METABOLISM STUDIES WITH LAYING HENS

By

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INTRODUCTION

Research work with broilers has demonstrated that energy, protein, and B-vitamin levels must be increased if modern broiler rations are to be utilized efficiently. However very little attention has been paid to the vitamin requirements of hens when a high-energy, high-protein type layer ration is fed. In the past few years it has been shown in practical feeding experience with laying hens that certain stress factors including exposure to disease, egg production, temperature change and management errors seem to increase the hen's requirement for certain nutrients. The effect of these stress factors can be counteracted by increasing vitamin levels, by increasing protein levels and quality, and by adding high levels of antibiotics to the layer ration. However, it is not understood at the present time just how these higher nutrient levels act in counteracting stress. The purpose of the metabolism study which is reported in this thesis was to obtain more basic information from a metabolism standpoint as to the effect of different nutrient levels on ration utilization.

LITERATURE REVIEW

Feed Utilization

It is well known that a deficiency of the B-vitamins causes a decrease in feed consumption and feed utilization. This was demonstrated by Tsukie and Tokado (1922) in their work with dogs and by Kennedy and Dutcher (1922) in their work with cats, when these animals were fed rations deficient in B-vitamins. When B-vitamins were added to the deficient rations, it was noted that appetite and metabolic processes were stimulated. On the other hand it was observed by Arvay (1926) with rats and by Kon and Drummond (1927) with pigeons that there was no difference in the utilization of diets supplemented with B-vitamins as compared to the same diets that were deficient in these factors.

Later, Althausen et al. (1946) and Sure and Romans (1948) in their work with rats showed that B-vitamins increased food consumption, efficiency of utilization and growth. Althausen et al. (1946) found that thiamine had by far the greatest influence in improving the alimentary functions of the body. Pantothenic acid and riboflavin had a less marked effect, and pyridoxine was found to be the least important in this respect. However, Sure and Easterling (1949) in their work with rats found that pyridoxine produced a marked effect on economy of food utilization. Supplementary niacin was found to be necessary by Scott and co-workers (1946) and Sarma and Elvehjem (1946) in order to get normal chick growth and feed utilization from high-energy rations containing high levels of corn or corn grits.

It was demonstrated by Althausen et al. (1946b) that the addition of B-vitamins to rations deficient in these factors increased the basal metabolism rate and decreased the respiratory quotient of rats. Later, Malaguzzi-Valeri et al. (1950) noted that large doses of thiamin and niacin increased the basal metabolism rate by 20 percent. However, pantothenic acid and pyridoxine were demonstrated by Althausen et al. (1946 b) and Allardyce and Singer (1947) to have little if any effect on the basal metabolic rate.

Although supplementary B-vitamins seem to increase the basal metabolism rate, Allardyce and Singer (1947) found that certain B-vitamins countered the effect of desiccated thyroids so far as elevation of the basal metabolic rate and losses of weight were concerned. It was shown that riboflavin was the most effective in countering the effect of the desiccated thyroids with thiamine second, p-amino benzoic acid third, and nicotinic acid fourth.

Carbohydrate Metabolism

It is now well known that thiamine, riboflavin, nicotinamide and the pyridoximers function catalytically as prosthetic groups of enzymes or as coenzymes. Of these thiamine was the first to be considered as having some relation to carbohydrate metabolism, and is still regarded as the vitamin most concerned. The brain tissue of thiamine-deficient pigeons was found by Gavilescu and Peters (1931) to be incapable of oxidizing glucose at the normal rate. However, it was shown that the deficient brain tissue greatly increased its rate of respiration when thiamine was added in vitro. Later Peters and Thompson (1934) found that thiamine-deficient tissue was unable to oxidize pyruvic acid because of the low rate of respiration. As a result it has been shown that when there is a thiamine deficiency there is always a high level of blood pyruvate and lactate. It was further demonstrated that when thiamine was added, the blood levels of pyruvate and lactate were restored to normal at the same time the toxic symptoms were corrected. Pyruvic acid metabolism depends upon the enzyme carboxylase, which in turn depends upon the coenzyme co-carboxylase for its action. Lahman and Schuster (1937) demonstrated that thiamine pyrophosphate is the primary constituent of this coenzyme.

