ESTABLISHING PRECISE DAILY NUTRIENT REQUIREMENTS FOR LAYING HENS

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ESTABLISHING PRECISE DAILY NUTRIENT

REQUIREMENTS FOR LAYING HENS

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INTRODUCTION

The egg-producing segment of the poultry industry is more important economically than the meat-producing segment. Despite this fact much less is known about the specific requirements and nutritional problems of the laying hen than of the growing broiler or turkey. No area of study in the field of poultry nutrition research appears to be yielding such contradictory results as that pertaining to the nutrient requirements of laying hens. These nutrient requirements are normally expressed as a percentage of the total ration or in units per pound, with little consideration being given to the many factors which are known to influence total feed intake and consequently affect the requirements for protein, amino acids, energy and vitamins.

There has been considerable research and discussion concerning the protein requirement of laying hens. Available research findings suggest that any level of dietary protein between 11 and 18 percent is adequate for normal egg production. Many factors have probably contributed to this wide range of suggested protein levels. For instance, there is no doubt of the intrinsic importance of a proper ratio of metabolizable energy and digestible protein for chicks and other animals. This same mechanism must be important with hens, but it has not been clearly demonstrated. Other factors which regulate the protein requirement include total feed intake, level of egg production, amino acid balance and availability, and vitamin levels as well as dietary energy level.

Since the laying hen has not been very sensitive in her response to energy:protein ratios, the determination of energy requirements has been a very complex problem. The energy content of the diet has a very definite effect on feed consumption and yet it has been shown that hens can maintain their body weight and a normal rate of egg production on energy levels ranging from 740 to 1025 Calories of productive energy per pound.

Research data on the vitamin requirements of laying hens are very limited. The general consensus of opinion is that the vitamin requirements become more exacting as egg production is increased to higher levels and as less feed is required to produce a dozen of eggs. However, the type of contradictions that are found in protein and energy requirements are also found in the vitamin requirements.

The primary objective of this study was to develop a more precise method of determining and expressing the nutritive requirements of the laying hen. It was reasoned that more consideration would be given to total nutrient intake, nutrient interrelationships and other factors which govern nutrient requirements, if these requirements were expressed on a nutrient intake basis and determined through a technique which would allow nutrient intake control.

REVIEW OF LITERATURE

Protein Requirements of Laying Hens

The problem of providing the correct quality and quantity of protein is one of the major problems involved in formulating diets for laying hens. Protein apparently exerts more control over the rate of egg production than does any other single nutrient. Therefore, the protein level of experimental diets must be adequate or the effects of other nutrients cannot be measured accurately. On the other hand, from a practical viewpoint, protein is expensive and it would be desirable to hold the protein level low enough to prevent any possible waste.

Heuser (1941) reviewed available research data on the protein requirements of laying hens. In a discussion of the requirements, he suggested that there was much confusion over the protein requirements reported to that time. Unfortunately, this confusion has not been eliminated with the protein requirement studies which have been conducted since 1941.

In an investigation of the protein requirement of New Hampshire pullets, Berg et al. (1952) fed diets which contained protein levels of 11, 13 and 15 percent. The data from this study indicated that a 13 percent protein diet was adequate to maintain egg production and body weight. Thornton et al. (1956) and Thornton et al. (1957) also concluded that the protein level required for egg production might be as low as 13 percent. These workers reported no differences in egg production, feed efficiency or maintenance of body weight among groups of White Leghorn hens fed 11, 13, 15 and 17-percent-protein diets. Egg weight was the only factor measured which was significantly reduced by the 11 percent protein diet. Miller et al. (1957) obtained good egg production from hens fed experimental diets which contained 12.5 to 13 percent of protein. They found that a level of protein in the diet as low as 12.5 percent did not affect egg weight. Additional support for the 13 percent level of protein for laying hens was reported by Adams et al. (1958). Their data on various levels of protein fed to Leghorn layers in floor pens indicated that the optimal level of protein appeared to be between 12 and 14 percent. Other studies conducted during this same period of time show the protein requirement to be higher than 13 percent.

Two experiments were conducted by Ringrose et al. (1954) in which protein levels of 15 and 18 percent were fed to meat-type New Hampshire pullets. These research workers reported that the small differences in egg production among hens subjected to the two treatments tended to favor the 15 percent level of protein. In the following year Heywang et al. (1955) reported the results of two experiments which were designed to study the effect of hot weather on the dietary level of protein required by White Leghorn hens. These experiments involved six dietary protein levels which ranged from 11.5 to 19.3 percent. Considered collectively, the data from both experiments indicated that no increase in egg production would occur if the protein level in the diet during hot weather was greater than 15 percent.

Lee et al. (1944) noted that the egg production of Leghorns showed more tendency to decline, during periods of hot weather, in pens receiving a low protein (13 percent) diet. These investigators stated that results which would be acceptable to the commercial poultryman were obtained from laying hens on a diet containing 13 percent of protein. However, they also pointed out that approximately 16 percent of protein in the total ration was necessary for maximum egg production. MacIntyre and Aitken (1957) reported data from two feeding trials in which dietary protein levels of 20.0 to 21.3 percent were compared with levels of 15.4 to 16.5 percent. These data show that 15.4 to 16.3 percent of protein gave the better results. Hochreich et al. (1958) reported that a dietary level of 17 percent of protein was required to maintain maximum egg production and feed efficiency when the feed contained an energy level in excess of 950 Calories of productive energy per pound.

Studies reported by Reid et al. (1951) add to the confusion over the percentage of protein required by laying hens. These research workers reported that White Leghorn hens laid fewer eggs when fed 13- or 15-percent-protein diets than when they were fed 18-percent-protein diets. In another experiment at the same station McDaniel et al. (1957) supported these findings. In the latter experiment, a six percent improvement in feed conversion was obtained with cage layers when the dietary protein level was raised from 17 to 18 percent.

The varied conditions under which the protein requirement of laying hens had been determined led to some investigation into the factors which could affect protein requirement. Milton and Ingram (1957) studied the effect of temperature, age, breed, system of management and rate of egg production upon the protein requirement. Under the simulated summer conditions an 18-percent-protein diet gave the best results. Diets which contained 16 and 18 percent protein produced the greatest number of eggs with old hens, but 14 percent of protein appeared to be adequate for pullets. Thornton and Whittet (1960) carried out an experiment in which type of management, dietary energy level and genetic background were considered as factors which could influence the protein requirement for egg production. Comparable egg production rates were obtained with protein levels of 13, 15 and 17 percent under all conditions of the experiment.

Amino Acid Requirements of Laying Hens

A discussion of the protein requirement of laying hens would not be complete without including related data on amino acid requirements. The protein in the diet of laying hens must be in an available form and furnish adequate quantities of certain amino acids. Grau and Taylor (1948) and Ingram et al. (1950) demonstrated that an amino acid deficiency causes a hen to cease egg production in four to six days. This would indicate that the ability of the hen to draw protein from her body stores for egg production is very limited. These investigations also revealed that zein is not suitable for the study of amino acid requirements, even when it is supplemented with the amino acids that are deficient in zein. Apparently the amino acids present in zein are not in an available form.

In a review article written by Almquist (1952) only four amino acids were listed as being essential for laying hens. The inconsistencies that were reported in the protein requirement data to 1952 contributed greatly to the confusion over specific amino acid requirements. One of the facts pointed out by Almquist in his review was that the amino acid requirements are directly dependent upon the protein level of the diet. In recent years, work on amino acid requirements has been done largely with purified or semi-purified diets.

Arginine and Glycine: Menge et al. (1956) compared a synthetic layer hen diet low in arginine and glycine to a complete practical diet. They also compared the practical diet with the synthetic diet supplemented with 0.4 percent of glycine, 0.3 percent of arginine and a combination of the two. The practical control diet supported a 64 percent egg production rate for a 24-week period, while the synthetic diet low in glycine and arginine supported only a 47 percent egg production rate. Rates of 48, 54 and 60 percent egg production were obtained from the hens receiving 0.4 percent of glycine, 0.3 percent of arginine, and a combination of the two, respectively. These findings provide evidence which indicates that arginine and glycine are necessary for egg production. However, Johnson and Fisher (1956) reported that glycine was not required for egg production.

Cystine and Methionine: A quantitative estimation of the methionine requirement of laying hens has been made by using peanut meal supplemented with lysine and tryptophan as the source of protein, Ingram *et al.* (1951b). It was shown that the requirement of the laying hen for methionine was not more than 0.38 percent of the rations, and that the combined methionine and cystine requirement was not more than 0.63 percent. Leong and McGinnis (1952) reported that the level of methionine required to support egg production, body weight gain and egg size appeared to approximately 0.28 percent in the presence of 0.25 percent of cystine. These findings are in agreement with Ingram and co-workers who stated that a total of no more than 0.63 percent of dietary cystine and methionine was required by laying hens. Data which gave additional support to this work were presented by Little (1957). He found the methionine requirement for egg production to be no greater than 0.225 percent of the ration, whereas levels of 0.25 and 0.32 percent of methionine were required to support egg size and body weight, respectively. The cystine level used in these diets was 0.26 percent. Similar results which also supported these findings were reported by Johnson and Fisher (1958).

Isoleucine and Threonine: Miller et al. (1954), used blood meal as the main source of protein in the ration, and estimated the isoleucine requirement of the laying hen to be 0.53 percent. This estimate of the isoleucine requirement was later substantiated by Johnson and Fisher (1958).

Semi-purified diets in which crude casein and crystalline amino acids provided all of the dietary protein were used by Adkins et al. (1958) to make a quantitative estimation of the threonine requirement of the laying hen. In order to maintain body weight and egg size, it appeared that the L-threonine requirement was approximately 0.42 percent of the diet.

Lysine and Tryptophan: A corn-corn gluten meal ration was employed by Ingram et al. (1951a) to study the lysine and tryptophan requirements of the laying hen. It was shown by these investigators that the requirement for L-tryptophan did not exceed 0.15 percent of the ration, and that the requirement for L-lysine did not exceed 0.52 percent of the ration. Likewise, Little (1957), using the same type of basal diet, found the L-tryptophan requirement to be approximately 0.142 percent of the ration and the L-lysine requirement to be approximately 0.488 percent.

Johnson and Fisher (1956) conducted a study to determine the relative importance of eleven amino acids in laying hen nutrition. Through the use of a diet of free amino acids, it was concluded that amino acids could be classified according to their order of essentiality, as follows: arginine, glutamic acid, histidine, isoleucine, leucine, lysine, methionine, phenylalenine, threonine, tryptophan and valine. These research workers also concluded that all eleven amino acids were essential for egg production.

The minimal dietary levels of amino acids required to support egg production were reported by Johnson and Fisher (1958) and are shown in Table I. Laying hens were fed an amino acid-free diet to which minimal quantities of amino acids were added in amounts sufficient to maintain egg production. The minimal levels of the essential amino acids were based on the composition of whole-egg protein and a lysine requirement of 0.5 percent.

TABLE I

AMINO ACID REQUIREMENTS OF LAYING HENS BASED ON THE COMPOSITION OF WHOLE-EGG PROTEIN AND A LYSINE REQUIREMENT OF 0.50 PERCENT

Amino acid	Whole egg Gm./16 gm. N	Ratio	Calculated requirement (Percentage of diet)
Cystine	2.3	0.33	0.16
Histidine	2.4	0.35	0.18
Isoleucine	6.9	1.00	0.50
Leucine	9.4	1.36	0.68
Lysine	6.9	(1.00)	0.50
Methionine	3.3	0.48	0.24
Phenylalanine	5.8	0.84	0.42
Threonine	5.0	0.72	0.36
Tryptophan	1.6	0.23	0.12
Tyrosine	4.1	0.59	0.30
Valine	7.4	1.07	0.54

An experiment was designed by Johnson and Fisher (1959) to confirm their previous findings. A layer hen diet composed of practical feed ingredients was used. A practical corn-soybean oil meal diet which contained 15.7 percent of protein was compared to two low-protein diets (10.4 and 11.3 percent). The 10.4-percent-protein diet contained wheat as the main source of protein, while the 11.3-percent-protein diet contained corn as the principal protein contributor. The wheat-diet (10.4-percent-protein) supported egg production at a rate equal to that obtained with the practical corn-soybean oil meal diet (15.7-percent-protein). The corn-diet (11.3-percent-protein) was inferior in all respects to the wheat-diet which contained 10.4 percent of protein. This was thought to be caused by an incorrect relationship of non-essential to essential amino acids or to differences in the protein digestibility of the two rations. It was concluded that the good results which were obtained with the 10.4-percent-protein diet confirmed under practical feeding conditions the previous estimation of the minimal essential amino acid requirement obtained by using a diet consisting of free amino acids.

Fisher et al. (1960) reported another experiment which dealt with amino acid balance in low-protein diets for laying hens. In this experiment it was found that gelatin (a good source of arginine) when added to a 12.3-percent-protein layer diet significantly improved body weight, egg size and egg production. It was observed that free glutamic acid decreased body weight and egg production. It was pointed out that amino acid balance becomes more and more critical as the level of dietary protein is reduced. This research work brings out another discrepancy in the suggested dietary protein requirement of laying hens. Although 10.4- and 12.3-percent-protein diets have not actually been recommended by these workers, the fact remains that good results were obtained with these low dietary protein levels.

It would appear from the research work that has been reported by Johnson and Fisher that amino acid requirements for laying hens have been pretty well established. However, there has already been some research work which casts doubt on the amino acid requirements that were suggested by these workers. Adkins et al. (1959) were unable to formulate a synthetic diet which would sustain egg production and body weight gain in laying hens. The amino acid levels and ratios that were reported by Johnson and Fisher were used as a basis for the formulating of these diets. More time and research work will determine whether or not their percentage recommendations for the amino acids in laying hen diets are adequate.

The Energy Requirements of Laying Hens

The importance of an adequate energy level in feeds for poultry was first established in experiments where young growing chickens were used as the experimental animal. In some early research work with dietary energy level, Hoagland and Snider (1941) obtained maximum growth with chicks fed diets which contained 30 percent of added fat. Scott et al. (1947) reported data which showed that rations high in energy promoted more rapid growth and better feed conversion in broilers than rations lower in energy. It was apparent from these and other early studies that as the dietary energy level was increased, the feed required to produce a pound of growing chicken was reduced.

The energy requirement of laying hens and its relation to productive efficiency has received concentrated attention only during recent years. Hill (1956) studied the relation of dietary energy level to efficiency of egg production and found that a linear relationship existed between energy level and efficiency of egg production. Hill et al. (1956) reported that a ration which contained 830 Calories of productive energy per pound, when compared to a ration containing 930 Calories per pound, increased feed consumption by approximately 12 percent. These research workers showed from their data that feed intake was reduced when fat was used to increase the energy content of the diet. This reduction in feed intake amounted to 2 percent for each 1 percent of added fat. Nine experiments were conducted over a six-year period by Berg et al. (1956) in which diets varying from 1100 to 1367 Calories of metabolizable energy (approximately 750 to 922 Calories of productive energy) per pound of feed were compared. The results of these experiments indicated also that the efficiency of egg production was related to energy content of the ration. A relationship was observed between efficiency of feed conversion and dietary energy, in which efficiency decreased 6.17 percent for each decrease of 100 Calories of metabolizable energy per pound of ration. Even though less feed was consumed by the hens fed the high-energy diets, their body weight increase was greater than that of the hens fed the low-energy diets. Egg production was not affected by the energy level of the diet. Berg and Bearse (1956) reported the results of an experiment in which two diets that contained approximately 1148 and 1331 Calories of metabolizable energy (approximately 769 and 895 Calories of productive energy) per pound were compared. The high-energy diet promoted the greatest efficiency of feed utilization, and energy level had no effect on the rate of lay. Data from an experiment conducted by Anderson et al. (1957) showed that a high-energy ration (884 Calories of productive energy per pound) did not result in any greater egg production than a low-energy ration (723 Calories per pound), but efficiency of feed utilization was significantly greater for the hens fed the high-energy rations.

The evidence thus far presented shows that an increase in the dietary energy level of laying hens will reduce total feed consumption and thus improve feed efficiency. However, Bolton (1958) has presented evidence which indicated that egg production and the efficiency of protein and energy utilization were improved when hens were fed low-energy diets. The hens that received a high-energy diet produced an average of 243.9 eggs per year, while the hens fed a low-energy diet produced 247.9 eggs. In this study, the efficiency of energy utilization was measured by comparing the gross energy of the eggs laid with the intake of metabolizable energy. The efficiency of energy utilization, expressed as a percentage, was 20.6 and 22.0 on the high- and low-energy rations, respectively. The crude protein in the eggs, as a percentage of the intake of digestible crude protein was 28.6 and 30.2, respectively.

A recent investigation by Petersen et al. (1960) produced data that are contrary to the reports in either of the two preceding paragraphs. The studies presented in the first of these two paragraphs indicate that dietary energy level has little influence on the rate of egg production, while the research work of Bolton (1958) in the second paragraph shows that hens fed low-energy diets will produce more eggs than hens fed high-energy diets. The work reported by Petersen and co-workers showed that the egg production of hens fed a low-energy diet (650 Calories of productive energy per pound) was not equal to that obtained from hens fed a high-energy diet (910 Calories). Despite these differences regarding the effect of dietary energy level on egg production, all of the reported experiments show that total feed intake is reduced when dietary energy level is increased.

The effect of high levels of dietary energy and protein on the performance of laying hens was studied by MacIntyre and Aitken (1957) and Price et al. (1957). These workers concluded that neither high energy nor high protein had any influence on rate of egg production, egg weight, specific gravity of the eggs, albumin height or incidence of blood and meat spots in the eggs.

Energy-Protein Interrelationships

In experiments with chicks, the energy level of the ration has been shown to influence the protein level required in the ration, Donaldson _et al._ (1956). A protein-energy interrelationship affecting the rate of production has been observed by Berg and Bearse (1957). Hens fed a low-energy-14-percent-protein diet supported a rate of lay comparable to that of hens fed a high-energy diet which contained 18 percent of protein.

Experimental results obtained by Frank and Waibel (1960) supported the earlier findings of Berg and Bearse (1957). White Leghorn hens in cages were fed diets which contained 10.2, 12.4, 14.9, 19.9 and 29.9 percent of protein in a high-energy (984-1250 Calories of productive energy per pound) and a low-energy (634-947 Calories of productive energy per pound) series of diets. Hens fed the low-energy diets with 12.4 percent of protein laid at a normal rate, while 14.9 percent of protein was required by the hens fed the high-energy series of diets. Thornton and Whittet (1960) reported that an 11-percent-protein diet supported a rate of egg production comparable to a 17-percent-protein diet when the dietary energy level was reduced from 900 to 700 Calories of productive energy per pound.

McDaniel _et al._ (1957) found that the addition of each 88 Calories of productive energy to a cage layer diet which contained 17 percent of protein brought about a 12.2 percent increase in feed efficiency. The same energy increment in an 18-percent-protein diet brought about only an 8.2 percent reduction in feed required per dozen eggs.

Because protein is usually the first limiting nutrient when energy is increased in poultry rations, the protein-energy relationship has been commonly expressed as a Calorie-protein ratio. The Calorie-protein ratio means that for a particular purpose there should be approximately so many Calories of energy for each percent of protein. An optimum Calorie-protein ratio for broiler rations has been established by Combs and Romoser (1955); Leong et al. (1955); Donaldson et al. (1955) and Donaldson et al. (1956) to be 42:1 in terms of productive energy. The Calorie-protein ratio for laying hens has not been this well established. Combs and Romoser (1955) suggested that the Calorie-protein ratio of layer-breeder rations should be approximately 55:1 in terms of productive energy. However, protein quality, fat content of the ration, level of egg production, body size, sex, environmental temperature and exercise were given as conditions which could alter the optimum Calorie-protein ratio. Data reported by Miller et al. (1957) showed that the Calories of productive energy in the diets of laying pullets could range from 31 to 86 for each percent of protein, without altering egg production. Although the Calorie-protein ratio may aid in maintaining the balance between energy and protein, Wilgus (1957) has pointed out that it is nothing more than a tool to be used in ration formulation since it does not express quantities of either protein or energy.

Energy-Fiber Interrelationships

The dietary energy level of rations formulated from practical ingredients is governed to a large extent by the quantity of the fibrous ingredients used. Evidence reported by Richardson et al. (1956) indicated that when fibrous materials were added to broiler rations the growth rate of the birds was significantly depressed. However, the addition of 12 percent of fat to a diet which contained 8.3 percent of crude fiber counteracted the growth depressing effect of the fiber. Essentially these same results were obtained by Marz et al. (1956). These workers found that chicks fed a 10-percent-fiber diet (450 Calories of productive energy) weighed significantly less at 8 weeks of age than chicks fed a 5-percent-fiber diet (900 Calories of productive energy).

Hill and Dansky (1954) studied energy levels ranging from 975 to 505 Calories of productive energy per pound of chick ration. This range in energy levels was obtained by substituting pulverized oat hulls for grain components at levels up to 40 percent of the diet. In several experiments, maximum growth rate was obtained by the ration which contained 505 Calories of productive energy per pound. These workers concluded that maximum growth rate at this low dietary energy level was made possible by a marked increase in feed consumption. These findings were verified by Griminger et al. (1957) when they found that the replacement of carbohydrate by non-nutritive fiber in purified or near-purified diets increased the voluntary food intake of chicks which consumed such a diet. Saito et al. (1959) found that the addition of cellulose to a diet which contained a low level of crude fiber was beneficial to chick growth. This was particularly true where the basal diet was deficient in nutrients required for normal growth.

The energy-fiber interrelationship has been expressed by Marz et al. (1957) as an energy:volume ratio. In this study a basal diet which contained 900 Calories of productive energy per pound was diluted with sand to obtain energy levels of 800, 700, 600, 500, 400, 300 and 200 Calories. These diets were measured for density and fed to growing male chicks. It was shown that neither energy nor density alone proved to be a satisfactory criterion for measuring the adequacy of a grower diet. In this experiment 0.79 Calorie per cubic centimeter of diet was adequate for maintenance of rapid growth, while 0.57 Calorie was inadequate.

Although experiments in this area have been less extensive with laying hens than with growing chickens, the facts appear to be similar. Heuser et al. (1945) reported that rations which contained feed ingredients of a fibrous nature, such as oats and wheat by-products, were not utilized as efficiently by White Leghorns for egg production and body weight gain as less fibrous feed which contained crushed wheat. Simultaneously Bird and Whitson (1946), using Rhode Island Reds, demonstrated the extreme efficiency differential between low- and high-energy rations that were formulated so that 10 percent of oats, 15 percent of wheat standard middlings, 20 percent of wheat bran and 6 percent of alfalfa meal replaced ground wheat. It was concluded that egg production was approximately equal on the high- and low-energy diets, but that the hens fed the fibrous diet had to eat more feed in order to maintain their production. Similar results have been obtained in a feeding experiment with White Holland turkey pullets, Dymsza et al. (1954). Pelleted diets that contained 5, 10, 15 and 20 percent of crude fiber in combination with calculated productive energy levels of 882, 670, 460 and 249 Calories per pound, respectively, were fed in this experiment. Efficiency of feed utilization, as measured by pounds of feed consumed per dozen of eggs, was progessively better as the dietary energy level was increased and total caloric intake increased.

Lillie et al. (1951) reported that the egg production of layers was reduced when the fiber level of the diet was raised. The critical nature of diet density was shown by feeding diets in a pelleted form as well as a non-pelleted form. When oat hulls were added to non-pelleted diets fed to Rhode Island Red pullets at levels of 32, 48 and 64 percent, the resulting egg production was 62, 36 and 25 percent, respectively. The egg production rates of the pullets fed the pelleted feed were 68, 61 and 55 percent, respectively. This work was supported by Cowlishaw and Eyles (1958) when they reported that diets which contained high levels of fiber would support a high level of egg production only when the nutrient density of the diet was sufficient as not to restrict energy intake below optimum. The ability of the hen to compensate for inadequate dietary nutrient levels was demonstrated in this experiment. It was observed that feed intake by weight was greater on a high-fiber diet (20 percent) than on a low-fiber diet (10 percent).

Vitamin Requirements of Laying Hens

Experiments designed to study the quantitative vitamin requirements of laying hens have been limited to the relatively small number of vitamins shown to be the most critical for growing chickens. As would be expected with laying hens, since protein and energy requirements have not been thoroughly established, the results of the vitamin requirement studies have been variable. Thayer _et al._ (1956) fed New Hampshire layers graded levels of niacin, riboflavin, pantothenic acid and folic acid in high- and low-energy basal rations. They found that niacin, riboflavin, pantothenic acid and folic acid levels above the National Research Council (1954) recommended requirements were needed in the high-energy rations for maximum egg production and economy of feed conversion. In support of higher vitamin supplementation, Adams _et al._ (1958) reported that a corn-soybean oil meal ration which contained 10 percent of protein gave as satisfactory egg production as did a higher protein diet, when all known vitamins were added.

In contrast to these findings, Berg and Bearse (1956) found that the increasing of the level of B-vitamins in high-energy layer rations tended to suppress egg production. The egg production was calculated on a hen-day basis. This suppression was not obvious when egg production was calculated and expressed on a per-bird-housed basis. It was suggested that the added vitamins only prolonged the life of non-laying hens. Anderson et al. (1957) also reported that vitamin supplementation of high- or low-energy rations had no effect on the rate of egg production. There was, however, a tendency for the body weight of the experimental layers to increase following vitamin supplementation.

Vitamin B_{12}: Reid et al. (1951) found that egg production was increased when additions of aureomycin and vitamin B_{12} were made to layer diets. Berg et al. (1952) fed rations which were formulated from vegetable sources and were calculated to contain 11, 13 and 15 percent of protein. The 11- and 13-percent-protein rations, when supplemented with three micrograms of vitamin B_{12} per 100 grams of feed, gave results comparable to that of the 15-percent-protein ration which contained no added vitamin B_{12}. These workers concluded that vitamin B_{12} enhanced the utilization of low-protein rations by laying hens.

Pantothenic Acid: Gillis et al. (1942) obtained normal egg production from laying hens that were fed diets which contained 750 micrograms of pantothenic acid per 100 grams of laying ration. Maximum hatchability was obtained with diets which contained at least 1750 micrograms of pantothenic acid per 100 grams. In a later experiment Gillis et al. (1947) reported that hens required not more than 150 micrograms of pantothenic acid per 100 grams of diet for weight maintenance and egg production, and that approximately 800 micrograms per 100 grams of diet appeared adequate for good hatchability.

Folic Acid: The folic acid requirement of White Leghorn hens for egg production has been reported by Taylor (1947) to be no more than 12 micrograms per 100 grams of diet. Sunde et al. (1950) concluded that the folic acid requirement of layers was higher than 0.25 milligrams per kilogram of ration. When these two requirements are put in the same terms, the latter is over twice as large as the former.

Riboflavin: Hill et al. (1954) found the level of riboflavin necessary to maintain egg production and body weight in White Leghorns to be about one milligram per pound of diet. The minimum level of riboflavin that would sustain normal hatchability was 1.7 milligrams per pound of diet. On the other hand, Gleaves et al. (1961) reported that one milligram of riboflavin per pound of diet was inadequate to maintain egg production and body weight in laying hens. These workers suggested that the minimum dietary riboflavin level in layer hen diets should be at least 1.5 milligrams per pound.

Niacin: Briggs et al. (1946) fed layers a basal ration considered to be deficient in niacin. This ration caused the hen to lose body weight and brought about a decline in egg production and hatchability. When this basal ration was supplemented with 227 milligrams of niacin per pound, body weight, egg production and hatchability returned to normal. Gleaves et al. (1961) found no difference in egg production, feed consumption or body weight of hens fed diets which contained 10, 15, 20 or 25 milligrams of niacin per pound. However, the eggs produced by the hens fed the two lower levels of niacin weighed slightly less than the eggs from the hens fed the two higher levels.

Choline: The laying hen has not been found to require supplemental choline, Lucas et al. (1946). However, Reid et al. (1957) presented data which indicate that the addition of choline to a fat-containing layer diet aids in preventing a condition called fatty liver disease. They suggested that 400 grams of choline chloride per pound be incorporated into cage layer diets which contain added fat. It was concluded by Balloun (1956) that the choline requirement of the breeder hen is not over 500 milligrams per pound of diet.

Vitamin A: Sherwood and Fraps (1932) reported that rations which are normally fed to laying hens apparently do not supply enough vitamin A for body maintenance and high egg production, unless the hens have access to green grass or similar green feed. It was estimated by these workers that a pullet with an average egg production of 20 eggs per month would require 1363 units of vitamin A per day for maintenance and egg production. On the basis of egg production, hatchability performance and mortality, Taylor et al. (1947) placed the pro-vitamin A requirement for laying hens at 2000 I. U. per pound of feed.

Vitamin D: The vitamin D requirement of pullets for egg production was determined by Couch et al. (1947) to be from 38 to 76 A.O.A.C. chick units per 100 grams of diet. At least 38 A.O.A.C. chick units of vitamin D were required to maintain fertility and hatchability. Older hens, however, were reported to require a minimum of 76 A.O.A.C. chick units of vitamin D per 100 grams of diet for egg production.

Interrelationships of Vitamins with Vitamins and with Other Nutrients

A vitamin-vitamin interrelationship between vitamin B_{12} and pantothenic acid was reported by Balloun and Phillips (1957). In experiments conducted with hens confined to community-type cages, both the vitamin B_{12} and pantothenic acid levels in the diet were found to influence pantothenic acid storage in the eggs produced. A vitamin B_{12} deficiency in the layer diet intensified a pantothenic acid deficiency on low-panthenic acid diets.

The pantothenic acid deficiency was measured by hatchability, growth and viability of progeny, and pantothenic acid storage in the eggs produced.

The presence of an interaction between riboflavin and niacin in laying hen diets was reported by Gleaves et al. (1961). This interaction was statistically significant for egg production, egg weight, feed consumption and body weight. Egg production, egg weight and feed consumption were all greater at the lowest level of riboflavin (1.0 milligram per pound) when this level was fed in combination with the highest level of niacin (25 milligrams per pound).

The existence of a vitamin-protein interrelationship in laying hen nutrition was reported by Berg et al. (1952). An all-vegetable layer diet which contain 11 percent of protein was compared to a layer diet which contained 15 percent of protein. Hens performed better on the 15-percent-protein diet until three micrograms of vitamin B_{12} per 100 grams of feed were added to these diets. After vitamin B_{12} was added, the 11-percent-protein diet gave results comparable to the 15-percent-protein layer diet.

In research work with growing chicks, Olsen et al. (1959) found an inverse relationship between protein level and vitamin A storage in the liver. This relationship indicates a higher vitamin A requirement at a higher level of protein. These workers also reported that an increase in the energy level of the diet resulted in better chick growth and a greater storage of vitamin A in the liver. However, they suggested that the increased storage might be due to the higher fat content of the diets rather than to the higher energy level as such.

Regulation of Food Intake

In general, the literature which has been cited in this review indicates that nutrient requirements are dependent upon total food intake and the factors which control food intake. Therefore, the next few pages will be devoted to a discussion of the regulators of food intake in poultry and other animals. There are three major theories pertaining to the regulation of food intake in animals, all of them consistent with the known functions of the hypothalamus.

Glucostatic theory: This mechanism, suggested by Mayer (1953) and Mayer (1955), relates appetite and satiety to the level and availability of blood sugar. Consideration is given to the fact that the difference in the amount of sugar (delta glucose) in arterial and venous blood is an indication of the rate at which it is being used by body tissues. When delta glucose is high, hunger is absent; when delta glucose diminishes, hunger returns. This has been shown to occur in humans on ordinary diets. The idea was supported also by studies with rats in which injected glucose reduced food intake by an amount greater than the energy of the glucose, whereas other substances such as fat and sucrose did not.

