VITAMIN REQUIREMENTS FOR HATCHABILITY AND EARLY CHICK GROWTH

A THESIS

by

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INTRODUCTION

The nutrient allowances presently recommended for growing chicks have been established using dietary protein levels of eighteen percent and a growth period of zero to eight weeks. This tends to mask the higher growth rate and possibly higher nutrient requirements of the initial four week period. Current research indicates that growth rate and efficiency of feed utilization can be improved by increasing the protein content and otherwise providing better nutritional balance of the ration. This raises the question of the adequacy of presently recommended vitamin allowances under the conditions of higher growth rate and feed efficiency now possible.

The influence of parental nutrition on the vitamin content of the egg, hatchability and growth of chicks fed deficient diets has been observed repeatedly. Little evidence has been advanced, however, to demonstrate that these differences in egg reserves can affect the growth of chicks fed rations of the recommended vitamin content.

It is the purpose of this study to investigate the vitamin requirements of the chick during the initial month of life and to determine if they may be modified by more rapid rate of growth and different vitamin reserves in the newly-hatched chick.

REVIEW OF LITERATURE

The importance of the poultry industry draws special attention to those aspects of nutrition which are of greatest economic significance. As might be expected then, studies on the vitamin requirements of the fowl have concentrated mainly on those vitamins which are present in diets of natural feedstuffs in quantities insufficient for optimum growth and reproduction. Vitamins A, D_3, B_{12}, riboflavin, pantothenic acid, choline and niacin must all be added to such diets (47) as natural supplements like fish oil, yeast, alfalfa and dried milk powder or as synthetics in the crystalline form. From the practical viewpoint efficient utilization of the protein, fat and carbohydrate depends mainly on the adequate fortification of the ration with these vitamins. The other vitamins required by chickens - thiamin, biotin, inositol, folic acid and vitamin E - are all abundantly contained in corn, wheat and soybean meal which constitute the major portion of most practical rations (47). This circumstance and the desire to have results directly applicable to poultry husbandry has encouraged investigation on the former vitamins in this study.

It is widely believed that the chick's vitamin requirements are generally dependent on growth rate. Petersen (110), considering the higher growth rates now possible through the use of high energy rations and dietary antibiotic, questions whether our old concepts of protein, energy and vitamin requirements for growing birds will prove valid under these new conditions. Ewing (47) believes that at these accelerated growth rates, the requirements for protein, vitamins and energy become more critical.

Evidence in support of this correlation between growth rate and nutrient requirement comes from a number of sources. The chick growth assay for many vitamins is based on the fact that one of the primary effects of a vitamin deficiency is a slowing or cessation of growth. Since increasing concentrations of the vitamin under study permit correspondingly greater growth, it is concluded that each successive increment in growth rate is conditioned by or actually requires a higher dietary concentration of the vitamin. Thus a given vitamin requirement is relative to the growth rate possible with the vitamin supplemented basal ration. Heuser (65) clearly demonstrated that the riboflavin requirement of the chick is not constant but decreases with age. There is a direct correlation between riboflavin requirement and growth rate expressed as the instantaneous rate of gain. Similarly a slower growth rate has been offered as the reason why White Leghorns are less susceptible to thiamin (79), riboflavin (80) and vitamin E (72) deficiency than faster growing New Hampshire and Barred Rock chicks. While none of these observations are direct evidence of an association between growth rate and vitamin requirement, it would appear advisable to verify the adequacy of recommended allowances for chicks showing growth rates higher than were possible five years ago.

The foregoing suggests that in studies on chick vitamin requirements, a basal ration should be used which will sustain the greatest possible growth when adequately fortified with the vitamin under study. Recent work is in general agreement on the basic composition of such rations. Singsen (130), Almquist (2) and Lloyd (85) have found that a dietary protein level of 20 to 21 percent is optimum for growth and feed efficiency of birds up to eight weeks of age. Although higher protein levels gave better initial response, the advantage was lost by the eighth week. Almquist (1) reported that 9 to 13 percent of this protein should be of animal origin, preferably fish meal which has a greater biological value than meat meal (116, 118). Titus (146) concludes that to assure maximum growth and feed efficiency a chick ration should contain 20 to 21 percent protein, approximately 20 percent of which should be animal in origin.

The well known superiority of animal protein sources was formerly believed due to a more efficient complement of amino acids. Almquist (1,4), however, showed that an all-vegetable protein ration supplemented with amino acids could not equal the growth promoting value of a diet containing animal protein. In 1948 vitamin B_{12} was isolated from fish and liver meal and was immediately hailed as the long-sought "animal protein factor." Ott (105) and Briggs (20), using simplified corn-soybean meal diets, have demonstrated that vitamin B_{12} and animal protein are equally effective as supplements to an all-vegetable ration. The recent evidence of Sunde (144) and Carlson (25) although in general agreement with these results, suggests that animal protein contains additional unidentified growth factors. While excellent growth can now be obtained with all-vegetable protein diets containing vitamin B_{12} it is premature to recommend the complete omission of animal protein from chick rations.

The energy requirement of chicks is another phase of poultry nutrition which has received considerable attention in the past few years. Carrick and Roberts (27, 28) found that replacing 20 percent corn with equal parts of wheat bran, wheat middlings or light oats in chick rations reduced growth and feed efficiency. Robertson (121) explained similar results with the belief that the high fiber content of these feedstuffs acted as inert diluent in the ration. Fraps (48) showed that an inverse relationship existed between fiber and productive energy content of many feedstuffs. The theory that the growth depressing effect of high fiber diets was due to corresponding decrease in productive energy received partial confirmation by the work of Robertson (120). The addition of soybean oil to a high fiber ration increased growth almost to that obtained on a high energy diet. Fraps (48) and Robertson (121) placed the productive energy requirement of growing chicks at approximately 800 Calories per pound of feed. Although energy levels above this value have no effect on body weight, there is a progressive increase in carcass fat (42), which may affect market quality.

These findings have resulted in the formulation of high energy rations designed to produce maximum growth and greatest feed efficiency in growing chicks. In general one may describe a high energy ration as one which contains 20 to 21 percent protein of good quality, less than 4 percent fiber, productive energy in excess of 900 Calories per pound and generous fortification with vitamin supplements. The feedstuff content of two commercial high energy rations is presented in Table V together with the composition of the basal ration used in the present study. The calculated chemical composition (Table VIII) indicates how closely these diets adhere to the basic high energy formula. The high concentration of many vitamins in these rations suggests the hesitancy of feed manufacturers and nutritionists alike to assume that the recommended vitamin allowances are adequate for chicks fed high energy rations. Ewing (47) says "Until further research provides a basis for re-evaluating the vitamin requirements of chicks at these higher growth rates, the practice of amply fortifying high energy rations with vitamin supplements seems fully justified."

The current N.R.C. recommended vitamin allowances are based on estimates of chick requirements made, for the most part, before the development of high energy diets. For clarity the individual and N.R.C. (36) recommendations are summarized in Table I. Since Bird (16) published an extensive review of studies on vitamin requirements conducted before 1947 the present summary considers mainly those estimates which have been made since that time. Hogan (70) has emphasized that many of the inconsistencies in these estimates are due to differences in genetic background, environment, diet and initial reserves of the birds. Although these variables complicate evaluation of the data in Table I, it is evident that many of the individual estimates of requirement approach or even exceed the N.R.C. recommendation. The chief danger in adopting a "recommended allowance" would appear to lie in underestimating the actual requirement.

Hogan (70) has suggested that one source of discrepancy between estimates of chick vitamin requirements is difference in the initial reserves of the bird. Despite considerable indirect evidence in favour of this view, few investigators have studied the direct influence of inherited reserves on the vitamin requirements of the young chick.

In the train of events which correlate breeder diet with reserves, the first essential step is the ability of the bird to transfer vitamins to the egg in proportion to the quantity present in its diet. Sherwood (128) found that the quantity of vitamin A in the egg and breeder diet are directly related. Russell (123) confirmed these findings and showed that a similar relationship exists for vitamin D. The influence of the dietary level of riboflavin (102), pantothenic acid (108) and vitamin B_{12} (94) on the egg content of these vitamins has also been demonstrated. Lucas (87) was unable to impair the hatchability of eggs by feeding diets low in choline to the breeding stock and concluded that the mature bird possessed the ability to synthesize this vitamin. The work of Ringrose (117) showed no correlation between the amount of choline in the egg and breeder diet.

For this influence of the hens' diet to be evident in the reserves of the chick, it is of course essential that no distortion in the vitamin content of the egg occur between the time that the egg is laid and the time, immediately before hatching, when the fully developed embryo draws the unabsorbed portion of the yolk within its body. Snell and Quarles (136) studied the quantities of riboflavin and pantothenic acid in eggs and concluded that little change occurs during incubation. Pearson (108) proved that there is no difference between the pantothenic acid content of eggs and the amount in chicks hatched therefrom. According to Parrish (106) approximately 25 percent of the initial vitamin A content of eggs is lost during incubation, 25 percent is found in the embryo and the remaining 50 percent in the unabsorbed yolk. Studies of hatchability and chick livability have provided indirect evidence that the vitamin D content of the egg is not distorted during embryonic development. In contrast, the quantity of niacin in eggs increases almost 20 times by embryonic synthesis (41, 136). This synthesis prevents the niacin level of the breeder diet from influencing the niacin reserves and niacin requirement of the young chick.

Considering the embryonic synthesis of niacin, it is not surprising that niacin-low breeder diets cannot decrease hatchability. Actually, impaired hatchability produced by vitamin deficiency is physiological evidence of the relationship between vitamin content of the breeder diet and the egg. The criterion of maximum hatchability has been generally used to establish the requirements for reproduction. Numerous estimates of these requirements, together with the N.R.C. (36) recommended allowances are summarized in Table II. The use of maximum hatchability to measure the vitamin requirements of breeding stock may result in the underestimation of actual requirements for reproduction. Lillie and Briggs (83, 84) have shown that a level of folic acid in the breeder diet which maintained hatchability was insufficient to permit normal growth of chicks fed a high energy ration. Clandinin (32) noted that chicks from dams fed a low-riboflavin diet could not grow as rapidly as chicks from dams which had received dietary riboflavin, despite the fact that both lots of chicks were fed a high energy ration. Evidently the vitamin requirement to produce healthy chicks is equal to and probably greater than the requirement for hatchability.

There are many reports showing that the composition of the hens' diet influences growth and livability of chicks fed rations deficient in the vitamin under study. Chick livability on vitamin A deficient diets is dependent on the level of vitamin A contained in the breeder ration (11, 127). Norris (103) and Clandinin (32) showed that the riboflavin content of the breeder ration is reflected in the growth of progeny fed a riboflavin deficient diet. Similarly the hens' intake of pantothenic acid (52) and vitamin B_{12} (68) determines the growth of chicks on rations low in these vitamins.

Direct evidence that these differences in vitamin reserves of the young chick can modify its requirements is almost non-existent. Milligan (94) reports that chick requirement for vitamin B_{12} depends on the quantity of B_{12} supplement in the maternal diet. The requirement for vitamin D by turkey poults was found by Stadelman (137) to be altered by the vitamin D content of the breeder ration. Poults receiving the recommended allowance of vitamin D, showed normal growth and calcification when the breeder ration contained no supplementary vitamin.

The present study is intended to test the adequacy of currently recommended vitamin allowances for rapidly growing chicks of differing initial vitamin reserves. Differences in chick reserves are to be produced by decreasing the vitamin intake of half the breeding stock to a level slightly below that recommended for good hatchability.

TABLE I: SUMMARY OF THE VITAMIN REQUIREMENTS FOR CHICK GROWTH

Vitamin	Units/lb.	N.R.C. Recommended Allowance	Individual Estimates of Requirement
A	I.U.	2000.0	765 (115), 1200 (145), 1350 (56), 1820 (74)
D_3	AOAC	180.0	77 (30), 82 (8), 226 (64), 272 (29)
B_{12}	γ	-	2.0 (43), 5.6 (99), 12.1 (104)
Riboflavin	mg.	1.6	1.1 (13), 1.4 (33), 1.5 (103), 1.5 (17)
Pantothenic Acid	mg.	5.0	2.7 (10), 3.4 (93), 4.6 (33), 6.3 (75)
Niacin	mg.	8.0	8.2 (21), 14 (33)
Choline	mg.	700.0	454 (63), 454 (76), 680 (114)
Pyridoxine	mg.	1.6	1.4 (22), 1.6 (71), 1.8 (86)
Biotin	mg.	0.045	0.07(62), 0.07(97)
Folic Acid	mg.	-	0.14(119), 0.23(5), 0.45(88), 0.68(82)
Thiamin	mg.	0.9	0.36(7), 0.68(77), 0.91(95)
Inositol	Required for growth, but actual quantity unknown (151, 61)		
E	Required for growth, but actual quantity unknown (18, 107)		
Ascorbic Acid	Synthesized by the embryo, chick and hen (112, 26, 60)		

TABLE II: SUMMARY OF THE VITAMIN REQUIREMENTS FOR HATCHABILITY

Vitamin	Units/lb.	N.R.C. Recommended Allowance	Individual Estimates of Requirement
A	I.U.	3300.0	2040 (3), 2480 (67), 4160 (122), 4540 (129)
D_3	AOAC	450.0	225 (14), 270 (31), 350 (98), 350 (35)
B_{12}	γ	-	0.8 (109), 0.9 (153), 2.0 (94)
Riboflavin	mg.	1.3	1.0 (103), 1.0 (73), 1.1 (44), 1.6 (111)
Pantothenic Acid	mg.	5.0	4.5 (54), 7.7 (53)
Pyridoxine	mg.	1.6	0.9 (39)
Biotin	mg.	0.07	0.07 (40) 0.09(34)
Folic Acid	mg.	-	0.15 (83) 0.23(143), 0.45(38)
Choline	Limited synthesis by the hen. Requirement less than 540 mg./lb.(87,117)		
E	Required by hen but relative quantity unknown (9,24)		
Thiamin	Deposited in egg but unnecessary for hatchability (45, 126)		
Inositol	Deposited in egg but requirement for hatchability undemonstrated(136, 152)		
Niacin	Synthesized by the embryo (41, 136)		
Ascorbic Acid	Synthesized by the embryo, chick and hen (20, 60, 112)		

EXPERIMENTAL

1. Breeding Stock

One hundred and twenty Barred Plymouth Rock pullets were confined to the laying house with floor litter and fed a commercial mash for a standardization period of one month. The birds were then randomized into two pens of sixty each and maintained for an additional month on a high nutrient ration (H.N. Table III) to ensure nutritional uniformity.