As thiamine is required for the oxidation of pyruvic acid, Magyar (1949) found that the phosphorylation process is required for the absorption and utilization of the B-vitamins. When phosphorylation was increased, the urinary output of thiamine and riboflavin was increased. When the phosphorylation was decreased, the urinary output of thiamine and riboflavin was decreased. It was also demonstrated that heavy carbohydrate intake decreased the excretion of thiamine and riboflavin due to the exhaustion of the phosphorylation mechanism.

The first knowledge of the role of riboflavin as a part of an enzyme came as a result of the attempt of Warburg and co-workers (1935) to isolate the enzyme responsible for the oxidation of glucose. It was discovered to be the prosthetic group of the yellow oxidation enzyme, which is present in all living cells. Riboflavin also occurs as the prosthetic group of several other oxidizing enzymes besides the yellow enzyme. These enzymes carry electrons during fat and carbohydrate oxidation as do a number of enzymes which oxidize amino acids. In any case the flavin molecule functions by being alternately oxidized and reduced.

The decreased ability of the tissues of pantothenic acid-deficient rats to oxidize pyruvates has been found to indicate that this vitamin may be a general catalyst for the metabolism of acetyl groups, (Pilgrim and Elvehjem, 1944). The coenzyme form of pantothenic acid was discovered by Lipmann and co-workers (1947) to be a dinucleatide, containing in addition to the usual components a sulphur bearing moiety. This coenzyme was found to be a part of coenzyme A, which causes the union of acetic acid with choline to form acetyl choline. Coenzyme A, as suggested earlier in the case of pantothenic acid, is also concerned with acetylation reactions, (Scott, 1953).

The function of biotin in intermediary metabolism was demonstrated by Lardy and co-workers (1947) to be concerned with the fixation of carbon dioxide with pyruvate to form oxalacetate which plays a vital role in the synthesis of amino acids.

Naicin in the form of a nicotinamide was found by Warburg and co-workers (1931) to be present in coenzyme I and coenzyme II, and as such functions in the metabolism of carbohydrates. The catalytic effect of both coenzymes depends upon its ability to accept electrons from the dehydrogenase and transfer them to the flavin enzymes. The nicotinamide portion of the molecule is the electron acceptor.

It has been demonstrated that niacin can be dispensed with in the ration if the amino acid tryptophan is present at high enough levels. This was shown by Krehl and co-workers (1945), who demonstrated that either niacin or tryptophan would correct a growth depression in rats fed a low-protein diet containing forty percent of corn. These findings were further supported by the work of Dan (1946). Sengal and co-workers (1946), Spector and Mitchell (1946), and Hundley (1947). Briggs (1945) and Hundley (1947) reported that tryptophan seems to be interchangeably active in reversing growth depressions produced by niacin or tryptophan-low diets.

The action of niacin in promoting growth in the presence of an apparent deficiency of tryptophan was explained by Krehl, Sarma, Teply and Elvehjem (1946) and Spector and Mitchell (1946) as being due to increased efficiency of utilization of tryptophan. However, Hundley (1947) concluded that tryptophan is an important precursor of niacin, and that a niacin deficiency is produced when the diet contains insufficient tryptophan to compensate for the lack of preformed niacin. Certain other amino acids have been demonstrated by Groschke and Briggs (1946) and Krehl, Henderson, de la Huerga and Elvehjem (1946) to have a marked growth-inhibiting action which can be overcome by niacin.