Thermostatic theory: This theory was suggested by Brobeck (1948) and Strominger and Brobeck (1953) and postulates that food intake is regulated as one means of temperature control. It was supported by experiments in which rats were fed diets differing in fat and protein content. In these experiments, with all diets, the amounts of food eaten appeared to produce a relatively constant specific dynamic action (extra heat production associated with eating). In support of this theory is the lowering of food intake which occurs when environmental temperature rises. It is known that the hypothalamus is concerned with food intake, and it is postulated that the hypothalamus integrates the effects of temperature and circulating metabolites to control food intake.

Lipostatic theory: In this theory, which is concerned primarily with long-term regulation of food intake, Kennedy (1952) suggested that the state of fat stores of animals governs their rate of food consumption. This theory helps to explain why there appears to be a body weight which is characteristic of the animal, the environment and the feeding program.

Hill (1957) pointed out that each of these proposed mechanisms has shortcomings. It is not possible to explain satisfactorily by means of the glucostatic theory the appetite-satisfying value of a high-protein diet. Research work conducted by Fryer et al. (1955) showed that the satiety values of different diets for humans do not agree with their effects on blood glucose. In studies in which reducing diets were fed to young men, the highest satiety value was obtained with a diet high in protein, high in fat, and low in carbohydrate; the poorest satiety was observed with a diet high in carbohydrate and low in protein. The effects of these diets on blood glucose were exactly the opposite of that predicted by the glucostatic theory. The thermostatic theory helps to explain the effect of protein, since this nutrient has a high specific dynamic action. However, this theory does not account for the effects of thyroxin, which increase both body temperature and food consumption. Integrating these various views is difficult, but it appears that each of them has value and that none of them is capable of explaining all aspects of appetite.

Dietary bulk has been shown to be a factor which affects feed consumption. A series of experiments with normal as well as with cropectomized chicks was carried out by Fisher and Weiss (1956) to study the effect of fiber *per se* on feed consumption. This work indicated that fiber *per se* was an important factor which influences feed consumption independently of the energy level of the diet. Fiber *per se*, up to a given dietary level, stimulated feed consumption; but beyond that level feed consumption remained relatively constant. It was found that efficiency of feed utilization was not sacrificied when fiber was added (simultaneously with fat) to high-energy diets, but it was actually improved. Couch and Isaacks (1957) were successful in restricting the protein and energy intake in growing pullets by substituting 18.2 percent of oat hulls for an equivalent amount of milo. The pullets fed this diet weighed approximately the same at 16 weeks of age as did pullets fed a high-energy type ration on a 70 percent restricted basis. While the fibrous bulk was restricting the total nutrient intake of these pullets, the inherent reduction of dietary energy level which accompanied the substitution of oat hulls for milo was increasing feed consumption. Meyer (1958) concluded that the addition of cellulose to the diet of *ad libitum*- and pair-fed growing rats increased protein needs of the rats as measured by gains in the fat-free body. This was attributed to a loss of metabolic fecal nitrogen induced by the dietary cellulose addition. It was demonstrated that 30 percent of cellulose added to the diet resulted in a need for about 1.85 percent of additional crude casein. Evidence was presented by Sibbald *et al.* (1957b) which suggested that fiber in the diet of growing rats tended to depress digestible energy consumption. A decrease in digestible energy might be the reason Meyer (1958) concluded that high fiber levels reduced protein efficiency.

From the same experiment mentioned above, Sibbald concluded that variations in the food consumption of weanling rats which were fed rations that contained varying nitrogen sources were largely associated with the digestible energy content of the rations. A significant difference in the digestible energy consumption of rats between nitrogen sources was attributed to the quality of the nitrogen sources. The higher the quality of the nitrogen source the greater the digestible energy consumption. Sibbald et al. (1957a) also concluded that variations in the food consumption of weanling rats, which were fed rations containing a mixture of indispensable amino acids and diammonium citrate, were largely associated with the digestible energy content of the diets. The facts presented in the two previous paragraphs were pretty well summed up by Hill (1956) when he stated that the basic factor underlying performance differences between energy levels is the fact that animals tend to regulate their feed consumption to meet energy needs, up to the limit of their capacity or willingness to consume feed.

In a study of the effect of food preferences on nutrient intake, Young and Lafortune (1957) concluded that, contrary to common belief, food dislikes in college women seemed to have little influence on the adequacy of the diet. The greatest effect on adequacy of nutrient intake seemed to lie in the lack of ingestion in sufficient quantities of the choice food items.

Factors mediating food and liquid intake in chickens were studies by Jacobs et al. (1957). It was concluded that under the conditions of their experiments the chicken could discriminate among sucrose solutions, saccharine solutions and water. The chickens preferred sucrose solution and avoided saccharine. This preference for sucrose was not shown to be related to its caloric value. The presence of sucrose in the drinking water did not produce any measurable effect on rate of weight increase or amount of food intake. Kare et al. (1957) presented data that showed the chick to have a sense of taste. The response to a variety of sweet and bitter flavors suggested that the broad classifications of taste recognized by man were not applicable to the fowl, but that the sense of taste in the fowl was more than rudimentary.

An interesting experiment by Lepkovsky et al. (1960) on food intake, water intake and body water regulation of chickens showed that feeding chickens with or without water did not greatly influence their food intake. This was probably due in part to the fact that the crop of the chickens was able to adjust its water content to water supply. There was more water in the crop content of chickens fed with water than in the crop content of chickens fed without water.

EXPERIMENTAL PROCEDURE

General

The study which is reported in this thesis involved three feeding trials which were conducted in a windowless cage layer house located on the Oklahoma State University Poultry Farm. Environmental conditions were partially controlled within the cage house throughout each of the three trials. Temperature and ventilation were regulated with the use of a furnace, water cooler, air ducts and fans engineered specifically for this house. Temperature varied from a low of 60 degrees Fahrenheit during the winter months to a high of approximately 90 degrees Fahrenheit during the summer months. Since the house had no windows, artificial light was supplied by incandescent lamps which were controlled with automatic time clocks. The hens in all three trials in this experiment were given 14 hours of continuous light and 10 consecutive hours of darkness per day.

The nutrient composition of the experimental diets was calculated from chemical analyses, published nutrient levels for the various feedstuffs or from the nutrient level guarantees by the feedstuff manufacturer. Because of limited ingredient storage space, feed ingredients were purchased several times during the course of the experiment. In order to avoid fluctuations in dietary protein level, due to possible variation in protein content of different batches of ingredients, periodic chemical analyses were run on each ingredient to determine protein level. Fortunately there were not many fluctuations in ingredient protein levels. The few fluctuations that were present were adjusted in the experimental diets by increasing or decreasing the amount of the ingredient which had changed in protein level. Based upon these protein values, subsequent adjustments were made in the dietary amino acid levels. All amino acid calculations were based upon average values reported by Block and Weiss (1956). The metabolizable energy, calcium, phosphorus and crude fiber levels were based upon values presented by Titus (1955).

In Trials II and III, dry volume of each ingredient was determined and taken into consideration in the formulation of the experimental diets. Dry volume of each feed ingredient was expressed in milliliters per gram of ingredient. Volume was determined by pouring 454 grams of a feed ingredient lightly into a 1,000 milliliter volumetric flask from which a reading of the volume was taken. Measurements were taken on four replicate samples and the average volume of the four was used in the formulation of the experimental diets. The volumes of all experimental diets were measured, after they had been mixed, to verify the exact volume of each diet. It is of interest to note that the combined volume measurements of the ingredients in a diet gave an excellent estimate of the actual volume of the mixed diet.

Commercial hybrid laying hens were housed in the windowless cage house in individual wire cages. Each cage was equipped with an automatic waterer, a feeder and a feed storage container. Egg production, egg weight and mortality were recorded daily. In Trials I and II, every egg was weighed individually. However, in Trial III, eggs were individually weighed for the first month of the test period only. After the first month until the close of the trial, eggs were individually weighed only during four consecutive days of each week. The average egg weight obtained in this manner was used as an estimate of the average weight of all eggs produced during that week.

Individual body weight and feed consumption data were collected and recorded every 14 days in Trials I and II and every 28 days in Trial III. The hens were supplied feed and water ad libitum in all feeding trials.

The IBM 650 electronic computer was utilized to make all summary and statistical computations. Egg production, egg weight, body weight, feed consumption and mortality data were punched on IBM cards at the end of each experimental period. A program was written for the computer to summarize and compute the following variables for each replicate and for each treatment:

(1) hens per treatment,
(2) average number of eggs produced,
(3) percentage egg production,
(4) average egg weight,
(5) total body weight gain or loss,
(6) daily feed consumption,
(7) daily protein consumption,
(8) daily energy consumption,
(9) daily vitamin-mineral concentrate consumption,
(10) units of protein per unit of egg,
(11) Calories per unit of egg,
(12) units of vitamin-mineral concentrate per unit of egg, and
(13) average daily volume of feed consumed.

A complete randomized experimental design was used for Trials I and II. At the beginning of the series of feeding trials, each cage was assigned a number which remained the same during each of the three trials. Using a set of random numbers, each replication of the experimental diets was assigned a cage number. The hens were individually selected for health and vigor, and randomly distributed into the cages. After the randomization procedure was completed each hen was wing-badged with a number which corresponded to the cage number. An analysis of variance as outlined by Snedecor (1956), was applied to all data which were collected from Trials I and II and computed on the IBM 650.

An experiment (unpublished data) that was designed to measure the uniformity of performance of laying hens in the windowless cage house was conducted simultaneously with Trials I and II. This experiment revealed a slight position gradient in the performance of hens from the North to the South side of the house. Because of this gradient, Trial III was set up in a randomized block design. The hens and experimental diets were randomly assigned to each block in a manner similar to that previously described. Hens were selected for each block on the basis of egg production during the first four weeks immediately following the beginning of lay. Groups of hens with similar production backgrounds were placed in each block. The Doolittle analysis of variance as described by Dwyer (1951) was applied to the data from Trial III for each period and for the overall summary of 13 four-week periods.

Data on efficiency of nutrient utilization that are presented in this thesis are expressed as units of nutrient per unit of egg. However, it was found with individual hen data that efficiency of nutrient utilization expressed in this manner was often of infinite magnitude and could not be defined. Non-producing hens that continued to eat had an efficiency of nutrient utilization of infinity. In order to apply statistics to efficiency data of this kind, it had to be expressed as unit of egg per unit of nutrient. The efficiency of nutrient utilization for non-producing hens expressed as unit of egg per unit of nutrient is zero. Because of the high incidence of non-producing hens, all data on efficiency of nutrient utilization were converted to the latter method of expression for statistical analysis. This conversion was accomplished by writing a program for the IBM 650 which made the necessary calculations.

The IBM 650 program that was available for the statistical analyses would not process and handle negative numbers. Therefore, it was necessary to add a constant to the body-weight-change data of each hen. A constant of 5,000 was chosen since no one hen could lose 5,000 grams of body weight and remain alive. This adjustment allowed the body-weight-change data to be always positive in the statistical analysis. The constant was finally removed from body-weight-change data which are reported herein.

FEEDING TRIALS

Trial I

Purpose

The purpose of this feeding trial was twofold: (1) to determine the effect of high-energy, high-protein diets upon egg production and body-weight-change of high producing layers; and (2) to measure the effect of high-energy and high-protein diets upon daily feed consumption. Although dietary energy has been shown to influence feed consumption more than dietary protein, this experiment was not designed to study their individual effects.

Procedure

This feeding trial consisted of 8 different experimental diets, with six replicates per diet. A replicate consisted of one DeKalb-131 pullet. The pullets were 24 weeks old when the trial was initiated on November 12, 1958. Data were collected for 5 two-week periods and the trial was terminated on January 21, 1959.

The composition of the eight experimental diets that were used in this study is shown in Table II. Protein levels of the diets were 24.6, 23.2, 22.0, 20.8, 19.5, 18.2, 16.9 and 15.6 percent, respectively, while the corresponding energy levels were 2043, 1937, 1834, 1730, 1621, 1510, 1404 and 1300 Calories of metabolizable energy per pound. A Calorie:protein ratio of 83:1, in terms of metabolizable energy, was constant for all diets.

TABLE II

COMPOSITION OF THE EIGHT EXPERIMENTAL DIETS, TRIAL I

Diet number	1	2	3	4	5	6	7	8
Ingredients	Percent of total diet							
Polyethylene spheres[1]	----	----	----	----	----	----	5.2	11.6
Starch	1.0	8.0	15.7	23.6	32.3	39.8	38.5	35.0
Corn oil	30.0	25.4	20.5	15.5	10.0	5.0	4.0	4.0
Dehydrated alfalfa meal (17% protein)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Oat mill feed	14.05	13.75	13.10	12.35	11.50	10.85	10.00	9.35
Dried whole egg solids	19.0	18.0	17.0	16.0	15.0	14.0	13.0	12.0
Drackett[2]	4.8	4.5	4.3	4.0	3.8	3.5	3.3	3.0
Casein[3]	7.1	6.8	6.4	6.0	5.6	5.3	4.9	4.5
Gelatin	1.0	0.9	0.85	0.8	0.75	0.7	0.65	0.6
Live yeast culture	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2
Dried condensed fermented corn extractives[4]	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2
Delactosed dried whey	2.0	1.8	1.7	1.6	1.5	1.4	1.3	1.2
Dried condensed fish solubles	3.0	2.7	2.5	2.4	2.3	2.1	2.0	1.8
Dicalcium phosphate (18% phosphorus)	5.0	5.5	5.5	5.5	5.5	6.0	6.0	6.0
Calcium carbonate	2.5	2.5	2.5	2.5	2.2	2.0	2.0	2.0
Trace mineral mix[5]	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Salt (Na cl)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Vitamin concentrate[6]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Coliver[7]	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Gelusil[8]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Calculated analyses								
Crude protein (percent)	24.6	23.3	22.0	20.8	19.5	18.2	16.9	15.6
Calories (M.E.)[9] per pound	2043	1937	1834	1730	1621	1510	1404	1300
Calorie-protein ratio	83	83	83	83	83	83	83	83
Calcium (percent)	2.46	2.59	2.58	2.57	2.45	2.51	2.50	2.49
Total phosphorus (percent)	1.18	1.26	1.24	1.23	1.21	1.29	1.28	1.26
Crude fiber (percent)	5.16	4.92	4.68	4.43	4.19	3.94	8.90	15.05

Footnotes to Table II

1. Polyethylene spheres--colorless polyethylene pellets, 1/8 inch spheres. Eastman Chemical Products, Incorporated, Kingsport, Tennessee.
2. Drackett-assay C-1 protein. Archer-Daniels-Midland, Kansas City, Missouri.
3. Casein--Hy-Case, a salt-free product. Sheffield Chemical Company, Incorporated, Norwich, New York.
4. Dried condensed fermented corn extractives--C.F.S. No. 3, Clinton Corn Processing Company, Clinton, Iowa.
5. Trace mineral mix--adds per pound of finished ration: manganese 27.5 mg., iodine 0.88 mg., cobalt 0.59 mg., iron 8.3 mg., copper 1.65 mg., and zinc 1.52 mg. Calcium Carbonate Company, Carthage, Missouri.
6. Vitamin concentrate--refer to Table III.
7. Coliver--a cold-process cod liver extract, Silmo Chemical Company, Vineland, New Jersey
8. Gelusil--anti-acid adsorbent which adds 0.57 gm. of magnesium-trisilicate and 0.28 gm. of aluminum-hydroxide per pound of finished diet.
9. (M.E.)--metabolizable energy, Titus (1955).

TABLE III

COMPOSITION OF THE VITAMIN CONCENTRATE, TRIAL I

Vitamins	Units	Adds per pound of finished diet
Vitamin A	U.S.P.	10,000.0
Vitamin D_3	I.C.U.	1,000.0
Vitamin E	I.U.	50.0
Vitamin K_3	Mg.	0.3
Vitamin B_{12}	Mcg.	3.0
Riboflavin	Mg.	2.0
Niacin	Mg.	20.0
Pantothenic acid	Mg.	4.0
Pyridoxine	Mg.	2.0
d-Biotin	Mg.	1.0
Choline	Mg.	600.0
Thiamin	Mg.	4.0
Folic acid	Mg.	0.8
Ascorbic acid	Mg.	22.5
Inositol	Mg.	227.0

It was impractical to go above 25 percent of protein since this level of protein required 2075 Calories of energy per pound of diet to obtain the desired Calorie:protein ratio. Amino acid ratios for Diet 1 and all other diets in this Trial (Table IV) were based upon research work reported by Johnson and Fisher (1956). Diet number 1 was formulated with a protein level of 24.6 percent and an energy level of 2043 Calories per pound. Diets 2 through 8 were formulated using 95, 90, 85, 80, 75, 70 and 65 percent, respectively, of all protein-contributing ingredients of Diet 1. In order to reduce energy level with each decrease in protein level, without altering amino acid ratios, the percentage of corn oil in the diets was decreased and the percentage of starch was increased. Polyethylene spheres were added to Diets 7 and 8 as an inert filler to maintain the proper weight.

Results

A summary of the data on egg production, egg weight, body-weight-change, feed consumption and efficiency of feed utilization obtained per hen during the 10-week laying period are given in Table V. The analyses of variance of these data are presented in Tables VI, VII, VIII, IX, X and XI. The differences in egg production per hen among all diets were significant only at the 0.10 level of probability (Table VI). This significance was probably due to the fact that hens fed Diet 1 laid fewer eggs than did the hens fed any other diet in Trial I. Differences in egg weight were even less significant statistically (Table VII)than those in egg production.

As could be expected, the higher dietary energy and protein levels produced greater body weight gains than the lower dietary energy and protein levels. These differences were significant at the 0.01 level of probability (Table VIII). Hens that were fed Diet 8 lost an average of 15 grams while

TABLE IV

CALCULATED AMINO ACID COMPOSITION[1]
OF ALL EXPERIMENTAL DIETS, TRIAL I

Amino acid	Gm. per 16 gm. of nitrogen	Amino acid	Gm. per 16 gm. of nitrogen
Arginine	7.64	Threonine	4.93
Histidine	2.59	Leucine	10.21
Lysine	7.19	Isoleucine	7.16
Tyrosine	4.04	Valine	6.66
Tryptophan	1.46	Glutamic acid	11.18
Phenylalanine	6.06	Aspartic acid	5.91
Cystine	1.35	Glycine	5.14
Methionine	2.37	Alanine	2.59
Serine	6.86	Proline	6.66

[1]Calculated amino acid composition - all amino acid calculations were based upon average values as given in Block and Weiss (1956).

TABLE V

AVERAGE EGG PRODUCTION, EGG WEIGHT, BODY WEIGHT CHANGE, FEED CONSUMPTION, AND EFFICIENCY OF FEED UTILIZATION PER HEN, TRIAL I

Diet number	1	2	3	4	5	6	7	8
Number of days on experiment	70	70	70	70	70	70	70	70
Number of surviving hens per diet (replicates)	5	6	6	6	6	6	5	6
Average number of eggs produced	39.0	47.5	52.5	41.5	49.2	48.5	46.8	45.5
Percent egg production	55.7	67.9	75.0	59.3	70.2	69.3	66.9	65.0
Egg weight (gm.)	55.3	57.1	55.1	54.8	58.0	55.9	54.4	52.8
Total body weight gain or loss (gm.)	399	430	386	302	644	321	190	-15
Daily feed consumption (gm.)	66.4	75.4	75.4	74.5	92.1	96.3	83.5	77.2
Daily protein consumption (gm.)	16.3	17.6	16.6	15.5	18.0	17.5	14.1	12.1
Daily calorie (M.E.)[1] consumption	299	322	305	284	329	321	258	221
Gm. protein per gm. egg	0.53	0.45	0.40	0.48	0.44	0.45	0.39	0.35
Calories (M.E.)[1] per gm. egg	9.7	8.3	7.4	8.7	8.1	8.3	7.1	6.4

[1](M.E.)-Metabalizable Energy, Titus (1955).

TABLE VI

ANALYSIS OF VARIANCE OF THE EGG PRODUCTION DATA, TRIAL I

Source of variation	d.f.	Sums of squares	Mean squares	F value	Probability Level
Total sum of squares	45	2,813.41	-----	----	------
Diet	7	726.28	103.75	1.88	P> 0.10
Error (among individuals within diets)	38	2,087.13	54.92	----	------

TABLE VII

ANALYSIS OF VARIANCE OF THE EGG WEIGHT DATA, TRIAL I

Source of variation	d.f.	Sums of squares	Mean squares	F value	Probability level
Total sum of squares	45	726.63	-----	----	------
Diet	7	108.58	15.51	0.95	P>0.25
Error (among individuals within diets)	38	618.05	16.26	----	------

TABLE VIII

ANALYSIS OF VARIANCE OF THE BODY WEIGHT GAIN OR LOSS DATA, TRIAL I

Source of variation	d.f.	Sums of squares	Mean squares	F value	Probability level
Total sum of squares	45	3,685,173.74	------	----	------
Diet	7	1,508,940.70	215,562.96	3.76	P > 0.01
Error (among individuals within diets)	38	2,176,233.04	57,269.29	----	------

TABLE IX

ANALYSIS OF VARIANCE OF THE FEED CONSUMPTION DATA, TRIAL I

Source of variation	d.f.	Sum of squares	Mean squares	F value	Probability level
Total sum of squares	45	8,903.48	-----	----	------
Diet	7	3,948.92	564.13	4.33	P > 0.01
Error (among individuals within diets)	38	4,954.56	130.38	----	------

TABLE X

ANALYSIS OF VARIANCE OF THE PROTEIN EFFICIENCY DATA, TRIAL I

Source of variation	d.f.	Sum of squares	Mean squares	F value	Probability level
Total sum of squares	45	10.92	----	----	------
Diet	7	3.56	0.51	2.63	P >0.05
Error (among individuals within diets)	38	7.36	0.19	----	------

TABLE XI

ANALYSIS OF VARIANCE OF THE ENERGY EFFICIENCY DATA, TRIAL I

Source of variation	d.f.	Sum of squares	Mean squares	F value	Probability level
Total sum of squares	45	0.0326	-----	----	------
Diet	7	0.0108	0.0015	2.50	P >0.05
Error (among individuals within diets)	38	0.0218	0.0006	----	------

hens fed Diet 5 gained 644 grams. Although hens that were fed Diets 1, 2, 3 and 4 gained 300 to 400 grams, it was expected that they would gain more than hens fed Diet 5. This along with the fact that egg production was lowest on Diet 1 tends to indicate that some nutritive imbalance was present in Diets 1 through 4.

Average daily feed consumption was reduced significantly ($P > 0.01$, Table IX) by the higher energy and protein levels of Diets 1 through 4. Hens that were fed Diet 1 consumed the least amount of feed. This lower feed consumption accounts for some of the difference in egg production and body weight gain among hens fed the experimental diets. Theoretically, if all nutrients were balanced, this reduced feed consumption should not affect egg production and body-weight-change since the nutrients were more highly concentrated in Diets 1 through 4.

One of the most interesting findings of this trial was that, regardless of the protein and energy levels of the experimental diets, protein and energy consumption among all hens was about the same. With the exceptions of those hens that were fed Diets 7 and 8, protein consumption was approximately 16 grams per bird per day and energy consumption was approximately 300 Calories per day (Table V). Intake values which are given for Diets 7 and 8 may not be representative. The polyethylene spheres which were used as filler were picked over by the hens and remained in the feed container and were weighed back at the end of the experiment. Had the hens consumed these diets without picking over the polyethylene, protein and energy consumption values might have been closer to those of the other diets.

The efficiency of protein and energy utilization was significantly different ($P > 0.05$, Tables X and XI) among hens that were fed the different experimental diets. This is in agreement with the observations that egg production was lower on the diets with higher levels of energy and protein, and that energy consumption and protein consumption were nearly equal for all diets.

In an attempt to find a possible explanation for lower egg production and reduced body weight gains among hens that were fed Diets 1 through 4, vitamin consumption per hen was calculated for each of the 8 experimental diets and a standard diet (Table XII). In calculating the vitamin intake levels of the standard diet the assumption was made that laying hens would consume 114 grams of a practical-type diet per day. The vitamin requirements recommended by Titus (1955) were used in making these daily vitamin intake calculations. A comparison of these intake values with the vitamin intake values of the experimental diets shows that vitamin A, vitamin B_{12} and pantothenic acid consumption rates per hen were low in all diets and that riboflavin, pyridoxine, choline and thiamin consumption rates were low in Diets 1 through 4.

Trial II

Purpose

In Trial I daily feed consumption appeared to be dependent upon the levels of dietary protein and energy. Hens were able to compensate for low dietary levels of protein and energy by consuming more feed, and similarly hens that were fed extremely high levels of protein and energy compensated by consuming less feed. Ability of the hen to regulate nutrient intake has contributed greatly to the confusion over the exact percentages or proportions of nutrients required in layer hen diets. Regardless of dietary energy and protein levels, approximately 16 grams of protein and 300 Calories of energy were consumed per hen per day. These results suggest

TABLE XII

AVERAGE DAILY VITAMIN CONSUMPTION, TRIAL I

Diet number			1	2	3	4	5	6	7	8
Vitamins	Units	Standard[1]								
Vitamin A	I.U.	3,000.00	1463.00	1661.00	1661.00	1641.00	2029.00	2121.00	1839.00	1700.00
Vitamin D_3	I.C.U.	88.00	146.00	166.00	166.00	164.00	203.00	212.00	184.00	170.00
Vitamin E	I.U.	6.25	7.31	8.30	8.30	8.20	10.14	10.60	9.19	8.50
Vitamin K_3	Mg.	0.05	0.04	0.05	0.05	0.05	0.06	0.06	0.06	0.05
Vitamin B_{12}	Mcg.	0.75	0.44	0.50	0.50	0.49	0.61	0.64	0.55	0.51
Riboflavin	Mg.	0.45	0.29	0.33	0.33	0.33	0.41	0.42	0.37	0.34
Niacin	Mg.	1.88	2.93	3.33	3.33	3.29	4.06	4.25	3.68	3.40
Pantothenic acid	Mg.	1.13	0.59	0.66	0.66	0.65	0.81	0.85	0.74	0.68
Pyridoxine	Mg.	0.38	0.29	0.33	0.33	0.33	0.41	0.42	0.37	0.34
d-Biotin	Mg.	0.02	0.15	0.17	0.17	0.16	0.20	0.21	0.18	0.17
Choline	Mg.	125.00	85.86	97.50	97.50	96.33	119.09	124.53	107.97	99.83
Thiamin	Mg.	0.75	0.59	0.66	0.66	0.65	0.81	0.85	0.74	0.68
Folic acid	Mg.	0.06	0.12	0.13	0.13	0.13	0.16	0.17	0.15	0.14
Ascorbic acid	Mg.	----	3.29	3.74	3.74	3.69	4.57	4.78	4.14	3.83
Inositol	Mg.	----	33.20	37.70	37.70	37.25	46.05	48.15	41.75	38.60

[1]Standard - calculated from Titus (1955). The assumption was made that the hens would consume 114 grams of a practical type ration in one day.

that the customary method of basing nutrient requirements on a percentage or a proportion of the total diet does not adequately consider daily nutrient requirements of laying hens.

It was obvious from Trial I and other research work which has been reported in the literature that in order to measure the effects of different nutrient intake levels it would be necessary to control feed consumption. The assumption was made that excess dietary volume would reduce the hen's ability to compensate for low levels of dietary nutrients, while a low dietary volume would allow hens to consume higher levels of the nutrients.

Trial II was initiated to determine if feed intake could be controlled by regulating volume, with enough precision to study the effects of graded intake levels of protein and energy. In addition, this trial was designed to obtain more data on daily nutrient intake and to check the validity of daily nutrient intake as a basis for the determination of nutrient requirements for laying hens.

Procedure

Trial II consisted of 8 experimental diets, each replicated 9 times, with each laying hen serving as a replicate. DeKalb-131 layers which were ten months of age were placed on the experimental diets April 2, 1959. These hens were laying at a rate of approximately 55 percent when the trial started. Data were collected for 6 two-week periods and the trial was terminated on November 29, 1960.

Although data from Trial I did not furnish a complete daily nutrient intake standard, it provided valuable information as to the daily intake requirement of laying hens for protein and energy. The experimental diets that were fed in Trial II were formulated on a per-hen-per-day basis, using

TABLE XIII

INGREDIENT COMPOSITION[1] OF THE EIGHT EXPERIMENTAL DIETS, TRIAL II

Diet number	1	2	3	4	5	6	7	8
Ingredients				Grams of ingredient[2]				
Polyethylene fluff[3]	28.4	30.6	33.8	34.5	34.6	29.7	24.7	23.5
Oat mill feed[4]	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Starch	17.7	21.8	25.9	31.9	6.1	11.9	23.5	29.3
Dried whole egg solids	30.8	27.7	24.6	21.4	30.8	30.8	30.8	30.8
Corn oil	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin concentrate[5]	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate (18% phosphorus)	4.5	4.5	5.0	5.0	4.5	4.5	4.5	4.5
Calcium carbonate	3.5	3.5	3.1	3.1	3.5	3.5	3.5	3.5
Trace mineral mix[6]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Salt (Na cl)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Total weight (gms.)	106.5	109.7	114.0	117.5	101.1	102.0	108.6	113.2
Total volume (ml.)	300	300	300	302	300	294	295	295
Desired daily nutrient consumption								
Crude protein (gm.)	16.14	14.57	12.98	11.39	16.14	16.14	16.14	16.14
Calories (M.E.)[7]	288	297	309	318	229	254	318	356
Calorie-protein ratio	17.8	20.4	23.8	27.9	14.2	15.7	19.7	22.1
Calcium (gm.)	2.64	2.63	2.61	2.60	2.64	2.64	2.64	2.64
Total phosphorus (gm.)	1.14	1.12	1.19	1.17	1.14	1.14	1.14	1.14
Crude fiber (gm.)	32.6	34.8	38.0	38.7	38.8	33.9	28.9	27.7

Footnotes to Table XIII

1. Ingredient composition--the experimental diets were calculated on a per hen, per day basis and they are presented on this basis.
2. Grams of ingredient--calculated to meet the desired daily nutrient consumption which is listed at the bottom of Table XIII and in Table XIV.
3. Polyethylene fluff--"Alathon" 10, E. I. DuPont De Nemours and Company, Incorporated, St. Louis 1, Missouri.
4. Oat mill feed--Red-3 higrade oat mill by-product, National Oats Company, Cedar Rapids, Iowa.
5. Vitamin concentrate--refer to Table XIV.
6. Trace mineral mix--see footnote 5, Table II.
7. (M.E.)--metabolizable energy, Titus (1955).

TABLE XIV

COMPOSITION OF THE VITAMIN CONCENTRATE FED IN TRIAL II AND THE DAILY INTAKE OF EACH VITAMIN WHEN THE CONCENTRATE IS CONSUMED AT THE RATE OF ONE GRAM PER HEN PER DAY

Vitamins	Units	One gram of concentrate supplies the following:
Vitamin A	U.S.P.	4,405.00
Vitamin D_3	I.C.U.	220.00
Vitamin E	I.U.	11.00
Vitamin K_3	Mg.	0.07
Vitamin B_{12}	Mcg.	1.45
Riboflavin	Mg.	0.88
Niacin	Mg.	4.40
Pantothenic acid	Mg.	0.88
Pyridoxine	Mg.	1.32
d-Biotin	Mg.	0.71
Choline	Mg.	132.59
Thiamin	Mg.	4.40
Folic acid	Mg.	0.44
Ascorbic acid	Mg.	11.01
Inositol	Mg.	50.00

TABLE XV

CALCULATED AMINO ACID COMPOSITION[1]
OF ALL EXPERIMENTAL DIETS, TRIAL II

Amino acid	Gm. per 16 gm. of nitrogen	Amino acid	Gm. per 16 gm. of nitrogen
Arginine	9.59	Threonine	5.89
Histidine	2.30	Leucine	12.60
Lysine	7.88	Isoleucine	8.79
Tyrosine	3.19	Valine	6.99
Tryptophan	1.70	Glutamic acid	12.60
Phenylalanine	7.30	Aspartic acid	5.80
Cystine	2.30	Glycine	3.80
Methionine	2.70	Alanine	2.59
Serine	8.40	Proline	4.49

[1]Calculated amino acid composition - all amino acid calculations were based upon average values as given in Block and Weiss (1956).

the daily nutrient intake standard that was based upon the data from Trial I. An examination of the 8 experimental diets, the composition of which is given in Table XIII, will help explain this per-hen-per-day method of formulation. In the formulation of any diet for laying hens, it is relatively simple to combine all required nutrients within the quantity of feed which a hen will consume in one day. Since total weight of the daily diets was not important until the weight as well as dietary volume exceeded that quantity which could be consumed per hen per day, dietary weight was not considered to be an important factor in the formulation of these diets. The quantities of feed ingredients that were necessary to provide the desired daily nutrient intake of all nutrients were combined, then total volume of the combined ingredients was calculated. Polyethylene fluff, which is an inert source of volume, was added to raise the volume of each daily diet to 300 milliliters. Vitamin concentrate supplementation to all diets was constant and increased above that used in Trial I (Table XIV).