At the end of this period the breeding stock was placed on two nutritional planes. The high nutrient diet (H.N.) was a mixture of mash and whole grain in the ration of 1:1 while the low nutrient diet (L.N. 1) used the same components in the ratio of 1:2. After two months on this regimen the low nutrient ration was diluted still further to a 1:3 mixture (L.N. 2) to increase the difference between the two planes.

Calculated composition of the three breeder rations (Table VI) reveals that dilution of the mash nutrients with whole grain in the ration of 1:2 or 1:3 has little effect upon crude protein or ether-extract. These components are present in all three diets in quantities sufficient to meet the requirements for egg production, the major consideration, since it has been shown that neither chemical composition nor hatchability of the egg is affected by variations in the dietary intake of these macronutrients (66, 147). The vitamin levels in the lowest nutrient group are all in excess of or bordering on the adequate when one considers the safety margins of 66 percent, 45 percent and 20 percent included in the N.R.C. recommended allowances (36) for vitamins A, D_3 and the B complex. Thus it may be expected that the dietary treatment of the breeding stock would have little effect on the hatchability of the eggs produced. It should be recalled that the main objective of the two nutritional levels was the production of chicks with differing vitamin reserves. Examination of the breeder diets with this point in mind reveals that the second low nutrient diet contained approximately one half the vitamin A, D and riboflavin of the high nutrient ration. The much smaller differences in pantothenic acid, choline, niacin and B_{12} content of the rations might not be clearly reflected in the reserves of these vitamins in the day old chick.

The feed was provided *ad libitum* and fresh water was accessible at all times. Each group of sixty pullets was mated with five New Hampshire males. Egg production and hatchability during the first four months of the experiment was recorded in Appendix i. The experiment was terminated at six months due to cessation of egg production as the result of the summer heat.

2. Chick Feeding Trials

On the day of hatching, chicks were randomized into groups of twenty, weighed and leg banded. In Experiments III to VI inclusive each group of chicks was composed of 10 high nutrient and 10 low nutrient chicks. Each treatment group was housed in a compartment of an electrically heated battery brooder with raised, wire screen floors. Feed and water were supplied *ad libitum*.

The basal diets used in Experiments I to VI are set out in Table IV. Proximate chemical analysis and calculated vitamin content of the rations are presented in Table VII. Preparation of samples and procedures for moisture, crude protein, ether-extract, crude fibre, calcium and ash are described in AOAC Book of Methods (6). Phosphorus was determined after wet digestion with nitric and sulphuric acids by the method of Bertramson (12).

The chicks were weighed to the nearest gram at weekly intervals. To prevent bias due to feed content of the birds, weighings were made in the early morning (nine o'clock) before appreciable feed had been consumed. At the same time unused feed was weighed and the amount consumed during the week obtained by difference. Considerable care was taken in recovering spilled feed from the dropping pans since this wastage is both large and variable and may, if unconsidered, cause serious inaccuracies in the feed consumption data.

3. Analysis of Data

The four-week weight gains were analysed by the method of Snedecor and Cox (135) using within group variance to test the significance between groups. This procedure was first applied to the analysis of chick growth data by Titus and

Hammond (148) and has been used with little modification since 1935. It would appear advisable to consider in some detail the validity of this method when it is applied to chick experiments in which all birds receiving a given treatment are housed in a single cage and fed from a common feed supply.

A basic assumption in the analysis of variance is that treatments have been allotted at random to individuals of a population. The replicates receiving the treatments must be randomized through every phase of the experiment, i.e., selecting, housing, feeding, weighing, etc., to ensure that they are influenced by all variation due to factors other than treatment, which can by chance alone produce a difference in growth between samples of a population.

In most chick experiments this prerequisite is ignored by placing all birds receiving the same treatment in one cage. Quite obviously a bias applied to any cage is attributed to "treatment." This procedure would be justifiable if it were known that different cages of birds would respond alike to the same dietary treatment. Hill (69) was the first to show that groups of birds in different cages, receiving the same diet, could grow at significantly different rates. Campbell and Emslie (23) demonstrated that there is sometimes a greater variance between replicated groups than there is between individuals within the groups. This clearly demonstrated that conclusions on the effect of treatments based on within-group variance might be erroneous.

The obvious solution would be to house each bird in a separate cage and to assure that in all other respects they were true replicates. This however is not feasible with chicks due to their need for high environmental temperature during the first four weeks and their apparent need for companionship. Campbell and Emslie (23) suggested the replication of entire groups and the use of a variance between replicated groups to test the difference between treatment means. This procedure requires the use of at least four groups for each treatment in order that the mean be correctly estimated and sufficient degrees of freedom provided to ensure a sensitive test of treatment differences. An alternative procedure is the replication of treatments at different times and strict adherence to the principle of repeating an observation at least twice before any conclusion as to its biological significance is made.

In the present experiments unreplicated groups have been used in every instance, due mainly to a lack of chicks and space. This must be borne in mind when interpreting results, for as noted above, treatments could be "significantly different" through group bias. Rather than place undue emphasis on a single experiment, the groups receiving similar treatment effects will be judged by the response of similar groups in various experiments. In cases where a treatment has been applied only once, a significant response will be considered only as indication.

In summary, the data in each experiment have been analysed by the method of Snedecor and Cox (135). Within-group variance being used as an estimate of error in testing the difference between groups. When a significant (P = .05) group effect has been observed at least twice it has been considered as due to the dietary treatment.

The method of measuring "feed efficiency" also deserves a few words of explanation. The present project in common with most poultry nutrition work uses the ratio of feed consumed to body weight gained as an expression of feed efficiency.

From the nutritional viewpoint feed consumption and growth data are equally important and should be equally reliable. Treatment which alters the metabolic efficiency is as significant as that which affects growth. Individual feed consumption data permit the statistical evaluation of differences in "appetite" and the correction of weight gains to equal feed intake by analysis of covariance (149).

In chick experiments, where all birds receiving one treatment are housed in a single cage, only group consumption is obtainable. Statistical evaluation of the effect of treatment on feed intake and correction of gains is impossible. The feed/gain ratio thus provides only a crude estimate of the biological efficiency of a ration. The most obvious limitation is the disregard of a maintenance feed requirement; assuming that a constant proportion of feed consumed is transferred into new growth. Fraps (49) estimated the maintenance of chicks from zero to four weeks at 5.7 to 8.4 percent of body weight per day. Since total feed consumption during this period is 10 to 14 percent of body weight per day (37), almost 60 percent of feed eaten is needed to maintain existing weight; the remaining feed is utilized for new growth. Any increase in feed consumption produces a disproportionate increase in feed available for growth, as the maintenance requirement is essentially unaltered. As one may then expect a higher growth rate may be accompanied by "apparent" increase in feed efficiency (59). Conversely, the gradual increase in feed/gain ratio with advancing age is attributed to the increasing proportion of consumed feed required for maintenance of the increasing body weight (90). Whenever a treatment increases or decreases both weight gain and feed intake it is difficult to draw valid conclusions regarding feed efficiency. Greater gain with equal feed consumption or equal gain with lower feed consumption may be attributed to greater feed efficiency.

From the practical standpoint the feed/gain ratio has considerable value. To poultry producers, more interested in economic than nutritional significance, a feed/gain ratio describes the quantity of feed necessary to produce a unit weight of bird in a specified time. It is in this economic sense that the term "feed efficiency" is used throughout this work. Since no data are available on the variability in feed efficiency between replicated groups it is difficult to judge when feed/gain ratios of treatment groups are actually different. The present work arbitrarily considers a 10 percent deviation from the basal ratio as significant.

TABLE III: COMPOSITION OF MASH AND WHOLE GRAIN BREEDER MIXTURES

Mash Ingredients	Pounds*	Whole Grain Ingredients	Pounds
Ground Wheat	20.0		
Ground Barley	20.0		
Ground Oats	10.0	Wheat	50.0
Wheat Middlings	10.0	Oats	20.0
Wheat Bran	7.5	Barley	30.0
Soybean Meal	5.0		100.0 lbs.
Linseed Meal	5.0		
Fish Meal	6.0		
Dehydrated Alfalfa	7.5		
Dried Milk Powder	1.0		
Dried Brewer's Yeast	1.0		
Salt	1.0		
Rock Phosphate	2.5		
Calcium Carbonate	3.0		
Fish Oil 2400 A-400 D_3	0.5		
$MnSO_4$	11.4 gms.		
Riboflavin Merck No. 54	6 gms.		
Choline Cl 25%	240 gms.		
APF Merck No. 3	20 gms.		
	100 lbs.		

*Weight of ingredients in pounds unless otherwise stated.

TABLE IV: COMPOSITION OF CHICK BASAL RATIONS

Ingredients	Basal Ration*			
	R-1	R-2	R-3	R-4
Ground Yellow Corn	30.0	30.0	30.0	30.0
Ground Wheat	24.5	-	25.0	23.0
Wheat Middlings	20.0	10.0	20.0	20.0
Ground Oats	-	10.0	-	-
Ground Barley	-	20.0	-	-
Soybean Oil Meal (44%)	10.0	15.0	10.0	17.5
Fish Meal (65%)	6.0	2.5	2.5	-
Meat Meal (51%)	-	3.0	3.0	-
Milk Powder	2.0	2.0	2.0	2.0
Dried Brewer's Yeast	2.0	2.0	2.0	2.0
Dehydrated Alfalfa	2.0	2.0	2.0	2.0
Limestone	2.0	2.0	2.0	2.0
Rock Phosphate	1.0	1.0	1.0	1.0
Salt	0.5	0.5	0.5	0.5
Manganese Sulphate	11.4 gm.	11.4 gm.	11.4 gm.	11.4 gm.
Riboflavin Merck No. 54	0.5 gm.	0.5 gm.	0.5 gm.	0.5 gm.
Choline CL 25%	144.0 gm.	144.0 gm.	-	-
Fish Oil (2400A - 400D_3)	-	100.0 gm.	-	-
Dry Vit. D_3 mixture (9,000,000 AOAC/lb.)	9.0 gm.	-	9.0 gm.	9.0 gm.
	100.0	100.0	100.0	100.0

*Weight of ingredients in pounds unless otherwise stated.

TABLE V: COMPOSITION OF THREE HIGH ENERGY RATIONS

Ingredients	New England* Conference	Borden Co.*	Macdonald* R-1
Ground Yellow Corn	63.0	55.0	30.0
Corn Gluten Meal	2.5	-	-
Ground Wheat	-	-	24.5
Wheat Middlings	-	10.0	20.0
Soybean Oil Meal 44%	20.0	20.0	10.0
Fish Meal 60%	2.5	7.5	6.0
Liver Meal	1.0	-	-
Meat Scrap 51%	5.0	-	-
Brewer's Yeast	-	-	2.0
Butyl Fermentation Product	2.0	-	-
Dried Milk Powder	-	-	2.0
Dehydrated Alfalfa Meal	2.0	3.7	2.0
Ration AyD (Vit. A + D)	-	1.5	-
Dry D_3 (9,000,000 AOAC/lb.)	0.03	-	0.02
Steamed Bone Meal	1.0	1.0	1.0
Ground Limestone	0.5	1.0	2.0
Iodized Salt	0.5	0.5	0.5
Manganese Sulphate	0.025	0.025	0.025
Riboflavin No. 54	-	-	0.50 gm.
Nicotinic Acid	0.9 gm.	-	-
Choline Chloride 25%	128.9 gm.	-	144.0 gm.
	100.0	100.0	100.0

*Weight of ingredient in pounds unless otherwise stated.

TABLE VI: CALCULATED CHEMICAL COMPOSITION OF THE BREEDER RATIONS

Component	High Nutrient	Low Nutrient	Low Nutrient	N.R.C. (1946) Recommended Allowances
Mash: Grain	1:1	1:2	1:3	
Crude Protein %	16.5	15.5	15.0	15-16
Ether Extract %	2.9	2.8	2.8	3.0
Crude Fiber %	6.7	6.5	6.4	7.0
Vit. A IU/lb.	4900.0	3270.0*	2450.0*	3300.0
Vit. D_3 AOAC/lb.	454.0	303.0*	227.0*	450.0
Riboflavin mg./lb.	2.2	1.7	1.3	1.3
Pantothenic mg./lb.	5.3	4.5*	4.1*	5.0
Choline mg./lb.	809.0	639.0	634.0	540.0
Niacin mg./lb.	27.0	26.0	25.5	NIL
Vit. B_{12} γ /lb.	4.7	3.4	2.8	1-2

*Below recommended level.