The blood sugar levels of rats with hyperglycemia induced by a high-carbohydrate, low-fat diet and by glucose injections were reported by Ling and Chow (1954) to have been lowered following the administration of vitamin B_{12}. They concluded that vitamin B_{12} plays an important role in carbohydrate and lipid metabolism.

The kinds and amounts of B-vitamins needed in the diet have been indicated by Mannering and co-workers (1944) and Krehl, de la Huerga and Elvehjem (1946) to depend upon the kind of carbohydrate present in the diet. When the riboflavin (Mannering and co-workers, 1944) or niacin (Krehl, de la Huerga and Elvehjem, 1946 and Hundley, 1949) intake is below normal, rats fed a ration high in dextrin or corn starch show greater weight increases than do animals receiving rations high in sucrose, fructose, lactose, cellulose, or lard. Hundley (1949) concluded that the high niacin requirement observed when sucrose is fed is due principally to its fructose content.

Funk (1914) suggested that the composition of the diet of an animal with respect to carbohydrate and fat directly influences the B-vitamin requirement. Later, Evans and Lenkovsky (1929) demonstrated that rats fed diets high in fat grew faster than those fed diets high in carbohydrates. They concluded that fat spared the anti-neuritic vitamin (thiamine). Since that time Stirn and co-workers (1939), Arnold and Elvehjem (1939), and Banerji (1940) have accumulated evidence to indicate that in animals the requirement for thiamine is decreased as the fat content is increased. However, Reinhold and co-workers (1944) observed that a "sparing action" of fat was observed only when the fat was fed in conjunction with low carbohydrate intake. When, on the other hand, diets high in fat were conpared with diets in which the carbohydrate content was not strikingly different, no "sparing action" was detected. The "sparing action" of fat seems to be related to a decrease in the carbohydrates in the diet. Less thiamine is required as thiamine functions primarily in carbohydrate metabolism.

High levels of fat in the diet of the rat was shown by Salmon (1947) to have a niacin-sparing effect as the energy metabolism shifted from carbohydrates to fat. This indicates that niacin, like thiamine, functions primarily in carbohydrate metabolism. Mannering and co-workers (1941), Tange (1941), and Czaczkes and Guggenheim (1946) noted that rats fed high fat diets required higher levels of riboflavin as the fat level increased. Later, Mannering and co-workers (1944) concluded that the increase in the riboflavin requirement when high fat diets were fed was due to the influence of the fat in decreasing the intestinal synthesis of riboflavin. This conclusion was based on the fact that when sucrose, which also does not enhance intestinal synthesis, was substituted for dextrin the same reduced growth rate resulted. Reiser and Pearson (1949) also found that more riboflavin is required when the diet is high in fat. They observed that chicks fed unsaturated fatty acids required more riboflavin than those fed the saturated ones. They concluded that unsaturated fatty acids interfere more with the intestinal synthesis of riboflavin than do saturated fatty acids. It has been shown by Lehninger and Kennedy (1948) that riboflavin seems to have a role in the oxidative catabolism of fatty acids, and an increased requirement might be expected on a high fat diet.

Sure and Roman (1948) found that B-vitamins caused a marked increase in fat synthesis which resulted in an increase in body weight. Pyridoxine was shown by Conti and Sbragia (1949) and Sure et al. (1949) to be one of the vitamins which favors fat formation.