In order to obtain some information as to the daily amino acid requirements of layers, a single source of protein was fed in this trial. It was reasoned that the protein source should be one which would furnish an available and adequate supply of amino acids. Based upon reasoning and previous research work reported by Johnson and Fisher (1956), dried whole egg solids was chosen as the source of protein. The amino acid ratios of the experimental diets were calculated and are presented in Table XV. The protein intake levels for Trial II were originally planned to be 16, 14, 12 and 10 grams and the metabolizable energy intake levels were to be 350, 325, 300, 250 and 225 Calories per day.

It will be noted in Table XIII that the dietary levels of protein and energy are not the same as those which were originally planned. In the formulation of these experimental diets, there were two factors which caused the discrepancy between the planned nutrient levels and those which were actually fed: (1) Oat mill feed was added to the diets at a constant level as a source of volume and crude fiber. It was originally decided not to consider the protein and energy that was contributed by this oat mill feed. However, in the final calculations, the quantities of protein and energy that were contributed by oat mill feed were added to that contributed by the other ingredients and this increased the level of dietary protein and energy above that which was planned. (2) In the early stages of the development of this method of diet formulation, it was difficult to avoid the customary procedure of considering dietary weight. Dietary energy levels were calculated as though the ingredient quantities were expressed in pounds rather than grams. The Calorie-per-pound values of the total combined ingredients were then divided by 454 to arrive at Calories-per-gram. Then the Calories-per-gram were multiplied by 113.5 which is considered to be a standard figure for daily feed consumption per hen. The author failed to consider that 113.5 grams might not be consumed per day and that unequal daily feed intake weights would result from maintaining a constant volume with graded nutrient levels. The Calories-per-gram should have been multiplied by the actual weights of the daily diets, which represent the desired daily feed intake. Consequently the actual corrected levels of protein and energy in the experimental daily diets turned out to be 11.39, 12.98, 14.57 and 16.14 grams and 229, 254, 318 and 356 Calories. This method of energy calculation is one that can be followed if the proper diet weights are considered in the calculations.

A subsequent procedure which was used was to calculate the total Calories of energy and grams of nutrient contributed by each ingredient and then add the nutrient values together for the various ingredients to arrive at the total nutrient level in the daily diet. Energy content of feed ingredients is normally given as Calories per pound; therfore, the latter method of diet formulation on a daily nutrient intake basis requires that Calories per pound of feedstuff be converted to Calories per gram. This can be accomplished by dividing Calories per pound of feedstuff by 454. It should be remembered with this method of diet formulation that the quantity of each ingredient which is added to the diet is equivalent to the desired daily intake of that ingredient.

Both the summary and the statistical analyses for Trial II were divided into two separate studies (Table XVI) to facilitate comparisons among the graded protein and graded energy intake levels. Study 1 (protein study) included Diets 1, 2, 3 and 4 which contained 16.14, 14.57, 12.98 and 11.39 grams of protein, respectively. Due to the two factors which were described previously, the respective levels of metabolizable energy were 288, 297, 309 and 318 Calories. Study 2 (energy study) included Diets 5, 6, 1, 7 and 8 which contained 229, 254, 288, 318 and 356 Calories of metabolizable energy, respectively. The level of protein was constant at 16.14 grams in all five diets of Study 2.

Study 1

Results

Table XVII gives a summary of egg production, egg weight, body weight change, feed consumption and efficiency of feed utilization for the hens in Study 1. Egg production did not appear to be influenced by the single source of protein or by the graded levels of protein intake in this study. Although there were some sizable differences among diets, there was so much

TABLE XVI

EXPERIMENTAL DESIGN OF THE TWO STUDIES MADE IN TRIAL II

Diet number	Protein, Study 1 1	2	3	4
Daily protein intake (gm.)	16.14	14.57	12.98	11.39
Daily Calorie (M.E.)[1] intake	288	297	309	318
Calorie-protein ratio	17.8	20.4	23.8	27.9

Diet number	Energy, Study 2 5	6	1	7	8
Daily protein intake (gm.)	16.14	16.14	16.14	16.14	16.14
Daily Calorie (M.E.)[1] intake	229	254	288	318	356
Calorie-protein ratio	14.2	15.7	17.8	19.7	22.1

[1](M.E.) - metabolizable energy, Titus (1955).

TABLE XVII

AVERAGE EGG PRODUCTION, EGG WEIGHT, BODY WEIGHT CHANGE, FEED CONSUMPTION AND EFFICIENCY OF FEED UTILIZATION PER HEN, STUDY 1, TRIAL II

Diet number	1	2	3	4
Number of days on experiment	84	84	84	84
Number of surviving hens per diet (replicates)	8	8	8	8
Average number of eggs produced	38.4	48.1	50.4	40.5
Percent egg production	45.7	57.3	60.0	48.2
Egg weight (gm.)	58.9	58.0	57.7	57.9
Total body weight gain or loss (gm.)	-42	-227	-278	-214
Daily feed consumption (gm.)	96.4	94.2	102.5	99.5
Daily protein consumption (gm.)	14.6	12.5	11.7	9.7
Daily Calorie (M.E.)[1] consumption	245	240	243	229
Gm. protein per gm. egg	0.54	0.38	0.34	0.30
Calories (M.E.)[1] per gm. egg	9.1	7.2	7.2	7.2
Vitamin concentrate consumed (gm.)	0.91	0.86	0.90	0.85
Milliliters consumption of total diet	270	254	267	259

[1] (M.E.) - metabolizable energy, Titus (1955).

individual hen variation that these differences were not statistically significant. However, the difference in body weight change, among hens that were fed the various experimental diets, were significant ($P > 0.025$, Table XVIII). All diets were nutritionally inadequate to maintain the body weight of the hens, but hens that were fed the highest level of protein lost less body weight than those that were fed the lowest level of protein. With the exception of the hens that were fed Diet 4, body weight loss per hen decreased as dietary protein was increased. These hens lost only 214 grams as compared to 278 grams lost by hens that were fed Diet 3.

The extreme volume of the experimental diets apparently restricted feed intake more than was desired in this study. Total feed consumption did not reach the expected level; consequently, the consumption of both protein and energy was lower than calculated. Even though protein consumption values were all lower than was calculated, there was a definite gradation in protein consumption. Protein consumption levels for the four experimental diets were calculated to be 16.14, 14.57, 12.98 and 11.39 grams. The respective actual consumption levels were 14.6, 12.5, 11.7 and 9.7 grams.

Differences in efficiency of protein utilization were highly significant among hens that were fed the various experimental diets (Table XIX). The grams of protein that were required to produce each gram of egg increased steadily as dietary protein was increased. There was very little difference in efficiency of energy utilization among hens fed the various experimental diets. This indicates that the differences in dietary energy levels were not serious enough to influence the results of the protein study.

Study 2

Results

Performance data for the hens in Study 2 are summarized in Table XX.

TABLE XVIII

ANALYSIS OF VARIANCE OF THE BODY WEIGHT GAIN OR LOSS DATA, STUDY 1, TRIAL II

Source of variation	d.f.	Sum of squares	Mean squares	F value	Probability level
Total sum of squares	31	836,966.00	------	----	------
Diet	3	237,190.50	79,063.50	3.69	P > 0.025
Error (among individuals within diets)	28	599,775.50	21,420.55	----	------

TABLE XIX

ANALYSIS OF VARIANCE OF THE PROTEIN EFFICIENCY DATA, STUDY 1, TRIAL II

Source of variation	d.f.	Sum of squares	Mean squares	F value	Probability level
Total sum of squares	31	25.41	----	----	------
Diet	3	8.57	2.86	4.77	P > 0.01
Error (among individuals within diets)	28	16.84	0.60	----	------

TABLE XX

AVERAGE EGG PRODUCTION, EGG WEIGHT, BODY WEIGHT CHANGE, FEED CONSUMPTION AND EFFICIENCY OF EGG PRODUCTION PER HEN, STUDY 2, TRIAL II

Diet number	5	6	1	7	8
Number of days on experiment	84	84	84	84	84
Number of surviving hens per diet (replicates)	7	9	8	8	9
Average number of eggs produced	40.4	30.6	38.4	44.6	46.8
Percent egg production	48.1	36.4	45.7	53.1	55.7
Egg weight (gm.)	54.2	60.2	58.9	58.8	56.7
Total body weight gain or loss (gm.)	-194	-144	-42	-40	-81
Daily feed consumption (gm.)	89.7	87.0	96.4	87.2	87.8
Daily protein consumption (gm.)	14.4	13.2	14.6	13.0	12.6
Daily Calorie (M.E.)[1] consumption	201	212	245	235	244
Gm. protein per gm. egg	0.55	0.59	0.54	0.42	0.39
Calories (M.E.)[1] per gm. egg	7.7	9.5	9.1	7.5	7.6
Vitamin concentrate consumed (gm.)	0.89	0.85	0.91	0.80	0.78
Milliliters consumption of total diet	269	252	270	236	228

[1](M.E.) - metabolizable energy, Titus (1955).

There were no statistically significant differences among the diets for any of the variables tested. However, hens that were fed the higher energy levels tended to lose less body weight than those fed the lower energy levels.

Feed consumption, protein consumption, protein efficiency and vitamin concentrate consumption figures were all nearly equal for the five experimental diets. With the exception of Diet 1, the gradations in actual energy consumption followed the predetermined pattern, but the volume of feed consumed per hen generally decreased as dietary energy was increased. Although it was less obvious, this pattern also existed with the small energy differences present in Study 1 (Table XVII). From the standpoint of diet formulation, these data indicated that as dietary energy was increased dietary volume should have been decreased.

A feed volume of 300 milliliters per day restricted feed intake too drastically in this study as had been the case in Study 1. The hens were unable to consume an average of 300 milliliters per day on any diet that was fed in Trial II. One possible explanation was that the hens were all relatively old and laying at a low rate of production when the trial was initiated. Low egg production would tend to reduce feed consumption. Another factor which contributed to the low overall volume of feed consumption per hen is evident from the data in Table XXI. The data on volume of feed consumed per hen for each period and for all eight experimental diets that were fed in Trial II are found in Table XXI. These data indicated that hens needed an adjustment period of approximately four weeks in order to reach a maximum rate of consumption of the high volume diets. Hens reached maximum feed consumption on all diets during Periods 3, 4 and 5. During the sixth period, feed consumption per hen again decreased. In Periods 3, 4

TABLE XXI

AVERAGE DAILY VOLUME OF FEED CONSUMPTION PER HEN, TRIAL II, SUMMARIZED BY PERIODS

Period number	1	2	3	4	5	6
Diet number	Milliliters of feed consumption					
1	205	265	285	304	284	277
2	209	234	275	237	335	237
3	218	256	324	333	293	181
4	199	241	294	294	299	228
5	189	250	319	274	330	253
6	222	265	287	244	289	202
7	198	273	262	241	241	199
8	201	238	256	215	245	214

and 5 the hens were attempting to consume enough feed to compensate for the low nutrient levels of the experimental diets. It was reasoned that the high volume of these diets forced the hens to reduce feed consumption during Period 6. They were unable to continue consuming an extremely high volume of feed for long periods of time.

The data collected in Trial II gave evidence that volume could be used with some degree of accuracy in controlling feed intake of laying hens. However, two major changes in procedure were indicated by these data: (1) volume of the diet evidently should have started low and increased gradually until the hens were on full-feed, and (2) as dietary energy was increased the dietary volume should have been reduced.

In addition to the findings relative to dietary volume, there were also some indications that relatively small gradations in protein and energy intake could have some influence on the overall performance of laying hens. This was particularly true in body-weight-change of the hens, which was influenced by both protein and energy intake.

Trial III

Purpose

Since Trials I and II were of short duration and involved only small numbers of hens, it was considered desirable to repeat these experiments. Trial III was designed to examine more thoroughly a combination of essentially the same factors that were studied in Trials I and II. The specific purposes of this trial were:

(1) to study the effects of dietary volume upon nutrient intake and to improve the volume technique of nutrient intake control,

(2) to determine the effects of a range of protein intakes upon the performance of laying hens,

(3) to determine the effects of a range of metabolizable energy intake upon the performance of laying hens,

(4) to study the effects of a range of vitamin-mineral concentrate intakes upon the utilization of other nutrients in the diet and upon the overall performance of layers, and

(5) to study interactions among the factors listed above.

Procedure

This feeding trial comprised 29 experimental diets, each replicated 11 times. Three hundred and nineteen Kimber Chik, 5-month-old pullets were fed the experimental diets. The trial was initiated on December 1, 1959 and data which are to be reported herein were collected for 13 four-week periods. Data to be reported in a later publication were collected for 6 additional four-week periods. In order to maintain an adequate number of replicates throughout the entire experiment, hens that died during the first four periods were replaced.

The overall experimental design for Trial III is presented in Table XXII. Twenty-nine combinations of three intake levels of protein (13, 16 and 19 grams), three intake levels of metabolizable energy (250, 300 and 350 Calories), and five intake levels of vitamin-mineral concentrate (0.215, 0.640, 1.065, 1.490 and 1.915 grams) were set up as the intake levels. Table XXIII shows the specific vitamin and mineral consumption per hen that was desired for each of the five vitamin-mineral concentrate levels.

Nine basals (Table XXIV) were formulated on a per-hen-per-day basis. This method of formulation was described under the procedure for Trial II. The basals furnished the desired volumes and nine combinations of three intake levels of protein and three intake levels of energy. To comply with the findings of Trial II the volume of all basals was standardized at approximately 250 milliliters at the start of Trial III. Feed consumption data

TABLE XXII

OVERALL EXPERIMENTAL DESIGN, TRIAL III

Daily protein consumption		Daily vitamin-mineral concentrate[1] consumption (gm.)				
Grams	Calories	Diet Number	Diet Number	Diet Number	Diet Number	Diet Number
13	250	(1) 0.215	(2) 0.640	(3) 1.065		
13	300		(4) 0.640	(5) 1.065	(6) 1.490	
13	350			(7) 1.065	(8) 1.490	(9) 1.915
16	250	(10) 0.215	(11) 0.640	(12) 1.065	(13) 1.490	
16	300		(14) 0.640	(15) 1.065	(16) 1.490	
16	350		(17) 0.640	(18) 1.065	(19) 1.490	(20) 1.915
19	250	(21) 0.215	(22) 0.640	(23) 1.065		
19	300		(24) 0.640	(25) 1.065	(26) 1.490	
19	350			(27) 1.065	(28) 1.490	(29) 1.915

[1]Vitamin-mineral concentrate - see Table XXVII for the composition of this concentrate.

TABLE XXIII

DESIRED VITAMIN AND MINERAL CONSUMPTION PER HEN, PER DAY FOR EACH OF THE FIVE VITAMIN MINERAL-CONCENTRATE LEVELS, TRIAL III

Vitamin or mineral	Units	Vitamin-mineral concentrate level Grams 0.215	0.640	1.065	1.490	1.915
Vitamin A	U.S.P.	505.14	1503.67	2502.20	3500.73	4499.25
Vitamin D_3	I.C.U.	75.77	225.55	375.33	525.11	674.88
Vitamin E	I.U.	0.38	1.13	1.87	2.62	3.37
Vitamin K_3	Mg.	0.19	0.56	0.94	1.31	1.69
Vitamin B_{12}	Mcg.	0.51	1.50	2.50	3.50	4.50
Riboflavin	Mg.	0.25	0.75	1.25	1.74	2.24
Niacin	Mg.	2.02	6.02	10.01	14.00	18.00
Pantothenic acid	Mg.	0.51	1.50	2.50	3.50	4.50
Pyridoxine	Mg.	0.51	1.50	2.50	3.50	4.50
d-Biotin	Mg.	0.02	0.06	0.10	0.13	0.17
Choline	Mg.	31.57	93.98	156.38	218.79	281.20
Thiamin	Mg.	0.76	2.25	3.75	5.24	6.74
Folic acid	Mg.	0.13	0.38	0.63	0.88	1.13
Ascorbic acid	Mg.	0.63	1.88	3.13	4.38	5.63
Inosital	Mg.	3.16	9.40	15.63	21.87	28.04
Para amino benzoic acid	Mg.	0.25	0.75	1.25	1.74	2.24
Manganese	Mg.	1.75	5.21	8.67	12.13	15.59
Iodine	Mg.	0.05	0.16	0.27	0.37	0.48
Cobalt	Mg.	0.04	0.11	0.18	0.25	0.33
Iron	Mg.	1.38	4.10	6.82	9.54	12.26
Copper	Mg.	0.10	0.31	0.51	0.72	0.92
Zinc	Mg.	1.43	4.27	7.10	9.94	12.77

TABLE XXIV

INGREDIENT COMPOSITION[1] OF THE NINE BASALS USED TO FORMULATE THE EXPERIMENTAL DIETS, TRIAL III

Basal	A	B	C	D	E	F	G	H	I
Ingredients	Grams of ingredient[2]								
Stabilized animal tallow	8.0	10.0	12.0	8.0	10.0	12.0	7.0	10.0	12.0
Starch	12.5	21.2	30.4	4.4	13.2	21.8	--	5.0	13.7
Ground yellow corn	12.2	12.2	12.2	15.0	15.0	15.0	17.8	17.8	17.8
Oat mill feed	24.4	24.4	24.4	30.0	30.0	30.0	35.6	35.6	35.6
Dehydrated alfalfa (17% protein)	1.6	1.6	1.6	2.0	2.0	2.0	2.3	2.3	2.3
Herring fish meal (74.6% protein)	2.5	2.5	2.5	3.0	3.0	3.0	3.7	3.7	3.7
Soybean oil meal (50% protein)	8.5	8.5	8.5	10.5	10.5	10.5	12.5	12.5	12.5
Blood meal (84% protein)	3.3	3.3	3.3	4.0	4.0	4.0	4.8	4.8	4.8
Gelatin	1.6	1.6	1.6	2.0	2.0	2.0	2.3	2.3	2.3
Delactosed dried whey	1.6	1.6	1.6	2.0	2.0	2.0	2.3	2.3	2.3
Dried condensed fermented corn extractives[3]	1.6	1.6	1.6	2.0	2.0	2.0	2.3	2.3	2.3
Dicalcium phosphate (18% phosphorus)	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3	5.3
Calcium carbonate	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Salt (Na cl)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
dl-Menthionine	0.12	0.12	0.12	0.14	0.14	0.14	0.17	0.17	0.17
Polyethylene fluff[4]	18.0*	14.5*	11.6*	13.9*	11.2*	8.5*	6.8*	4.8*	2.1*
Total weight (gm.)	105.2*	112.4*	120.3*	106.2*	114.3*	122.2*	106.9*	112.9*	120.4*
Total volume (me.)	255*	251*	250*	253*	253*	253*	251*	250*	250*
Calculated nutrient analyses									
Crude protein (gm.)	13.06	13.06	13.06	16.02	16.02	16.02	19.07	19.07	19.07
Calories (M.E.)[5]	250	300	350	250	300	350	255	300	350
Calorie-protein ratio	19.1	23.0	26.8	15.6	18.7	21.8	13.4	15.7	18.4
Calcium (gm.)	2.82	2.82	2.82	2.86	2.86	2.86	2.91	2.91	2.91
Available phosphorus (gm.)	1.08	1.08	1.08	1.14	1.14	1.14	1.14	1.14	1.14
Crude fiber (gm.)	26.9*	23.4*	20.5*	24.9*	22.2*	19.5*	19.9*	17.9*	15.2*

Footnotes to Table XXIV

1. Ingredient composition--the basals were calculated on a per hen, per day basis and they are presented on this basis.
2. Grams of ingredient--calculated to meet the desired daily protein and energy consumptions that are listed in Table XXII.
3. Dried condensed fermented corn extractives--C.F.S. No.3, Clinton Corn Processing Company, Clinton, Iowa.
4. Polyethylene fluff--"Alathon" 10, E. I. DuPont De Nemours and Company, Incorporated, St. Louis 1, Missouri.
5. (M.E.)--metabolizable energy, Titus (1955).

*Refer to Table XXV for adjustments that were made in these values after the first twenty eight-day period of the experiment.

were examined at the end of each of the first three experimental periods to determine what volume adjustments should be made in order to control nutrient intake better. Then, based upon feed consumption data, volume adjustments were made in the basals for Periods 2, 3 and 4. Volume of the basals was adjusted by adding or removing polyethylene fluff. Therefore, total weight and fiber level were changed with each adjustment. The volume adjustments and the subsequent weight and fiber changes are tabulated in Table XXV. The volume was not changed after the beginning of the fourth period.

The experimental diets were composed of a certain specific calculated quantity of one of the basals plus one of the desired intake levels of vitamin-mineral concentrate. The quantity of basal diet used after each volume adjustment, and quantity of vitamin-mineral concentrate combined with the basal, are shown in Table XXVI. The composition of the vitamin-mineral concentrate and the amino acid ratios for these diets are presented in Tables XXVII and XXVIII, respectively.

Again, as in Trial II, this trial was divided into separate studies for summarization and statistical purposes. The following five complete factorial studies were summarized and analyzed statistically:

(1) effects of energy and protein consumption upon the overall performance of layers,

(2) effects of protein and vitamin-mineral concentrate consumption with 250 Calories of metabolizable energy upon the performance of layers,

(3) effects of protein and vitamin-mineral concentrate consumption with 300 Calories of metabolizable energy upon the performance of layers,

(4) effects of protein and vitamin-mineral concentrate consumption with 350 Calories of metabolizable energy upon the performance of layers, and

TABLE XXV

VOLUME ADJUSTMENTS MADE IN THE BASALS FOR PERIODS TWO, THREE AND FOUR, TRIAL III

Basal	A	B	C	D	E	F	G	H	I
	Values for period two								
Grams of polyethylene fluff (Table XII, footnote 2)	20.7	10.6	6.0	18.0	11.2	7.0	16.0	9.4	--
Adjusted total weight (gm.)	107.9	108.5	114.7	110.3	114.3	120.7	116.9	117.5	118.3
Adjusted total volume (ml.)	270	230	220	275	253	245	300	275	239
Adjusted daily fiber consumption (gm.)	29.6	19.5	14.9	29.0	22.2	18.0	29.1	22.5	13.1
	Values for period three								
Grams of polyethylene fluff (Table XII, footnote 2)	20.7	14.5	11.6	18.0	11.2	8.5	16.0	13.2	--
Adjusted total weight (gm.)	107.9	112.4	120.3	110.3	114.3	122.2	116.9	121.3	118.3
Adjusted total volume (ml.)	270	251	250	275	253	253	300	296	239
Adjusted daily fiber consumption (gm.)	29.6	23.4	20.5	29.0	22.2	19.5	29.1	26.3	13.1
	Values for periods four through thirteen[1]								
Grams of polyethylene fluff (Table XII, footnote 2)	45.0	21.5	8.0	43.0	20.8	11.5	41.3	18.0	1.7
Adjusted total weight (gm.)	132.2	119.4	116.7	135.3	123.9	125.2	142.2	126.1	120.0
Adjusted total volume (ml.)	401	289	231	410	305	269	436	321	248
Adjusted daily fiber consumption (gm.)	53.9	30.4	16.9	54.0	31.8	22.5	54.4	31.1	14.8

[1]The volumes of the basal diets were held constant from the fourth through the thirteenth period of the experiment.

TABLE XXVI

COMPOSITION[1] OF THE TWENTY NINE EXPERIMENTAL DIETS, TRIAL III*

Diet number	Basal	Grams of basal diet period 1	2	3	4-13	Supplemental level of vitamin-mineral concentrate[2] Grams
1	A	105.2	107.9	107.9	132.2	0.215
2	A	105.2	107.9	107.9	132.2	0.640
3	A	105.2	107.9	107.9	132.2	1.065
4	B	112.4	108.5	112.4	119.4	0.640
5	B	112.4	108.5	112.4	119.4	1.065
6	B	112.4	108.5	112.4	119.4	1.490
7	C	120.3	114.7	120.3	116.7	1.065
8	C	120.3	114.7	120.3	116.7	1.490
9	C	120.3	114.7	120.3	116.7	1.915
10	D	106.2	110.3	110.3	135.3	0.215
11	D	106.2	110.3	110.3	135.3	0.640
12	D	106.2	110.3	110.3	135.3	1.065
13	D	106.2	110.3	110.3	135.3	1.490
14	E	114.3	114.3	114.3	123.9	0.640
15	E	114.3	114.3	114.3	123.9	1.065
16	E	114.3	114.3	114.3	123.9	1.490

*Continued on next page.

TABLE XXVI (Continued)

COMPOSITION[1] OF THE TWENTY NINE EXPERIMENTAL DIETS, TRIAL III

Diet number	Basal	Grams of basal diet period 1	2	3	4-13	Supplemental level of vitamin-mineral concentrate[2] Grams
17	F	122.2	120.7	114.3	123.9	0.640
18	F	122.2	120.7	114.3	123.9	1.065
19	F	122.2	120.7	114.3	123.9	1.490
20	F	122.2	120.7	114.3	123.9	1.915
21	G	106.9	116.9	116.9	142.2	0.215
22	G	106.9	116.9	116.9	142.2	0.640
23	G	106.9	116.9	116.9	142.2	1.065
24	H	112.9	117.5	121.3	126.1	0.640
25	H	112.9	117.5	121.3	126.1	1.065
26	H	112.9	117.5	121.3	126.1	1.490
27	I	120.4	118.3	118.3	120.0	1.065
28	I	120.4	118.3	118.3	120.0	1.490
29	I	120.4	118.3	118.3	120.0	1.915

[1]Composition - The weight given for each basal plus the weight of the vitamin-mineral concentrate is equivalent to the desired total daily feed consumption.

[2]Vitamin-mineral concentrate - The grams of concentrate for each diet are equivalent to the desired daily vitamin-mineral concentrate consumption given in Table XXII. See Table XXVII for the composition of the concentrate.

TABLE XXVII

COMPOSITION OF THE VITAMIN-MINERAL CONCENTRATE, TRIAL III

Vitamins and minerals	Units	Amount supplied by one gram of concentrate
Vitamin A	U.S.P.	2,349.48
Vitamin D_3	I.C.U.	352.42
Vitamin E	I.U.	1.76
Vitamin K_3	Mg.	0.88
Vitamin B_{12}	Mcg.	2.35
Riboflavin	Mg.	1.17
Niacin	Mg.	9.40
Pantothenic acid	Mg.	2.35
Pyridoxine	Mg.	2.35
d-Biotin	Mg.	0.09
Choline	Mg.	146.84
Thiamin	Mg.	3.52
Folic acid	Mg.	0.59
Ascorbic acid	Mg.	2.94
Inositol	Mg.	14.68
Para amino benzoic acid	Mg.	1.17
Manganese	Mg.	8.14
Iodine	Mg.	0.25
Cobalt	Mg.	0.17
Iron	Mg.	6.40
Copper	Mg.	0.48
Zinc	Mg.	6.67

TABLE XXVIII

CALCULATED AMINO ACID COMPOSITION[1]
OF ALL EXPERIMENTAL DIETS, TRIAL III

Amino acid	Gm. per 16 gm. of nitrogen	Amino acid	Gm. per 16 gm. of nitrogen
Arginine	6.21	Threonine	4.51
Histidine	2.91	Leucine	9.83
Lysine	6.64	Isoleucine	4.49
Tyrosine	2.98	Valine	6.18
Tryptophan	1.19	Glutamic acid	14.91
Phenylalanine	4.95	Aspartic acid	10.00
Cystine	1.26	Glycine	6.91
Methionine	1.37	Alanine	5.26
Serine	5.57	Proline	5.81

[1]All amino acid calculations were based upon average values as given in Block and Weiss (1956).

(5) effects of energy and vitamin-mineral concentrate consumption with 16 grams of protein upon the performance of layers.

Some diets appear in more than one analysis, and it is recognized that the results may be slightly biased, since the results of one diet have an equal influence on the results of two or more studies. However, for clarity of presentation of results the author felt that it was necessary to present the results of diets 3, 5, 7, 11, 12, 14, 15, 16, 18, 19, 23, 25 and 27 more than one time. The experimental design for each study will be presented immediately before the results of that study.

TABLE XXXIX

FACTORIAL DESIGN, STUDY 1[1], TRIAL III, THE EFFECTS OF ENERGY AND PROTEIN CONSUMPTION UPON THE OVERALL PERFORMANCE OF LAYERS

Desired daily protein cons. (gm.)	Desired daily Calorie (M.E.)[2] consumption		
	250	300	350
13	13-250 (3)*	13-300 (5)	13-350 (7)
16	16-250 (12)	16-300 (15)	16-350 (18)
19	19-250 (23)	19-300 (25)	19-350 (27)

[1]Study 1 - All experimental diets in this study were calculated to supply 1.065 grams of vitamin-mineral concentrate per hen per day.

[2](M.E.) - metabolizable energy, Titus (1955).

*The numbers in parentheses represent the diet numbers.

Study 1

Results

Survival data (Table XXX) show that livability was excellent in this study. The treatments did not adversely affect livability, with the possible exception of those diets that supplied 250 Calories of energy. Mortality was slightly higher among the hens that were fed Diets 2 and 23 than in the hens fed the other diets.

Both linear and quadratic effects of energy upon egg production were statistically significant at the 5 and 1 percent levels, respectively. These trends are easily delineated from the average egg production data in Table XXXI. The statistical analyses are summarized in Table XXXII. Hens fed the 300-Calorie diets produced more eggs than those fed either the 250- or 350-Calorie diets. This trend was present at all levels of protein intake, although it was not obvious with the 16-gram-protein diets until after the eighth period. Diet 15, which contained 16 grams of protein and 300 Calories of metabolizable energy, maintained egg production at a higher level than did any of the other diets. Diet 3, which contained 13 grams of protein and 250 Calories of metabolizable energy, did not support as high egg production as did the other diets. The effects of protein intake upon egg production were statistically significant only during the fourth and tenth experimental periods. In Period 4, this significance was probably due to an extremely low egg production of hens that were fed Diet 23, and in Period 10 egg production increased as dietary protein level was increased.