TABLE VII: CHEMICAL COMPOSITION OF BASAL RATIONS FOR CHICKS

(Per Cent Air-Dry Basis*)

Component		Ration R-1	R-2	R-3	R-4	N.R.C. (1946) Recommended Allowances
Protein (N x 6.25)	%	19.8	20.1	19.9	20.0	18-19
Ether Extract	%	2.8	2.6	3.0	2.8	3.0
Crude Fibre	%	3.2	6.0	3.9	3.7	7.0
Productive Energy	Cal./lb.	946.0	899.0	941.0	927.0	800.0
Calcium	%	1.42	1.44	1.54	1.61	
Phosphorus	%	.76	.78	.83	.80	
Vit. A	IU/lb.	1700.0	4100.0	1700.0	1700.0	2000.0
Vit. D_3	AOAC/lb.	180.0	400.0	180.0	180.0	180.0
Riboflavin	mg./lb.	1.6	1.6	1.6	1.6	1.6
Pantothenic Acid	mg./lb.	5.1	5.1	5.1	5.1	5.0
Choline	mg./lb.	792.0	855.0	553.0	779.0	700.0
Niacin	mg./lb.	26.6	19.5	26.5	26.3	8.0
Vit. B_{12}	γ /lb.	2.7	2.1	1.8	0.5	

*Vitamin content calculated from feedstuff tables.

TABLE VIII: CALCULATED CHEMICAL COMPOSITION OF THREE HIGH ENERGY RATIONS

Component		New England Conference	Borden Co.	Macdonald R-1	N.R.C. (1946) Recommended Allowance
Crude Protein	%	20.1	20.6	19.8	18-19
Ether Extract	%	4.6	3.4	2.8	3.0
Crude Fiber	%	4.3	4.8	3.2	7.0
Productive Energy	Cal./lb.	980.0	960.0	946.0	800.0
Calcium	%	1.37	1.24	1.50	
Phosphorus	%	0.87	0.72	0.78	
Vit. A	IU/lb.	5880.0	3890.0	1700.0	2000.0
Vit. D_3	AOAC/lb.	318.0	580.0	180.0	180.0
Riboflavin	mg./lb.	5.8	1.8	1.6	1.6
Pantothenic Acid	mg./lb.	5.9	5.1	5.1	5.0
Choline	mg./lb.	755.0	564.0	780.0	700.0
Niacin	mg./lb.	22.3	13.4	26.5	8.0

RESULTS

Experiment I: Vitamin Requirements for Maximum Growth of Chicks Fed a High Energy Ration

In view of the excessive addition of vitamins to high energy rations the first experiment was designed to determine if the currently recommended vitamin allowances for chick rations are sufficient to assure maximum growth on a high energy ration.

One hundred and sixty male chicks from the first experimental hatch were randomized into groups of twenty and allotted to compartments of the battery brooder. As the parent stock was all on the high nutrient breeder ration when the eggs were collected, there was no division of chicks into high and low nutrient stock.

The basal ration R-1 shown in Table IV contained five of the six vitamins under study at the recommended level. The inclusion of wheat in the basal ration resulted in a niacin content of three times the recommended amount. Proximate chemical analysis and calculated vitamin content are set out in Table VII. In each of six groups a quantity of one vitamin equal to the N.R.C. allowance was added to the basal; the last group was supplemented with one recommended allowance of all six vitamins. The dietary supplements, four-week weight gains and average feed consumption per bird are presented in Appendix ii. Growth curves are plotted in Fig. 1 and statistical analysis of the four-week gains recorded in Appendix viii.

The data indicate clearly that additional amounts of vitamins in the basal diet are without effect on the early growth rate of the chicks. The only noteworthy response was a growth depression upon the addition of extra choline. Since this effect was not repeated in the group receiving all supplements, its significance is uncertain. The present recommended allowances for vitamins A, D_3, riboflavin, choline and pantothenic acid appear adequate for maximum chick growth with the high energy basal ration employed in this trial. Similarly, the basal ration was not improved by the addition of further niacin.

The feed/gain ratios reveal that, although without effect on growth, vitamin A, riboflavin and particularly pantothenic acid supplements increased the efficiency of feed utilization.

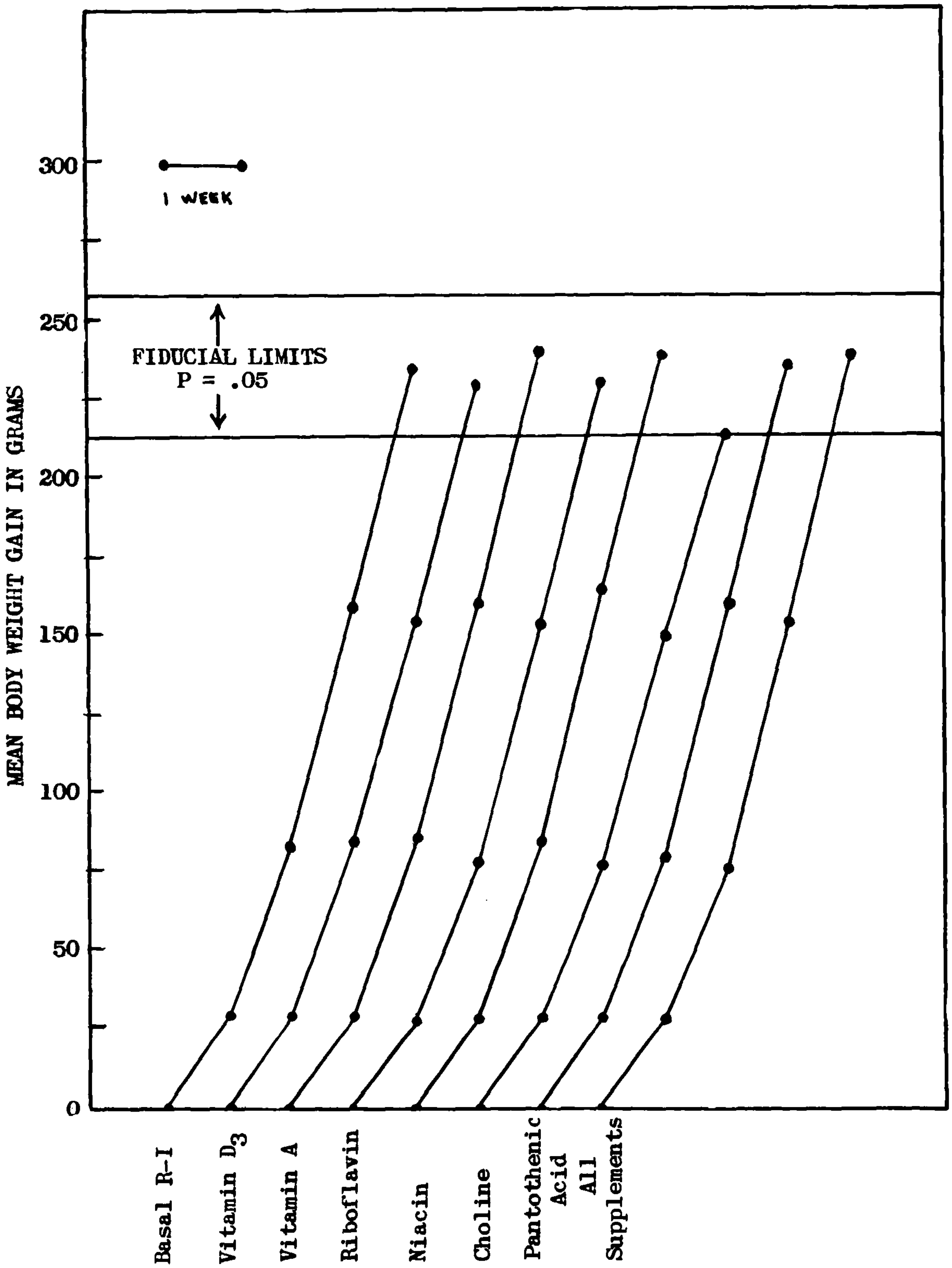


FIGURE I. THE EFFECT UPON GROWTH OF TWO-FOLD THE RECOMMENDED LEVEL OF CERTAIN VITAMINS IN A HIGH ENERGY CHICK RATION

Experiment II: Effect of an Animal Protein Factor Supplement Upon Chick Growth

During investigation on the vitamin B_{12} content of certain fermentation residues, Stoksted and Jukes (139) noted that the ferment produced by Streptomyces aureofaciens (Lederle Co.) stimulated chick growth to a greater degree than vitamin B_{12}, fish meal, fish solubles or distillers' solubles. In this laboratory (100) Animal Protein Factor (Lederle) supplement has been found to stimulate the growth of chicks fed a supposedly complete diet. The hitherto unattainable growth rates now possible through the use of this APF supplement could conceivably impose a stress on the vitamin requirements of the young chick. Experiment II was designed to find the level of Lederle APF conducive to most rapid early growth. The vitamin A and D content of the basal ration was doubled to insure that growth response to the APF would not be complicated by a marginal deficiency of these vitamins.

The breeding stock had been on the separate nutritional planes for two weeks when the eggs were collected for incubation. Inasmuch as the birds had received excellent rations for several months prior to this regimen, it appeared unlikely that any noticeable depletion of essential nutrients would have occurred in the space of fourteen days.

Four groups of twenty chicks were randomized from both high and low nutrient stock, allotted to compartments in the battery brooder and fed basal ration R-2 supplemented with nil, 0.25, 0.50 and 1.0 percent APF (Lederle) respectively. The feed consumption data and four-week gains are set out in Appendix iii with the growth curves plotted in Figure 2.

Analysis of variance (Appendix ix) reveals that chicks from the low nutrient dams grew significantly less than those from high nutrient dams in the APF (Lederle) supplemented Groups. It is difficult to interpret these differences in view of the short time that the breeding stock had been on the depletion regimen. Appendix i shows that during the first four months, egg production and hatchability were never impaired by the decreased vitamin intake, on the contrary there was a trend toward greater hatchability.

Equally striking was the enhanced growth in all groups receiving the APF (Lederle) supplement. The 0.5 percent level of APF stimulated 23 percent and the other two levels 13 percent more growth than the basal ration alone. A growth depressing effect at higher levels of APF (Lederle) was suggested by the better response of the 0.5 than 1.0 percent groups.

The increased feed consumption and unchanged feed/gain ratios revealed that the increased growth induced by APF (Lederle) was due to greater feed intake rather than more efficient feed utilization.

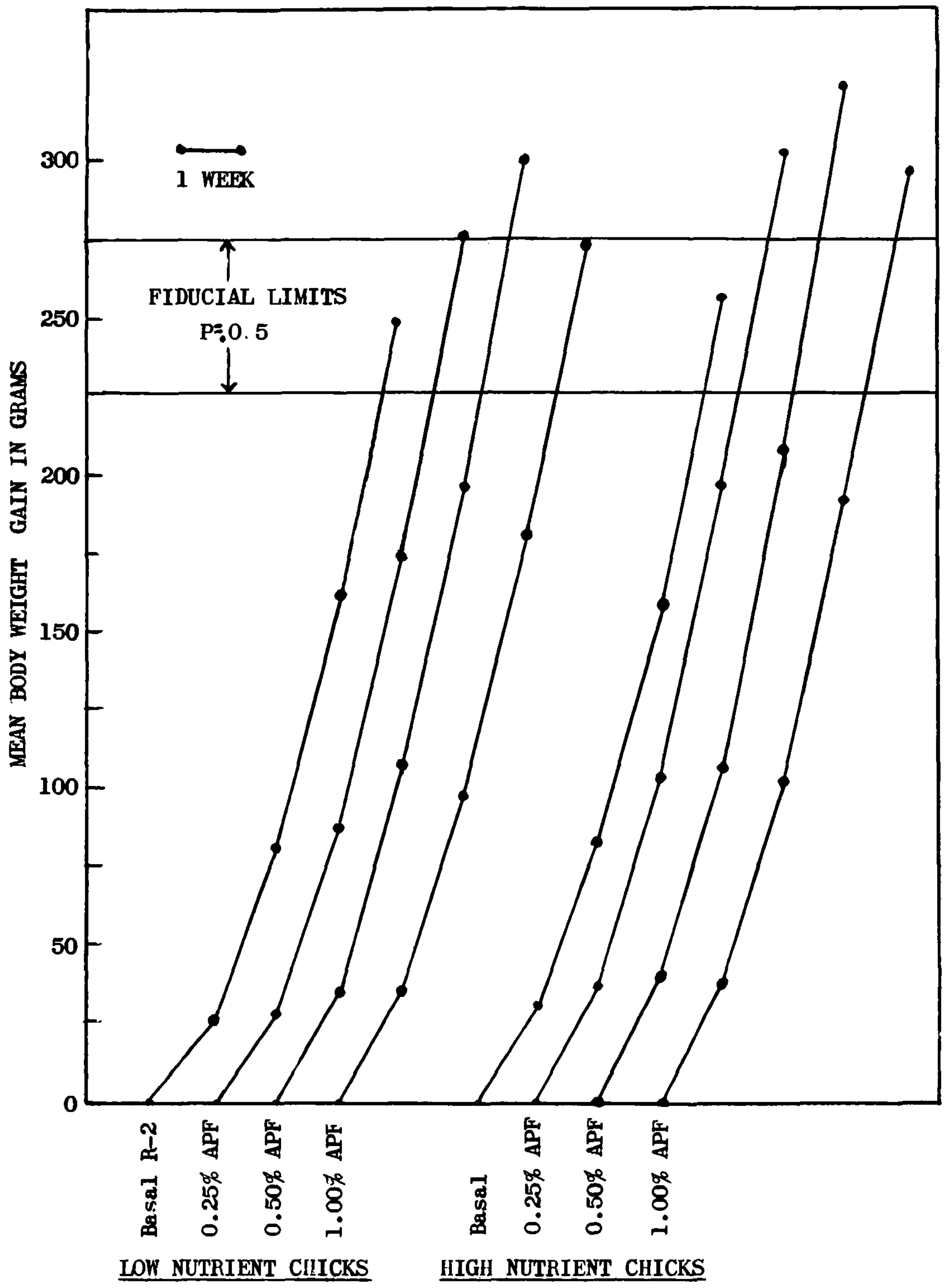


FIGURE 2. THE EFFECT UPON GROWTH OF VARIOUS LEVELS OF ANIMAL PROTEIN FACTOR SUPPLEMENT (A.P.F. LEDERLE) IN A HIGH ENERGY CHICK RATION

Experiment III: Effect of an APF Supplement on the Vitamin Requirements of the Chick

Experiment II established that addition of 0.5 percent APF (Lederle) to a basal ration containing adequate natural sources of all known vitamins would stimulate chick growth to an unprecedented degree. It must be remembered that the basal ration contained twice the recommended allowance of vitamins A and D. In the third experiment the basal level of these two vitamins was reduced to determine if the increased growth rate induced by APF (Lederle) would affect the vitamin requirements of the young chick.