Protein Metabolism

There is evidence which indicates that some of the B-vitamins affect protein metabolism. Reader and Drummond (1925) found that high protein levels without adequate B-vitamins resulted in poor growth in rats. High protein levels were demonstrated by Tscherkes (1926) to accelerate avitaminosis-B in rats. That B-vitamins are required in protein metabolism was further demonstrated by Richter and Barelare (1939). Their free-choice feeding experiments showed that rats given B-vitamins consumed more protein than was consumed by the controls. Kleiver and Jukes (1942) demonstrated that chicks fed diets deficient in riboflavin retained less protein than those fed the control diet. This was confirmed by Sarett et al. (1942) and Czaczkes and Guggenheim (1946), who found that less riboflavin was present in the urine of rats fed high-protein diets than was present in the urine of rats fed low-protein diets. Later Sarett et al. (1943) and Unna et al. (1944) found that the concentration of riboflavin in the liver varied directly with the level of protein in the diet and is independent of the vitamin intake. In spite of the ingestion of a liberal quantity of riboflavin Unna et al. (1944) and Czaczkes and Guggenheim (1946) found that rats maintained on a low protein diet were unable to retain riboflavin. Riboflavin, as demonstrated by Sure and Dichek (1941) in experiments with growing rats, has a profound effect on the utilization of food for the synthesis of tissue fat and protein. Inouye and Omori (1941) (1942) in their work with growing rats found that as the protein level was increased the riboflavin requirement was also increased. They found that an eighty-five percent protein diet required four times the normal requirement of riboflavin.

Pyridoxine was found by Sure et al. (1949) to increase the weight gain in rats by the synthesis of fats and proteins. Snell (1953) found that pyridoxine is an important constituent of the enzyme that catalyzes transformation and synthesis of amino acids. Injections of vitamin B_{12} were demonstrated by Wei-Juang and Thomas (1952) to aid in the conversion of the amino acid homocystine to methionine. Pantothenic acid, a constituent of the coenzyme for acetylation, was demonstrated by Lipmann et al. (1947) to be concerned in amino acid metabolism.

Mineral Metabolism

Although the B-complex vitamins have been reported to be related to perosis in chicks and poults, there has been little work done concerning their relationship to bone mineralization. Hogan and Shrewburg

(1930) observed that perosis was prevented by the addition of wheat or wheat middlings to the ration. This may have been due to certain B-vitamins present in the wheat. Choline was shown by Jukes (1940) to prevent perosis in turkey poults, while folic acid reduced the incidence of perosis in chicks. Later Scott (1951) reported that para-amino benzoic acid reduced the incidence of perosis in poults. Jukes and co-workers (1947) found that a deficiency of niacin, and Patrick and co-workers (1944) found that a deficiency of riboflavin produced perosis in poults. Patrick and Schweitzer (1952) found that riboflavin, vitamin B_{12}, pantothenic acid, niacin, pyridoxine, folic acid and para-amino benzoic acid increased the deposition of phosphorus in the tibia of chicks. Thiamine, however, was found to be ineffective in increasing bone phosphorus deposition.

Calcium utilization was reported by Bogert and Trail (1922) and Marshall (1931) to have been greatly improved by the B-vitamins. Since that time, however, there has been little indication that B-vitamins improve calcium utilization.

Choline's Effect on Metabolism

The most noticeable symptom of a deficiency of choline in the diet involves changes in the distribution of lipids in the animal's body. Choline-deficient diets were reported by Best and co-workers (1932) to produce "fatty livers" in rats. Lecithin or choline in the diet prevented this condition. Both the methionine and betaine content of the ration were found by Simmonds et al. (1943) to influence the choline requirement, since both are methyl donors. It was demonstrated by du Vigneaud et al. (1940) and Simmonds et al. (1943) that methionine can replace choline in the prevention of fatty livers in rats. However Jukes (1940) (1941) and Schaefer et al. (1951) have demonstrated that, unlike rats, chicks and poults are unable to utilize methionine, betaine or aminoethanol (a methylating substance) alone or together as an effective substitute for choline. However, they did demonstrate that mono-or dimethyl aminoethanol can substitute for choline. Vitamin B_{12} was indicated by Jukes et al. (1950) to have a role in chicks in the formation of methionine from homocystine. Jukes and Stokstad (1952) found no evidence of an interaction between choline, betaine, or homocystine in chicks until vitamin B_{12} was added to the ration.

The work of Griffith and Mulford (1941) suggested that choline is not only a methylating agent, but since it forms a part of the lecithin molecule, it aids in fat transportation as well.