The effect of protein intake upon egg production appeared to be dependent upon the level of dietary energy. The general trend with the 250-Calorie diets was for egg production to be highest on the 16-gram-protein diets. With 300 Calories of energy, egg production tended to increase

TABLE XXX

NUMBER OF HENS PER TREATMENT AT THE END OF EACH TWENTY-EIGHT-DAY PERIOD, STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Period number				Number of hens					
1	11	11	11	11	11	11	11	10	10
2	11	10	10	11	11	11	11	11	10
3	11	11	10	11	11	11	11	11	11
4	11	11	10	11	11	10	11	10	11
5	11	11	10	11	11	11	10	10	11
6	9	11	10	11	11	11	10	10	11
7	9	11	10	11	11	11	9	10	11
8	9	10	10	11	11	11	8	10	10
9	9	10	10	11	11	11	8	10	10
10	9	10	10	10	11	11	8	10	10
11	9	10	10	10	10	11	8	9	10
12	9	10	10	10	10	11	8	9	10
13	9	10	9	10	10	11	8	9	10

TABLE XXXI

AVERAGE EGG PRODUCTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Period number				Percent egg production					
1	72.4	77.9	73.9	79.2	78.2	80.8	76.0	76.0	81.4
2	74.0	74.0	66.1	74.4	75.6	75.6	71.1	76.9	68.2
3	69.5	80.2	78.2	75.3	72.7	75.6	76.0	78.6	66.2
4	63.6	71.8	74.3	66.9	72.4	70.8	29.9	76.8	72.4
5	55.2	75.0	71.1	69.2	67.5	70.5	36.1	74.6	70.5
6	54.8	61.7	66.1	60.7	60.4	65.3	57.9	67.5	58.8
7	56.0	68.5	72.1	59.1	64.3	66.6	42.1	72.5	57.1
8	56.7	64.3	63.9	68.5	64.3	63.0	58.5	69.3	50.0
9	54.0	62.5	57.5	62.3	65.3	60.1	57.1	66.1	54.3
10	48.0	58.2	53.9	52.1	66.6	55.8	58.0	68.9	60.7
11	45.6	53.9	55.4	44.3	64.3	58.4	51.8	63.5	60.7
12	30.2	55.4	55.4	48.6	64.6	52.6	36.6	67.1	52.9
13	21.0	49.3	49.2	35.0	64.6	48.1	38.8	55.6	50.4
Overall[1]	54.9	66.0	64.5	61.6	67.8	64.9	53.8	70.6	61.9

[1]Overall - based upon cumulative data for all periods.

TABLE XXXII

ANALYSIS OF VARIANCE OF EGG PRODUCTION DATA
BY PERIODS AND OVERALL, STUDY 1, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(96)	(95)	(93)	(92)	(89)	(89)	(88)	(86)	(86)	(85)	(85)
Blocks	10	145	318	95	120	154	134	139	156	180	116	170	204	426	10490
Energy: (E)	(2)														
E_L (Linear)	1	13	9	0	450**	347**	33	178*	8	1	17*	121	231*	292*	7044*
E_Q (Quadratic)	1	0	2	24*	189**	160**	9	149*	45	67	145*	110	372**	419**	15878**
Protein: (P)	(2)														
P_L	1	10	0	6	129**	55	0	86	5	3	103*	104	50	93	948
P_Q	1	15	19	0	46*	46	1	6	34	23	0	0	35	38	2240
Energy-protein interaction	(4)														
$E_L \times P_L$	1	3	5	71**	216**	64	22	0	54	10	2	0	17	35	26
$E_Q \times P_L$	1	14	1	17	7	32	0	0	2	0	12	15	5	22	45
$E_L \times P_Q$	1	0	0	5	304**	279**	20	125*	50	8	0	16	84	10	2292
$E_Q \times P_Q$	1	5	21	14	13	1	22	55	26	4	0	0	0	32	1002
Error		12	19	7	11	16	33	28	27	30	25	33	37	43	1401
Error d.f.[2]		78	78	79	78	77	75	74	71	71	70	68	68	67	67

[1]d.f. - the degrees of freedom for blocks and treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

with each increase in protein intake. The trend with the 350-Calorie diets was one of near equal egg production from hens fed the 13-gram-protein and 16-gram-protein diets. Until the ninth period, hens fed 19-gram-protein diets produced less eggs than hens fed either of the other two protein levels. From the tenth period through the thirteenth period, hens fed the 19-gram-protein diets tended to produce more eggs than hens fed the other protein intake levels. Even though these energy x protein interaction trends were present, they were statistically significant only in Periods 3, 4, 5 and 7.

A summary of the egg weight data for Study 1 is given in Table XXXIII. There were no significant differences in egg weight among hens fed the various experimental diets. Analyses of variance of the egg weight data are presented in Table XXXIV.

Body-weight-change data in Table XXXV show that as energy intake was increased body weight gain increased. Both linear and quadratic effects of energy upon body weight were significant (Table XXXVI) in the overall analysis. However, the quadratic effects have little meaning, since none were present in any of the period analyses. Either linear and/or quadratic effects of protein upon body weight were significant in Periods 1, 2, 4, 11 and 12 of Study 1. This meaning was also obscured since there was no discernible pattern of the effect and nothing was significant in the overall analysis. Energy x protein interactions were present in some periods, but again nothing was found in the overall analysis.

Daily feed consumption per hen (Table XXXVII) followed a pattern similar to that in Trial II. Approximately the first four weeks were required for the hens to reach their maximum feed consumption. This adjustment period was followed by higher feed intakes during Periods 2, 3, 4 and 5. Feed

TABLE XXXIII

AVERAGE EGG WEIGHT BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Period number	Egg weight in grams								
1	55.2	52.8	52.9	54.4	53.2	55.6	55.5	56.3	54.4
2	55.7	55.1	53.9	55.4	54.7	56.6	57.1	57.1	56.8
3	57.4	55.3	57.0	59.1	56.3	58.7	58.0	58.6	57.8
4	57.4	56.9	57.2	57.2	57.5	59.2	57.2	58.9	59.0
5	59.6	58.1	58.7	58.1	58.4	60.6	57.5	59.5	60.4
6	60.2	57.8	59.6	59.0	58.9	58.6	59.6	60.8	60.1
7	58.3	59.3	60.4	59.3	59.2	62.1	58.8	61.0	60.8
8	59.5	58.7	59.6	60.2	59.0	61.4	59.1	59.0	61.0
9	59.4	59.7	59.0	60.0	59.1	61.9	60.1	60.9	60.6
10	58.3	59.9	60.4	59.6	58.7	61.9	59.5	63.8	61.0
11	58.9	60.9	59.8	60.3	60.1	63.3	60.5	61.1	62.4
12	60.1	61.7	61.9	59.7	61.3	63.6	60.6	62.1	63.6
13	61.6	61.8	63.9	59.6	61.8	64.6	60.2	62.4	64.1
Overall[1]	58.1	57.9	58.5	58.4	58.1	60.2	58.4	59.7	59.8

[1]Overall - based upon cumulative data for all periods.

TABLE XXXIV

ANALYSIS OF VARIANCE OF EGG WEIGHT DATA BY PERIODS AND OVERALL, STUDY 1, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(96)	(95)	(93)	(92)	(89)	(89)	(88)	(86)	(86)	(85)	(85)
Blocks	10	114	119	158	94	675	337	741	1298	1462	901	2230	3098	2558	100
Energy: (E)	(2)														
E_L (Linear)	1	3	5	2	45*	440*	1	99	3	194	276*	94	711	456	23
E_Q (Quadratic)	1	11	2	37	0	39	156	76	56	8	3	241	299	238	10
Protein: (P)	(2)														
P_L	1	36*	63*	26	2	184	139	32	21	142	153	42	19	658	11
P_Q	1	0	0	12	5	90	0	0	321	117	12	27	5	473	1
Energy-protein interaction	(4)														
$E_L \times P_L$	1	3	3	1	32	524*	6	86	758*	64	142	263	59	878	0
$E_Q \times P_L$	1	0	7	0	0	8	180	13	23	124	38	0	526	9	0
$E_L \times P_Q$	1	0	11	11	4	260	17	67	8	7	9	19	80	58	0
$E_Q \times P_Q$	1	57**	21	34	22	67	26	285	157	28	0	0	40	141	5
Error		7	12	14	11	77	61	93	127	134	47	185	262	409	12
Error d.f.[2]		78	78	79	78	77	75	74	71	71	70	68	68	67	67

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE XXXV

AVERAGE BODY WEIGHT GAIN OR LOSS BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Period number	Grams body weight gain or loss								
1	-108	- 25	+ 23	- 89	- 62	+ 36	- 85	+ 74	+142
2	+ 20	+ 35	+ 24	+ 15	+120	+105	- 43	+ 25	- 1
3	- 45	+ 2	- 16	- 39	+ 30	+ 16	- 86	0	+ 92
4	- 92	- 4	+ 36	- 5	+ 3	+ 22	-142	- 57	- 5
5	+ 79	+ 35	+ 8	- 36	+ 26	+ 11	+ 42	+ 99	- 10
6	- 96	- 11	+ 57	- 85	- 21	+ 1	- 78	- 68	- 5
7	+ 58	- 52	- 1	+ 52	- 9	- 65	+ 3	- 4	- 36
8	+ 18	+ 62	+ 42	+ 35	- 39	+ 15	- 30	+161	+ 9
9	- 22	- 28	+ 21	- 85	+107	+ 11	+ 28	-110	+ 29
10	+ 6	+ 28	+ 25	- 14	+ 43	+ 30	+ 38	+ 18	+ 32
11	+ 62	+ 24	+ 30	+103	+ 29	+ 75	+ 16	+ 28	+ 35
12	+ 21	- 3	- 38	- 52	- 39	- 85	- 19	- 43	+ 10
13	- 38	+ 25	+ 40	+ 23	+ 17	+ 9	- 25	- 23	+ 20
Overall[1]	-157	+ 79	+251	-188	+209	+180	- 20	+110	+305

[1]Overall - based upon cumulative data for all periods.

TABLE XXXVI

ANALYSIS OF VARIANCE OF BODY WEIGHT GAIN OR LOSS DATA
BY PERIODS AND OVERALL, STUDY 1, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]					Mean squares ÷ 100									
Total d.f.[2]		(96)	(96)	(97)	(96)	(95)	(93)	(92)	(89)	(89)	(88)	(86)	(86)	(85)	(85)
Blocks	10	1378	1065	384	202	944	1421	1288	2356	1290	1696	465	857	624	20
Energy: (E)	(2)														
E_L (Linear)	1	4279**	338	1322**	1521**	109	1689**	879**	9	367*	83	14	53	182	297**
E_Q (Auadratic)	1	7	324	111	34	269	5	141	5	171	45	150	2	0	22*
Protein: (P)	(2)														
P_L	1	919**	180	62	379*	0	184	12	54	123	9	17	26	51	1
P_Q	1	390	1119**	24	540**	359	0	2	130	27	31	268*	443*	51	0
Energy-protein interaction	(4)														
$E_L \times P_L$	1	228	29	578**	2	10	140	1	0	56	1	17	236	25	8
$E_Q \times P_L$	1	55	98	0	27	126	1	269	155	810**	6	40	35	37	6
$E_L \times P_Q$	1	258	97	4	313*	101	0	187	401	763**	65	118	13	68	6
$E_Q \times P_Q$	1	7	84	163	113	478*	932*	1	165	188	6	19	110	230	6
Error		118	121	29*	67	104	136	75	129	80	103	61	66	140	3
Error d.f.[2]		78	78	79	78	77	75	74	71	71	70	68	68	67	67

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE XXXVII

AVERAGE DAILY FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Period number				Total grams of diet					
1	125.0	116.8	115.2	135.6	119.6	124.2	131.1	132.7	107.6
2	162.5	129.2	120.2	170.1	136.6	141.7	164.4	137.5	149.3
3	159.8	140.4	130.5	167.4	135.8	135.0	161.9	152.1	123.5
4	152.3	136.1	126.6	157.7	138.0	136.3	129.0	144.9	122.0
5	156.2	141.3	121.8	164.4	134.3	133.4	157.3	152.1	123.4
6	147.2	140.3	116.6	158.2	111.4	119.2	122.6	142.3	107.2
7	147.1	124.3	118.3	158.7	121.7	120.0	154.9	147.3	106.2
8	141.9	120.7	115.0	147.2	116.5	117.0	165.1	141.7	111.6
9	143.0	120.2	109.0	152.3	124.5	115.9	160.5	139.2	118.0
10	146.6	121.7	104.0	153.7	124.5	126.1	164.2	144.8	107.3
11	148.7	127.9	109.2	164.6	131.8	125.9	162.8	141.2	112.1
12	145.2	131.5	122.2	155.0	131.9	110.6	159.9	139.4	165.8
13	128.6	133.0	121.4	154.4	138.1	145.7	164.5	139.5	117.7
Overall[1]	146.8	129.7	117.7	156.9	127.9	127.0	156.1	143.2	118.4

[1]Overall - based upon cumulative data for all periods.

TABLE XXXVIII

ANALYSIS OF VARIANCE OF DAILY FEED CONSUMPTION DATA
BY PERIODS AND OVERALL, STUDY 1, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(96)	(95)	(93)	(92)	(89)	(89)	(88)	(86)	(86)	(85)	(85)
Blocks	10	5673	4575	2858	4832	3051	4321	3418	7679	6088	8997	4470	20511	8903	3935
Energy: (E)	(2)														
E_L (Linear)	1	3318**	23753**	17973**	5090**	17380**	28042**	22736**	18037**	19829**	23032**	24072**	5188	5745**	14130**
E_Q (Quadratic)	1	2	1413*	291	111	6	503	290	1006	761	637	457	1913	77	432*
P															
Protein: (P)	(2)														
P_L	1	504	775	78	739	306	79	494	2719*	3983**	2609*	1899*	8605*	2557	1404**
P_Q	1	514	1623*	38	1662*	91	1147	4	727	24	381	691	3978	2999	28
Energy-protein interaction	(4)														
$E_L \times P_L$	1	372	23	248	891	0	1971*	1236*	2058*	285	863	351	811	3654*	299
$E_Q \times P_L$	1	30	13	128	0	599	3518**	34	29	8	322	668	12	1085	219
$E_L \times P_Q$	1	393	73	685	1833*	456	410	1601*	54	27	241	98	601	789	79
$E_Q \times P_Q$	1	1710**	3654**	1460**	1660*	1541**	1855*	2834**	1578	173	2169*	1119*	6030*	696	840**
Error		209	270	204	272	216	394	290	546	480	409	256	1337	813	98
Error d.f.[2]		78	78	79	78	77	75	74	71	71	70	68	68	67	67

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

consumption leveled off after the fifth period and was fairly constant until the end of the trial. It could be assumed that these periods of increases and decreases in feed consumption would be correlated with egg production. However, in this trial this was not the case. Comparisons of egg production data (Table XXXI) with feed consumption data show that egg production was highest during Period 1 of the experiment while feed consumption was lowest during this same period. There was a gradual decline in egg production after Period 1, but feed consumption remained high for the next 4 consecutive experimental periods. During Period 1, while egg production was high and feed consumption was low, the hens were either losing body weight or just barely holding their own. The one exception was Diet 27, where the hens gained body weight. While feed consumption was highest during Period 2, most of the hens gained body weight and egg production decreased below the level of Period 1. After Period 2 there were no obvious correlations between feed consumption and body weight change. The overall feed consumption per hen was generally higher than the desired intake levels (Table XXXVII). However, the extremely high feed consumption per hen during Periods 2 through 5 was largely responsible for the high average overall feed consumption per hen.

An analysis of variance was also applied to the feed consumption data (Table XXXVIII). Although this analysis has little meaning in the interpretation of performance data, it was made in order to check the expected differences in feed consumption among hens fed the various experimental diets. The final adjusted weight of the experimental basals (Table XXV) generally increased as both protein and energy were increased. Therefore, a linear decrease in feed consumption per hen could be expected as protein and energy levels of the diet were increased. Both dietary protein and energy had significant (P>0.01) linear effects upon feed consumption. There was a significant energy quadratic x protein quadratic interaction effect upon feed consumption that was probably caused by Basal E, which was heavier than either Basal B or H. This resulted in a quadratic pattern for the weights of the three protein levels that were combined with 300 Calories of energy. Basal F was also heavier than either Basal C or I, which gave a quadratic curve for the three protein levels combined with 350 Calories of energy.

Table XXXIX contains a summary of the average daily volume of feed consumed per hen. The average daily volume of feed consumed per hen was generally higher in Study 1 of Trial III than in Trial II. This may have been due partly to a difference in the age at which hens were placed on the experimental diets. The hens in Study 1 were much younger and were laying at a higher rate than the hens in Trial II; therefore, they would probably consume more feed. The fact that the hens were allowed to become accustomed to the experimental diets before volume was increased may have contributed to the high volume of feed consumed per hen in Study 1.

Average daily consumption of metabolizable energy per hen is shown in Table XL. These data reflect the ability of laying hens to compensate for inadequate dietary energy. The hens were able to consume approximately 300 Calories of energy when fed 250-Calorie diets, even though volume was extremely high. Approximately 330 Calories were consumed by hens fed 300-Calorie diets, while the 350-Calorie diets were consumed at approximately the desired intake level. Efficiency of utilization of metabolizable energy (Table XLI), expressed as Calories of energy per gram of egg, did not follow a linear pattern with the three dietary energy levels. Hens that consumed 300 Calories per day utilized energy more efficiently than did hens that consumed 350 Calories. The most efficient utilization occured

TABLE XXXIX

AVERAGE DAILY VOLUME OF FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Period number	Milliliters of feed actually consumed								
1	300	257	242	325	263	261	302	292	226
2	406	271	228	425	301	283	427	328	236
3	399	309	274	419	299	283	421	350	247
4	457	327	253	473	345	300	400	362	256
5	469	339	244	493	336	293	488	380	259
6	442	337	233	475	278	262	515	356	225
7	441	298	237	476	304	264	480	368	223
8	426	290	230	442	291	257	512	354	234
9	429	288	218	457	311	255	498	348	248
10	440	292	208	461	311	277	509	362	225
11	446	307	218	494	329	277	505	353	235
12	436	316	244	465	330	243	496	348	348
13	386	319	243	463	345	321	510	349	247
Overall[1]	420	304	236	451	311	275	460	348	247

[1]Overall = based upon cumulative data for all periods.

TABLE XL

AVERAGE DAILY CONSUMPTION OF METABOLIZABLE ENERGY PER HEN, BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	12	23	5	15	25	7	18	27
Desired consumption (Cal.)	250			300			350		
Period number	Calories of energy actually consumed								
1	296	318	313	311	317	352	334	354	311
2	375	384	361	353	362	400	363	409	347
3	369	378	355	373	360	388	379	385	363
4	287	291	233	342	333	344	380	381	354
5	295	303	284	355	325	361	366	373	358
6	278	292	299	353	269	338	351	333	311
7	278	293	279	313	294	350	356	336	308
8	268	272	298	304	282	337	346	327	323
9	270	281	289	302	301	331	328	324	342
10	276	283	296	306	301	344	313	353	311
11	281	304	293	322	318	335	328	352	325
12	274	286	288	331	319	331	267	309	480
13	243	285	297	335	334	331	365	408	341
Overall[1]	294	306	300	331	316	349	352	347	344

[1]Overall - based upon cumulative data for all periods.

TABLE XLI

EFFICIENCY OF UTILIZATION OF METABOLIZABLE ENERGY, BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Period number	Calories of metabolizable energy per gram of egg								
1	7.4	7.5	8.6	7.4	7.6	7.9	7.4	8.2	7.0
2	9.1	8.7	10.2	9.3	8.8	9.5	8.9	9.1	9.0
3	9.2	8.4	8.5	8.5	8.8	8.7	8.1	8.4	9.5
4	7.9	8.4	9.0	7.6	8.0	9.1	13.6	7.6	8.3
5	9.0	8.2	8.8	7.6	8.2	8.7	13.7	8.1	8.4
6	8.4	9.9	8.9	8.1	7.6	8.7	8.7	8.2	8.8
7	8.5	7.7	8.2	8.4	7.7	8.1	11.3	7.9	8.9
8	7.9	8.0	9.1	6.6	7.4	8.5	8.6	8.2	10.6
9	8.4	8.1	9.7	7.5	7.8	8.7	8.4	8.2	10.4
10	9.9	8.8	9.6	9.1	7.7	10.2	8.6	6.9	8.4
11	10.4	9.8	9.9	11.4	8.2	9.5	9.4	8.6	8.6
12	15.1	9.7	10.7	9.9	8.0	9.2	13.0	8.0	14.3
13	18.7	11.0	11.6	13.7	8.4	13.1	12.7	9.6	10.6
Overall[1]	9.2	8.7	9.3	8.5	8.0	9.1	9.6	8.3	9.3

[1]Overall - based upon cumulative data for all periods.

when the hens consumed 330 Calories per day. This quadratic effect of energy consumption upon energy efficiency was significant in 5 of the 13 experimental period analyses and in the overall analysis (Table XLII). There was also a significant quadratic effect of protein consumption upon the efficiency of energy utilization. Energy was utilized most efficiently by hens that were fed Diets 12, 15 and 18, all of which contained 16 grams of protein. Energy was utilized less efficiently by hens fed the 13- and 19-gram-protein diets than by hens fed the 16-gram-protein diets.

Average daily protein consumption per hen (Table XLIII) for the desired protein intake levels of 13, 16 and 19 grams was approximately 14, 18 and 20 grams per day, respectively. The desired protein intake was achieved only when hens were fed 350-Calorie diets. Within each group of diets in which a specific protein intake was calculated, protein intake increased as the dietary energy intake level was decreased. This was particularly noticeable when hens were fed Diets 23, 25 and 27 that had been formulated to supply 19 grams of protein and 250,300 and 350 Calories of metabolizable energy, respectively. The actual protein consumptions per hen for the 250, 300 and 350 Calorie diets were 22.91, 20.36 and 18.74 grams, respectively.

Protein was utilized most efficiently by the hens that were fed diets that contained 13 grams of protein (Table XLIV). The linear effect of dietary protein upon protein efficiency was significant at the 1 percent level of probability (Table XLV). Both the linear and quadratic effects of energy intake upon protein utilization were significant.

TABLE XLII

ANALYSIS OF VARIANCE OF THE ENERGY EFFICIENCY DATA BY PERIODS AND OVERALL, STUDY 1, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(96)	(95)	(93)	(92)	(89)	(89)	(88)	(86)	(86)	(85)	(85)
Blocks	10	0.42	0.67	0.32	0.43	0.60	0.34	0.47	3.64	1.48	1.34	0.83	1.46	1.43	0.15
Energy: (E)	(2)														
E_L (Linear)	1	0.03	0.05	0.03	0.05	0.16	0.05	0.13	0.26	0.69*	0.00	0.07	0.21	0.38	0.01
E_Q (Quadratic)	1	0.03	0.07	0.01	0.40**	0.27*	0.00	0.57*	0.00	0.23	0.34	0.35	1.79**	1.65**	0.20**
Protein: (P)	(2)														
P_L	1	0.00	0.01	0.00	0.30*	0.16	0.06	0.51*	0.25	0.00	0.68*	0.45	0.08	0.39	0.00
P_Q	1	0.00	0.00	0.00	0.22*	0.40*	0.14	0.15	2.33*	0.27	0.06	0.00	0.97*	0.17	0.09*
Energy-protein interaction	(4)														
$E_L \times P_L$	1	0.18*	0.02	0.24**	1.41**	0.44*	0.00	0.05	0.07	0.02	0.06	0.03	0.07	0.07	0.01
$E_Q \times P_L$	1	0.04	0.00	0.02	0.04	0.03	0.19	0.01	0.41	0.01	0.10	0.18	0.03	0.14	0.00
$E_L \times P_Q$	1	0.04	0.06	0.00	1.20**	0.96**	0.02	0.18	0.00	0.01	0.00	0.18	0.19	0.00	0.02
$E_Q \times P_Q$	1	0.20*	0.02	0.01	0.02	0.00	0.01	0.16	0.85	0.12	0.29	0.00	0.18	0.25	0.00
Error		0.04	0.04	0.03	0.04	0.06	0.12	0.11	0.45	0.15	0.17	0.14	0.18	0.17	0.02
Error d.f.[2]		78	78	79	78	77	75	74	71	71	70	68	68	67	67

[1]d.f. - the degrees of freedom for blocks and for treatment are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE XLIII

AVERAGE DAILY CONSUMPTION OF PROTEIN PER HEN, BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Desired consumption (gm.)		13			16			19	
Period number	Grams of protein actually consumed								
1	15.50	13.55	12.44	20.34	16.87	16.27	23.34	22.30	16.90
2	19.66	15.38	13.46	24.66	19.26	18.70	26.95	26.42	18.90
3	19.33	16.28	14.09	24.27	19.15	17.68	26.55	24.64	19.76
4	14.93	14.84	14.18	18.61	17.80	17.58	17.93	19.27	19.28
5	15.30	15.40	13.64	19.40	17.33	17.21	21.87	20.24	19.49
6	14.43	15.29	13.06	18.67	14.37	15.38	23.07	18.93	16.94
7	14.42	13.55	13.25	18.73	15.70	15.48	21.53	19.59	16.77
8	13.90	13.16	12.88	17.37	15.03	15.09	22.95	18.85	17.63
9	14.01	13.10	12.20	17.97	16.06	14.95	22.31	18.52	18.64
10	14.37	13.26	11.65	18.13	16.06	16.27	22.82	19.25	16.95
11	14.58	13.94	12.23	19.43	17.00	16.24	22.62	18.78	17.71
12	14.23	14.34	13.69	18.29	17.02	14.26	22.22	18.54	26.19
13	12.60	14.50	13.60	18.21	17.82	18.79	22.87	18.55	18.60
Overall[1]	15.31	14.38	13.10	19.58	16.87	16.45	22.91	20.36	18.74

[1]Overall - based upon cumulative data for all periods.

TABLE XLIV

EFFICIENCY OF UTILIZATION OF PROTEIN BY PERIODS AND OVERALL[1], STUDY 1, TRIAL III

Diet number	3	5	7	12	15	18	23	25	27
Period number			Grams of protein per gram of egg						
1	0.39	0.33	0.32	0.47	0.41	0.36	0.55	0.52	0.38
2	0.48	0.38	0.38	0.60	0.47	0.44	0.66	0.60	0.49
3	0.48	0.37	0.32	0.55	0.47	0.40	0.60	0.53	0.52
4	0.41	0.36	0.33	0.49	0.43	0.42	1.05	0.43	0.45
5	0.47	0.35	0.33	0.48	0.44	0.40	1.05	0.46	0.46
6	0.44	0.43	0.33	0.52	0.40	0.40	0.67	0.46	0.48
7	0.44	0.33	0.30	0.53	0.41	0.37	0.87	0.44	0.48
8	0.41	0.35	0.34	0.42	0.40	0.39	0.66	0.46	0.58
9	0.44	0.35	0.36	0.48	0.42	0.40	0.65	0.46	0.57
10	0.51	0.38	0.36	0.58	0.41	0.47	0.66	0.38	0.46
11	0.54	0.42	0.37	0.73	0.44	0.44	0.72	0.48	0.47
12	0.79	0.42	0.40	0.63	0.43	0.43	1.00	0.45	0.78
13	0.97	0.48	0.43	0.87	0.45	0.61	0.98	0.54	0.58
Overall[1]	0.48	0.38	0.35	0.54	0.43	0.42	0.73	0.48	0.51

[1]Overall - based upon cumulative data for all periods.

TABLE XLV

ANALYSIS OF VARIANCE OF THE PROTEIN EFFICIENCY DATA
BY PERIODS AND OVERALL, STUDY 1, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(96)	(95)	(93)	(92)	(89)	(89)	(88)	(86)	(86)	(85)	(85)
Blocks	10	0.96	2.38	1.37	1.46	1.68	2.15	1.14	2.13	5.86	5.71	3.74	7.10	6.36	1.51
Energy: (E)	(2)														
E_L (Linear)	1	8.11**	4.72**	7.10**	8.61**	10.92**	5.83**	11.35**	0.59	1.26	5.80**	7.10**	8.97**	8.57**	5.04**
E_Q (Quadratic)	1	0.02	0.29	0.10	2.32**	1.85**	0.22	3.20**	0.98	2.10	2.47	2.74*	7.69**	7.49**	1.42*
Protein: (P)	(2)														
P_L	1	11.95**	8.42**	11.06**	14.05**	12.38**	5.79**	18.55**	0.13	7.76**	1.52	1.19	2.90*	0.65	6.50**
P_Q	1	0.77*	0.01	0.05	0.00	0.14	0.03	0.00	0.91	0.19	0.91	0.11	1.95	0.18	0.01
Energy-protein interaction	(4)														
$E_L \times P_L$	1	0.15	0.00	1.97**	1.89**	0.19	0.26	0.13	0.75	0.19	0.06	0.00	0.98	0.78	0.02
$E_Q \times P_L$	1	0.21	0.10	0.08	0.20	0.09	1.14	0.03	0.06	0.06	0.38	0.72	0.11	0.49	0.03
$E_L \times P_Q$	1	0.19	0.40	0.00	3.59**	2.95**	0.42	0.85	0.78	0.17	0.09	0.06	0.61	0.01	0.13
$E_Q \times P_Q$	1	0.69*	0.16	0.01	0.31	0.05	0.41	1.08	0.84	1.17	0.53	0.09	1.14	0.54	0.03
Error		0.15	0.18	0.10	0.11	0.20	0.52	0.37	0.70	0.64	0.68	0.62	0.69	0.67	0.28
Error d.f.[2]		78	78	79	78	77	75	74	71	71	70	68	68	67	67

[1] d.f. - the degrees of freedom for blocks and for treatment are the same for each period analysis and the overall analysis.

[2] Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE XLVI

FACTORIAL DESIGN, STUDY 2[1], TRIAL III, THE EFFECTS OF PROTEIN AND VITAMIN-MINERAL CONCENTRATE CONSUMPTION, WITH 250 CALORIES OF METABOLIZABLE ENERGY, UPON THE PERFORMANCE OF LAYERS

Desired daily protein cons. (gm.)	Desired daily vitamin-mineral concentrate consumption (gm.)		
	0.215	0.640	1.065
13	13-0.215 (1)*	13-0.640 (2)	13-1.065 (3)
16	16-0.215 (10)	16-0.640 (11)	16-1.065 (12)
19	19-0.215 (21)	19-0.640 (22)	19-1.065 (23)

[1]Study 2 - All experimental diets in this study were calculated to supply 250 Calories of metabolizable energy per hen per day.

*The numbers in parentheses represent the diet numbers.

Study 2

Results

Livability of laying hens was high throughout Study 2. The highest mortality occurred among hens that were fed Diets 3 and 23 (Table XLVII). However, these same hens and diets were included in Study 1 (see experimental design, Table XXII). There was more mortality among these hens in Study 1 than among those hens that were fed the other diets in this study. This was taken to mean that treatment had no effect upon mortality in either Study 1 or Study 2. Overall egg production in Study 2 varied from a low of 51.1 percent to a high of 64.1 percent (Table XLVIII). There were no significant effects of vitamin-mineral concentrate consumption upon egg production. However, the linear protein effects upon egg production were significant at the 1 percent level of probability in Periods 4, 5, 6, 12 and overall, but in Period 7 they were significant only at the 5 percent level (Table XLIX). Hens fed diets that contained 13 grams of protein produced more eggs than hens fed 16 grams of protein, and hens fed 16 grams of protein produced more eggs than hens fed 19 grams of protein. Vitamin x protein interactions were significant in some of the period analyses, but these interactions were not significant in the overall analysis.

The average egg weight for all diets was 58.6 grams (Table L). There were significant differences in egg weight for only three experimental periods among hens that were fed the experimental diets. Data in Table LI show the analysis of variance of egg weight data for Study 2. Significant vitamin-linear x protein-quadratic interaction occurred in Periods 1, 3, 6, 8, and 9. However, this interaction was not evident in the overall analysis.

