (2) is 1.2 mg of vit B_6 per lb, and (3) is 0.9 mg of vit B_6 per lb.

[b]Missing data dalculated for periods 3 and 4.

Feed Conversion Ratios

Table 23. Individual data and sums used in the analysis of variance

Square	Period	Individual data[a]			Sum
		Chick 1	Chick 2	Chick 3	
1	1	2.50 (1)	2.51 (2)	2.36 (3)	7.37
	2	2.27 (3)	2.24 (1)	2.08 (2)	6.59
	3	2.90 (2)	2.50 (3)	2.64 (1)	8.04
	4	3.09 (2)	2.91 (3)	3.50 (1)	9.50
	Sum	10.76	10.16	10.58	31.50
		Chick 4	Chick 5	Chick 6	
2	1	2.58 (3)	2.23 (2)	2.47 (1)	7.28
	2	2.42 (1)	2.10 (3)	2.32 (2)	6.84
	3	3.07 (2)	2.51 (1)	2.70 (3)	8.28
	4	3.35 (2)	2.83 (1)	3.36 (3)	9.54
	Sum	11.42	9.67	10.85	31.94
		Chick 7	Chick 8	Chick 9	
3	1	2.30 (3)	2.44 (2)	2.29 (1)	7.03
	2	2.12 (1)	2.42 (3)	2.04 (2)	6.58
	3	2.64 (2)	2.96 (1)	2.65 (3)	8.25
	4	2.73 (2)	3.16 (1)	3.65 (3)	9.54
	Sum	9.79	10.98	10.63	31.40
		Chick 10[b]	Chick 11	Chick 12	
4	1	2.31 (1)	2.39 (3)	2.32 (2)	7.02
	2	2.23 (3)	2.25 (2)	2.30 (1)	6.78
	3	2.75 (2)	2.75 (1)	2.76 (3)	8.26
	4	3.18 (2)	3.14 (1)	2.87 (3)	9.19
	Sum	10.47	10.53	10.25	31.25

[a]Treatments are shown in parenthesis: (1) is 0.6 mg of vit B_6 per lb, (2) is 1.2 mg of vit B_6 per lb, and (3) is 0.9 mg of vit B_6 per lb.

[b]Missing data calculated for Periods 3 and 4.

Change in Packed Cell Volume

Table 24. Individual data and sums used in the analysis of variance

Square	Period	Individual data[a]			Sum
		Chick 1	Chick 2	Chick 3	
1	1	-4.0 (1)	-10.5 (2)	-1.5 (3)	-16.0
	2	-4.0 (3)	-1.0 (1)	-0.5 (2)	-5.5
	3	5.5 (2)	7.0 (3)	0.5 (1)	13.0
	4	-6.0 (2)	-8.0 (3)	-4.0 (1)	-18.0
	Sum	-8.5	-12.5	-5.5	-26.5
		Chick 4	Chick 5	Chick 6	
2	1	-1.5 (3)	4.0 (2)	-6.0 (1)	-3.5
	2	-1.5 (1)	-5.0 (3)	-0.5 (2)	-7.0
	3	6.0 (2)	3.0 (1)	2.0 (3)	11.0
	4	-6.0 (2)	0.0 (1)	4.0 (3)	-2.0
	Sum	-3.0	2.0	-0.5	-1.5
		Chick 7	Chick 8	Chick 9	
3	1	-13.0 (3)	-15.5 (2)	3.5 (1)	-25.0
	2	-0.5 (1)	0.0 (3)	-3.5 (2)	-4.0
	3	-0.5 (2)	1.0 (1)	-2.0 (3)	-1.5
	4	0.0 (2)	-3.0 (1)	1.0 (3)	-2.0
	Sum	-14.0	-17.5	-1.0	-32.5
		Chick 10[b]	Chick 11	Chick 12	
4	1	-1.5 (1)	-1.7 (3)	-2.0 (2)	-5.2
	2	-3.0 (3)	-2.5 (2)	-3.0 (1)	-8.5
	3	4.0 (2)	2.5 (1)	-2.0 (3)	4.5
	4	-3.5 (2)	-1.0 (1)	4.0 (3)	-0.5
	Sum	-4.0	-2.7	-3.0	-9.7

[a]Treatments are shown in parenthesis: (1) is 0.6 mg of vit B_6 per lb, (2) is 1.2 mg of vit B_6 per lb, and (3) is 0.9 mg of vit B_6 per lb.

[b]Missing data calculated for Periods 2, 3, and 4.