In this and all subsequent growth trials, day-old male chicks were randomized into groups of twenty each containing ten high nutrient and ten low nutrient birds. The basal ration R-1, modified by the inclusion of 0.5 percent APF (Lederle) was supplemented with the same vitamins used in Experiment I. The four-week gains and feed consumption data are recorded in Appendix iv. Growth curves for each group are plotted in Figures 3a and 3b.

Statistical analysis of the gains (Appendix x) shows no difference between high and low plane chicks as there was in Experiment II. The high average gain of all groups (288 gms.) as compared to the average gain in Experiment I (234 gms.) suggests the beneficial effect of the APF Supplement included the basal ration. The response of the basal low nutrient chicks (291 gms.) approximated that observed on 0.5 percent

APF in Experiment II (301 gms.) and was not improved by addition of the vitamins. In contrast, the high nutrient basal (270 gms.) was far below its comparable group in Experiment II (327 gms.) and all vitamins except D_3 tended to increase growth. It is difficult to accept the implication that the presence of APF (Lederle) increased the vitamin requirements of high nutrient chicks but not those of low nutrient chicks. However, pending further investigation one must conclude that the addition of twice the recommended level of vitamins A, riboflavin, niacin and pantothenic acid to a high energy basal ration is without adverse effect and may in fact prove beneficial when the ration contains Lederle APF.

The improvement of feed efficiency with vitamin A, riboflavin and pantothenic acid supplements was greater in this trial than in Experiment I, indicating that these effects may be enhanced by the presence of APF (Lederle) supplements in the diet. Despite a reduction in growth, addition of vitamin D_3 to the basal ration also increased feed efficiency.

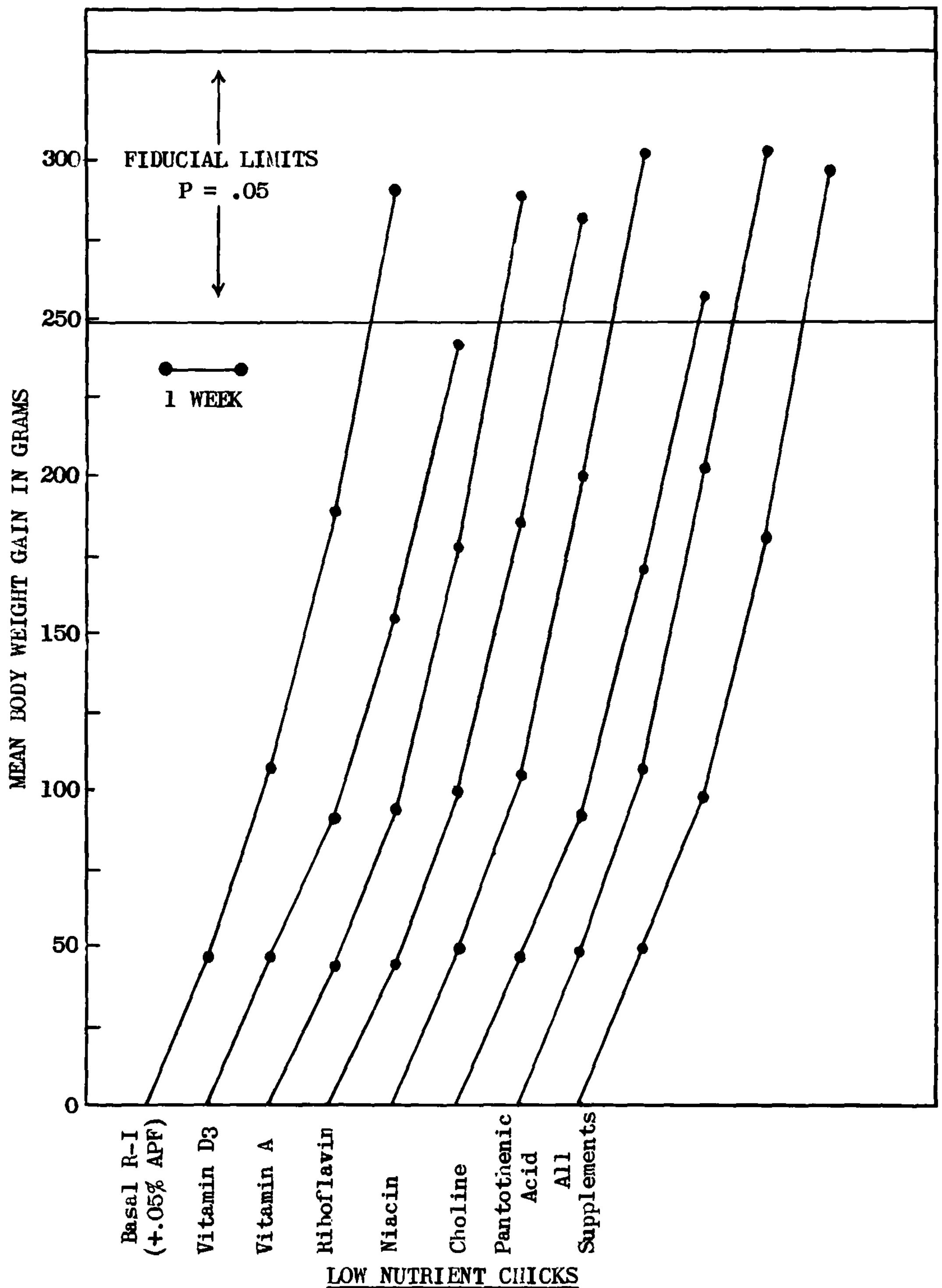
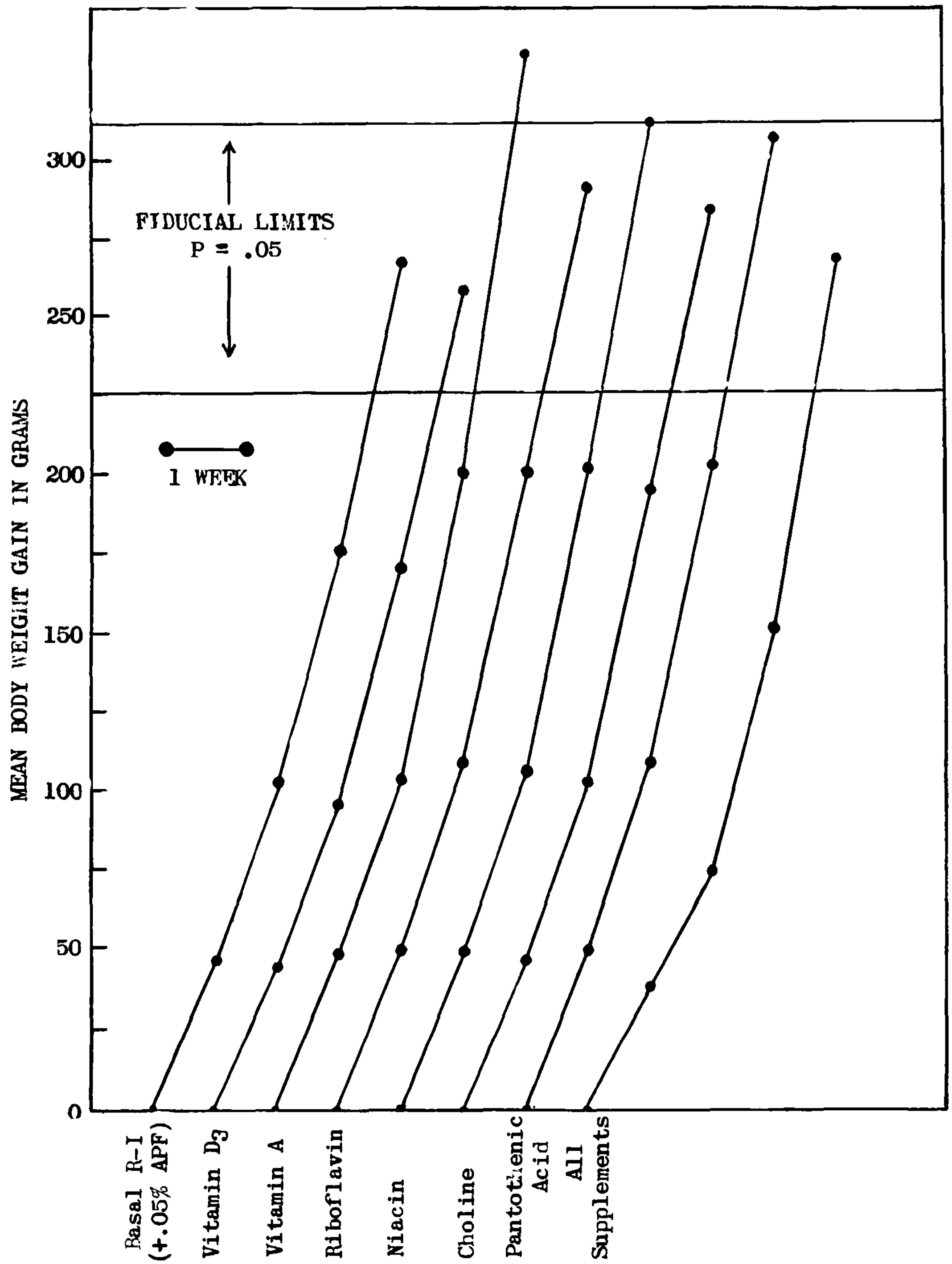


FIGURE 3a THE EFFECT UPON GROWTH OF TWO-FOLD THE RECOMMENDED LEVEL OF CERTAIN VITAMINS IN A HIGH ENERGY CHICK RATION CONTAINING A.P.F. (LEDERLE).



HIGH NUTRIENT CHICKS

FIGURE 3b. THE EFFECT UPON GROWTH OF TWO-FOLD THE RECOMMENDED LEVEL OF CERTAIN VITAMINS IN A HIGH ENERGY CHICK RATION CONTAINING A.P.F. (LEDERLE).

Experiment IV: Supplementary Value of APF, Vitamin B_{12} and Vitamin A in a High Energy Chick Ration

The results of previous experiments indicated that the growth stimulating factor in APF (Lederle) manifest in Experiment II, was distinct from vitamins A, D_3, riboflavin, niacin, choline and pantothenic acid (Experiment I) and was not present to any degree in fish or meat meal. This APF supplement was marketed primarily as a source of vitamin B_{12} and was reported by the Lederle Co. to contain the equivalent of 2 mg. vitamin B_{12} per pound. The fact that a more potent carrier (APF Merck - 3 mg. B_{12} per pound) was less effective in promoting chick growth suggested that the Lederle Co. product contained a growth promoting factor in addition to vitamin B_{12} (15, 100). To establish this point the supplementary values of APF (Lederle) and crystalline vitamin B_{12} were compared in Experiment IV. In addition two groups receiving APF and vitamin B_{12} were supplemented with vitamin A to check the apparent increased requirement shown in Experiment III.

Appendix v shows the supplements which were added to the basal ration (R-3 Table IV) with the corresponding four-week weight gains and feed consumption. Growth curves are presented in Figures 4a and 4b.

As in Experiment III analysis of variance (Appendix xi) shows no difference in growth between low and high nutrient chicks. The highly significant response to APF was not increased by addition of vitamin A, in contrast to the supplementary value of vitamin A in Experiment III. The inability of added vitamin B_{12} to increase growth demonstrates firstly that the basal ration was adequate in this factor and secondly that APF (Lederle) was not effective primarily through its vitamin B_{12} content. However the increased growth in the group receiving vitamins B_{12} plus A approximates that produced by APF (Lederle) and suggests an interrelationship between APF, vitamin A and vitamin B_{12}.

The basal ration was less efficiently utilized in this experiment than in previous tests. This fact may partially explain the dramatic increase in feed efficiency resulting from the addition of either APF (Lederle) or vitamin B_{12} to the ration. The beneficial value of the latter is more significant when one considers its negligible effect on growth.