Body-weight-change was not affected by the intake of dietary vitamin-

TABLE XLVII

NUMBER OF HENS PER TREATMENT AT THE END OF EACH TWENTY-EIGHT-DAY PERIOD, STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Period number				Number of hens					
1	11	11	11	11	11	11	11	11	11
2	11	10	11	11	10	11	11	11	11
3	11	11	11	10	10	11	11	11	11
4	11	10	11	10	10	11	11	11	10
5	11	11	11	11	10	11	11	11	10
6	11	11	9	11	10	11	11	11	10
7	11	11	9	11	10	11	11	11	9
8	11	10	9	11	10	11	11	11	8
9	11	10	9	11	10	11	11	11	8
10	11	10	9	11	10	10	11	11	8
11	11	10	9	11	10	10	11	11	8
12	11	10	9	11	10	10	11	11	8
13	11	10	9	11	10	10	11	11	8

TABLE XLVIII

AVERAGE EGG PRODUCTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Period number				Percent egg production					
1	74.0	76.0	72.4	71.8	71.4	79.2	85.1	77.9	76.0
2	76.6	69.2	74.0	77.9	75.9	74.4	78.2	70.1	71.1
3	77.9	70.5	69.5	70.5	71.4	75.3	77.6	73.7	76.0
4	64.3	59.1	63.6	58.4	52.1	66.9	30.5	41.9	29.9
5	66.2	56.5	55.2	63.6	54.3	69.2	18.8	51.9	36.1
6	66.6	59.7	54.8	56.8	59.3	60.7	42.2	44.2	57.9
7	62.7	62.7	56.0	57.1	55.0	59.1	57.5	50.3	42.1
8	66.9	69.6	56.7	53.6	53.9	68.5	69.5	65.6	58.5
9	62.7	70.4	54.0	54.2	56.8	62.3	61.4	65.6	57.1
10	47.7	60.0	48.0	61.7	47.1	52.1	51.6	53.6	58.0
11	50.0	58.2	45.6	57.1	50.4	44.3	33.8	49.0	51.8
12	57.5	57.9	30.2	43.2	44.3	48.6	24.0	41.9	36.6
13	54.5	62.5	21.0	32.1	30.7	35.0	33.8	38.0	38.8
Overall[1]	63.7	64.1	54.9	58.3	55.7	61.6	51.1	55.7	53.8

[1]Overall - based upon cumulative data for all periods.

TABLE XLIX

ANALYSIS OF VARIANCE OF EGG PRODUCTION DATA
BY PERIODS AND OVERALL, STUDY 2, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.		(98)	(97)	(97)	(97)	(96)	(94)	(93)	(91)	(91)	(90)	(90)	(90)	(90)	(90)
Blocks	10	175	242	159	319	162	313	157	185	169	266	687	367	235	14402
Vitamins: (V)	(2)														
V_L (Linear)	1	2	25	4	9	24	7	45	1	0	1	0	8	80	193
V_Q (Quadratic)	1	3	33	10	3	10	8	0	0	38	0	47	102	103	944
Protein: (P)	(2)														
P_L	1	38	0	13	1016**	715**	213**	123*	1	1	3	79	294**	144	13677**
P_Q	1	13	8	5	207*	391*	37	4	70	34	1	16	23	116	242
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	12	4	10	0	169*	146*	14	0	5	2	69	313**	252*	4307
$V_Q \times P_L$	1	15	4	1	0	0	0	10	57	44	121*	26	56	202*	4384
$V_L \times P_Q$	1	35	1	22	12	127	10	24	84*	20	38	92	6	81	247
$V_Q \times P_Q$	1	13	0	7	154	299**	4	22	86*	35	12	19	51	9	2062
Error		18	11	10	40	38	26	29	19	19	28	25	39	44	1331
Error d.f.[2]		80	79	79	79	78	76	75	73	73	72	72	72	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE L

AVERAGE EGG WEIGHT BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Period number				Egg weight in grams					
1	55.6	53.7	55.2	53.9	54.8	54.4	56.6	53.6	55.5
2	57.0	55.2	55.7	55.5	56.8	55.4	58.3	55.5	57.1
3	58.8	56.4	57.4	56.9	57.6	59.1	57.7	57.0	58.0
4	58.4	57.6	57.4	56.5	57.6	57.2	58.0	57.1	57.2
5	60.9	57.9	59.6	58.1	58.7	58.1	59.3	57.7	57.5
6	61.7	60.0	60.2	59.0	59.3	59.0	61.5	58.2	59.6
7	61.7	59.6	58.3	58.9	59.9	59.3	60.0	59.0	58.8
8	61.6	59.3	59.5	59.7	59.5	60.2	61.3	59.4	59.1
9	61.4	58.1	59.4	59.4	60.3	60.0	60.8	58.6	60.1
10	59.7	59.1	58.3	58.9	59.1	59.6	59.7	59.2	59.5
11	60.2	59.5	58.9	59.8	60.5	60.3	60.5	59.0	60.5
12	61.4	60.4	60.1	60.6	61.0	59.7	63.6	59.6	60.6
13	63.8	60.2	61.6	62.4	61.0	59.6	65.1	62.2	60.2
Overall[1]	60.1	58.0	58.1	58.1	58.6	58.4	59.7	57.9	58.4

[1]Overall - based upon cumulative data for all periods.

TABLE LI

ANALYSIS OF VARIANCE OF EGG WEIGHT DATA
BY PERIODS AND OVERALL, STUDY 2, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(97)	(97)	(97)	(96)	(94)	(93)	(91)	(91)	(90)	(90)	(90)	(90)	(90)
Blocks	10	89	103	328	733	927	1209	1158	1129	642	1021	2007	4534	3967	71
Vitamins: (V)	(2)														
V_L (Linear)	1	1	11	77	14	242	4	41	7	127	7	178	71	334	6
V_Q (Quadratic)	1	22	11	1	39	57	644*	2	68	5	86	39	572	9	8
Protein: (P)	(2)														
P_L	1	1	16	0	2	2347*	138	21	35	1	250	13	769	62	1
P_Q	1	12	9	48	28	1050	1	153	219	333*	58	100	498	171	4
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	1	0	9	2	254	0	11	98	1	6	79	593	2148*	4
$V_Q \times P_L$	1	13	11	55	308*	1203	152	546*	16	187	85	175	185	510	8
$V_L \times P_Q$	1	32*	31	207*	139	31	713*	61	976**	639**	29	566	158	858	20
$V_Q \times P_Q$	1	7	15	32	64	190	167	0	111	48	3	56	0	6	0
Error		8	12	46	76	367	150	125	118	82	85	205	404	454	9
Error d.f.[2]		80	79	79	79	78	76	75	73	73	72	72	72	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

mineral concentrate (Table LII). The analyses of variance of body-weight-change data (Table LIII) show that the linear effects of protein upon body-weight-change were significant for some period analyses and the overall analysis. There were also several period analyses that gave significant mean squares for the quadratic effects of protein. However, these trends do not always go in the same direction. In Period 2, hens fed 19 grams of protein lost more weight than those fed 13 grams of protein; the opposite effect occurred in Period 8. Hens that were fed 16 grams of protein lost more weight in some periods than either of the other two groups, and in different periods hens fed 16 grams of protein gained more than either of the other groups. Thus, both the linear and quadratic effects were cancelled in the overall analysis. These changes in effects were probably responsible for the significant vitamin-quadratic x protein-quadratic interaction that occurred in Periods 4, 5, 7 and 9.

There were no significant effects of vitamin-mineral concentrate consumption upon total feed consumption among hens fed the various experimental diets (Tables LIV and LV). The linear effects of protein consumption upon total feed consumption were significant at the 1 percent level of probability. Hens that consumed the most protein tended to consume the most feed. As dietary protein was increased, total feed consumption increased. However, this was a function of the experimental design, because as dietary protein was increased, total weight of the experimental diets increased. This same trend is shown in the average volume of feed consumption per hen (Table LVI).

The average daily consumption of vitamin-mineral concentrate per hen (Table LVII) was generally higher than the desired intake level. Nevertheless the primary objective of the nutrient-intake-control technique was realized because there were three distinct intake levels. The linear effects

TABLE LII

AVERAGE BODY WEIGHT GAIN OR LOSS BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Period number			Grams body weight gain or loss						
1	- 80	-110	-108	-105	- 72	- 90	- 90	- 58	- 85
2	+ 25	+ 10	+ 20	+ 24	+ 18	+ 15	- 48	+ 15	- 43
3	- 47	- 61	- 45	- 71	- 70	- 39	- 75	- 89	- 86
4	- 57	-128	- 92	- 48	- 74	- 5	- 85	- 66	-142
5	+ 55	+ 83	+ 79	+ 8	+ 63	- 36	+ 69	- 5	+ 42
6	- 89	- 62	- 96	- 76	- 92	- 85	- 62	- 85	- 78
7	+ 53	+ 21	+ 58	+ 80	- 26	+ 51	+ 60	+131	+ 3
8	- 3	+ 27	+ 18	+ 2	+103	+ 35	- 25	- 29	- 30
9	- 73	- 61	- 22	- 16	- 55	- 85	- 51	- 78	+ 28
10	+ 26	0	+ 6	- 14	- 7	- 14	- 18	+ 10	+ 38
11	+ 67	+ 89	+ 62	+ 53	+ 63	+103	+ 45	+ 49	+ 16
12	+ 10	+ 14	+ 21	- 59	+ 10	- 52	+ 18	+ 6	- 19
13	- 44	- 25	- 38	+ 70	+ 5	+ 23	+ 33	+ 29	- 25
Overall[1]	-155	-207	-157	-142	-140	-189	-230	-169	-449

[1]Overall - based upon cumulative data for all periods.

TABLE LIII

ANALYSIS OF VARIANCE OF BODY WEIGHT GAIN OR LOSS DATA BY PERIODS AND OVERALL, STUDY 2, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares ÷ 100													
Total d.f.[2]		(98)	(97)	(97)	(97)	(96)	(94)	(93)	(91)	(91)	(90)	(90)	(90)	(90)	(90)
Blocks	10	758	751	402	646	1315	1418	509	431	779	1005	919	598	439	15
Vitamins: (V)	(2)														
V_L (Linear)	1	1	1	4	50	40	14	105	80	29	8	10	9	129	1
V_Q (Quadratic)	1	37	49	40	45	20	1	10	176*	130	0	6	154	0	3
Protein: (P)	(2)														
P_L	1	78	309**	173**	14	228	6	103	278*	48	5	150	24	409*	11*
P_Q	1	0	113	20	614**	421*	4	70	532**	9	65	37	354	427*	3
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	31	3	4	13	73	4	69	20	22	118	4	54	90	1
$V_Q \times P_L$	1	78	29	0	10	38	64	11	76	0	22	65	108	63	0
$V_L \times P_Q$	1	3	95	12	1	69	9	519**	138	146	4	53	73	84	1
$V_Q \times P_Q$	1	38	109	24	656**	613**	10	837**	61	688**	0	111	14	30	2
Error		46	31	13	77	62	60	69	39	42	43	63	94	89	2
Error d.f.[2]		80	79	79	79	78	76	75	73	73	72	72	72	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LIV

AVERAGE DAILY FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Period number				Total grams of diet					
1	126.8	109.8	125.0	128.7	133.1	135.6	139.1	132.5	131.1
2	155.9	157.2	162.5	154.4	168.6	170.1	167.6	171.8	164.4
3	158.7	151.9	159.8	157.1	157.4	167.4	168.9	175.1	161.9
4	160.5	139.7	152.3	147.6	154.2	157.7	142.3	143.9	120.0
5	155.7	149.1	156.2	158.8	160.6	164.4	153.4	163.5	157.3
6	155.9	145.4	147.2	147.7	153.5	158.2	176.8	156.9	166.0
7	153.3	145.5	147.1	154.7	153.0	158.7	168.6	164.7	154.9
8	139.0	143.2	141.9	142.8	140.4	147.2	147.5	151.0	165.1
9	143.2	149.7	143.0	147.7	142.9	152.3	165.5	165.0	160.5
10	146.9	147.2	146.6	153.6	143.0	153.7	157.9	156.2	164.2
11	142.7	153.6	148.7	165.7	155.5	164.6	154.7	170.1	162.8
12	154.9	156.4	145.2	154.2	160.1	155.0	156.3	172.0	159.9
13	157.8	161.1	128.6	153.0	157.1	154.4	166.9	175.3	164.5
Overall[1]	150.1	146.7	146.8	151.2	152.1	156.9	158.9	161.4	156.1

[1]Overall - based upon cumulative data for all periods.

TABLE LV

ANALYSIS OF VARIANCE OF DAILY FEED CONSUMPTION DATA
BY PERIODS AND OVERALL, STUDY 2, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(97)	(97)	(97)	(96)	(94)	(93)	(91)	(91)	(90)	(90)	(90)	(90)	(90)
Blocks	10	3915	3806	2901	3767	5450	6773	3992	7487	5643	2953	5846	7143	7301	2066
Vitamins: (V)	(2)														
V_L (Linear)	1	13	665	34	235	194	142	362	1105	0	24	288	53	1266	11
V_Q (Quadratic)	1	764	266	25	138	1	997	87	210	8	465	253	1850	2112	7
Protein: (P)	(2)														
P_L	1	3116**	1451	2314*	2551	351	4688**	3387**	2576**	5824**	2216*	3039**	1719	5346**	1858**
P_Q	1	567	37	124	1563	574	549	3	541	1047	166	951	37	342	27
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	103	264	179	71	44	5	89	552	4	79	12	700	1670	50
$V_Q \times P_L$	1	1175*	337	93	1701	238	45	26	179	619	347	755	4	465	14
$V_L \times P_Q$	1	229	370	0	241	88	2294*	151	64	28	58	1003	11	530	37
$V_Q \times P_Q$	1	306	362	1861	272	535	619	696	193	161	1	297	230	464	163
Error		287	410	521	752	385	414	370	382	318	453	351	471	571	152
Error d.f.[2]		80	79	79	79	78	76	75	73	73	72	72	72	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LVI

AVERAGE DAILY VOLUME OF FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Period number	Milliliters of feed actually consumed								
1	304	263	300	309	319	325	320	305	302
2	390	393	406	386	421	425	436	447	427
3	397	380	399	393	394	419	439	455	421
4	481	419	457	443	462	473	441	446	400
5	467	447	469	476	482	493	476	507	488
6	468	436	442	443	461	475	548	486	515
7	460	436	441	464	459	476	523	510	480
8	417	430	426	428	421	442	457	468	512
9	430	449	429	443	429	457	513	411	498
10	441	442	440	461	429	461	489	484	509
11	428	461	446	497	466	494	480	527	505
12	465	469	436	462	480	465	485	533	496
13	473	483	386	459	471	463	517	543	510
Overall[1]	432	422	420	436	437	451	471	479	460

[1]Overall - based upon cumulative data for all periods.

TABLE LVII

AVERAGE DAILY CONSUMPTION OF VITAMIN-MINERAL CONCENTRATE PER HEN, BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	10	21	2	11	22	3	12	23
Desired cons. (gm.)	0.215			0.640			1.065		
Period number	Grams of vitamin-mineral concentrate actually consumed								
1	0.364	0.365	0.394	0.944	1.135	1.123	1.794	1.927	1.850
2	0.435	0.423	0.436	1.319	1.386	1.341	2.273	2.330	2.140
3	0.443	0.431	0.439	1.275	1.294	1.368	2.235	2.293	2.108
4	0.260	0.232	0.216	0.673	0.647	0.649	1.223	1.238	0.969
5	0.252	0.249	0.233	0.719	0.674	0.738	1.254	1.291	1.181
6	0.253	0.232	0.269	0.701	0.645	0.707	1.182	1.242	1.247
7	0.248	0.243	0.256	0.701	0.643	0.743	1.182	1.246	1.163
8	0.225	0.224	0.224	0.690	0.590	0.681	1.139	1.156	1.240
9	0.232	0.232	0.252	0.722	0.600	0.744	1.148	1.196	1.206
10	0.238	0.241	0.240	0.710	0.601	0.704	1.177	1.206	1.233
11	0.231	0.260	0.235	0.740	0.653	0.767	1.194	1.292	1.222
12	0.251	0.242	0.238	0.754	0.673	0.776	1.166	1.217	1.201
13	0.256	0.240	0.254	0.776	0.660	0.790	1.033	1.212	1.235
Overall[1]	0.284	0.278	0.283	0.829	0.786	0.856	1.414	1.456	1.415

[1]Overall = based upon cumulative data for all periods.

of vitamin-mineral concentrate consumption upon the efficiency of utilization of vitamin-mineral concentrate were highly significant (Tables LVIII and LIX). The quadratic effects of vitamin-mineral concentrate consumption upon the efficiency of utilization of vitamins and minerals were also highly significant (P>0.01). Efficiency of utilization of vitamin-mineral concentrate improved as dietary concentrate was decreased, but not in a straight line. The efficiency of utilization of 0.215 grams of concentrate was three times better than the utilization of 0.640 grams, while 0.640 grams was utilized only two times more efficiently than 1.065 grams.

Dietary protein also influenced the utilization of vitamin-mineral concentrate. As dietary protein was decreased, the efficiency of utilization of vitamin-mineral concentrate improved. This was significant at the 1 percent level of probability. Vitamin-linear x protein-linear interactions were also highly significant. The effect of protein upon the efficiency of vitamin-mineral utilization decreased as dietary vitamin-mineral concentrate increased.

Data on the average daily consumption of protein per hen for Study 2 are given in Table LX. The three protein intake levels were approximately 15, 19 and 23 grams per day. The efficiency of utilization of protein (Table LXI) was a linear function of protein intake (P>0.01, Table LXII). Vitamin-linear x protein-linear interaction effects were significant at the 1 percent level of probability. This can be explained by the fact that protein efficiency acted in opposite directions in the 13- and 19-gram-protein diets. With the 13-gram-protein diets, protein efficiency improved as the dietary vitamin-mineral concentrate was decreased; but with the 19-gram-protein diets, protein efficiency improved as the dietary vitamin-mineral concentrate was increased.

TABLE LVIII

EFFICIENCY OF UTILIZATION OF VITAMIN-MINERAL CONCENTRATE BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Period number			Grams of concentrate per gram of egg						
1	0.009	0.023	0.045	0.010	0.029	0.045	0.008	0.027	0.044
2	0.010	0.035	0.045	0.010	0.032	0.057	0.010	0.034	0.053
3	0.010	0.033	0.057	0.011	0.031	0.052	0.010	0.033	0.048
4	0.007	0.020	0.033	0.007	0.022	0.032	0.012	0.027	0.056
5	0.006	0.022	0.038	0.007	0.021	0.032	0.021	0.025	0.057
6	0.006	0.020	0.036	0.007	0.018	0.035	0.010	0.028	0.036
7	0.006	0.019	0.036	0.007	0.019	0.036	0.071	0.025	0.048
8	0.005	0.017	0.034	0.007	0.018	0.028	0.005	0.017	0.036
9	0.006	0.018	0.036	0.007	0.018	0.032	0.007	0.019	0.035
10	0.008	0.020	0.043	0.007	0.022	0.039	0.008	0.022	0.036
11	0.008	0.021	0.044	0.008	0.021	0.048	0.012	0.027	0.039
12	0.007	0.022	0.064	0.009	0.025	0.042	0.016	0.032	0.055
13	0.007	0.021	0.080	0.012	0.035	0.058	0.012	0.033	0.053
Overall[1]	0.007	0.022	0.044	0.008	0.024	0.040	0.009	0.027	0.045

[1]Overall - based upon cumulative data for all periods.

TABLE LIX

ANALYSIS OF VARIANCE OF THE VITAMIN-MINERAL CONCENTRATE EFFICIENCY DATA BY PERIODS AND OVERALL, STUDY 2, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	overall
Source of variation	d.f.[1]	Mean squares ÷ 100													
Total d.f.[2]		(98)	(97)	(97)	(97)	(96)	(94)	(93)	(91)	(91)	(90)	(90)	(90)	(90)	(90)
Blocks	10	11	60	21	82	41	44	36	106	76	70	100	44	54	6
Vitamins: (V)	(2)														
V_L (Linear)	1	1402**	1360**	1409**	1438**	1393**	1715**	2140**	3065**	2173**	1694**	1252**	1058**	1132**	1486**
V_Q (Quadratic)	1	199**	254**	156**	200**	168**	242**	280**	387**	247**	219**	128**	923**	87**	188**
Protein: (P)															
P_L	1	0	1	0	166**	312**	125**	29*	2	10	2	56**	176**	74*	26**
P_Q	1	6	0	2	34	96**	9	0	79*	12	22	21	2	41	0
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	2	2	1	84**	315**	125**	8	5	8	2	41*	176**	68*	18**
$V_Q \times P_L$	1	2	1	0	5	18	14	3	5	7	13	0	6	1	1
$V_L \times P_Q$	1	6	0	6	32	144**	2	5	156**	20	25	39*	3	35	0
$V_Q \times P_Q$	1	0		0	8	27	1	1	15	5	6	1	13	12	1
Error		2	7	2	11	9	7	5	11	8	8	8	12	12	1
Error d.f.[2]		80	79	79	79	78	76	75	73	73	72	72	72	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LX

AVERAGE DAILY CONSUMPTION OF PROTEIN PER HEN,
BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Desired cons. (gm.)	13			16			19		
Period number	Grams of protein actually consumed								
1	15.72	13.61	15.50	19.30	19.96	20.34	24.75	23.59	23.34
2	18.86	19.02	19.66	22.39	24.44	24.66	27.48	28.17	26.95
3	19.20	18.39	19.33	22.79	22.83	24.27	27.70	28.72	26.55
4	15.72	13.69	14.93	17.42	18.19	18.61	19.78	20.00	17.93
5	15.26	14.61	15.30	18.74	18.95	19.40	21.33	22.73	21.87
6	15.28	14.25	14.43	17.43	18.11	18.67	24.57	21.80	23.07
7	15.02	14.25	14.42	18.25	18.06	18.73	23.44	22.89	21.53
8	13.62	14.04	13.90	16.85	16.57	17.37	20.51	20.99	22.95
9	14.04	14.67	14.01	17.42	16.87	17.97	23.00	22.93	22.31
10	14.40	14.43	14.37	18.13	16.88	18.13	21.95	21.71	22.82
11	13.99	15.05	14.58	19.56	18.34	19.43	21.51	23.65	22.62
12	15.18	15.33	14.23	18.19	18.90	18.29	21.73	23.91	22.22
13	15.47	15.79	12.60	18.05	18.54	18.21	23.19	24.36	22.87
Overall[1]	15.52	15.18	15.31	18.81	18.97	19.58	23.15	23.50	22.91

[1]Overall - based upon cumulative data for all periods.

TABLE LXI

EFFICIENCY OF UTILIZATION OF PROTEIN BY PERIODS AND OVERALL[1], STUDY 2, TRIAL III

Diet number	1	2	3	10	11	12	21	22	23
Period number			Grams of protein per gram of egg						
1	0.38	0.33	0.39	0.50	0.51	0.47	0.51	0.57	0.55
2	0.43	0.50	0.48	0.52	0.57	0.60	0.60	0.72	0.66
3	0.42	0.46	0.48	0.57	0.55	0.55	0.62	0.68	0.60
4	0.42	0.40	0.41	0.53	0.61	0.49	1.12	0.84	1.05
5	0.38	0.45	0.47	0.51	0.60	0.48	1.91	0.76	1.05
6	0.37	0.40	0.44	0.52	0.52	0.52	0.95	0.85	0.67
7	0.39	0.38	0.44	0.54	0.55	0.53	0.68	0.77	0.87
8	0.33	0.34	0.41	0.53	0.52	0.42	0.48	0.54	0.66
9	0.36	0.36	0.44	0.54	0.49	0.48	0.62	0.60	0.65
10	0.51	0.41	0.51	0.50	0.61	0.58	0.71	0.68	0.66
11	0.46	0.43	0.54	0.57	0.60	0.73	1.05	0.82	0.72
12	0.43	0.44	0.79	0.70	0.70	0.63	1.42	0.96	1.00
13	0.44	0.42	0.97	0.90	0.99	0.87	1.05	1.03	0.98
Overall[1]	0.41	0.41	0.48	0.56	0.58	0.54	0.76	0.73	0.73

[1]Overall - based upon cumulative data for all periods.

TABLE LXII

ANALYSIS OF VARIANCE OF THE PROTEIN EFFICIENCY DATA
BY PERIODS AND OVERALL, STUDY 2, TRIAL III

Period number Source of variation	d.f.[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	overall
		Mean squares													
Total d.f.[2]		(98)	(97)	(97)	(97)	(96)	(94)	(93)	(91)	(91)	(90)	(90)	(90)	(90)	(90)
Blocks	10	0.66	3.74	2.54	4.01	11.41	3.26	1.94	3.68	1.78	2.85	4.23	3.54	2.29	0.71
Vitamins: (V)	(2)														
V_L (Linear)	1	0.03	2.10*	0.05	0.21	0.01	0.00	0.67	0.39	0.00	0.00	0.01	0.48	2.30*	0.09
V_Q (Quadratic)	1	0.07	1.25	0.00	0.12	0.02	0.03	0.01	0.02	0.31	0.02	0.32	0.67	1.16	0.01
Protein: (P)	(2)														
P_L	1	13.02**	8.64**	6.97**	34.42**	31.85**	24.20**	20.83**	13.35**	14.90**	5.50**	13.42**	18.59**	12.86**	15.23**
P_Q	1	1.28*	0.06	0.27	0.67	2.35*	0.06	0.06	1.86*	0.67	0.00	0.00	0.00	2.74*	0.08
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	0.05	0.38	0.39	0.00	2.74*	2.18*	0.00	0.00	0.40	0.03	0.87	4.73**	4.13**	0.45*
$V_Q \times P_L$	1	0.95*	0.35	0.01	0.00	0.10	0.04	0.00	0.45	0.14	1.33	0.12	0.82	3.09*	0.24
$V_L \times P_Q$	1	0.22	0.00	0.43	0.08	0.66	0.18	0.31	3.73**	0.85	0.42	0.47	0.52	1.97	0.09
$V_Q \times P_Q$	1	0.00	0.00	0.00	1.44	2.95*	0.02	0.13	1.79*	0.32	0.22	0.48	0.64	0.13	0.06
Error		0.19	0.41	0.17	0.43	0.46	0.34	0.35	0.39	0.27	0.43	0.39	0.51	0.51	0.07
Error d.f.[2]		80	79	79	79	78	76	75	73	73	72	72	72	72	72

[1]d.f. - the degrees of freedom for blocks and for treatment are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LXIII

FACTORIAL DESIGN, STUDY 3[1], TRIAL III, THE EFFECTS OF PROTEIN AND VITAMIN-MINERAL CONCENTRATE CONSUMPTION, WITH 300 CALORIES OF METABOLIZABLE ENERGY, UPON THE PERFORMANCE OF LAYERS

Desired daily protein cons. (gm.)	Desired daily vitamin-mineral concentrate consumption (gm.)		
	0.640	1.065	1.490
13	13-0.640 (4)*	13-1.065 (5)	13-1.490 (6)
16	16-0.640 (14)	16-1.065 (15)	16-1.490 (16)
19	19-0.640 (24)	19-1.065 (25)	19-1.490 (26)

[1]Study 3 - All experimental diets in this study were calculated to supply 300 Calories of metabolizable energy per hen per day.

*The numbers in parentheses represent the diet numbers.

Study 3

Results

There were no noticeable differences in hen livability among the experimental diets (Table LXIV). The 300-Calorie diets fed in this study were all nutritionally adequate for the hens to maintain good egg production for the twelve-month experimental period (Table LXV). There were no significant main effects of either protein or vitamin-mineral concentrate intake upon egg production (Table LXVI). Hens that were fed diets that contained 13 grams of protein maintained the highest egg production when the protein was conbined with 1.490 grams of vitamin-mineral concentrate. The highest egg production occurred on the 16-gram-protein diets that contained 0.640 grams of vitamin-mineral concentrate. Hens fed 19 grams of protein produced more eggs on 1.065 grams of vitamin-mineral concentrate. These interactions, plus the fact that slightly more eggs were produced on the diets that contained 13 and 19 grams of protein than on the 16-gram diets, probably account for the significant vitamin-linear x protein-quadratic interaction that occurred in Periods 5, 7 and overall.

Slightly heavier eggs were produced with each increase in dietary protein (Table LXVII). Eggs were approximately one gram heavier from hens that were fed 16 grams of protein than from hens fed 13 grams. Likewise, eggs from the hens fed 19 grams of protein were approximately one gram heavier than those fed 16 grams. Table LXVIII shows that the linear effect of dietary protein upon egg weight was significant at the 1 percent level of probability. It should be noted that most of this effect was established during the first 4 experimental periods. There were no significant effects of dietary vitamin-mineral concentrate upon egg weight.

Overall differences in body weight change of hens among the experimental

TABLE LXIV

NUMBER OF HENS PER TREATMENT AT THE END OF EACH TWENTY-EIGHT-DAY PERIOD, STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Period number	Number of hens								
1	11	11	11	11	11	11	11	10	11
2	10	10	11	11	11	11	11	11	11
3	10	11	11	10	11	11	11	11	10
4	10	11	11	10	11	11	11	10	10
5	10	11	10	10	11	11	11	10	10
6	10	11	10	10	11	11	11	10	10
7	10	11	10	10	11	11	11	10	10
8	10	10	10	10	11	11	11	10	10
9	10	10	10	9	11	11	11	10	10
10	10	10	10	9	11	11	11	10	10
11	10	10	10	9	10	11	11	9	10
12	9	10	10	8	10	11	11	9	8
13	9	10	10	8	10	11	11	9	8

TABLE LXV

AVERAGE EGG PRODUCTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Period number				Percent egg production					
1	77.9	77.9	78.2	77.3	78.2	74.7	79.5	76.0	84.4
2	72.4	74.0	78.6	78.6	75.6	64.3	76.3	76.9	80.8
3	75.0	80.2	79.2	76.4	72.7	68.2	76.0	78.6	75.4
4	70.0	71.8	74.7	72.5	72.4	67.9	71.1	76.8	77.5
5	71.8	75.0	73.9	75.7	67.5	65.6	68.5	74.6	76.4
6	69.6	61.7	73.6	69.6	60.4	61.0	66.2	67.5	71.8
7	72.1	68.5	72.1	73.9	64.3	57.5	66.6	72.5	73.9
8	66.1	64.3	68.6	67.1	64.3	55.2	64.9	69.3	70.0
9	67.1	62.5	66.4	64.7	65.3	60.4	63.0	66.1	70.4
10	61.8	58.2	63.9	61.9	66.6	53.2	58.4	78.6	68.2
11	52.5	53.9	63.2	68.3	64.3	46.4	58.8	63.5	62.5
12	56.0	55.4	62.5	62.3	64.6	51.6	50.3	67.1	54.3
13	55.0	49.3	60.0	55.8	64.6	46.1	48.4	55.6	57.6
Overall[1]	66.9	66.0	70.6	70.1	67.8	59.4	64.2	70.6	71.4

[1]Overall - based upon cumulative data for all periods.