Folic Acid in Metabolism

Folic acid was found by Daniel and co-workers (1946) to be needed as much by the chick for the prevention of perosis as are manganese, choline, and biotin. Later Schaefer and co-workers (1950) reported that the addition of folic acid and vitamin B_{12} to the ration reduced the requirement for choline. They also demonstrated that folic acid was essential in the prevention of perosis. In the presence of both vitamin B_{12} and folic acid, they found that the level of choline chloride necessary to prevent perosis in the chick was further reduced. Later Young and co-workers (1955) found that even lower levels of choline chloride was necessary for maximum growth and perosis prevention when folic acid was adequate. The livers of folic acid-deficient chicks were reported by Dinning and co-workers (1951) to have a reduced ability to form methionine from homocystine plus choline or betaine.

EXPERIMENTAL PROCEDURE

General

The experiment which formed the basis of this thesis was conducted with 80 Single Comb White Leghorn pullets which were hatched from eggs produced on the Oklahoma Agricultural and Mechanical College Poultry Farm. The experiment began October 27, 1954 and terminated August 15, 1955. During the nine and a half month experimental period, data were collected on egg production, egg weight, hen weight, daily feed consumption and mortality.

The pullets were trapnested from mid-September 1954 until mid-October 1954 in order to have egg production records as a basis for selection. They were selected on the basis of egg production, physical condition and sexual maturity. From these pullets, four groups of 20 birds each were selected at random.

The all-mash rations employed in this experiment were formulated from common feedstuffs as shown in Table I. They all contained approximately 16 percent of crude protein.

The experimental rations and their computed compositions are shown in Table I and Table III. The four all-mash rations were designated as low-energy 1 NRC, high-energy 1 NRC, high-energy 2 NRC and high-energy 2 NRC + NB. The National Research Council's (1946) allowances were used as the basis for determining the levels of vitamin A, vitamin D, riboflavin and pantothenic acid which were to be fed in each ration. The low-energy 1 NRC ration was supplemented with vitamin D only. The computed ration levels of riboflavin, niacin, choline, pantothenic acid

Table I Composition of rations fed in metabolism studies

Ingredients	High energy 1 NRC Percent	High energy 2 NRC Percent	High energy 2 NRC + NB Percent	Low energy 1 NRC Percent
Ground Yellow corn	56.0	56.0	56.6	45.0
Ground oats	8.0	8.0	8.0	14.0
Wheat shorts	12.0	12.0	6.0	12.0
Wheat bran				6.0
Dehydrated alfalfa meal (17% protein)	3.0	3.0	3.0	6.0
Fish meal (60% protein)	3.0	3.0	6.0	3.0
Soybean oil meal (44% protein)	8.1	8.1	6.0	6.0
Meat and bone scrap (50% protein)	3.0	3.0		3.0
Dried brewers yeast			1.2	
Dried whey			1.8	
Dried fish solubles			1.8	
Dried butyl solubles			1.8	
Dicalcium phosphate	0.9	0.9	2.4	
Calcium carbonate	3.7	3.7	3.0	4.4
Salt	0.3	0.3	0.3	0.6
Trace mineral mix[1]			0.03	
Vitamin mixture[2]				
Feed grade fat[3]			1.8	
Manganese sulphate[4]	8.0 gm	8.0 gm		5.4 gm
Vitamin D (3000 ICU/gm)				12.0 gm

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[1] Description in Table II.

[2] Vitamins were added to each of the high-energy rations in the amounts shown on page 14.

[3] Marco B-75, manufactured by Marco Chemical Company, Fort Worth, Texas.

[4] Feedgrade 70% $MnSo_4$.

and folic acid are those provided by the feedstuffs themselves with no additional supplementation. The high-energy 1 NRC ration contained these vitamins at the levels which were recommended by the National Research Council (1946).