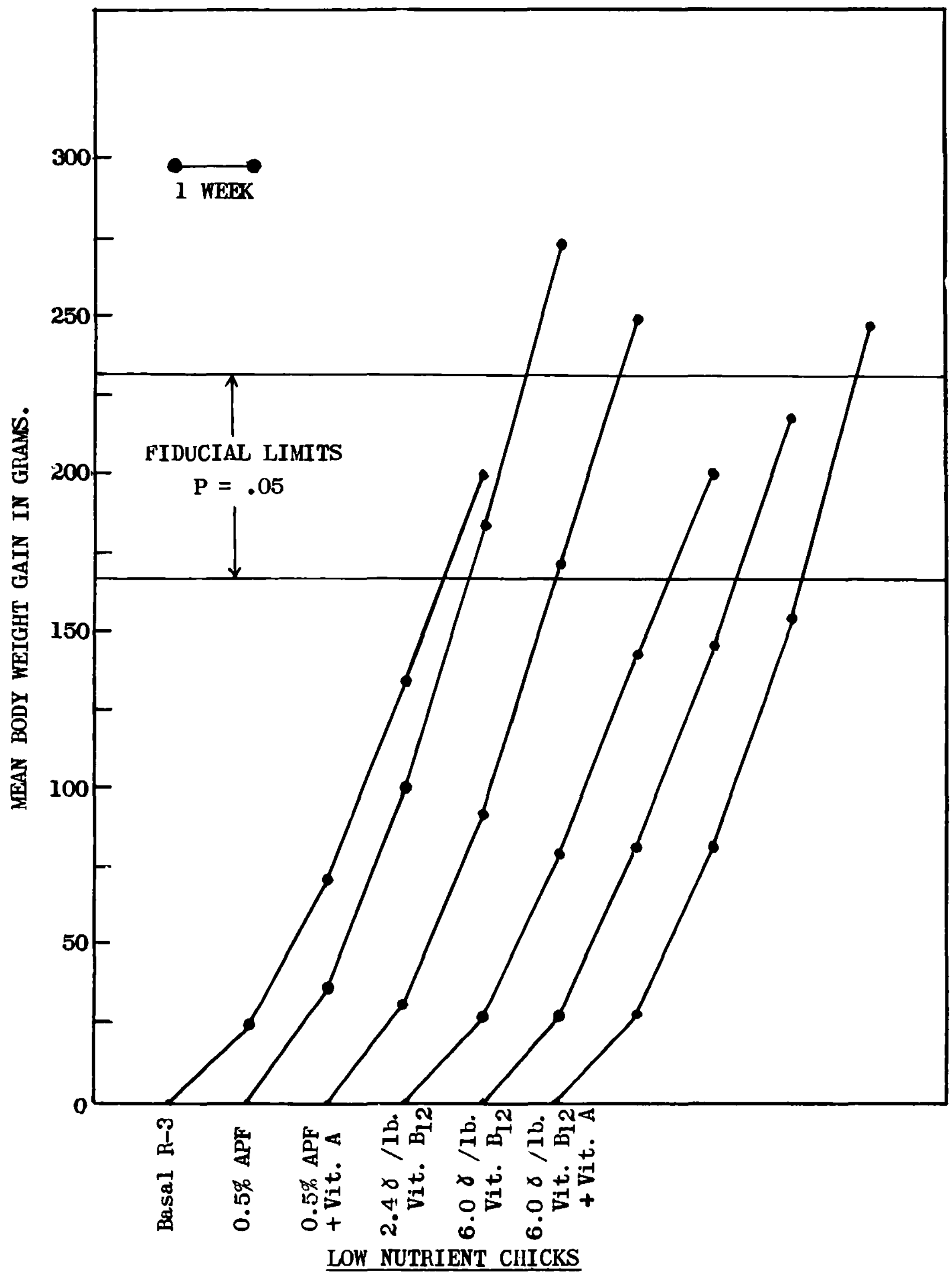


FIGURE 4a. THE EFFECT UPON GROWTH OF VITAMIN B_{12}, A.P.F AND VITAMIN A SUPPLEMENTS IN A HIGH ENERGY CHICK RATION.

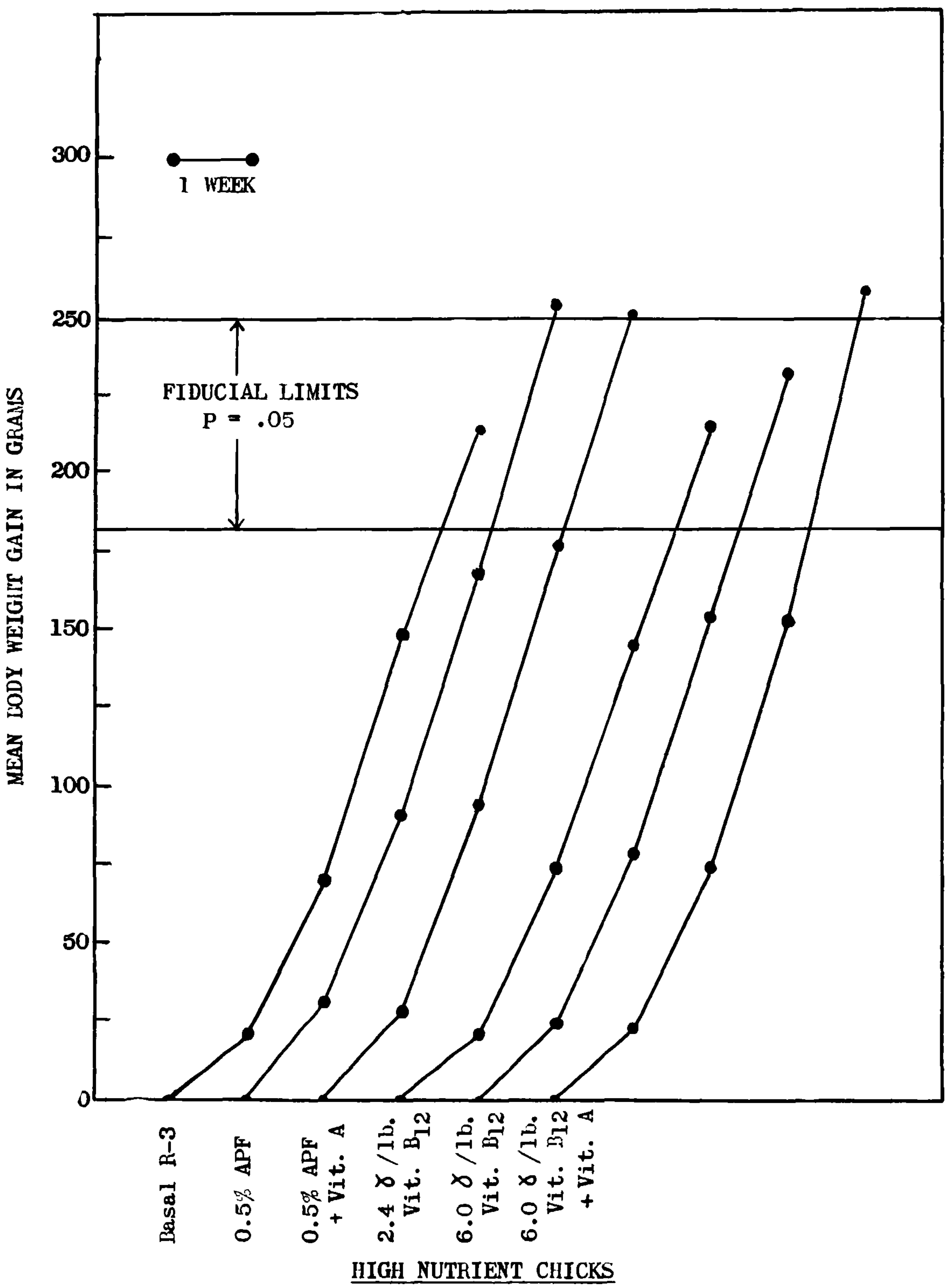


FIGURE 4b. THE EFFECT UPON GROWTH OF VITAMIN B_{12} A.P.F AND VITAMIN A SUPPLEMENTS IN A HIGH ENERGY CHICK RATION

Experiment V: Supplementary Value of Crystalline Aureomycin and Vitamin B_{12} in a High Energy Chick Ration

The report of Stokstad and Jukes (139) substantiated in this laboratory by the results of Experiments II and IV left no doubt that APF (Lederle) contained a chick growth stimulating factor in addition to vitamin B_{12}. The effectiveness of this supplement in a ration adequate in the known vitamins naturally aroused interest regarding the identity of its active component (s).

The organism Streptomyces aureofaciens used to produce an antibiotic aureomycin, also synthesized vitamin B_{12}. Since the crude mash, after removal of aureomycin, was dried and marketed as an APF supplement (Lederle), it seemed possible that its growth promoting property might be due to residual antibiotic. This possibility was investigated in Experiment V using crystalline aureomycin. Several workers (50, 105) reported that all animal protein could be excluded from high energy rations without adverse effect provided an APF supplement was used to supply adequate vitamin B_{12}. The availability of the crystalline vitamin enabled this claim to be rigorously tested in Experiment V.

The supplements to basal rations R-3 and R-4, with corresponding four-week gains and feed consumption data are set out in Appendix vi. Growth curves are plotted in Figures 5a and 5b.

Statistical analysis of the four-week gains (Appendix xii) reveals no difference between low and high nutrient chicks although the breeding stock had been on separate nutritional planes for three and one-half months when eggs were collected for incubation.

The enhanced growth of chicks fed APF (Lederle) appears due to its residual aureomycin, as the crystalline antibiotic produced the same dramatic response. Addition of vitamin B_{12} to an aureomycin-supplemented ration stimulated no additional growth, further demonstrating that the value of APF (Lederle) in chick rations containing adequate animal protein is not due to the vitamin B_{12} content.

The inclusion of 2 γ. vitamin B_{12} per pound in a vegetable protein ration was equivalent in terms of chick growth to the addition of 2.5 percent fish meal plus 3.0 percent meat meal. Further increase in the vitamin B_{12} content of the basal ration was ineffective. These results show that all of the animal protein in a high energy ration of the composition fed in this experiment can be replaced by vitamin B_{12} without appreciably affecting growth.