TABLE LXVI

ANALYSIS OF VARIANCE OF EGG PRODUCTION DATA
BY PERIODS AND OVERALL, STUDY 3, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]							Mean squares							
Total d.f.[2]		(98)	(98)	(95)	(94)	(93)	(93)	(93)	(92)	(91)	(91)	(89)	(88)	(86)	(86)
Blocks	10	215	278	124	83	125	194	151	84	459	246	96	183	164	11233
Vitamins: (V)	(2)														
V_L (Linear)	1	1	1	3	4	0	0	11	3	0	1	8	0	2	18
V_Q (Quadratic)	1	2	0	7	3	1	41	1	1	0	22	7	60	15	181
Protein: (P)	(2)														
P_L	1	4	11	3	11	0	0	0	2	1	20	29	1	1	411
P_Q	1	8	23	45*	13	28	39	62*	46	9	11	0	5	1	2374
Vitamin-protein interation	(4)														
$V_L \times P_L$	1	4	1	4	1	7	0	12	2	14	13	8	0	4	221
$V_Q \times P_L$	1	0	6	1	3	4	13	4	11	12	49	35	48	115	1731
$V_L \times P_Q$	1	0	48	21	23	55*	54	97*	48	9	44	137*	99	35	8370*
$V_Q \times P_Q$	1	28	68	5	6	8	1	16	22	8	17	90*	9	49	3346
Error		13	20	9	8	9	19	15	18	17	15	22	31	30	1203
Error d.f.[2]		80	80	77	76	75	75	75	74	73	73	71	70	68	68

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LXVII

AVERAGE EGG WEIGHT BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Period number	Egg weight in grams								
1	51.7	52.8	52.1	54.1	53.2	54.7	54.7	56.3	55.2
2	54.0	55.1	52.5	55.5	54.7	56.3	56.9	57.1	56.0
3	56.5	55.3	55.0	57.2	56.3	58.1	58.4	58.6	59.2
4	56.2	56.9	54.9	58.7	57.5	59.0	58.7	58.9	58.1
5	58.1	58.1	58.5	59.2	58.4	59.6	59.6	59.5	59.8
6	59.1	57.8	57.9	59.9	58.9	60.5	59.9	60.8	60.4
7	59.0	59.3	58.5	59.8	59.2	60.0	60.7	61.0	61.8
8	58.3	58.7	58.6	60.0	59.0	60.9	60.1	59.0	61.2
9	59.1	59.7	58.5	59.9	59.1	61.1	60.7	60.9	60.5
10	59.0	59.9	58.6	59.9	58.7	60.7	60.5	63.8	59.1
11	59.8	60.9	59.6	61.4	60.1	61.6	61.0	61.1	61.8
12	61.0	61.7	60.5	61.9	61.3	63.5	61.3	62.1	62.3
13	62.5	61.8	61.3	62.0	61.8	63.4	62.0	62.4	62.8
Overall[1]	57.7	57.9	57.1	58.9	58.1	59.7	59.3	59.7	59.5

[1]Overall - based upon cumulative data for all periods.

TABLE LXVIII

ANALYSIS OF VARIANCE OF EGG WEIGHT DATA,
BY PERIODS AND OVERALL, STUDY 3, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(98)	(95)	(94)	(93)	(93)	(93)	(92)	(91)	(91)	(89)	(88)	(86)	(86)
Blocks	10	52	428	131	89	72	469	427	80	3165	200	63	1058	1383	36
Vitamins: (V)	(2)														
V_L (Linear)	1	6	12	0	9	1	0	48	11	0	1	0	94	2	0
V_Q (Quadratic)	1	1	36	6	0	2	155	15	10	0	1	0	3	163	0
Protein: (P)	(2)														
P_L	1	152**	312**	164**	93**	31	200*	77	38	34	24	23	11	36	30*
P_Q	1	1	16	0	19	0	22	115	8	676*	3	2	60	72	10
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1		48	6	1	1	5	4	0	0	7	0	349	126	0
$V_Q \times P_L$	1	13	92	2	16	0	86	8	13	8	16	11	84	252	1
$V_L \times P_Q$	1	2	2	0	1	0	7	36	2	8	0	0	53	74	0
$V_Q \times P_Q$	1	5	7	2	5	0	45	105	1	9	24	1	152	191	2
Error		5	39	11	8	9	48	48	14	101	17	11	195	201	7
Error d.f.[2]		80	80	77	76	75	75	75	74	73	73	71	70	68	68

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

diets were not significant (Tables LXIX and LXX). However, the linear protein effects upon body weight were significant in 5 of the experimental periods. As in Study 2, these effects do not all go in the same direction. Hens fed 19 grams of protein gained the most weight in Period 1; these same hens lost the most weight in Period 4. There were many other examples of a reversal of body weight change from period to period. These reversals account for the statistically significant vitamin x protein interactions of one type or another that occurred in 7 of the period analyses.

As the level of dietary protein was increased, there was a linear increase in feed consumption (Table LXXI). A possible reason for this was explained under the results of Study 2. Hens that were fed 16 grams of protein during Periods 6, 7, 8 and 10 consumed significantly less feed than those fed either 13 or 19 grams of protein. This quadratic effect of dietary protein upon feed consumption was significant at the 5 percent level of probability (Table LXXII). In this study, slightly over 300 milliliters of feed were consumed per hen per day (Table LXXIII).

Average daily consumption of vitamin-mineral concentrate per hen for Study 3 is given in Table LXXIV. The grams of concentrate actually consumed were slightly higher than desired for each of the three intake levels. Efficiency of utilization of the vitamin-mineral concentrate is shown in Table LXXV. Both linear and quadratic effects of dietary vitamin-mineral concentrate upon the efficiency of utilization of vitamin-mineral concentrate were highly significant (Table LXXVI). As dietary vitamin-mineral concentrate was increased, the efficiency with which it was utilized decreased. Again, as in Study 2, the efficiency of utilization of vitamin-mineral concentrate decreased faster with the first increase in dietary concentrate than with the second increase. The quadratic effect of protein upon vitamin and min-

TABLE LXIX

AVERAGE BODY WEIGHT GAIN OR LOSS BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Period number			Grams body weight gain or loss						
1	-103	- 25	- 85	- 22	- 62	- 43	+ 19	+ 74	+ 28
2	+118	+ 35	+ 61	+ 78	+120	+ 76	+ 60	+ 25	+ 65
3	- 34	+ 2	+ 41	- 9	+ 30	+ 15	- 5	0	- 50
4	+ 31	- 4	- 49	- 25	+ 3	- 19	- 40	- 57	- 31
5	+ 37	+ 35	+ 26	+ 56	+ 26	+ 35	+ 18	+ 99	+102
6	- 13	- 11	- 36	+ 5	- 21	- 40	- 6	- 68	- 19
7	+ 4	- 52	+ 42	- 6	- 9	- 72	- 20	- 4	- 1
8	+ 61	+ 62	- 52	+ 38	- 39	+ 94	+ 33	+161	+ 63
9	- 16	- 28	+ 22	+ 4	+107	+ 9	- 9	-110	- 8
10	+ 33	+ 28	+ 34	+ 61	+ 43	+ 38	+ 34	+ 18	+ 46
11	- 15	+ 24	- 20	+ 27	+ 29	+ 16	+ 38	+ 23	+ 26
12	- 31	- 3	- 1	- 43	- 39	- 65	- 57	- 43	-131
13	+ 19	+ 25	- 7	+ 31	+ 17	+ 14	0	- 23	+ 19
Overall[1]	+ 83	+ 79	- 26	+202	+209	+ 38	+ 63	+110	+141

[1]Overall - based upon cumulative data for all periods.

TABLE LXX

ANALYSIS OF VARIANCE OF BODY WEIGHT GAIN OR LOSS BY PERIODS AND OVERALL, STUDY 3, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares ÷ 100													
Total d.f.[2]		(98)	(98)	(95)	(94)	(93)	(93)	(93)	(92)	(91)	(91)	(89)	(88)	(86)	(86)
Blocks	10	2235	1530	620	287	109	006	532	1408	347	612	455	938	635	84
Vitamins: (V)	(2)														
V_L (Linear)	1	83	33	56	71	49	103	9	8	38	3	14	35	8	5
V_Q (Quadratic)	1	168	55	55	5	10	43	26	66	156	27	33	58	1	2
Protein: (P)	(2)														
P_L	1	1949**	45	71	193*	236**	20	5	94	0	0	176*	568*	33	1
P_Q	1	144	242	71	36	48	7	90	2	520**	56	14	14	59	9
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	1	73	350*	189*	247**	3	12	506	35	1	1	133	38	6
$V_Q \times P_L$	1	304*	271	37	3	4	29	416**	854*	478**	0	56	1	17	0
$V_L \times P_Q$	1	113	86	1	125	217**	14	263*	57	37	17	2	34	5	2
$V_Q \times P_Q$	1	8	40	6	3	3	177	65	393	137	23	11	1	55	8
Error		76	82	51	37	29	106	43	159	54	32	39	84	91	5
Error d.f.[2]		80	80	77	76	75	75	75	74	73	73	71	70	68	68

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LXXI

AVERAGE DAILY FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Period number				Total grams of diet					
1	111.4	116.8	108.5	118.0	119.6	117.0	124.5	132.7	131.2
2	149.8	129.2	133.7	144.5	136.6	135.5	137.5	149.3	152.9
3	146.2	140.4	143.0	142.2	135.8	141.4	150.7	152.1	153.3
4	138.6	136.1	133.4	145.7	138.0	145.6	137.9	144.9	150.6
5	151.8	141.3	140.1	145.0	134.3	144.2	142.2	152.1	157.8
6	143.0	140.3	136.7	132.3	111.4	120.5	145.5	142.3	164.9
7	134.4	124.3	129.8	132.5	121.7	120.9	139.3	147.3	151.0
8	129.4	120.7	126.9	129.0	116.5	118.9	129.0	141.7	143.0
9	126.6	120.2	124.5	131.1	124.5	123.6	133.8	139.2	142.9
10	126.8	121.7	123.7	118.1	124.5	122.4	144.3	144.8	139.2
11	127.4	127.9	127.3	136.7	131.8	126.9	132.9	141.2	151.2
12	130.6	131.5	131.8	134.2	131.9	133.8	125.8	139.4	130.6
13	137.8	133.0	136.8	143.0	138.1	140.4	124.1	139.5	134.5
Overall[1]	134.9	129.7	130.5	135.0	127.9	130.1	136.0	143.2	146.5

[1]Overall - based upon cumulative data for all periods.

TABLE LXXII

ANALYSIS OF VARIANCE OF DAILY FEED CONSUMPTION DATA
BY PERIODS AND OVERALL, STUDY 3, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(98)	(95)	(94)	(93)	(93)	(93)	(92)	(91)	(91)	(89)	(88)	(86)	(86)
Blocks	10	2738	3227	9054	1001	10	2129	1817	1690	6188	7749	1660	4005	2251	492
Vitamins: (B)	(2)														
V_L (Linear)	1	13	168	8	77	19	6	72	0	50	52	93	53	120	12
V_Q (Quadratic)	1	466	345	203	112	399	1782	371	241	287	13	8	76	6	157
Protein: (P)	(2)														
P_L	1	4893**	1331*	1197	1151**	599	1558	3799**	2153**	3014*	4532**	2853**	1	263	1168**
P_Q	1	156	222	1343	191	872	12422**	3404**	2170**	256	2486*	178	94	899	594*
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	250	2703**	68**	870*	1960**	1633	721	721	302	0	878	26	312	562
$V_Q \times P_L$	1	76	490	0	172	41	876	95	33	32	56	1	2	40	0
$V_L \times P_Q$	1	38	723	39	214	310	530	1054	1647*	760	329	655	439	897	540
$V_Q \times P_Q$	1	1	66	107	24	221	613	13	22	50	2	537	212	182	0
Error		234	290	981	140	222	533	299	238	598	583	225	360	393	149
Error d.f.[2]		80	80	77	76	75	75	75	74	73	73	71	70	68	68

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LXXIII

AVERAGE DAILY VOLUME OF FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Period number	Milliliters of feed actually consumed								
1	245	257	239	260	263	257	274	292	289
2	314	271	281	318	301	298	303	328	336
3	322	309	315	313	299	311	347	350	353
4	333	327	320	364	345	364	345	362	376
5	364	339	336	362	336	360	356	380	394
6	343	337	328	331	278	301	364	356	412
7	323	298	311	331	304	302	348	368	378
8	310	290	305	323	291	297	322	354	358
9	304	288	299	335	311	309	335	348	357
10	304	292	297	295	311	306	361	362	348
11	306	307	305	342	329	317	332	353	378
12	313	316	316	336	330	334	314	348	327
13	331	319	328	358	345	351	310	349	336
Overall[1]	316	304	306	327	311	316	332	348	357

[1]Overall - based upon cumulative data for all periods.

TABLE LXXIV

AVERAGE DAILY CONSUMPTION OF VITAMIN-MINERAL CONCENTRATE PER HEN, BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	14	24	5	15	25	6	16	26
Desired cons. (gm.)	0.640			1.065			1.490		
Period number	Grams of vitamin-mineral concentrate actually consumed								
1	0.898	0.948	1.001	1.569	1.480	1.777	2.040	2.190	2.464
2	1.237	1.160	1.117	1.780	1.690	2.021	2.580	2.537	2.897
3	1.178	1.142	1.163	1.885	1.680	1.956	2.689	2.648	2.761
4	0.743	0.750	0.698	1.216	1.181	1.220	1.987	1.742	2.682
5	0.813	0.747	0.720	1.262	1.150	1.281	2.088	1.726	2.810
6	0.767	0.681	0.736	1.253	0.953	1.198	2.037	1.442	2.937
7	0.721	0.682	0.705	1.110	1.042	1.240	1.933	1.447	2.690
8	0.693	0.664	0.653	1.078	0.998	1.193	1.891	1.424	2.547
9	0.679	0.691	0.677	1.073	1.065	1.172	1.856	1.479	2.545
10	0.680	0.608	0.730	1.087	1.066	1.219	1.843	1.466	2.479
11	0.683	0.704	0.673	1.142	1.128	1.189	1.896	1.519	2.693
12	0.700	0.691	0.636	1.175	1.129	1.173	1.964	1.602	2.327
13	0.739	0.737	0.628	1.188	1.182	1.174	2.039	1.681	2.395
Overall[1]	0.815	0.794	0.780	1.301	1.212	1.383	2.073	1.762	2.637

[1]Overall - based upon cumulative data for all periods.

TABLE LXXV

EFFICIENCY OF UTILIZATION OF VITAMIN-MINERAL CONCENTRATE BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Period number	Grams of concentrate per gram of egg								
1	0.022	0.038	0.050	0.023	0.036	0.054	0.023	0.042	0.053
2	0.032	0.044	0.063	0.027	0.041	0.071	0.026	0.046	0.064
3	0.028	0.042	0.062	0.026	0.042	0.067	0.026	0.042	0.062
4	0.019	0.030	0.048	0.018	0.028	0.044	0.017	0.027	0.060
5	0.020	0.029	0.048	0.017	0.029	0.044	0.018	0.029	0.061
6	0.019	0.035	0.048	0.016	0.026	0.040	0.019	0.029	0.068
7	0.017	0.027	0.046	0.015	0.027	0.042	0.017	0.029	0.059
8	0.018	0.029	0.048	0.016	0.026	0.042	0.017	0.029	0.059
9	0.017	0.029	0.048	0.018	0.028	0.041	0.018	0.029	0.060
10	0.019	0.031	0.049	0.016	0.027	0.045	0.021	0.029	0.062
11	0.022	0.035	0.050	0.017	0.029	0.054	0.019	0.031	0.070
12	0.020	0.034	0.052	0.018	0.028	0.049	0.021	0.028	0.069
13	0.021	0.039	0.055	0.021	0.030	0.057	0.021	0.034	0.066
Overall[1]	0.021	0.035	0.051	0.019	0.031	0.050	0.020	0.033	0.062

[1] Overall - based upon cumulative data for all periods.

TABLE LXXVI

ANALYSIS OF VARIANCE OF THE VITAMIN-MINERAL CONCENTRATE EFFICIENCY DATA
BY PERIODS AND OVERALL, STUDY 3, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(98)	(95)	(94)	(93)	(93)	(93)	(92)	(91)	(91)	(89)	(88)	(86)	(86)
Blocks	10	697	270	649	400	616	1116	837	1462	2938	5735	832	995	1042	532
Vitamins: (V)	(2)														
V_L (Linear)	1	10337**	7415**	8494**	20281**	20007**	19617**	24710**	22325**	21180**	23994**	18328**	13886**	11477**	13457**
V_Q (Quadratic)	1	721**	188*	307**	202*	245**	748**	432**	385*	357	452	241	5	152	224**
Protein: (P)	(2)														
P_L	1	66	19	0	100	3	0	76	4	120	93	68	9	3	0
P_Q	1	14	20	1	45	182*	826**	186	137	16	1575*	617*	584	202	144*
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	0	149*	12	346**	245**	110	24	143	18	17	451*	43	9	65
$V_Q \times P_L$	1	10	1	0	4	73	29	19	12	36	4	5	34	219	3
$V_L \times P_Q$	1	10	1	23	2	54	100	141	1	63	1337	279	226	12	10
$V_Q \times P_Q$	1	57	168*	49	76	15	274*	17	9	396	15	11	77	4	1
Error		19	30	38	29	28	63	48	77	161	360	97	170	166	21
Error d.f.[2]		80	80	77	76	75	75	75	74	73	73	71	70	68	68

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LXXVII

AVERAGE DAILY CONSUMPTION OF PROTEIN PER HEN, BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Desired cons. (gm.)	13				16			19	
Period number	Grams of protein actually consumed								
1	12.92	13.55	12.59	16.64	16.87	16.49	20.92	22.30	22.04
2	17.82	15.38	15.91	20.38	19.26	19.11	24.34	26.42	27.06
3	16.96	16.28	16.59	20.05	19.15	19.94	24.41	24.64	24.84
4	15.11	14.84	14.54	18.80	17.80	18.78	18.34	19.27	20.03
5	16.54	15.40	15.27	18.70	17.33	18.60	18.92	20.24	20.91
6	15.59	15.29	14.90	17.07	14.37	15.54	19.35	18.93	21.93
7	14.65	13.55	14.14	17.09	15.70	15.59	18.53	19.59	20.09
8	14.10	13.16	13.83	16.64	15.03	15.34	17.15	18.85	19.02
9	13.80	13.10	13.57	17.30	16.06	15.94	17.80	18.52	19.00
10	13.82	13.26	13.48	15.23	16.06	15.79	19.19	19.25	18.52
11	13.89	13.94	13.87	17.63	17.00	16.37	17.68	18.78	20.11
12	14.23	14.34	14.37	17.31	17.02	17.26	16.73	18.54	17.38
13	15.02	14.50	14.91	18.45	17.82	18.11	16.50	18.55	17.88
Overall[1]	14.97	14.38	14.47	17.82	16.87	17.14	19.22	20.36	20.79

[1] Overall - based upon cumulative data for all periods.

TABLE LXXVIII

EFFICIENCY OF UTILIZATION OF PROTEIN BY PERIODS AND OVERALL[1], STUDY 3, TRIAL III

Diet number	4	5	6	14	15	16	24	25	26
Period number	Grams of protein per gram of egg								
1	0.32	0.33	0.21	0.40	0.41	0.40	0.48	0.52	0.47
2	0.46	0.38	0.39	0.47	0.47	0.53	0.56	0.60	0.60
3	0.40	0.37	0.38	0.46	0.47	0.50	0.55	0.53	0.56
4	0.38	0.36	0.35	0.44	0.43	0.47	0.44	0.43	0.44
5	0.40	0.35	0.35	0.42	0.44	0.48	0.46	0.45	0.46
6	0.38	0.43	0.35	0.41	0.40	0.42	0.49	0.46	0.51
7	0.34	0.33	0.33	0.39	0.41	0.45	0.46	0.44	0.44
8	0.37	0.35	0.34	0.41	0.40	0.46	0.44	0.46	0.44
9	0.35	0.35	0.35	0.47	0.42	0.43	0.47	0.46	0.42
10	0.38	0.38	0.36	0.41	0.41	0.49	0.54	0.38	0.46
11	0.44	0.42	0.37	0.42	0.44	0.57	0.49	0.48	0.52
12	0.42	0.42	0.38	0.45	0.43	0.53	0.54	0.45	0.51
13	0.44	0.48	0.41	0.53	0.45	0.62	0.55	0.54	0.49
Overall[1]	0.39	0.38	0.36	0.43	0.43	0.48	0.50	0.48	0.49

[1]Overall - based upon cumulative data for all periods.

TABLE LXXIX

ANALYSIS OF VARIANCE OF THE PROTEIN EFFICIENCY DATA
BY PERIODS AND OVERALL, STUDY 3, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(98)	(95)	(94)	(93)	(93)	(93)	(92)	(91)	(91)	(89)	(88)	(86)	(86)
Blocks	10	5.65	2.83	2.63	0.85	1.56	4.12	2.95	4.52	11.61	14.62	2.43	3.12	2.77	1.27
Vitamins: (V)	(2)														
V_L (Linear)	1	0.04	0.01	0.12	0.03	0.00	0.05	0.19	0.01	0.00	0.47	0.10	0.13	0.27	0.01
V_Q (Quadratic)	1	0.34	0.17	0.02	0.18	0.15	0.24	0.07	0.01	0.01	0.01	0.10	0.99	0.01	0.09
Protein: (P)	(2)														
P_L	1	21.61**	10.26**	11.32**	2.90**	4.67**	4.21**	7.69**	5.57**	7.24*	5.53**	2.62**	3.01*	1.85	5.51**
P_Q	1	0.20	0.02	0.36	1.45**	0.96**	0.06	1.43*	1.01	1.60	0.04	0.09	0.01	0.42	0.29
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	0.04	0.66	0.08	0.23	0.23	0.18	0.00	0.06	0.28	0.20	0.90	0.12	0.07	0.07
$V_Q \times P_L$	1	0.02	0.03	0.01	0.04	0.02	0.75	0.01	0.02	0.23	0.14	0.65	0.72	1.82	0.14
$V_L \times P_Q$	1	0.00	0.11	0.32	0.10	0.44	0.35	0.75	0.06	0.01	3.11*	1.53*	0.97	0.03	0.17
$V_Q \times P_Q$	1	0.22	1.00*	0.22	0.07	0.32	0.29	0.41	0.43	0.10	0.21	0.72	0.00	1.22	0.19
Error		0.15	0.23	0.21	0.11	0.12	0.29	0.24	0.31	0.46	0.78	0.25	0.57	0.58	0.10
Error d.f.[2]		80	80	77	76	75	75	75	74	73	73	71	70	68	68

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

eral utilization was also significant ($P > 0.05$). Hens that were fed 16 grams of protein utilized the vitamin-mineral concentrate more efficiently than those fed the other two protein levels.

Protein consumption and efficiency of utilization of protein are given in Tables LXXVII and LXXVIII, respectively. Protein consumption levels were all above the desired intake levels of 13, 16 and 19 grams. Nevertheless, variation in protein consumption within each of the three groups was relatively small. Actual protein consumption per hen for each of the respective intake levels was approximately 14.6, 17.3 and 20.2 grams. There were no significant effects of dietary vitamin-mineral concentrate upon the utilization of protein (Table LXXIX). Only the expected linear effect of increasing dietary protein levels had any significant influence upon the efficiency of utilization of protein.

TABLE LXXX

FACTORIAL DESIGN, STUDY 4[1], THE EFFECTS OF PROTEIN AND VITAMIN-MINERAL CONCENTRATE CONSUMPTION, WITH 350 CALORIES OF METABOLIZABLE ENERGY, UPON THE PERFORMANCE OF LAYERS

Desired daily protein cons. (gm.)	Desired daily vitamin-mineral concentrate consumption (gm.)		
	1.065	1.490	1.915
13	13-1.065 (7)*	13-1.490 (8)	13-1.915 (9)
16	16-1.065 (18)	16-1.490 (19)	16-1.915 (20)
19	19-1.065 (27)	19-1.490 (28)	19-1.915 (29)

[1]Study 4 - All experimental diets in this study were calculated to supply 350 Calories of metabolizable energy per hen per day.

*The numbers in parentheses represent the diet numbers.

Study 4

Results

Livability, egg production and egg weight data are presented in Tables LXXXI, LXXXII and LXXXIV, respectively. There were no statistically significant differences in egg production or egg weight among hens that were fed the various experimental diets (Tables LXXXIII and LXXXV). There was a significant vitamin-linear x protein-linear interaction effect upon the rate of egg production in Periods 3, 7 and 8 and upon egg weight in Periods 7 and 8, but these interactions were not significant in the overall analysis. With all experimental diets fed in this trial, the hens maintained high egg production and egg weight. Although the egg weight differences were not statistically significant, hens that were fed the 16- and 19-gram-protein diets produced slightly heavier eggs than hens fed the 13-gram-protein diet.

Average body-weight-gain per hen was higher in this study than in Studies 2 and 3 (Table LXXXVI). This was to be expected since the hens in this study were fed diets that contained 350 Calories of metabolizable energy, while the diets fed in Studies 2 and 3 contained only 250 and 300 Calories, respectively. Dietary vitamin-mineral concentrate had no significant effect upon body-weight-change (Table LXXXVII). The linear effect of protein upon body weight gain was significant in the overall analysis and in the analyses for Periods 1, 3, 4 and 11. Body weight gain increased as dietary protein was increased. However, the real meaning of this was clouded by a significant vitamin-linear x protein-quadratic interaction that occurred in the analysis for Period 12 and in the analysis of the overall data.

Feed consumption data and the analysis of variance for these data are presented in Tables LXXXVIII and LXXXIX, respectively. Both linear and

TABLE LXXXI

NUMBER OF HENS PER TREATMENT AT THE END OF EACH TWENTY-EIGHT-DAY PERIOD, STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Period number				Number of hens surviving					
1	10	11	11	11	11	11	10	11	11
2	10	11	11	11	11	9	10	11	11
3	10	11	11	11	11	11	11	11	11
4	10	11	11	11	11	11	11	11	11
5	10	11	11	11	11	11	11	11	11
6	10	11	11	11	11	11	11	11	11
7	10	11	11	11	11	11	11	11	11
8	10	11	11	11	11	11	10	11	11
9	10	11	10	11	11	10	10	11	11
10	10	11	10	11	11	10	10	11	11
11	10	10	10	11	10	10	10	11	11
12	9	9	10	11	10	10	10	11	11
13	9	9	10	11	10	10	10	10	11

TABLE LXXXII

AVERAGE EGG PRODUCTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Period number				Percent egg production					
1	73.9	77.3	70.5	80.8	79.2	80.2	81.4	80.2	82.8
2	66.1	75.3	71.8	75.6	74.0	74.4	68.2	77.6	78.6
3	78.2	76.6	65.6	75.6	75.0	76.3	66.2	76.9	76.9
4	74.3	74.4	66.2	70.8	71.1	74.4	72.4	68.8	70.5
5	71.1	72.4	64.3	70.5	70.1	75.0	70.5	69.8	72.1
6	66.1	69.2	56.5	65.3	61.0	72.1	58.8	63.6	68.2
7	72.1	64.6	56.2	66.6	62.7	72.4	57.1	64.0	65.6
8	63.9	55.2	51.0	63.0	57.8	66.6	50.0	58.8	65.3
9	57.5	45.4	56.8	60.1	55.5	62.9	54.3	57.8	56.8
10	53.9	46.1	57.5	55.8	46.1	57.1	60.7	52.6	57.1
11	55.4	53.6	60.4	58.4	43.9	55.7	60.7	51.3	53.6
12	55.4	60.3	52.1	52.6	58.9	58.2	52.9	50.0	58.8
13	49.2	45.2	46.8	48.1	46.1	55.7	50.4	50.3	55.2
Overall[1]	64.5	63.2	59.8	64.9	61.9	68.1	61.9	63.2	66.3

[1]Overall - based upon cumulative data for all periods.

TABLE LXXXIII

ANALYSIS OF VARIANCE OF EGG PRODUCTION DATA
BY PERIODS AND OVERALL, STUDY 4, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(97)	(97)	(97)	(97)	(96)	(94)	(94)	(92)	(91)	(90)	(90)
Blocks	10	36	170	50	61	46	146	298	311	60	377	228	141	204	6303
Vitamins: (V)	(2)														
V_L (Linear)	1	1	28	0	5	0	7	0	4	2	0	3	9	19	629
V_Q (Quadratic)	1	1	17	16	0	0	0	2	12	34	134*	91	1	17	22
Protein: (P)	(2)														
P_L	1	69*	15	0	1	3	0	4	4	7	19	2	5	30	346
P_Q	1	10	5	9	1	5	11	28	44	33	3	13	4	1	725
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	6	6	116**	8	15	73	122*	162*	3	6	26	20	7	154
$V_Q \times P_L$	1	13	12	6	11	17	68	10	3	9	0	17	7	1	460
$V_L \times P_Q$	1	1	26	1	22	12	3	2	1	6	0	4	33	2	29
$V_Q \times P_Q$	1	1	2	7	1	2	11	41	15	29	8	2	10	8	135
Error		4	18	9	7	11	22	25	29	42	32	34	35	45	1737
Error d.f.[2]		78	78	79	79	79	79	79	78	76	76	74	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degree of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LXXXIV

AVERAGE EGG WEIGHT BY PERIODS AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Period number	Egg weight in grams								
1	52.9	52.8	53.8	55.6	54.5	55.5	54.4	55.0	54.3
2	53.9	55.1	54.1	56.6	56.0	55.1	56.8	57.1	56.1
3	57.0	56.1	56.1	58.7	58.3	57.4	57.8	57.4	56.8
4	57.2	57.7	57.9	59.2	57.8	58.6	59.0	58.5	58.0
5	58.7	57.8	58.8	60.6	59.6	59.4	60.4	58.7	58.4
6	59.6	59.0	59.8	58.6	59.8	60.5	60.1	59.5	60.5
7	60.4	59.9	59.2	62.1	61.0	60.9	60.8	59.5	60.2
8	59.6	59.3	60.2	61.4	60.7	60.5	61.0	59.7	60.2
9	59.0	57.5	59.0	61.9	60.5	61.4	60.6	59.1	63.3
10	60.4	58.2	59.4	61.9	60.6	62.4	61.0	59.7	61.0
11	59.8	59.4	60.9	63.3	62.9	62.8	62.4	61.2	62.2
12	61.9	60.8	62.3	63.6	63.0	64.2	63.6	62.8	62.8
13	63.9	61.1	63.5	64.4	62.1	60.4	64.1	63.0	63.0
Overall[1]	58.5	58.0	58.5	60.2	59.3	59.6	59.8	59.0	59.4

[1] Overall - based upon cumulative data for all periods.

TABLE LXXXV

ANALYSIS OF VARIANCE OF EGG WEIGHT DATA
BY PERIODS AND OVERALL, STUDY 4, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(97)	(97)	(97)	(97)	(96)	(94)	(94)	(92)	(91)	(90)	(90)
Blocks	10	208	75	118	141	144	725	1050	1516	1006	1053	7957	1417	1624	147
Vitamins: (V)	(2)														
V_L (Linear)	1	0	14	17	0	10	13	3	48	341	89	188	236	10	6
V_Q (Quadratic)	1	4	23	0	3	20	0	28	0	1084*	461	126	63	74	10
Protein: (P)	(2)														
P_L	1	34	74	8	13	3	120	0	4	786	9	34	13	285	6
P_Q	1	39	0	42	6	33	24	278	467	404	39	0	32	82	15
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	2	0	2	3	4	46	342*	620*	14	47	71	88	85	0
$V_Q \times P_L$	1	0	1	1	3	0	37	38	53	869	8	52	406	568	0
$V_L \times P_Q$	1	5	19	1	3	0	91	9	199	45	16	6	17	53	0
$V_Q \times P_Q$	1	5	16	0	0	5	1	3	18	70	300	104	51	432	7
Error		13	22	15	12	14	55	86	156	234	132	101	145	391	12
Error d.f.[2]		78	78	79	79	79	79	79	78	76	76	74	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LXXXVI

AVERAGE BODY WEIGHT GAIN OR LOSS BY PERIODS AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Period number	Grams body weight gain or loss								
1	+ 25	- 42	- 44	+ 36	+ 53	+ 43	+142	+ 59	+121
2	+ 24	+ 42	+ 46	+105	+ 66	+150	- 1	+106	+ 42
3	- 16	+ 21	+ 23	+107	+ 42	+ 36	+ 92	+ 33	+ 35
4	+ 36	+ 35	+ 50	+ 20	+ 25	+ 18	- 5	- 20	- 16
5	+ 8	+ 17	+ 53	+ 11	+ 13	+ 33	- 10	+ 30	+ 23
6	+ 52	+ 59	- 70	+ 1	+ 25	+ 19	- 5	- 33	- 32
7	- 1	- 34	- 4	- 65	- 56	- 38	- 36	- 24	+ 15
8	+ 42	- 5	- 19	+ 15	+ 66	+ 57	+ 9	+ 20	+ 44
9	+ 21	- 25	+ 21	+ 11	+ 38	+ 31	+ 29	+ 15	- 9
10	+ 25	- 7	+ 99	+ 30	- 5	+ 44	+ 32	+ 16	+ 25
11	+ 30	- 36	- 25	+ 75	+ 40	+ 17	+ 35	+ 80	+ 43
12	- 42	- 3	- 24	- 85	+ 17	- 24	+ 10	- 48	+ 8
13	+ 40	-167	- 53	+ 9	- 16	+ 30	+ 20	+ 31	+ 18
Overall[1]	+251	-121	+ 53	+180	+310	+413	+305	+263	+318

[1] Overall - based upon cumulative data for all periods.