Table II Trace mineral mix used in the rations fed in metabolism studies

Trace mineral	1 lb of trace mineral premix supplies per ton feed
Manganese	27.5 mg/lb.
Iodine	0.88 mg/lb.
Cobalt	0.59 mg/lb.
Iron	18.30 mg/lb.
Copper	1.65 mg/lb.
Zinc	1.52 mg/lb.

The three high-energy rations were supplemented with vitamin A, vitamin D, choline, riboflavin, niacin, pantothenic acid, and folic acid as follows.

		High-energy 1 NRC	High-energy 2 NRC and high-energy 2 NRC + NB
Vitamin A	I.U./lb.	2080.00	2080.00
Vitamin D	I.U./lb.	360.00	360.00
Choline	mg/lb.	341.00	341.00
Riboflavin	mg/lb.	0.33	1.60
Niacin	mg/lb.	0.00	4.00
Pantothenic acid	mg/lb.	1.36	6.80
Folic acid	mg/lb.	0.67	1.96

The high-energy 1NRC ration contained more vitamin A (Table III) than was recommended by the National Research Council because the vitamin A provided by the feed ingredients exceeded the recommended allowances for the entire ration. Niacin was provided at a level of eight milligrams per pound of ration as recommended by the National Research Council for growing

Table III Computed analysis of rations fed in metabolism studies

	High energy 1 NRC	High energy 2 NRC	High energy 2 NRC + NB	Low energy 1 NRC
Protein percent	16.3	16.3	15.8	16.5
Metabolizable energy (calories/lb.)	1256.0	1256.0	1318.0	1183.0
Vitamin A IU/lb.	5341.0	6600.0	4032.0	6206.0
Vitamin D ICU/lb.	375.0	750.0	450.0	360.0
Riboflavin mg/lb.	1.5	2.6	2.23	1.2
Niacin mg/lb.	13.8	16.0	16.4	19.6
Choline mg/lb.	500.0	500.0	419.0	416.0
Pantothenic acid mg/lb.	5.0	10.0	12.0	4.79
Vitamin B-12 added mg/lb.	0.5	2.0	3.3	0.0
Vitamin E added mg/lb.	0.0	0.0	0.8	0.0
Folic acid mg/lb.	1.3	2.6	2.6	0.7

chicks. The high-energy 1 NRC ration exceeded this amount, as was the case with vitamin A. because the feed ingredients in the ration contained an excess of niacin. Niacin was added to the other two high-energy rations in sufficient quantities to supply niacin levels of two times the recommended allowances of eight milligrams per pound. The high-energy 2 NRC + NB ration was further improved in nutritive value by increasing the metabolizable energy through the addition of fat; by improving the overall protein quality by using a variety of high quality animal and vegetable protein concentrates; and by providing calcium, phosphorus, and trace minerals in adequate quantities and in a form readily available to the laying hen.

The low-energy ration, the same ration that was fed to the Oklahoma Agricultural and Mechanical College laying flock in 1949 (Table III) contained approximately 1188 calories of metabolizable energy per pound calculated from the data of Titus (1955). The two high-energy rations contained approximately 1256 calories of metabolizable energy per pound. This ration was fed with both 1 NRC and 2 NRC vitamin levels. The high energy 2 NRC + NB ration, containing two percent of fat thus supplying 1318 calories of metabolizable energy per pound. New feed was mixed about every three weeks and a representative sample was taken for chemical analysis.

The birds were transferred to the cage house in mid-October although the experiment did not begin until October 27, 1954. A few days before the experiment began each pullet was leg-banded, wormed with nicotine sulphate and deloused with sodium floride. During the first two weeks of the experimental period each pullet was again wormed and deloused. Each pullet was weighed at the beginning of the experiment.