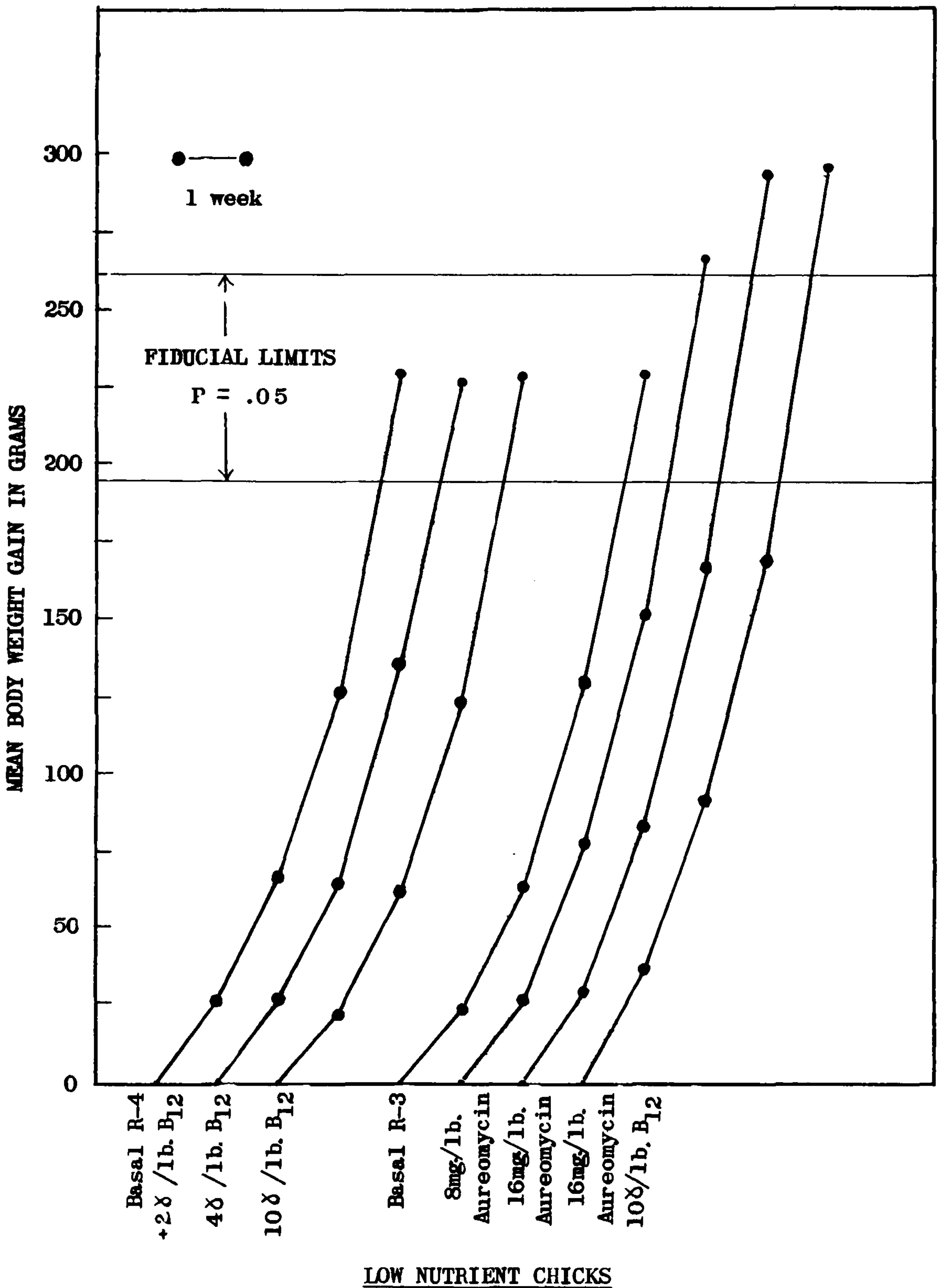


FIGURE 5a. THE EFFECT UPON GROWTH OF VITAMIN B_{12} AND AUREOMYCIN SUPPLEMENTS IN A HIGH ENERGY CHICK RATION

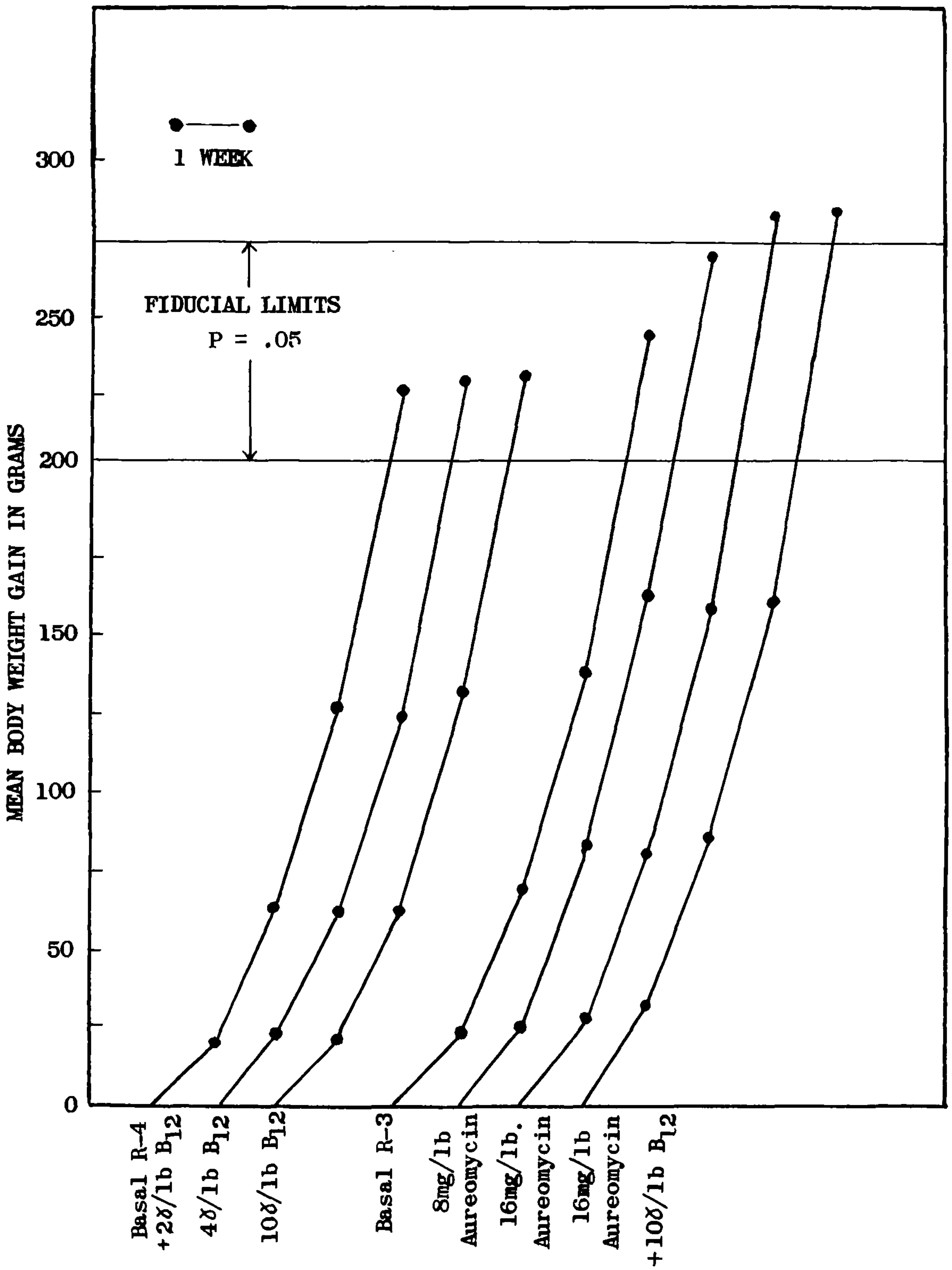


FIGURE 5b. THE EFFECT UPON GROWTH OF VITAMIN B_{12} AND AUREOMYCIN SUPPLEMENTS IN A HIGH ENERGY CHICK RATION

Experiment VI: Vitamin Requirements for Maximum Growth of Chicks Fed a High Energy Ration

The breeding stock had been on two different nutritional planes for five months when eggs were collected for the sixth and final hatch. In previous experiments the only recommended vitamin allowance which bordered on inadequacy was that for vitamin A. Supplementation of the basal rations employed in Experiments III and IV with vitamin A increased the four-week weight gains. To finalize this series of feeding trials, the same vitamin supplements used in Experiments I and III were added to the basal ration. Two additional groups were supplemented with vitamin B_{12} and vitamin B_{12} plus vitamin A to repeat if possible the results of Experiment IV.

Vitamin supplements added to basal ration R-3 are listed in Appendix vii with weight gains and feed consumption data. Growth curves are shown in Figures 6a and 6b.

High nutrient chicks grew more rapidly than low nutrient birds when results of the entire experiment were analysed (Appendix xiii); however, since in any single treatment the difference was far from significant, it could not be said that any supplement reduced this effect. It may be stated that during the six month test period lowering the nutritional plane of one-half the breeding stock did not decrease vitamin reserves of the chick to a degree necessitating vitamin requirements above the recommended allowance.

The addition of vitamin A alone or as part of a supplementary mixture stimulated the growth of high and low nutrient chicks alike. This result coupled with those of Experiments III and IV would indicate that the basal ration was slightly inadequate with respect to vitamin A. The addition of vitamin D_3, riboflavin and pantothenic acid supplements to the basal ration had no effect on growth and one must conclude, as in Experiment I that the recommended allowances for these vitamins are adequate for maximum early growth. The fortification of the basal diet with niacin and vitamin B_{12} was also ineffective.

In contrast to Experiments I, III, and IV, additional riboflavin and pantothenic acid did not improve feed efficiency. Vitamin A supplements however did induce better feed utilization while stimulating growth.