TABLE LXXXVII

ANALYSIS OF VARIANCE OF BODY WEIGHT GAIN OR LOSS DATA
BY PERIODS AND OVERALL, STUDY 4, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares ÷ 100													
Total d.f.[2]		(96)	(96)	(97)	(97)	(97)	(97)	(97)	(96)	(94)	(94)	(92)	(91)	(90)	(90)
Blocks	10	1787	3744	542	167	1041	1367	235	2008	599	3166	1518	504	999	43
Vitamins: (V)	(2)														
V_L (Linear)	1	140	152	0	0	189	305	109	4	3	108	190	122	82	1
V_Q (Quadratic)	1	223	41	0	3	0	118	57	1	27	401	4	25	67	11
Protein: (P)	(2)														
P_L	1	2640**	26	301*	472*	19	219	1	54	5	41	638*	9	348	47*
P_Q	1	15	758*	0	12	0	86	34	220	104	12	93	51	7	13
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	62	8	240*	18	6	251	85	264	34	154	66	4	139	7
$V_Q \times P_L$	1	39	49	5	5	3	146	30	72	142	49	58	15	16	10
$V_L \times P_Q$	1	332	256	112	1	52	419	0	115	48	52	176	542*	9	21*
$V_Q \times P_Q$	1	34	379	22	4	13	7	0	9	78	34	8	144	178	16
Error		126	150	37	37	78	168	45	113	94	167	112	89	181	5
Error d.f.[2]		78	78	79	79	79	79	79	78	76	76	74	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE LXXXVIII

AVERAGE DAILY FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Period number				Total grams of diet					
1	115.2	106.4	104.7	124.2	120.3	126.2	107.6	106.4	109.5
2	120.2	124.4	120.8	141.7	130.1	133.6	118.1	126.4	127.7
3	130.5	131.8	134.9	135.0	132.2	136.9	123.5	127.6	128.6
4	126.6	128.1	122.9	136.3	129.6	137.4	122.0	124.6	123.3
5	121.8	124.7	180.1	133.4	127.3	133.6	123.4	112.7	120.7
6	116.6	115.3	97.2	119.2	113.1	122.6	107.2	115.5	120.8
7	118.3	109.7	107.7	120.0	113.1	124.4	106.2	113.1	117.3
8	115.0	102.5	98.4	117.0	114.6	130.5	111.6	111.2	119.1
9	109.0	95.2	102.0	115.9	125.7	128.9	118.0	112.1	117.6
10	104.0	95.2	105.4	126.1	100.5	122.2	107.3	110.8	199.1
11	109.2	101.9	105.3	125.9	106.1	120.3	112.1	111.9	113.0
12	122.2	118.1	107.3	110.6	129.2	124.5	165.8	144.7	188.9
13	121.4	160.1	105.4	145.7	120.3	129.6	117.7	116.8	122.7
Overall[1]	117.7	112.3	109.4	127.0	120.2	128.6	118.4	118.0	124.5

[1]Overall - based upon cumulative data for all periods.

TABLE LXXXIX

ANALYSIS OF VARIANCE OF DAILY FEED CONSUMPTION DATA
BY PERIODS AND OVERALL, STUDY 4, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(97)	(97)	(97)	(97)	(96)	(94)	(94)	(92)	(91)	(90)	(90)
Blocks	10	2473	5506	2312	1709	2157	2518	4281	9316	2467	10742	8182	42287	5957	1954
Vitamins: (V)	(2)														
V_L (Linear)	1	0	0	2	1	469	2	68	22	47	11	193	1415	1358	2
V_Q (Quadratic)	1	298	3	18	7	80	16	271	780	443	2386*	1034	1182	1691	203
Protein: (P)	(2)														
P_L	1	16	84	531	99	10	460	6	1276	2982*	982	827	42474**	1089	774*
P_Q	1	5076**	3105**	608	2157**	3699**	919	1177	2611*	4478**	2584*	1579	8017	5878*	1371**
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	448	227	0	54	318	2776**	1179	1554	113	30	35	3662	1000	352
$V_Q \times P_L$	1	1	343	16	352	1236*	965	71	62	612	401	263	54	274	344
$V_L \times P_Q$	1	39	821	105	19	571	15	26	559	1027	1158	443	4550	939	17
$V_Q \times P_Q$	1	139	4	13	142	29	171	353	735	165	1031	178	2854	16	9
Error		149	311	412	143	237	284	357	492	444	444	403	2572	1059	137
Error d.f.[2]		78	78	79	79	79	79	79	78	76	76	74	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

quadratic main effects of protein upon feed consumption were significant ($P > 0.05$ and $P > 0.01$, respectively). The desired feed intakes were 117, 123 and 120 grams (Table XXVI) for the three groups of diets (7, 8, 9), (18, 19, 20) and (27, 28, 29), respectively. The actual feed intakes for these respective groups were approximately 113, 125 and 120 grams. Since the weights of Diets 27, 28 and 29 were more than the weights of Diets 7, 8 and 9, and because Diets 18, 19 and 20 weighed more than either of the other two groups, it can be seen readily that the experimental design dictated both the linear and the quadratic main effect of protein upon feed intake. The volume of feed consumed per hen in this study was approximately 247 milliliters (Table XC). The weights of vitamin-mineral concentrate consumed per hen are summarized in Table XCI.

Since egg production was not improved by increased levels of dietary vitamin-mineral concentrate, the efficiency of utilization of vitamin-mineral concentrate decreased with each increase in dietary vitamin-mineral concentrate (Table XCII). Linear effects of dietary vitamin-mineral concentrate upon efficiency of utilization of the concentrate were significant at the 1 percent level of probability (Table XCIII). The vitamin quadratic effect upon efficiency of utilization of vitamins and minerals was again significant at the 5 percent level of probability. Although to a lesser degree than in Studies 2 and 3, efficiency of utilization of vitamins and minerals decreased faster with the first increase in dietary concentrate than with the second increase.

Average protein consumption per hen was very close to the desired levels (Table XCIV). Within each protein intake group, the efficiency of utilization of protein (Table XCV) was nearly equal. However, as dietary protein was increased, the efficiency of utilization of protein decreased.

TABLE XC

AVERAGE DAILY VOLUME OF FEED CONSUMPTION PER HEN BY PERIOD AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Period number	Milliliters of feed actually consumed								
1	242	224	220	261	253	265	226	223	230
2	228	236	230	283	260	267	236	253	255
3	274	277	283	283	278	287	247	255	257
4	253	256	246	300	285	302	256	262	259
5	244	249	216	293	280	294	259	237	254
6	233	231	194	262	249	270	225	243	254
7	237	219	215	264	249	274	223	237	246
8	230	205	197	257	252	287	234	234	250
9	218	190	204	255	276	283	248	235	247
10	208	190	211	277	221	269	225	233	231
11	218	204	211	277	234	265	235	235	237
12	244	236	215	243	284	274	348	304	397
13	243	212	211	321	265	285	247	245	258
Overall[1]	236	226	220	275	260	279	247	246	260

[1] Overall - based upon cumulative data for all periods.

TABLE XCI

AVERAGE DAILY CONSUMPTION OF VITAMIN-MINERAL CONCENTRATE PER HEN, BY PERIODS AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	18	27	8	19	28	9	20	29
Desired cons. (gm.)	1.065			1.490			1.915		
Period number	Grams of vitamin-mineral concentrate actually consumed								
1	1.445	1.535	1.441	1.870	2.081	1.869	2.376	2.840	2.466
2	1.569	1.771	1.529	2.273	2.275	2.247	2.838	3.005	2.924
3	1.636	1.668	1.598	2.316	2.288	2.273	3.060	3.079	2.945
4	1.156	1.160	1.075	1.637	1.542	1.537	2.019	2.198	1.953
5	1.112	1.135	1.087	1.593	1.515	1.389	1.773	2.138	1.912
6	1.065	1.014	0.945	1.473	1.346	1.424	1.596	1.962	1.914
7	1.080	1.021	0.935	1.402	1.346	1.394	1.769	1.991	1.858
8	1.050	0.996	0.983	1.310	1.363	1.372	1.616	2.087	1.886
9	0.995	0.986	1.040	1.216	1.495	1.382	1.674	2.062	1.863
10	0.950	1.073	0.945	1.217	1.196	1.366	1.731	1.955	1.741
11	0.997	1.071	0.987	1.302	1.263	1.379	1.729	1.925	1.789
12	1.116	0.941	1.460	1.510	1.538	1.784	1.761	1.991	2.992
13	1.109	1.240	1.037	1.354	1.432	1.440	1.731	2.073	1.944
Overall[1]	1.176	1.201	1.158	1.581	1.595	1.604	1.984	2.264	2.168

[1]Overall - based upon cumulative data for all periods.

TABLE XCII

EFFICIENCY OF UTILIZATION OF VITAMIN-MINERAL CONCENTRATE BY PERIODS AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Period number	Grams of concentrate per gram of egg								
1	0.037	0.046	0.063	0.034	0.048	0.064	0.033	0.043	0.055
2	0.044	0.055	0.073	0.041	0.055	0.073	0.039	0.051	0.066
3	0.037	0.054	0.083	0.038	0.052	0.070	0.042	0.051	0.067
4	0.027	0.038	0.053	0.028	0.038	0.050	0.028	0.038	0.048
5	0.027	0.039	0.047	0.027	0.036	0.048	0.026	0.034	0.045
6	0.028	0.037	0.047	0.027	0.037	0.045	0.027	0.038	0.046
7	0.025	0.036	0.053	0.025	0.035	0.045	0.027	0.034	0.048
8	0.028	0.040	0.053	0.026	0.039	0.052	0.032	0.040	0.048
9	0.029	0.045	0.050	0.027	0.045	0.053	0.032	0.040	0.052
10	0.029	0.045	0.051	0.032	0.043	0.055	0.026	0.044	0.050
11	0.030	0.041	0.047	0.029	0.046	0.055	0.027	0.044	0.054
12	0.033	0.041	0.054	0.028	0.041	0.053	0.043	0.057	0.082
13	0.035	0.049	0.058	0.040	0.050	0.062	0.032	0.045	0.056
Overall[1]	0.031	0.043	0.057	0.031	0.043	0.056	0.031	0.043	0.056

[1]Overall - based upon cumulative data for all periods.

TABLE XCIII

ANALYSIS OF VARIANCE OF THE VITAMIN-MINERAL CONCENTRATE EFFICIENCY DATA BY PERIODS AND OVERALL, STUDY 4, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(97)	(97)	(97)	(97)	(96)	(94)	(94)	(92)	(91)	(90)	(90)
Blocks	10	79	84	70	164	100	361	690	5827	403	936	857	746	529	103
Vitamins: (V)	(2)														
V_L (Linear)	1	2469**	1458**	2358**	5145**	4311**	4068**	5012**	6300**	3376**	4376**	3479**	2758**	1677**	3110**
V_Q (Quadratic)	1	13	1	9	102**	94	109	88	406	475	923*	564*	19	82	66*
Protein: (P)	(2)														
P_L	1	96**	57*	4	42	38	0	29	11	2	282	16	217	96	0
P_Q	1	24	2	6	9	6	12	90	1117	82	198	22	315	0	0
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	6	3	104**	0	5	21	256	370	12	287	130	7	2	0
$V_Q \times P_L$	1	14	6	0	0	10	39	3	102	14	36	0	6	103	4
$V_L \times P_Q$	1	5	14	10	30	11	0	26	1885*	305	188	0	131	8	0
$V_Q \times P_Q$	1	4	0	3	2	0	1	12	63	0	3	1	5	2	0
Error		7	13	12	12	25	59	65	387	125	145	104	120	113	15
Error d.f.[2]		78	78	79	79	79	79	79	78	76	76	74	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE XCIV

AVERAGE DAILY CONSUMPTION OF PROTEIN PER HEN, BY PERIODS AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Desired cons. (gm.)	13				16			19	
Period number			Grams of protein actually consumed						
1	12.44	11.50	11.31	16.27	15.76	16.54	16.90	16.71	17.19
2	13.46	13.93	13.53	18.70	17.17	17.64	18.90	20.23	20.43
3	14.09	14.23	14.57	17.68	17.32	17.93	19.76	20.42	20.58
4	14.18	14.35	13.77	17.58	16.72	17.72	19.28	19.69	19.48
5	13.64	13.96	12.09	17.21	16.43	17.24	19.49	17.80	19.07
6	13.06	12.91	10.88	15.38	14.59	15.82	16.94	18.25	19.09
7	13.25	12.29	12.07	15.48	14.59	16.05	16.77	17.86	18.53
8	12.88	11.48	11.02	15.09	14.78	16.83	17.63	17.57	18.81
9	12.20	10.66	11.42	14.95	16.21	16.62	18.64	17.71	18.59
10	11.65	10.67	11.81	16.27	12.97	15.77	16.95	17.50	17.36
11	12.23	11.41	11.80	16.24	13.69	15.52	17.71	17.67	17.85
12	13.69	13.23	12.01	14.26	16.67	16.06	26.19	22.86	29.85
13	13.60	11.87	11.80	11.79	15.52	16.71	18.60	18.45	19.39
Overall[1]	13.10	12.51	12.17	16.45	15.58	16.67	18.74	18.67	19.71

[1]Overall - based upon cumulative data for all periods.

TABLE XCV

EFFICIENCY OF UTILIZATION OF PROTEIN BY PERIODS AND OVERALL[1], STUDY 4, TRIAL III

Diet number	7	8	9	18	19	20	27	28	29
Period number			Grams of protein per gram of egg						
1	0.32	0.28	0.30	0.36	0.37	0.37	0.38	0.38	0.38
2	0.38	0.34	0.35	0.44	0.41	0.43	0.49	0.46	0.46
3	0.32	0.33	0.40	0.40	0.40	0 41	0 52	0.46	0.47
4	0.33	0.33	0.36	0.42	0.41	0.41	0.45	0.49	0.48
5	0.33	0.33	0.32	0.40	0.39	0.39	0.46	0.43	0.45
6	0.33	0.32	0.32	0.40	0.40	0.36	0.48	0.48	0.46
7	0.30	0.32	0.36	0.37	0.38	0.36	0.48	0.47	0.47
8	0.34	0.35	0.36	0 39	0.42	0.42	0.58	0.50	0.48
9	0.36	0.39	0.34	0.40	0.48	0.43	0.57	0.52	0.52
10	0.36	0.40	0.35	0.47	0.46	0.44	0.46	0.56	0.50
11	0.37	0.36	0.32	0.44	0.50	0.44	0.47	0.56	0.54
12	0.40	0.36	0.37	0.43	0.45	0.43	0.78	0.73	0.81
13	0.43	0.43	0.40	0.61	0.54	0.50	0.58	0.58	0.56
Overall[1]	0.35	0.34	0.35	0.42	0.42	0.41	0.51	0.50	0.50

[1]Overall - based upon cumulative data for all periods.

TABLE XCVI

ANALYSIS OF VARIANCE OF THE PROTEIN EFFICIENCY DATA
BY PERIODS AND OVERALL, STUDY 4, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(96)	(96)	(97)	(97)	(97)	(97)	(97)	(96)	(94)	(94)	(92)	(91)	(90)	(90)
Blocks	10	1.70	1.43	0.84	1.17	1.64	2.78	5.12	8.20	1.74	5.20	4.73	3.92	3.93	0.57
Vitamins: (V)	(2)														
V_L (Linear)	1	0.02	0.48	0.24	0.07	0.29	0.21	0.07	0.00	0.06	0.03	0.12	0.04	0.66	0.01
V_Q (Quadratic)	1	0.34	0.51	0.49	0.02	0.00	0.01	0.04	0.00	1.85	3.23	2.07	0.04	0.10	0.00
Protein: (P)	(2)														
P_L	1	8.90**	8.01**	12.68**	10.32**	12.40**	14.04**	15.90**	11.20**	9.72**	5.06*	10.87**	18.34**	0.36	11.74**
P_Q	1	1.54**	0.38	0.00	0.17	0.25	0.00	0.30	0.42	0.09	1.32	0.55	1.14	0.02	0.07
Vitamin-protein interaction	(4)														
$V_L \times P_L$	1	0.15	0.05	1.62**	0.04	0.05	0.23	1.88	2.26	0.00	1.01	1.07	0.02	0.01	0.00
$V_Q \times P_L$	1	0.38	0.11	0.11	0.05	0.10	0.68	0.25	0.00	0.46	0.64	0.08	0.07	0.78	0.10
$V_L \times P_Q$	1	0.01	0.15	0.00	0.30	0.07	0.15	0.01	0.55	1.66	0.34	0.07	0.21	0.03	0.00
$V_Q \times P_Q$	1	0.15	0.04	0.05	0.00	0.03	0.11	0.31	0.02	0.05	0.01	0.01	0.01	0.00	0.00
Error		0.11	0.22	0.21	0.08	0.24	0.43	0.48	0.61	0.85	0.90	0.68	0.73	0.88	0.13
Error d.f.[2]		78	78	79	79	79	79	79	78	76	76	74	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

This linear dietary protein effect upon the utilization of protein was significant at the 1 percent level of probability (Table XCVI).

Summary of Studies 2, 3 and 4

Results

Studies 2, 3 and 4 all involved experimental diets that contained graded levels of vitamin-mineral concentrate combined with graded levels of protein. Dietary protein levels of 13, 16 and 19 grams were fed in all three studies, but the three levels of vitamin-mineral concentrate that were fed in each study increased as dietary energy was increased. In Study 2, the three intake levels of protein were combined with 250 Calories of energy and graded vitamin-mineral concentrate intake levels of 0.215, 0.640 and 1.065 grams. The combinations of energy and vitamin-mineral concentrate that were fed in Studies 3 and 4 were 300 Calories with 0.640, 1.065 and 1.490 grams and 350 Calories with 1.065, 1.490 and 1.915 grams, respectively. The results of Studies 2, 3 and 4 may be compared under the assumption that as dietary energy is increased all other nutrients must be increased proportionately. If this assumption is not true, or if the proportionate increases in dietary vitamin-mineral concentrate are not correct, these comparisons will be of little value.

Livability of hens was generally good in all three studies. None of the experimental diets had any appreciable effect upon the mortality of the hens. Egg production was higher among the hens in Study 3 than among those in Studies 2 or 4. In Study 2, there was a significant linear effect of protein intake upon egg production. Hens that were fed diets that contained 13 grams of protein produced more eggs than those fed 16 or 19 grams of protein. There were no significant effects of dietary protein upon egg production in either of the other two studies. Data from Study 3 showed that as dietary protein was increased there was a linear increase in egg weight ($P > 0.05$). This trend was not evident in either of Studies 2 or 4. Egg weight was essentially the same in all three studies.

Body weight gain progressively increased from the 250-Calorie diets that were fed in Study 2 through the 350-Calorie diets that were fed in Study 4. Hens in Study 2 all lost weight. They weighed less at the close of the experiment than at the beginning. With the exception of hens that were fed Diet 6, the hens in Study 3 gained body weight. Hens that were fed Diet 8 were the only ones to lose weight in Study 4. Those hens that were fed Diets 7, 19, 20, 27, 28 and 29 in Study 4 gained more than one-half pound each. Linear effects of dietary protein upon body-weight-change were evident in all three studies. These effects were significant at the 5 percent level of probability in the overall analyses of Studies 2 and 4. However, vitamin x protein interaction effects upon body-weight-change were significant in all three studies. The effects of protein upon body weight were always linear, but not always in the same direction.

Because of the high volume of the diets that were fed in Study 2, and because the hens were apparently trying to compensate for low dietary energy, feed consumption was higher than in Studies 3 and 4. Feed consumption in Study 3 was also higher than in Study 4. The linear effects of protein upon feed consumption were significant in all three studies. Quadratic protein effects upon feed consumption were significant in Studies 2 and 4. A possible reason for both of these effects was explained under the results for the separate studies. The volume of feed consumed per hen per day was approximately 446, 324 and 250 milliliters in Studies 2, 3 and 4, respectively.

Vitamin-mineral concentrate consumption was higher in all studies than was expected, but there were five definite intake levels. Since the vitamin-mineral concentrate was consumed at higher levels than calculated, it was deemed unnecessary to present the specific vitamin and mineral intakes for each of the experimental diets. The calculated vitamin and mineral intakes in Table XXIII are minimums for the actual intake level. Linear and quadratic effects of dietary vitamin-mineral concentrate upon the efficiency with which the concentrate was utilized were significant in all three studies. The linear effects were to be expected, since increasing the level of dietary vitamin-mineral concentrate did not increase egg production. The quadratic effects were apparently caused by the efficiency of utilization of vitamin-mineral concentrate decreasing faster with the first increase in dietary concentrate than with the second increase. In Study 2, there were significant linear protein-intake effects upon the utilization of vitamins and minerals. As dietary protein was decreased, the efficiency of utilization of vitamin-mineral concentrate improved. There was also a significant vitamin-linear x protein-linear interaction in this study.

Protein consumption per hen increased with each protein intake group as dietary energy was reduced from Study 4 to Study 2. The protein intake levels were slightly over 15, 18 and 23 grams in Study 2, while the respective levels were approximately 14, 17 and 20 for Study 3 and 12.5, 16 and 19 for Study 4. As dietary protein was increased, the efficiency of utilization of protein decreased. This was to be expected, since the increased levels of protein did not improve egg production.

TABLE XCVII

FACTORIAL DESIGN, STUDY 5[1], TRIAL III, THE EFFECTS OF ENERGY AND VITAMIN-MINERAL CONCENTRATE CONSUMPTION, WITH 16 GRAMS OF PROTEIN, UPON THE PERFORMANCE OF LAYERS

Desired daily energy (M.E.)[2] cons. (Calories)	Desired daily vitamin-mineral concentrate consumption (gm.) 0.640	1.065	1.490
250	250-0.640 (11)*	250-1.065 (12)	250-1.490 (13)
300	300-0.640 (14)	300-1.065 (15)	300-1.490 (16)
350	350-0.640 (17)	350-1.065 (18)	350-1.490 (19)

[1]Study 5 - All experimental diets in this study were calculated to supply 16 grams of protein per hen per day.

[2](M.E.) - metabolizable energy, Titus (1955).

*The numbers in parentheses represent the diet numbers.

Study 5

Results

Livability of the hens in this study was not seriously affected by any of the experimental diets (Table XCVIII). Mortality was higher among the hens that were fed Diet 14 than among hens that were fed the other diets. Even in this case, the mortality was not severe. Egg production increased with each increase in dietary energy (Table XCIX). Hens that were fed diets that contained 300 Calories produced more eggs than those fed either the 250- or the 350-Calorie diets. Hens fed the 350-Calorie diets produced more eggs than hens fed the 250-Calorie diets; consequently, both the linear and quadratic effects of energy upon egg production were statistically significant ($P > 0.05$, Table C). There were no significant effects of dietary vitamin-mineral concentrate upon egg production. Vitamin x energy interactions were also non-significant in this study.

Egg weight increased slightly with each increase in dietary energy (Table CI). This trend was not statistically significant (Table CII) at the two common levels of probability reported herein, but it was significant at the 10 percent level of probability. Both linear and quadratic effects of dietary vitamin-mineral concentrate upon egg size were significant in the analysis for Period 12. However, there were also three types of vitamin x energy interaction significant in the same period analysis, which confused the meaning of any main effects that were significant.

When all experimental diets were considered, there was a linear trend for body-weight-gain to increase as dietary energy was increased (Table CII). The linear effects of energy consumption upon body weight gain were significant at the 1 percent level of probability (Table CIV). When overall body weight gains of the hens that were fed Diets 11, 14 and 17 were

TABLE XCVIII

NUMBER OF HENS PER TREATMENT AT THE END OF EACH TWENTY-EIGHT-DAY PERIOD, STUDY 5, TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Period number					Number of hens				
1	11	11	11	11	11	11	11	11	11
2	10	11	11	11	11	11	11	11	11
3	10	11	10	10	11	11	11	11	11
4	10	11	10	10	11	11	10	11	11
5	10	11	10	10	11	11	11	11	11
6	10	11	10	10	11	11	11	11	11
7	10	11	10	10	11	11	11	11	11
8	10	11	10	10	11	11	11	11	11
9	10	11	10	9	11	11	11	11	11
10	10	10	10	9	11	11	11	11	11
11	10	10	10	9	10	11	11	11	10
12	10	10	10	8	10	11	11	11	10
13	10	10	10	8	10	11	11	11	10

TABLE XCIX

AVERAGE EGG PRODUCTION PER HEN, IN PERIODS AND OVERALL[1], STUDY 5, TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Period number				Percent egg production					
1	71.4	79.2	63.3	77.3	78.2	74.7	75.0	80.8	79.2
2	75.9	74.4	63.0	78.5	75.6	64.3	74.4	75.6	74.0
3	71.4	75.3	68.9	76.4	72.7	68.2	71.1	75.6	75.0
4	52.1	66.9	49.3	72.5	72.4	67.9	64.9	70.8	71.1
5	54.3	69.2	45.0	75.7	67.5	65.6	64.6	70.5	70.1
6	59.3	60.7	47.5	69.6	60.4	61.0	61.7	65.3	61.0
7	55.0	59.1	60.0	73.9	64.3	57.5	62.3	66.6	62.7
8	53.9	68.5	59.3	67.1	64.3	55.2	59.1	63.0	57.8
9	56.8	62.3	57.1	64.7	65.3	60.4	57.5	60.1	55.5
10	47.1	52.1	53.6	61.9	66.6	53.2	55.2	55.8	46.1
11	50.4	44.3	52.9	68.3	64.3	46.4	54.5	58.4	43.9
12	44.3	48.6	47.5	62.3	64.6	51.6	54.5	52.6	58.9
13	30.7	35.0	38.2	55.8	64.6	46.1	50.0	48.1	46.1
Overall[1]	55.7	61.6	54.4	70.1	67.8	59.4	61.9	64.9	61.9

[1]Overall - based upon cumulative data for all periods.

TABLE C

ANALYSIS OF VARIANCE OF EGG PRODUCTION DATA BY PERIODS AND OVERALL, STUDY 5, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(97)	(95)	(95)	(95)	(95)	(95)	(95)	(94)	(93)	(91)	(91)	(90)	(90)
Blocks	10	147	249	95	113	101	217	228	279	666	255	154	402	579	21681
Vitamins: (V)	(2)														
V_L (Linear)	1	6	92*	5	0	20	54	16	9	5	20	105	0	1	2670
V_Q (Quadratic)	1	61	25	14	78	70	6	4	85	35	49	17	10	48	4838
Energy: (E)	(2)														
E_L	1	64*	23	6	199**	183**	55	48	0	0	5	16	110*	260**	8350*
E_Q	1	6	0	1	117*	83	30	30	9	67	153*	117*	121*	316**	8611*
Vitamin-energy interaction	(4)														
$V_L \times E_L$	1	32	23	9	17	46	25	3	7	1	47	28	0	20	99
$V_Q \times E_L$	1	29	1	6	68	158*	37	2	35	10	11	63	3	36	538
$V_L \times E_Q$	1	0	6	13	1	7	7	88	26	3	9	96	2	0	2148
$V_Q \times E_Q$	1	1	19	6	17	11	2	17	31	3	10	7	95	79	2104
Error		16	16	9	22	24	28	32	23	23	33	27	28	33	1885
Error d.f.[2]		80	79	77	77	77	77	77	77	76	75	73	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE CI

AVERAGE EGG WEIGHT BY PERIODS AND OVERALL[1], STUDY 5, TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Period number				Egg weight in grams					
1	54.8	54.4	54.2	54.1	53.2	54.7	54.9	55.6	54.5
2	56.8	55.4	54.6	55.5	54.7	56.3	56.0	56.6	56.0
3	57.6	59.1	57.2	57.2	56.3	58.1	58.0	58.7	58.3
4	57.6	57.2	57.8	58.7	57.5	59.0	59.1	59.2	57.8
5	58.7	58.1	64.0	59.2	58.4	59.6	60.1	60.6	59.6
6	59.3	59.0	58.6	59.9	58.9	60.5	60.6	58.6	59.8
7	59.9	59.3	59.1	59.8	59.2	60.0	60.7	62.1	61.0
8	59.5	60.2	59.2	60.0	59.0	60.9	60.5	61.4	60.7
9	60.3	60.0	59.0	59.0	59.1	61.1	60.6	61.9	60.5
10	59.1	59.6	59.5	59.9	58.7	60.7	60.9	61.9	60.6
11	60.5	60.3	59.8	61.4	60.1	61.6	61.7	63.3	62.9
12	61.0	59.7	60.5	61.9	61.3	63.5	62.2	63.6	63.0
13	61.0	59.6	61.8	62.0	61.8	63.4	60.7	64.6	62.1
Overall[1]	58.6	58.4	58.5	58.9	58.1	59.7	59.5	60.2	59.3

[1]Overall - based upon cumulative data for all periods.

TABLE CII

ANALYSIS OF VARIANCE OF EGG WEIGHT DATA
BY PERIODS AND OVERALL, STUDY 5, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean Squares													
Total d.f.[2]		(98)	(97)	(95)	(95)	(95)	(95)	(95)	(95)	(94)	(93)	(91)	(91)	(90)	(90)
Blocks	10	121	111	123	616	1287	1683	1064	647	5994	117	2021	1450	3170	56
Vitamins: (V)	(2)														
V_L (Linear)	1	0	9	0	4	28	7	2	52	39	83	1	344**	29	1
V_Q (Quadratic)	1	0	0	2	53	69	9	1	22	0	95	86	98*	157	0
Energy: (E)	(2)														
E_L	1	7	8	2	50	92	17	70	7	5	1	37	25	1039	29
E_Q	1	13	10	25	87	53	69	25	6	250	156	191	34	554	0
Vitamin-energy interaction	(4)														
$V_L \times E_L$	1	0	13	1	209	477	315	117	75	112	56	6	116*	154	0
$V_Q \times E_L$	1	1	0	22	41	38	77	303	3	56	15	73	315**	35	1
$V_L \times E_Q$	1	0	0	0	6	0	1	302	54	3	21	50	85*	367	0
$V_Q \times E_Q$	1	21	10	6	34	49	25	2	35	13	258	36	8	209	5
Error		6	10	12	84	131	81	127	47	154	117	177	18	337	8
Error d.f.[2]		80	79	77	77	77	77	77	77	76	75	73	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE CIII

AVERAGE BODY WEIGHT GAIN OR LOSS BY PERIODS AND OVERALL[1], STUDY 5, TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Period number				Grams body weight gain or loss					
1	- 72	- 90	- 44	- 22	- 62	- 43	- 19	+ 36	+ 53
2	+ 18	+ 15	- 25	+ 78	+120	+ 76	+ 37	+105	+ 66
3	- 70	- 39	- 2	- 9	+ 30	+ 15	+ 70	+ 16	+ 42
4	- 74	- 5	-111	- 25	+ 3	- 19	+ 60	+ 20	+ 25
5	+ 63	- 36	+ 84	+ 56	+ 26	+ 35	+ 5	+ 11	+ 13
6	- 92	- 85	- 64	+ 5	- 21	- 40	+ 41	+ 1	+ 25
7	- 26	+ 52	- 13	- 6	- 9	- 72	- 44	- 65	- 56
8	+103	+ 35	+ 73	+ 38	- 39	+ 94	- 55	+ 15	+ 66
9	- 55	- 85	- 53	+ 4	+107	+ 9	+ 4	+ 11	+ 38
10	- 7	- 14	+ 78	+ 61	+ 43	+ 38	+125	+ 30	- 5
11	+ 63	+103	- 7	+ 27	+ 29	+ 16	+ 37	+ 76	+ 40
12	+ 10	- 52	- 13	- 43	- 39	- 65	- 40	- 85	+ 17
13	+ 5	+ 23	- 45	+ 31	+ 17	+ 14	+ 19	+ 9	- 16
Overall[1]	-140	-189	-137	+202	+209	+ 58	+235	+180	+310

[1] Overall - based upon cumulative data for all periods.

TABLE CIV

ANALYSIS OF VARIANCE OF BODY WEIGHT GAIN OR LOSS DATA
BY PERIODS AND OVERALL, STUDY 5, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares ÷ 100													
Total d.f.[2]		(98)	(97)	(95)	(95)	(95)	(95)	(95)	(95)	(94)	(93)	(91)	(91)	(90)	(90)
Blocks	10	1363	934	526	272	362	1621	612	2955	1194	923	512	432	446	30
Vitamins: (V)	(2)														
V_L (Linear)	1	110	3	54	43	1	17	98	423	32	96	112	0	154	1
V_Q (Quadratic)	1	42	33	12	161*	360**	61	179*	462	60	142	345*	266*	56	1
Energy: (E)	(2)														
E_L	1	1396**	776*	996**	1378**	114*	1570**	561**	532	981**	109	1	74	17	201**
E_Q	1	81	704*	22	0	53	12	1	10	877**	23	157	96	96	17*
Vitamin-energy interaction	(4)														
V_L x E_L	1	52	115	242*	1	3	46	14	601*	28	1222**	123	148	6	0
V_Q x E_L	1	6	22	11	177*	400**	0	123*	55	545*	46	180	104	121	1
V_L x E_Q	1	297*	3	54	185*	140*	13	10	132	38	3	7	9	16	2
V_Q x L_Q	1	0	11	90	50	23	106	333*	304	164	2	18	234*	3	15
Error		66	118	43	35	26	116	31	139	89	97	66	58	113	4
Error d.f.[2]		80	79	77	77	77	77	77	77	76	75	73	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

compared, a linear trend due to energy level was obvious. These diets contained 0.640 grams of supplemental vitamin-mineral concentrate and 250 Calories of metabolizable energy. A comparison of Diets 12, 15 and 18 which contained 1.065 grams of vitamin-mineral concentrate and 300 Calories of metabolizable energy showed a quadratic effect of energy. Diet 15 produced the most body weight gain per hen. Diets 13, 16 and 19 also produced a linear effect of energy upon body weight gain. Body-weight-changes in hens that were fed Diets 12, 15 and 18 probably account for the quadratic effect of energy that was significant at the 5 percent level of probability in the overall analysis. Quadratic vitamin effects upon body weight gain were significant in the analyses of variance for Periods 4, 5, 7, 11 and 12. With 250 Calories of metabolizable energy, the second level of vitamin-mineral supplementation (1.065 grams) allowed less body weight loss per hen during periods 4, 5, 7 and 11 than either of the other two supplementation levels (0.640 or 1.490 grams). However, in Period 12 the second level of vitamin supplementation brought about the most body weight loss. In Periods 4, 11 and 12, the second level of vitamin supplementation with 300 Calories of metabolizable energy produced less body weight loss per hen than either of the other two supplementation levels. In Period 5, hens that were fed the second level of vitamins and 300 Calories of metabolizable energy gained less than hens fed the other two levels. In Period 7, body weight loss per hen increased as dietary vitamin-mineral concentrate was increased. Significant vitamin x energy interactions were most prevalent during the same periods (4, 5, 7, 11 and 12), so that the vitamin quadratic main effects were significant. The net effect of vitamin-mineral concentrate intake upon body weight change was not significant in the overall analysis.

Data on the average daily feed consumption per hen, analysis of variance of daily feed consumption data and average daily volume of feed consumption per hen are presented in Tables CV, CVI and CVII, respectively. These data show that as dietary energy was increased, both weight and volume of feed consumption per hen decreased. This trend was statistically significant at the 1 percent level of probability. The decrease in feed consumption per hen was not as great when the experimental diets were increased from 300 to 350 Calories as it was when the diets were increased from 250 to 300 Calories. Thus a quadratic curve was formed in Periods 1, 2, 6, 7 and 9 which was significant at the 5 percent level of probability. The net quadratic effect of energy upon feed consumption was also significant in the overall analysis. The effects of vitamins upon feed consumption per hen were significant only in Periods 9 and 11. During these periods slightly less feed was consumed among the hens that were fed 1.490 grams of vitamin-mineral concentrate. This same trend was evident in the overall analysis, even though it was not statistically significant.

Energy consumption was slightly higher than desired among hens that were fed diets that contained 250 and 300 Calories, and it was slightly lower than desired among hens that were fed diets that contained 350 Calories. The average level of energy intake for each of these respective groups was 293, 324 and 347 Calories (Table CVIII). Egg production increased enough with each dietary increase in energy to offset a linear decline in efficiency (Table CIX). However, the quadratic effect upon egg production which was brought about by the level of energy was evident again in the efficiency data. Energy was utilized most efficiently by the hens that were fed the 300-Calorie diets. This energy quadratic effect upon the efficiency of utilization of energy was significant at the 5 percent level of probability (Table CX). The effects of vitamins upon the utili-

TABLE CV

AVERAGE DAILY FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 5, TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Period number				Total grams of diet					
1	133.1	135.6	120.3	118.0	119.6	117.0	119.2	124.2	120.3
2	168.6	170.1	151.8	144.5	136.6	135.5	130.9	141.7	130.1
3	157.4	167.4	144.1	142.2	135.8	141.4	132.6	135.0	132.2
4	154.2	157.7	145.8	145.7	135.0	145.6	129.5	136.3	129.6
5	160.6	164.4	156.0	145.0	134.3	144.2	124.4	133.4	127.3
6	153.5	158.2	152.9	132.3	111.4	120.5	116.5	119.2	113.1
7	153.0	158.7	154.3	132.5	121.7	120.9	122.5	120.0	113.1
8	140.4	147.2	141.7	129.0	116.5	118.9	111.1	117.0	114.6
9	142.9	152.3	143.3	134.1	124.5	123.6	134.4	115.9	125.7
10	143.0	153.7	157.6	118.1	124.5	122.4	112.6	126.1	100.5
11	155.5	164.6	147.3	136.7	131.8	126.9	119.5	125.9	106.1
12	160.1	155.0	150.8	134.2	131.9	133.8	122.5	110.6	129.2
13	157.1	154.4	147.4	143.0	138.1	140.4	126.8	145.7	120.3
Overall[1]	152.1	156.9	147.0	135.0	127.9	130.1	123.3	127.0	120.2

[1]Overall - based upon cumulative data for all periods.

TABLE CVI

ANALYSIS OF VARIANCE OF DAILY FEED CONSUMPTION DATA
BY PERIODS AND OVERALL, STUDY 5, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(97)	(95)	(95)	(95)	(95)	(95)	(95)	(94)	(93)	(91)	(91)	(90)	(90)
Blocks	10	2153	4490	6180	3090	2353	1566	3672	10701	12797	5340	3700	8533	9083	3412
Vitamins: (V)	(2)														
V_L (Linear)	1	299	1121	370	123	10	469	815	79	817*	10	1553*	5	561	315
V_Q (Quadratic)	1	582	838	498	147	72	20	43	70	141	1512	1410*	928	1021	72
Energy: (E)	(2)														
E_L	1	1180*	13818**	8293**	6801**	15995**	23241**	21178**	12373**	6212**	22187**	22259**	17367**	6394*	11741**
E_Q	1	1159*	2173*	488	27	211	4470**	2960**	918	1149**	2092	416	358	1	731*
Vitamin-energy interaction	(4)														
$V_L \times E_L$	1	529	735	426	183	146	16	280	17	203	1868	69	618	29	11
$V_Q \times E_L$	1	175	452	2025	740	762	1104	340	727	1375**	87	475	36	5	387
$V_L \times E_Q$	1	50	178	43	61	278	958	311	878	38	355	124	360	634	166
$V_Q \times E_Q$	1	31	310	63	395	425	112	7	15	1166**	924	268	435	2105	77
Error		210	375	560	242	259	476	382	487	117	674	240	382	740	147
Error d.f.[2]		80	79	77	77	77	77	77	77	76	75	73	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

TABLE CVII

AVERAGE DAILY VOLUME OF FEED CONSUMPTION PER HEN, BY PERIODS AND OVERALL[1], STUDY 5, TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Period number				Milliliters of feed actually consumed					
1	319	325	289	260	263	257	250	261	253
2	421	425	379	318	301	298	262	283	260
3	394	419	360	313	299	311	278	283	278
4	462	473	438	364	345	364	285	300	285
5	482	493	468	362	336	360	274	293	280
6	461	475	459	331	278	301	256	262	249
7	459	476	463	331	304	302	269	264	249
8	421	442	425	323	291	297	245	257	252
9	429	457	430	335	311	309	296	255	276
10	429	461	473	295	311	306	248	277	221
11	466	494	442	342	329	317	263	277	234
12	480	465	452	336	330	334	270	243	284
13	471	463	442	358	345	351	279	321	265
Overall[1]	437	451	423	327	311	316	267	275	260

[1]Overall - based upon cumulative data for all periods.

TABLE CVIII

AVERAGE DAILY CONSUMPTION OF METABOLIZABLE ENERGY PER HEN, BY PERIODS AND OVERALL[1], STUDY 5 TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Desired cons. (Calories)			250		300			350	
Period number	Calories energy actually consumed								
1	312	318	282	313	317	310	340	354	343
2	381	384	343	383	362	359	378	409	375
3	356	378	326	377	360	375	378	385	377
4	284	291	269	352	333	352	362	381	363
5	296	303	288	350	325	348	348	373	356
6	283	292	282	320	269	291	326	333	316
7	282	293	285	320	294	292	343	336	317
8	259	272	261	312	282	287	311	327	321
9	264	281	264	324	301	299	376	324	352
10	264	283	291	285	301	296	315	353	281
11	287	304	272	330	318	307	334	352	297
12	295	286	278	324	319	323	343	309	362
13	290	285	272	346	334	339	355	408	337
Overall[1]	296	306	286	334	316	321	347	357	338

[1]Overall - based upon cumulative data for all periods.

TABLE CIX

EFFICIENCY OF UTILIZATION OF METABOLIZABLE ENERGY BY PERIODS AND OVERALL[1], STUDY 5, TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Period number	Calories of metabolizable energy per gram of egg								
1	8.0	7.4	8.2	7.5	7.6	7.6	8.3	7.9	8.0
2	8.8	9.3	10.0	8.8	8.8	9.9	9.1	9.5	9.1
3	8.6	8.5	8.3	8.6	8.8	9.5	9.2	8.7	8.6
4	9.5	7.6	9.4	8.3	8.0	8.8	9.4	9.1	8.8
5	9.3	7.6	10.0	7.8	8.2	8.9	9.0	8.7	8.5
6	8.1	8.1	10.1	7.7	7.6	7.9	8.7	8.7	8.7
7	8.6	8.4	8.0	7.2	7.7	8.5	9.1	8.1	8.3
8	8.1	6.6	7.4	7.7	7.4	8.6	8.7	8.5	9.1
9	7.7	7.5	7.8	8.4	7.8	8.1	10.8	8.7	10.5
10	9.5	9.1	9.1	7.7	7.7	9.2	7.4	10.2	10.1
11	9.4	11.4	8.6	7.9	8.2	10.7	9.9	9.5	10.8
12	10.9	9.9	9.7	8.4	8.0	9.9	10.1	9.2	9.2
13	15.5	13.7	11.5	10.0	8.4	11.6	11.7	13.1	11.8
Overall[1]	9.1	8.5	9.0	8.1	8.0	9.1	9.4	9.1	9.2

[1]Overall - based upon cumulative data for all periods.

TABLE CX

ANALYSIS OF VARIANCE OF THE ENERGY EFFICIENCY DATA
BY PERIODS AND OVERALL, STUDY 5, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(97)	(95)	(95)	(95)	(95)	(95)	(95)	(94)	(93)	(91)	(91)	(90)	(90)
Blocks	10	0.48	0.20	2.02	0.22	0.82	1.31	1.20	1.98	3.12	1.32	0.53	2.05	3.34	0.33
Vitamins: (V)	(2)														
V_L (Linear)	1	0.00	0.21*	0.95	0.01	0.04	0.09	0.01	0.01	0.14	0.55	0.16	0.00	0.08	0.00
V_Q (Quadratic)	1	0.10	0.01	0.56	0.16	0.11	0.52*	0.03	1.86	0.20	0.00	0.00	0.21	0.10	0.08
Energy: (E)	(2)														
E_L	1	0.00	0.01	1.59	0.04	0.00	0.01	0.00	0.16	1.75*	0.06	0.02	0.15	0.45	0.02
E_Q	1	0.10	0.01	0.56	0.16	0.11	0.52*	0.14	0.06	0.07	1.95*	0.42	0.58	1.26**	0.17*
Vitamin-energy interaction	(4)														
$V_L \times E_L$	1	0.04	0.03	1.56	0.02	0.17	0.28	0.00	0.00	0.05	0.08	0.05	0.00	0.01	0.01
$V_Q \times E_L$	1	0.07	0.00	0.85	0.21	0.39	0.10	0.02	0.05	0.03	0.02	0.40	0.02	0.17	0.00
$V_L \times E_Q$	1	0.00	0.00	1.45	0.00	0.01	0.01	0.40	0.54	0.16	0.29	0.35	0.01	0.01	0.02
$V_Q \times E_Q$	1	0.00	0.08	0.21	0.13	0.09	0.03	0.06	0.26	0.06	0.24	0.06	0.11	0.56*	0.05
Error		0.06	0.04	1.04	0.10	0.10	0.11	0.14	0.47	0.26	0.32	0.12	0.15	0.14	0.04
Error d.f.[2]		80	79	77	77	77	77	77	77	76	75	73	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

zation of energy was significant at the 5 percent level of probability (Table CX). The effects of vitamins upon the utilization of energy were significant only in Periods 2 and 6.

Since egg production was not increased by increasing vitamin-mineral concentrate levels, there was a significant linear decline in the efficiency of utilization of vitamin-mineral concentrate as it was increased in the layer diets (Tables CXI, CXII, and CXIII). Again, as in Studies 2, 3 and 4, the efficiency of utilization of vitamin-mineral concentrate decreased more from the second level of vitamin and mineral supplementation to the first than from the third to the second levels. Consequently, the quadratic effects of dietary vitamins and minerals were significant at the 1 percent level of probability. As dietary energy was increased, the efficiency of utilization of vitamins and minerals was improved significantly. However, this was to be expected, since less vitamin-mineral concentrate was consumed by hens fed the higher energy levels. Egg production was enough higher among hens that were fed the second level (300 Calories) of energy supplementation than among hens that were fed the third level (350 Calories), that there was not much difference in efficiency of utilization of vitamin-mineral concentrate between the two energy levels. The fact that the hens fed these supplemental levels of energy utilized the vitamin-mineral concentrate with approximately the same degree of efficiency, and that the 250-Calorie diets were utilized at a considerably lower degree of efficiency than the other two, resulted in a significant ($P > 0.05$) quadratic effect of energy upon the efficiency of utilization of vitamin-mineral concentrate.

TABLE CXI

AVERAGE DAILY CONSUMPTION OF VITAMIN-MINERAL CONCENTRATE PER HEN, BY PERIODS AND OVERALL[1], STUDY 5, TRIAL III

Diet number	11	14	17	12	15	18	13	16	19
Desired cons. (gm.)	0.640			1.065			1.490		
Period number	Grams of vitamin-mineral concentrate actually consumed								
1	1.135	0.948	0.884	1.927	1.480	1.535	2.395	2.190	2.081
2	1.386	1.160	0.982	2.330	1.690	1.771	2.911	2.537	2.275
3	1.294	1.142	0.984	2.293	1.680	1.668	2.765	2.648	2.288
4	0.647	0.750	0.663	1.238	1.181	1.160	1.603	1.742	1.542
5	0.674	0.747	0.637	1.291	1.150	1.135	1.714	1.726	1.515
6	0.645	0.681	0.596	1.242	0.953	1.014	1.680	1.446	1.346
7	0.643	0.682	0.627	1.246	1.042	1.021	1.696	1.447	1.346
8	0.590	0.664	0.569	1.156	0.998	0.996	1.557	1.424	1.363
9	0.600	0.691	0.688	1.196	1.065	0.986	1.575	1.479	1.495
10	0.601	0.608	0.576	1.206	1.066	1.073	1.732	1.466	1.196
11	0.653	0.704	0.612	1.292	1.128	1.071	1.619	1.519	1.263
12	0.673	0.691	0.627	1.217	1.129	0.941	1.657	1.602	1.538
13	0.660	0.737	0.649	1.212	1.182	1.240	1.619	1.681	1.432
Overall[1]	0.786	0.794	0.700	1.456	1.212	1.201	1.898	1.762	1.595

[1]Overall - based upon cumulative data for all periods.

TABLE CXII

EFFICIENCY OF UTILIZATION OF VITAMIN-MINERAL CONCENTRATE BY PERIODS AND OVERALL[1], STUDY 5, TRIAL III

Diet number	11	12	13	14	15	16	17	18	19
Period number	Grams of concentrate per gram of egg								
1	0.029	0.045	0.070	0.023	0.036	0.054	0.021	0.034	0.048
2	0.032	0.057	0.085	0.027	0.041	0.071	0.027	0.041	0.055
3	0.031	0.052	0.070	0.026	0.042	0.067	0.024	0.038	0.052
4	0.022	0.032	0.056	0.018	0.028	0.044	0.017	0.028	0.038
5	0.021	0.032	0.060	0.017	0.029	0.044	0.016	0.026	0.036
6	0.007	0.018	0.035	0.060	0.016	0.027	0.040	0.016	0.027
7	0.019	0.036	0.048	0.015	0.027	0.042	0.017	0.025	0.035
8	0.018	0.028	0.044	0.016	0.026	0.042	0.016	0.026	0.039
9	0.018	0.032	0.048	0.018	0.028	0.041	0.020	0.027	0.045
10	0.022	0.039	0.054	0.016	0.027	0.045	0.017	0.031	0.043
11	0.021	0.048	0.051	0.017	0.029	0.054	0.018	0.029	0.046
12	0.025	0.042	0.058	0.018	0.028	0.049	0.018	0.028	0.041
13	0.035	0.058	0.069	0.021	0.030	0.057	0.021	0.040	0.050
Overall[1]	0.024	0.040	0.060	0.019	0.031	0.050	0.019	0.031	0.043

[1]Overall - based upon cumulative data for all periods.

TABLE CXIII

ANALYSIS OF VARIANCE OF VITAMIN-MINERAL CONCENTRATE EFFICIENCY DATA
BY PERIODS AND OVERALL, STUDY 5, TRIAL III

Period number		1	2	3	4	5	6	7	8	9	10	11	12	13	Overall
Source of variation	d.f.[1]	Mean squares													
Total d.f.[2]		(98)	(97)	(95)	(95)	(95)	(95)	(95)	(95)	(94)	(93)	(91)	(91)	(90)	(90)
Blocks	10	209	120	1433	572	1040	2131	1439	3958	2502	1832	848	2788	3857	442
Vitamins: (V)	(2)														
V_L (Linear)	1	9528**	8716**	5564**	14380**	17173**	19618**	19170**	18563**	13684**	22745**	16759**	12157**	6851**	11954**
V_Q (Quadratic)	1	244**	310**	533	267	388	1022**	894*	29	272	1519	914*	169	148	375**
Energy: (E)	(2)														
E_L	1	1239**	1079**	238	1074**	1462**	962*	1555**	1015	0	1035	978**	2365**	2710**	1055**
E_Q	1	135*	35	5	166	123	325	255	38	116	2394*	544*	594	1185**	212*
Vitamin-energy interaction	(4)														
$V_L \times E_L$	1	71	86	523	7	3	73	2	16	52	242	56	70	246	27
$V_Q \times E_L$	1	11	10	127	81	168	8	7	0	82	71	211	13	55	2
$V_L \times E_Q$	1	2	7	340	33	94	16	560	653	201	755	432	72	0	41
$V_Q \times E_Q$	1	35	81	95	86	64	24	27	491	126	401	46	124	650*	55
Error		28	24	168	136	116	145	172	535	236	591	135	202	160	44
Error d.f.[2]		80	79	77	77	77	77	77	77	76	75	73	73	72	72

[1]d.f. - the degrees of freedom for blocks and for treatments are the same for each period analysis and the overall analysis.

[2]Total d.f. and error d.f. - mortality may change the degrees of freedom from period to period. The total d.f. are listed above the mean squares for each period and the error d.f. are listed below the mean squares for each period.

*Significant at the 5 percent level.

**Significant at the 1 percent level.

DISCUSSION

Method of Nutrient Intake Control

The effects of varied energy intake levels upon feed consumption were not completely counteracted by dietary volume regulation in either Trial II or Trial III. However, it has been shown by these feeding trials that the intake of nutrients by laying hens can be regulated within workable limits with dietary volume control. Data from Trial II indicated that as dietary energy and protein were increased there should have been an accompanying decrease in dietary volume. It was also indicated that approximately four weeks were required for hens to reach maximum feed consumption when fed high-volume diets.

The volume adjustments that were suggested by the results of Trial II were made in Trial III, and the value of dietary volume for nutrient intake control was improved. Based upon the results of Trial III, it is recommended that dietary volume be increased to 500 milliliters for experimental diets when the desired intake is 250 Calories or less. During the time hens are adjusting to high volume diets, volume of the daily diet intake should not be over 250 milliliters. Immediately following this period of adjustment the hens attempted to compensate for low nutrient intake by consuming large quantities of feed; therefore dietary volume should be doubled for approximately 8 weeks following the period of adjustment.

Although the influence of protein upon feed consumption was small as compared to the effect of energy, there was a definite trend for feed consumption to increase as dietary protein was increased. The linear effects of dietary protein upon feed consumption were statistically significant in Studies 1, 2, 3 and 4 of Trial III. Therefore, as dietary protein is increased, dietary volume should be increased. Level of dietary vitamin-mineral concentrate appeared to exert little influence upon feed intake. However, some of the period analyses indicated that high levels of vitamin intake tended to lower feed consumption.

An interesting aspect of the nutrient intake data is that nutrients that add density to diets tend to lower feed consumption in terms of both weight and volume. Supplemental fat and vitamin-mineral concentrate both increase the density of layer diets and each additional level of these nutrients resulted in a decrease in feed consumption (Tables CV and CVII). Perhaps the increasing of density is as effective in reducing feed consumption as the increasing of energy or vitamin intake. Considerable research remains to be done in the area of nutrient intake control. However, it is the judgement of the author that the findings of these feeding trials which concern nutrient requirements are much more reliable than the findings of those feeding trials where nutrient intake has not been regulated.

Protein and Energy

Data from Feeding Trial I indicated that regardless of the percentage of dietary protein, laying hens would consume approximately 16 grams of protein per day. Feeding Trial II was designed to control nutrient intake, in order that comparisons could be made in the performance of laying hens that had consumed different protein levels. In this study, protein intakes of 14.6, 12.5, 11.7 and 9.7 grams per day (Table XVII) were obtained. Although there were no significant differences in egg production or egg weight among the hens fed different experimental diets, hens that consumed 14.6 grams of protein per day lost less body weight than hens that consumed lower levels of protein. The daily protein requirement for layers was examined more thoroughly in Trial III. Experimental diets were designed to furnish 16 grams of protein per hen per day, and they actually furnished 17 grams per hen per day. Hens that consumed 17 grams of protein maintained a higher rate of egg production for the overall experimental period than did hens that consumed either 14 or 20 grams of protein per day. Neither egg weight nor body weight change was influenced by dietary protein, except in Study 3 of Trial III where there was a linear increase in egg weight as dietary protein was increased. Based upon these data, it is recommended that a minimum of 16-17 grams of protein should be supplied to laying hens each day.

There was some evidence from Study 1 of Trial III that protein requirements might depend to some extent on the age of laying hens. Hens that were fed 19 grams of dietary protein produced fewer eggs than did those fed either 13 or 16 grams during the first nine experimental periods. From the ninth through the thirteenth period, hens that were fed 19 grams of dietary protein produced more eggs than did hens that were fed the other protein intake levels. Results of Study 2 of Trial III indicate that when hens are fed diets that will not permit an energy consumption per hen of over 300 Calories per day, high dietary protein and vitamin-mineral concentrate levels will allow the hens to produce eggs at a higher rate than will low dietary levels of these nutrients. Hens that consumed 19 grams of protein and 1.065 grams of vitamin-mineral concentrate per day maintained a higher rate of egg production than hens that were fed either 13 or 16 grams of protein combined with vitamin-mineral concentrate levels of 0.215 and 0.640 grams.

In the feeding trials reported in this thesis, daily consumption of metabolizable energy exerted more influence upon egg production than did either protein or vitamin-mineral concentrate consumption. It was observed in Trial I that hens would consume approximately 300 Calories per day regardless of the number of Calories per pound of diet. Egg production was highest when 320-330 Calories of metabolizable energy were consumed per hen per day. These findings were verified by the results of Trial III. Both the linear and the quadratic effects of energy intake upon egg production were statistically significant in Studies 1 and 5 of Trial III. As energy intake per hen was increased, egg production increased.

Body-weight-change of the hens was greatly influenced by daily energy intake. As daily energy intake was increased there was a linear increase in body weight. The linear effects of energy intake upon body-weight-change were statistically significant in Studies 1 and 5 of Trial III. There was some evidence that energy intake per hen had an effect upon egg weight. As energy intake per hen was increased in Study 5 of Trial III, there was a linear increase in egg weight.

From the previous discussion it is evident that laying hens perform best when fed diets that furnish 16 to 17 grams of protein and 320-330 Calories of metabolizable energy per hen per day. In terms of daily requirements per hen, this is a Calorie:protein ratio that ranges from 19.4:1 to 20:1. Efficiency of utilization of energy, expressed as Calories of energy per gram of egg, did not show a linear decrease with each increase in dietary energy. Hens that consumed 300 Calories per day utilized the energy more efficiently than hens that consumed 350 Calories, but the most efficient utilization occurred when 330 Calories were consumed per hen per day. Protein was utilized most efficiently by hens that were fed diets that contained 13 grams of protein. The efficiency of protein utilization progressively decreased as protein intake increased. Linear effects of protein intake upon the efficiency of protein utilization were significant at the 1 percent level of probability in Study 1 of Trial III.

Protein-energy interrelationships were evident in Study 1 of Trial III. The effects of protein intake upon egg production varied depending upon the energy intake level that was fed. The general trend with the 250-Calorie diets was for egg production to be highest on the 16-gram-protein diets. With 300-Calorie diets, egg production tended to increase as dietary protein intake increased. Near equal egg production was obtained from hens that were fed 13 or 16 grams of protein combined with 350 Calories of energy, while those fed 19 grams of protein produced fewer eggs than did either of the other two groups. These energy x protein interaction trends were significant in Periods 3, 4, 5 and 7 of Study 1.

Vitamins and Minerals

Data from Trial I indicate that intake of vitamins might play an important role in the overall performance of laying hens. Hens that were fed Diet 1 in Trial I consumed a smaller quantity of vitamins and produced fewer eggs than hens fed the other experimental diets. Body weight gains were less for those hens that were fed Diets 1, 2, 3 and 4 than for those hens that were fed Diet 6. It was postulated that differences in vitamin intake per hen per day might be a reason for these differences in performance. However, data from Trial III do not verify this possibility. With the possible exception of the hens in Study 2, vitamin-mineral concentrate intake had no significant influence upon egg production, egg weight or feed consumption in Trial III. In Study 2, hens that were fed a vitamin-mineral concentrate at a level of 1.065 grams per hen per day maintained a slightly higher rate of egg production than hens that were fed the vitamin-mineral concentrate at levels of 0.215 or 0.640 grams. However, these hens were allowed to consume only 300 Calories of energy, and the higher rate of egg production was obtained from hens that received 1.065 grams of vitamin-mineral concentrate when it was combined with 19 grams of protein. It is possible that the higher protein and vitamin-mineral concentrate levels were being utilized as energy in this particular situation.

In Study 5, there was some evidence that the intake of vitamin-mineral concentrate had some influence upon feed consumption. Feed consumption per hen was slightly less when the hens were fed the highest vitamin-mineral concentrate intake level of 1.490 grams. Even though vitamin-mineral concentrate consumption levels were generally higher than desired in Trial III, less vitamins were consumed by the hens in Trial III than by the hens in Trial I. Therefore, it is doubtful that vitamin intake had any effect upon the performance of the hens in Trial I. The efficiency of utilization of vitamin-mineral concentrate, expressed as grams of concentrate per gram of egg, decreased as dietary vitamin-mineral concentrate was increased.

Vitamin x protein interactions were significant in Studies 2, 3 and 4 of Trial III. The effects of these interactions were particularly evident in the body-weight-change data of this trial. During any single experimental period, body weight gain might increase with each increase in dietary intake of vitamin-mineral concentrate. However, the opposite might happen during the next experimental period. The net effects of level of intake of vitamin-mineral contrate upon body-weight-change were generally not significant in the overall statistical analyses.

Trial III, which is being continued for 6 additional experimental periods beyond the scope of this study, may yet reveal some interesting facts concerning the effects of vitamin-mineral concentrate intake upon the overall performance of layers. It is the opinion of the author that pullets which have been grown on diets high in vitamins and minerals need small quantities of supplemental vitamins until their first year of egg production is completed.

SUMMARY

A technique of nutrient intake control was developed for use in laying hen diets. Although the effects of varied energy intake levels upon feed consumption were not completely counteracted by manipulations of dietary volume, definite gradations in the intake of protein, energy and vitamin-mineral concentrate were obtained. Protein intake per hen per day was approximately 14, 18 and 20 grams in diets which were calculated to supply 13, 16 and 19 grams, respectively. Hens were able to consume approximately 300 Calories of metabolizable energy per hen per day on diets that were calculated to furnish 250 Calories. Approximately 330 Calories were consumed by hens fed 300-Calorie diets, while the 350-Calorie diets were consumed at approximately the desired level. Diets that were calculated to supply 0.215, 0.640, 1.065, 1.490 and 1.915 grams of vitamin-mineral concentrate per hen per day actually supplied 0.25, 0.8, 1.3. 1.6 and 2.1 grams, respectively.

Generally, the performance of laying hens in these feeding trials was best when they consumed 16 to 17 grams of protein per hen per day. The most efficient protein utilization occurred when each hen consumed 14 grams of protein per day. Protein intake had no appreciable effect upon body-weight-change. In Study 1 of Trial III, it was found that as dietary protein intake was increased, there was a linear increase in egg weight. It was also found in Trial II that dried whole egg solids, fed as the only source of dietary protein, would maintain egg production for at least a twelve-week production period.

Hens that consumed 320 to 330 Calories of metabolizable energy per hen per day produced more eggs than hens that consumed either 300 or 350 Calories. There was a significant trend for the efficiency of energy utilization to decrease as energy consumption increased, but the most efficient utilization of energy occurred when hens were fed diets that supplied 320 to 330 Calories of energy per day. Energy consumption had a significant influence upon body-weight-change. As energy consumption increased, body-weight-gain of the hens increased. In Study 5 of Trial III, egg weight tended to increase with each increase in energy intake.

There were no significant main effects of vitamin-mineral concentrate intake upon egg production, egg weight or feed consumption at the end of the 12-month experimental period reported herein. Vitamin x protein interaction effects upon body weight were significant in several analyses of variance for specific experimental periods. However, the effects of vitamin-mineral concentrate intake upon body-weight-change were not significant in any of the overall analyses. The efficiency of utilization of vitamin-mineral concentrate declined progressively as the consumption of the concentrate was increased.

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