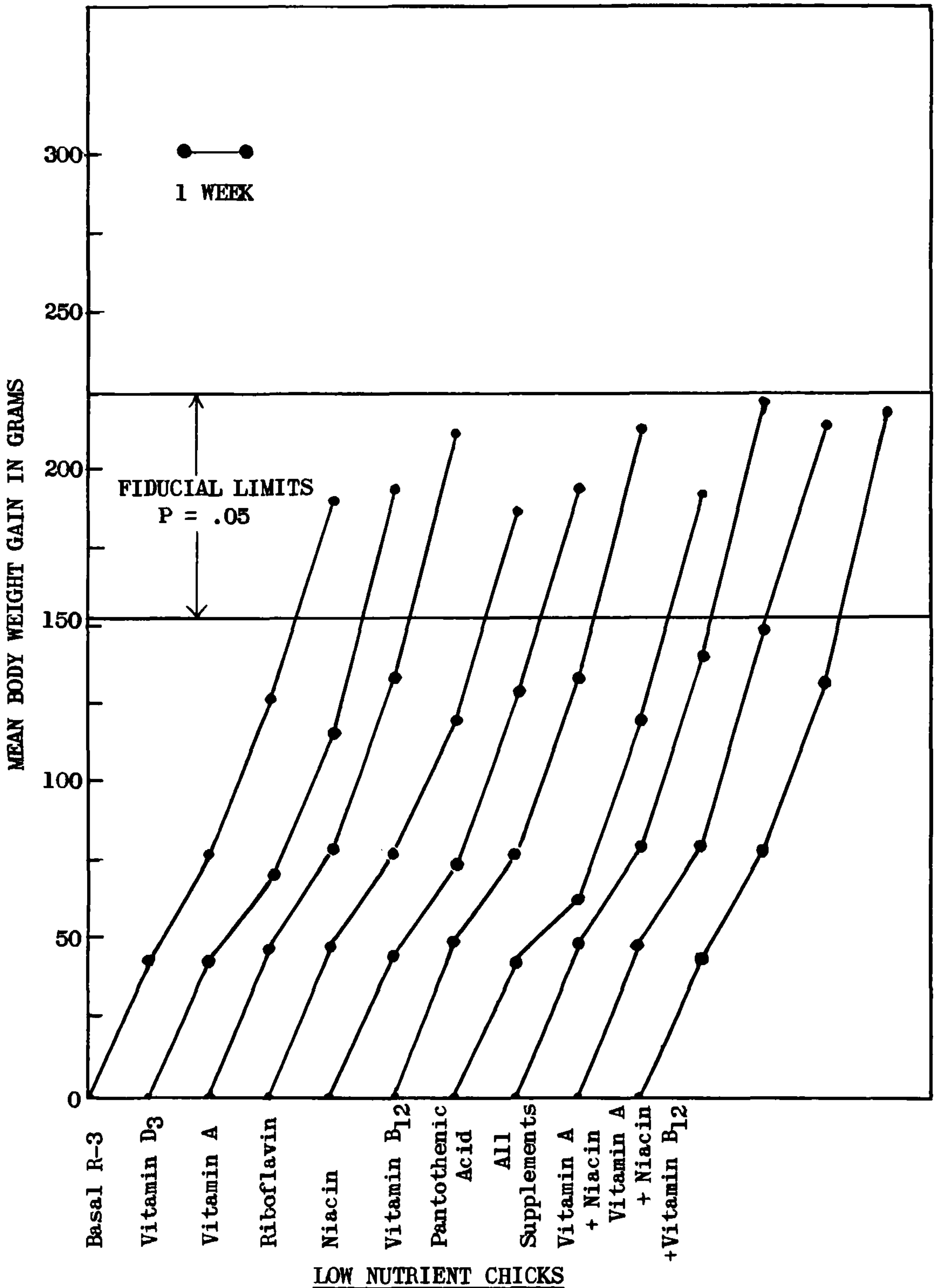


FIGURE 6a. THE EFFECT UPON GROWTH OF TWO-FOLD THE RECOMMENDED LEVEL OF CERTAIN VITAMINS IN A HIGH ENERGY CHICK RATION

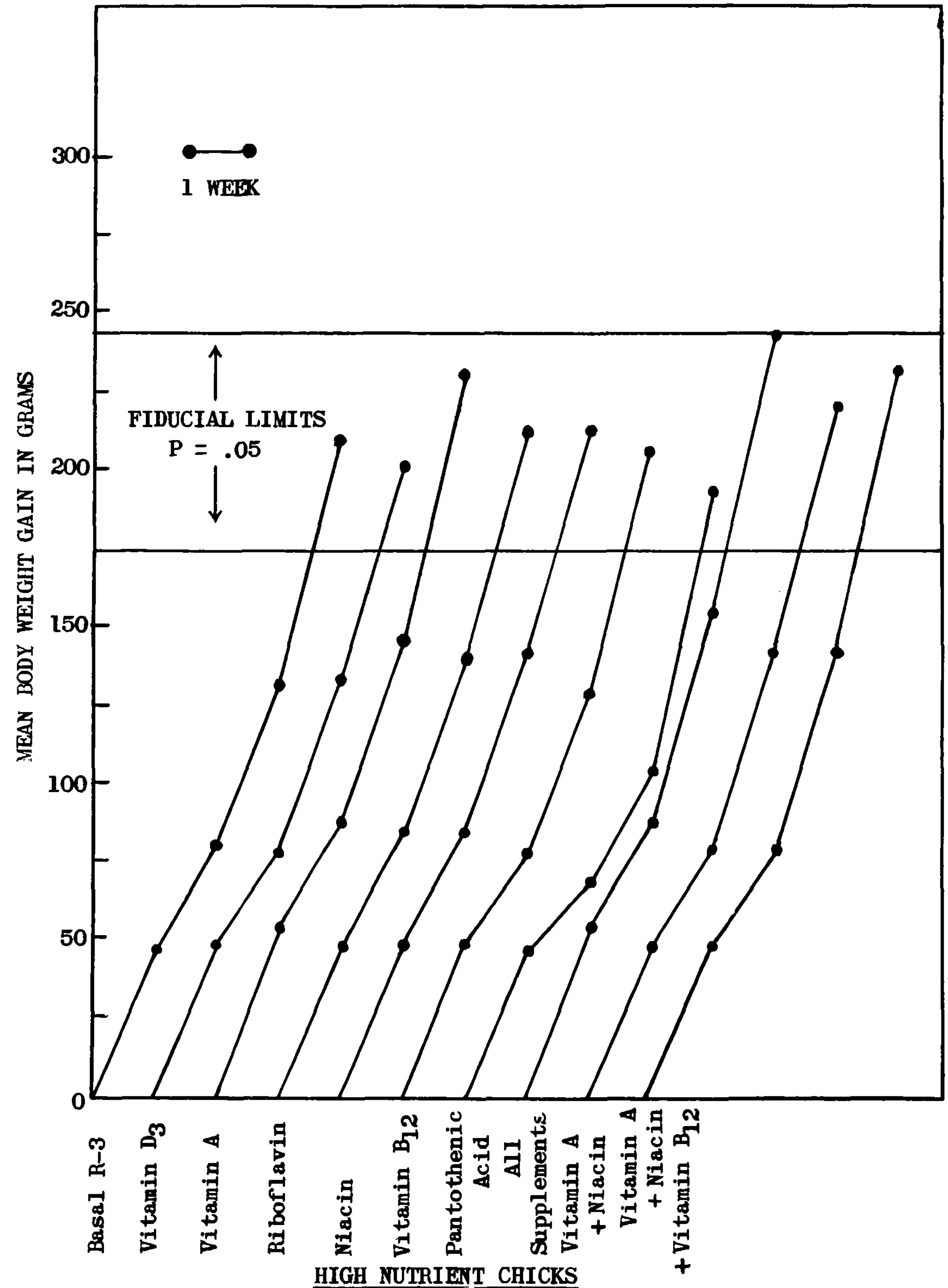


FIGURE 6b. THE EFFECT UPON GROWTH OF TWO-FOLD THE RECOMMENDED LEVEL OF CERTAIN VITAMINS IN A HIGH ENERGY CHICK RATION

TABLE IX: SUMMARY OF THE FOUR-WEEK B

Expt. No.	Basal Chick Diet	Hen Diet*	Basal Growth gms.	Mean Gain of Supplemented Groups from Basal Growth: Vit. A	Vit. D	Ribo-flavin	Niacin	Choline	Panto-thenic Acid	Vit Leve 1	APF 25%	APF 0.50%	APF 1.00%	Aureomycin Level 1	Aureomycin Level 2
6		L.N.	191	+11	+ 3	-2	+ 2		+ 1	+12					
	R-3	H.N.	209	+11	- 3	+1	+ 2		- 7	- 1					
		Av .	200	+11	0	-0.5	+2		- 3	+ 5.					
4		L.N.	201							0		+37	+25		
	R-3	H.N.	216							0		+17	+16		
		Av .	209							0		+27	+20.5		
5	R-4+	L.N.	234							- 2					
	2.0 γ	H.N.	229							+ 1					
	B_{12}/ lb.	Av .	232							- 0.					
5		L.N.	231											+16	+28
	R-3	H.N.	245											+10	+16
		Av .	238											+13	+22
1	R-1	Av .	237	+ 2	- 3	-3	+ 1	- 9	0						
2		L.N.	251									+20	+ 9		
	R-2	H.N.	258									+27	+16		
		Av .	254								.5	+23.5	+12.5		
3	R-1+	L.N.	291	0	-16	-3	+ 4	-11	+ 4						
	0.5%	H.N.	270	+24	- 4	+8	+16	+ 6	+15						
	APF	Av .	280	+12	-10	+2.5	+10	- 2.5	+ 9.5						

* L.N. - Low nut
H.N. - High nu

DISCUSSION

The section on analysis of data pointed out the danger of placing too great emphasis on results from unreplicated groups. To facilitate evaluation of the results from all experiments, the growth of supplemented groups have been recorded in Table IX as a percentage of the growth of corresponding basal groups. It will be noted that the table lists the various experiments in ascending order of basal growth.

The comparison of growth in different experiments shows a variation between experiments which is of much greater magnitude than the variation produced within any experiment by differences in maternal or chick diet. When one considers the genetic uniformity of the breeding stock, its diet and experimental management, and the similarity of chick diets in all experiments it is obvious that unknown environmental influences are producing substantial variations in the early growth of the chicks. It is of course impossible to say whether these influences occur during the pre- or post-hatching period. It is probable, however, that a considerable part of the variability in growth between lots of commercial broilers, hitherto attributed to genetic or nutritional sources, may in fact be due to environmental causes.

The main purpose of this project was to study the adequacy of recommended vitamin allowances for maximum chick growth during the first month of life. The results in Table IX show that twice the recommended level of riboflavin, pantothenic acid and vitamin D_3 in a high energy ration had no effect on growth. Slinger (133) recently published data confirming these indications that the recommended levels of riboflavin and pantothenic acid are ample for rapid chick growth. When one considers the great excess of certain vitamins in commercial broiler rations it is of special interest to note that twice the needed quantity of riboflavin, pantothenic acid or vitamin D_3 are without adverse effect on growth of chicks fed a diet of natural feedstuffs. When APF (Lederle) was included in the basal ration, additional D_3 appeared to depress growth (Experiment III). The possibility that this is due to an interaction between aureomycin in the APF (Experiment V) and the vitamin D_3 is heightened by recent evidence of Migicovsky (92) that antibiotic has a direct effect on calcium absorption. This effect is evident in vitamin D deficient chicks. Stokstad and Jukes (140), Scott (125), and Slinger (134) have shown that antibiotics or antibiotic feed supplements may aggravate or alleviate leg and hock disorders, depending on the basal diets employed.

Twice the recommended allowance of choline in the diet depressed chick growth in Experiments I and III. This observation was not studied in subsequent trials. However, Melass (91) observed a definite growth depression when 4.5, 9.0 or 18.0 gms. choline per pound was included in a chick starter. Since the highest level in the present work was 1.4 gm. per pound it would appear that choline toxicity can occur at lower concentrations than hitherto reported. These results may be due in part to the abundance in the basal ration of methionine and vitamin B_{12} which have definite choline sparing action (20, 51). Considering this toxicity of choline at relatively low dietary concentration it is concluded that the recommended allowance for this vitamin should be taken as an optimum rather than a minimum value.

It was previously pointed out that the niacin content of the basal ration used in this series of experiments was 20 to 25 mg. per pound, roughly three times the recommended allowance. From the ineffectiveness of further dietary niacin one can only conclude that the chick's requirement for this vitamin is not in excess of 20 to 25 mg. per pound. This finding is of some practical significance when one considers that the high corn content of the ration is likely to increase the niacin requirement (124). The Connecticut group (131) supplemented their high energy broiler ration with 15 mg. per pound principally to compensate for the low tryptophan content of the high corn diet. Mishler (96) reported that the addition of 22 mg. niacin per pound to an all-vegetable protein ration will depress growth. When animal protein was included in the diet this growth depression was counteracted. Results of the present experiments indicate that chicks fed diets containing animal protein can tolerate 34 mg. niacin per pound. Again, the results of the high nutrient group in Experiment III must be mentioned. Chicks which showed relatively poor growth despite the addition of APF (Lederle) to the ration, grew significantly faster when supplementary niacin was fed. This and other apparently beneficial effects of vitamin supplements in diets containing APF warrant confirmatory study.

Chicks from both nutritional stocks exhibited maximum growth on an all-vegetable protein ration containing 2.5 γ vitamin B_{12} per pound (Experiment V) and on a fish meal-soybean meal ration containing 1.8 γ vitamin B_{12} per pound (Experiment IV). Jukes (139) and Ott (104) placed the requirement of chicks at 8 to 12 γ and 7 to 10 γ vitamin B_{12} per pound respectively. However the maternal B_{12} depletion regimen and high protein diets employed by these workers would increase the vitamin B_{12} requirement considerably above that of normal chicks. Slinger (133) has recently claimed that over 10 γ vitamin B_{12} per pound is necessary for maximum early growth of undepleted chicks. The APF (Merck) supplement used in his study as the source of vitamin B_{12} and originally reported by the Merck Company to contain 12.5 mg. Vitamin B_{12} per pound has now been stated to contain only 3 mg. vitamin B_{12} per pound (150). Thus the estimate of Slinger should be approximately 3 γ vitamin B_{12} per pound. Norris (101) and Davis (43), using crystalline vitamin B_{12} placed the requirement at 1 to 2 γ per pound. Apparently much of the disagreement concerning the actual requirement was due to the use of stress factors and the incorrect potency rating of a standard B_{12} supplement. When consideration is given to these variables, current data indicate that the chick requirement for vitamin B_{12} is 2 to 3 γ per pound.

The recommended vitamin A allowance for early chick growth appeared slightly inadequate in two of three experiments. Although growth was not improved in Experiment I nor in the low nutrient group of Experiment III by doubling the recommended level of vitamin A, the same treatment significantly increased the growth of high nutrient chicks in Experiment III and of both high and low nutrient birds in Experiments IV and VI. The fact that "inadequacy" of the recommended allowance became more evident in later experiments suggests an increase in the vitamin A requirement with higher environmental temperatures. The final experiment in particular was conducted during a period of hot weather. Heywang (67) has demonstrated higher vitamin A requirement of hens for egg production and hatchability in the summer. Pending further study on this aspect of vitamin A metabolism it seems advisable to increase the recommended allowance for chicks, particularly those raised during the warmer months of the year.

Examination of the feed to gain ratios in all experiments reveals that dietary levels of vitamins A, B_{12}, riboflavin and pantothenic acid which are sufficient for maximum growth may be inadequate for most efficient feed utilization. Stevens (138) showed that a vitamin B_{12} supplement improved feed efficiency without increasing growth. In addition the involvement of riboflavin with feed utilization has been demonstrated by Record (113). These limited observations suggest that the vitamin requirements of chicks should be assessed with greater attention to the demands for maximum feed efficiency.

The second object of this work was to determine if alteration in the vitamin content of the breeder ration could produce differences in the vitamin reserves of chicks that would render the recommended vitamin allowances inadequate for maximum chick growth. It was desired to have vitamin levels in the low nutrient ration approaching but not below the minimum amounts necessary for good hatchability. The actual nutrient content of the breeder diets was considered on page 13. It was concluded that increasing the whole grain to mash ratio of the breeding ration from 1:1 in the high nutrient group to 3:1 in the low nutrient group would not lower the vitamin content of the diet below that necessary for good hatchability. This assumption was justified by the excellent egg production and hatchability of both breeding flocks in the four month period during which records were kept (Appendix i). It was apparent from Table VI that the greatest difference between the breeder diets lay in their content of vitamins A and D. This would be expected since whole grains are devoid of these two vitamins. The extensive synthesis of riboflavin in voided feces (19, 81), vitamin B_{12} in floor litter (57, 58, 78) choline in the hen (55, 87) and niacin in the developing embryo (41, 136) would tend to minimize the slight differences in the breeder ration content of these vitamins. Thus any effect of parental nutrition on the growth of the chick is more likely to be attributable to the difference in vitamin A or D intake of the breeding stock.

The four-week growth data show that dilution of the breeder mash nutrients has not affected the adequacy of the recommended vitamin allowances under study. When all groups in each experiment were considered, high nutrient chicks grew faster than low nutrient birds only in Experiments II and VI; where the differences were eight and six percent respectively. It is evident that the effect of breeder diet was small and did not increase with prolongation of feeding upon high and low nutrient planes. In no experiment was the growth of high and low nutrient basal groups significantly different; thus it was impossible to tell if any supplement was effective in counteracting the "deficiency" in low nutrient chicks. It may be judged from the results however, that no vitamin supplement consistantly tended to alleviate the deficiency. The example of vitamin A is most striking. The low nutrient breeder diet verged on deficiency of this vitamin. The chick basal diet contained insufficient amounts for maximum growth and yet, low nutrient chicks responded no better to supplementary vitamin A than did high nutrient chicks. It is concluded that although differences in vitamin intake of breeding stock can alter the growth rate and livability of chicks maintained on vitamin deficient rations, such changes in parental nutrition cannot seriously affect the growth rate of progeny fed an adequate diet. Further, the recommended allowances for vitamins D_3, B_{12}, choline, pantothenic acid and riboflavin appear adequate for maximum early chick growth even when the vitamin A, D_3, riboflavin and pantothenic acid intake of the parent stock has been reduced below the recommended level. This observation coupled with the continuance of good hatchability testifies to the adequacy of the N.R.C. recommendations of vitamins A, D_3, riboflavin and pantothenic acid for breeding stock.

The third consideration in this project was the relationship between growth rate and vitamin requirements. Stokstad and Jukes (139) reported that APF (Lederle) could accelerate chick growth when added to rations adequate in all known factors. Their results were verified in Experiment II. The adequacy of recommended vitamin allowances for chicks with APF stimulated growth rates was then studied (Experiment III). Unfortunately the results are contradictory. Low nutrient chicks showed the expected increase in growth yet derived no benefit from vitamin levels above the recommended allowance. High nutrient birds on the other hand gave lower responses to the APF and required supplementary vitamin A, riboflavin, niacin and pantothenic acid for maximum growth.

The question now raised is whether the increased requirements are due to a higher growth rate per se or directly due to some stress factor in the APF. Ershoff (46) has demonstrated that Lederle APF contains factor (s) distinct from vitamin B_{12} and aureomycin which can reduce the severity of thyroprotein toxicity in rats. Crystalline aureomycin significantly increased the growth of chicks without any apparent effect on their well being (Experiment V). Stokstad (141) and Davis (43) have shown no increase in the B_{12} requirements of chicks fed crystalline aureomycin. Slinger (132) and Machlin (89) report that dietary penicillin or aureomycin has a slight sparing action on the protein requirements of chicks. Stokstad (142) recently stated that the water-soluble vitamin requirements are not increased in aureomycin fed chicks despite the substantial growth acceleration. One may conclude from these results that the greater growth rate now possible through the use of well-balanced rations and dietary antibiotics does not appreciably increase the vitamin requirements of chicks.

SUMMARY

The adequacy of currently recommended vitamin allowances for growing chicks was studied in a series of four-week feeding trials, using day-old New Hampshire by Barred Plymouth Rock birds.

Two-fold the recommended levels of vitamin D_3, riboflavin, pantothenic acid or choline was without beneficial effect on the growth of chicks fed a high energy ration. Supplementing this ration with additional niacin and vitamin B_{12} likewise failed to increase growth. Despite the lack of growth stimulation, supplementary amounts of riboflavin, pantothenic acid and vitamin B_{12} improved the efficiency of feed utilization. In three out of four experiments, the recommended allowance for vitamin A appeared inadequate for maximum growth and feed efficiency.

The maintenance of one-half the breeding stock on a low nutrient diet by dilution of the mash with two to three times the normal quantity of whole grain had no marked effect on chick reserves that could be detected in the growth of progeny fed a diet containing the recommended vitamin allowances. Although, in the second and sixth experiments, chicks from low nutrient breeders grew significantly slower than those from high nutrient stock, the difference in final weight was only eight percent. In no case was there an indication that increasing the vitamin content of the chick ration could alter this slight difference.

The inclusion of Animal Protein Factor (Lederle Co.) supplement or crystalline aureomycin in the basal ration produced ten to twenty percent more body weight at four weeks. Despite the higher growth rates attained through the use of dietary antibiotics, there was no consistent evidence that the recommended vitamin allowances were inadequate.

The failure to demonstrate consistent differences in hatchability and early chick growth despite abnormal dilution of essential nutrients in the breeder ration, indicates that the recommended allowances of vitamins A, D_3, riboflavin and pantothenic acid for breeding stock are sufficient. The ability of supplementary amounts of vitamins B_{12}, riboflavin and pantothenic acid in the chick ration to increase feed efficiency, without affecting growth, demonstrates that recommended allowances for these vitamins, while adequate for maximum growth may not promote the most efficient feed utilization. On the other hand, under the conditions of these experiments, the recommended allowance of vitamin A for chicks proved generally inadequate for maximum growth and feed efficiency.

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APPENDIX i

EGG PRODUCTION AND HATCHABILITY DURING THE INITIAL FOUR MONTHS OF EXPERIMENT[1]

Month Eggs Produced	Month Eggs Hatched	Corresponding Chick Expt. No.	Percent Egg Production[2]			Percent Hatchability[3]		
			L.N.[4]	H.N.[5]	Difference H.N. L.N.	L.N.	H.N.	Difference H.N. L.N.
Dec. 1949	Jan. 1950	1	36.6	36.2	-0.4	65.9	66.5	+0.6
Jan. 1950	Feb. 1950	2	51.8	59.1	+7.3	78.8	74.9	-3.9
Feb. 1950	Mar. 1950	3	65.8	65.7	-0.1	70.0	65.9	-4.1
Mar. 1950	Apr. 1950	4	48.5	51.3	+2.8	75.5	70.4	-5.1
Apr. 1950	May 1950	5	No Records					
May 1950	June 1950	6	No Records					
		Average	50.7	53.1	+2.4	72.9	69.4	-3.1

1) These records were kept by J. Radford as part of his fourth-year project.

2) Calculated as average number of eggs per hen per 100 days.

3) Calculated as number of eggs hatched per 100 fertile eggs incubated.

4) Low nutrient breeder group.

5) High nutrient breeder group.

APPENDIX ii

EXPERIMENT I: EFFECT OF VITAMIN SUPPLEMENTS ON CHICK GROWTH RESPONSE TO A HIGH ENERGY RATION

Basal Ration: R-1

Lot No.	Supplement	Weight Gain 4 Weeks (gms.) 20 Chicks	Feed Consumption (gms.) 20 Chicks	Feed:Gain
1	None	237	522	2.20
2	Vit. D_3	230	489	2.12
3	Vit. A	241	514	2.13
4	Riboflavin	231	487	2.10
5	Niacin	240	533	2.22
6	Choline	215*	441	2.05
7	Pantothenic Acid	236	444	1.87
8	All Supplements	238	468	1.96
	Average (160 Chicks)	234	487	2.08

*Least Significant Difference at P = .05 per group of 20 chicks is 22 gms.

APPENDIX iii

EXPERIMENT II: SUPPLEMENTARY VALUE OF ANIMAL PROTEIN FACTOR (APF) TO A HIGH ENERGY RATION

Basal Ration: R-2

Lot No.	Supplement	Weight Gain 4 Weeks (gms.) L.N. 20 Chicks	Weight Gain 4 Weeks (gms.) H.N. 20 Chicks	Feed Consumption (gms.) L.N. 20 Chicks	Feed Consumption (gms.) H.N. 20 Chicks	Feed:Gain L.N.	Feed:Gain H.N.
1	None	251	258	518	542	2.06	2.10
2	0.25% APF	273	304	623	590	2.28	1.94
3	0.50% APF	301	327	625	648	2.08	1.98
4	1.00% APF	274	299	621	616	2.27	2.06
	Average (80 Chicks)	275	297	596	599	2.17	2.02

*Least Significant Difference at P = .05 for groups of 20 chicks = 23 gms.
40 chicks = 17 gms.
80 chicks = 12 gms.

APPENDIX iv

EXPERIMENT III: EFFECT OF ANIMAL PROTEIN FACTOR (APF) ON CHICK VITAMIN REQUIREMENTS

Basal Ration: R-1 Plus 0.5 per cent APF (Lederle)

Lot No.	Supplement	Weight Gain 4 Weeks (gms.) L.N. 10 Chicks	H.N. 10 Chicks	Average 20 Chicks	Feed Consumption (gms.) 20 Chicks	Feed:Gain
1	None	291	270	280	660	2.37
2	Vit. D_3	243*	260	251*	546	2.17
3	Vit. A	290	335*	313*	646	2.06
4	Riboflavin	282	292	287	636	2.21
5	Niacin	304	314*	309*	695	2.25
6	Choline	258	286	272	624	2.29
7	Pantothenic Acid	304	308	306	665	2.17
8	All Supplements	299	270	284	553	1.95
	Average (80 Chicks)	284	292	288	628	2.18

*Least Significant Difference at P = +.05 for groups of 10 chicks = 43 gms.
20 chicks = 30 gms.
80 chicks = 15 gms.

APPENDIX V

EXPERIMENT IV: SUPPLEMENTARY VALUE OF VITAMINS A, B_{12}, AND APF TO A HIGH ENERGY RATION

Basal Ration: R-3

Lot No.	Supplement	Weight Gain 4 Weeks (gms.) L.N. 10 Chicks	H.N. 10 Chicks	Average 20 Chicks	Feed Consumption (gms.) 20 Chicks	Feed:Gain
1	None	201	216	209	514	2.46
2	0.5% APF	275 *	254 *	265 *	559	2.11
3	0.5% APF + Vit. A	252 *	251 *	252 *	505	2.01
4	2.4 γ /lb. B_{12}	202	217	210	462	2.21
5	6.0 γ /lb. B_{12}	219	234	227	476	2.10
6	6.0 γ /lb. B_{12}+Vit.A	248 *	260	254 *	529	2.08
	Average (60 Chicks)	233	239	236	508	2.16

*Least Significant Difference at P = .05 for groups of 10 chicks = 34 gms.
20 chicks = 24 gms.
60 chicks = 14 gms.

APPENDIX vi

EXPERIMENT V: SUPPLEMENTARY VALUE OF AUREOMYCIN AND VITAMIN B_{12} TO A HIGH ENERGY RATION

Basal Ration: R-4 for Lots 1 - 3; R-3 for Lots 4 - 7

Lot No.	Supplement	Weight Gain 4 Weeks (gms.) L.N. 10 Chicks	H.N. 10 Chicks	Average 20 Chicks	Feed Consumption (gms.) 20 Chicks	Feed:Gain
1	2.0 γ/lb. B_{12}	234	229	232	479	2.08
2	6.0 γ/lb. B_{12}	229	232	231	431	1.87
3	10.0 γ/lb. B_{12}	230	233	232	507	2.19
4	None	231	245	238	499	2.10
5	8 mg./lb. Aureomycin	268*	270	269*	539	2.00
6	16 mg./lb. Aureomycin	297*	283*	290*	572	1.97
7	16 mg./lb. Aureomycin 10.0 γ/lb. B_{12}	297*	284*	291*	592	2.03
	Average (70 Chicks)	255	254	255	517	2.03

*Least Significant Difference at P = .05 for groups of 10 chicks = 32 gms.

20 chicks = 22 gms.

70 chicks = 12 gms.

APPENDIX vii

EXPERIMENT VI: EFFECT OF VITAMIN SUPPLEMENTS ON CHICK GROWTH RESPONSE TO A HIGH ENERGY RATION

Basal Ration: R-3

Lot No.	Supplement	Weight Gain 4 Weeks (gms.) L.N. 10 Chicks	H.N. 10 Chicks	Average 20 Chicks	Feed Consumption (gms.) 20 Chicks	Feed:Gain
1	None	191	209	200	467	2.34
2	Vit. D_3	196	202	199	487	2.45
3	Vit. A	213	232	222	473	2.13
4	Riboflavin	187	212	199	459	2.31
5	Niacin	195	213	204	503	2.46
6	Vit. B_{12}	214	207	210	489	2.33
7	Pantothenic Acid	193	195	194	453	2.33
8	All Supplements	223	245	234*	516	2.20
9	Vit. A + Niacin	216	222	219	523	2.39
10	Vit. A + Niacin + Vit. B_{12}	219	234	227*	525	2.31
	Average (100 Chicks)	205	217	211	490	2.32

*Least Significant Difference at P = .05 for groups of 10 chicks = 35 gms.
20 chicks = 24 gms.
100 chicks = 11 gms.

APPENDIX viii

ANALYSIS OF VARIANCE FOR EXPERIMENT I

Source of Variation	Sum of Squares	D/F	Variance	F
Total	196,632	151		
Supplements	10,387	7	1,481	1.15
Remainder	126,245	144	1,292	

$$\text{Least Significant Difference} = \frac{\text{Error Variance} \times 2}{N} \times t$$

When t at P .05 for 144 D/F = 1.98, and

N (number per group) = 20

$$\text{L.S.D.} = \frac{1292 \times 2}{20} \times 1.98 = 22$$

APPENDIX ix

ANALYSIS OF VARIANCE FOR EXPERIMENT II

Source of Variation	Sum of Squares	D/F	Variance	F
Total	296,072	159		
Between 8 Subgroups	93,777	7		
Breeder Rations	19,893	1	19,893	14.9**
Supplements	63,370	3	23,123	17.4**
Nil vs APF	51,750	1	51,750	38.8**
Levels APF	17,620	2	8,810	6.6**
Interaction	4,514	3	1,505	1.1
Remainder (Error)	202,295	152	1,331	
Without APF	39,140	38	1,030	
With APF	163,155	114	1,431	1.4

Least Significant Difference at P = .05 for groups of 20 = 23
40 = 17
80 = 12

**Significant at P = .01

APPENDIX x

ANALYSIS OF VARIANCE FOR EXPERIMENT III

Source of Variance	Sum of Squares	D/F	Variance	F
Total	407,896	159		
Between 16 Subgroups	84,111	15		
Breeder Diet	2,544	1	2,544	1.1
Supplements	61,304	7	8,758	3.89**
Interaction	20,263	7	2,895	1.29
Remainder (Error)	323.785	144	2,248	

Least Significant Difference at P = .05 for groups of 10 = 43
20 = 30
80 = 15

**Significant at P = .01

APPENDIX xi

ANALYSIS OF VARIANCE FOR EXPERIMENT IV

Source of Variance	Sum of Squares	D/F	Variance	F
Total	220,747	119		
Between 12 Subgroups	63,800	11	5,800	4.00**
Breeder Diet	1,030	1	1,030	1.00
Supplements	59,168	5	11,834	8.14**
Diet and Supplements	3,602	5	720	1.00
Remainder	156,947	108	1,453	
Without APF	98,023	72	1,361	
With APF	58,924	36	1,636	1.20

Least Significant Difference at P = .05 for groups of 10 = 34

20 = 24

60 = 14

**Significant at P = .01

APPENDIX xii

ANALYSIS OF VARIANCE FOR EXPERIMENT V

Source of Variance	Sum of Squares	D/F	Variance	F
Total	231,968	129		
Between 14 Subgroups	97,791	13		
Breeder Diet	35	1	35	1.0
Supplements	94,780	6	15,797	12.9**
Interaction	2,984	6	497	1.0
Remainder (Error)	141,726	116	1,222	
Without Antibiotic	60,610	65	932	
With Antibiotic	81,116	51	1,590	1.70

Least Significant Difference at P = .05 for groups of 10 = 32
20 = 22
70 = 12

**Significant at P = .01

APPENDIX xiii

ANALYSIS OF VARIANCE FOR EXPERIMENT VI

Source of Variance	Sum of Squares	D/F	Variance	F
Total	310,022	199		
Between 20 Subgroups	45,756	19	2,408	1.64
Breeder Diet	7,345	1	7,345	5.00*
Supplements	33,598	9	3,733	2.54**
Interaction	4,813	9	535	1.00
Remainder (Error)	264,266	180	1,468	

Least Significant Difference at P = .05 for groups of 10 = 35

20 = 24

100 = 11

*Significant at P = .05

**Significant at P = .01