The pullets were housed in a 12' x 60' cage house. Ventilation was manually controlled and the temperature and humidity varied with atmospheric conditions. Supplemental lights were used when necessary to provide a day length of about 13 to 15 hours. Each cage was equipped with an individual feeder and an automatic water supply whose temperature was kept above freezing by an electric water heater. There were 48 cages facing south and another 48 cages facing north. The cages on the south were numbered 1 through 48 and the cages on the north were numbered 49 through 96. The birds in the lot that were to be fed a high-energy ration with the normal NRC allowances of vitamins were placed in cages numbering from 1 to 20. The second lot that was to be fed the same high-energy ration with approximately double the normal NRC allowances was placed in cages numbering 29 to 48. The third lot that was fed the high-energy 2 NRC + NB ration with approximately double the NRC allowances was placed in cages numbering from 49 to 68. The fourth lot that was fed the low-energy ration with the normal NRC allowances was placed in cages numbering from 77 to 96. There were 16 cages left in the center of the cage house, eight cages on each side. These cages, known as metabolism cages, had a hanging shelf beneath them on which pyrex glass trays were placed in order to collect droppings. During the three days which fecal samples were collected, ethyl alcohol was added to each tray in order that the bacterial action of the droppings be kept at a minimum. It was assumed that the droppings voided during each three day fecal collection were representative of the feed eaten during the same time interval.

At the end of each three-day collection the droppings were placed in polyethylene bags, and then quick frozen. Later these bags were stored at a temperature well below freezing until such time as they could be analysed. The droppings were collected about once each month. As there were only 16 of these metabolism cages, only four pullets from each of the four lots could be placed in the metabolism cages. This means there were five groups of 16 birds each. They were placed in these cages in a rotation of three days for each group of the 16 hens. They were placed in the metabolism cages on Sunday and Wednesday and taken out of the cages on Wednesday and Saturday. It required two and a half weeks to complete one collection period.

The bird's weight in pounds and the consumption of feed in grams were recorded for each bird in the metabolism cages.

Sample Selection

If the effect of ration on metabolism were to be measured adequately, it was highly desirable that these pullets be as uniform as possible. Seven pullets from each of the four treatments were selected on the basis of total weight of eggs produced. It was noted later that the average body weight of the pullets in the four treatments was approximately the same. This was important as the body weight of the hen and the rate of egg production are the most important factors that affect the amount of feed required per unit weight of egg.

Due to the large number of samples to be analysed, only samples of droppings collected in November, December, April, and June were used. The November and December collections were used to establish a basis for comparison during subsequent collection periods. The April collection was selected, as this was a period when the temperature began to rise. It is well known that the temperature is a controlling factor in the efficiency of feed utilization. The June collection was selected, as it was the last collection period available at the time of analysis.

Analysis

The feed and droppings were analyzed using standard feed analysis methods as outlined by A.O.A.C. (1950). Phosphorus was analysed by a variation of the A.O.A.C. method known as the Koenig-Johnson (1942) method. The samples were analyzed for total dry matter, protein, calcium and phosphorus. The energy of the feed and droppings were analyzed with the aid of an Emerson bomb calorimeter, in which the standard method of energy determination by the use of the bomb calorimeter was used. After the frozen droppings were thawed they were homogenized with a Waring blender for about five minutes. Water was added to the mixture until it reached a volume of exactly one liter. The mixture was homogenized again and a 300 ml. aliquot was quickly taken by means of a transfer pipette and then transferred to an aluminum foil evaporating dish, which was placed on either a steam plate or a hot plate overnight. Most of the liquid was removed in this way; but in order that the samples be completely dry, they were placed in a forced-draft drying-oven at about 130^{o} F. for approximately eight hours. The dry weight was taken and from here on the procedure was that of routine feed analysis. In order to obtain adjusted means for each constituent which was based upon a uniform level of feed intake, an analysis of covariance as outlined by Snedecor (1946) was applied to the percentage of utilization data. In order to obtain adjusted means for each constituent based upon a uniform level of feed intake, the data from each lot and each collection period were analyzed separately.

The Byerly Partition Method

The data collected was also analysed by the use of the Byerly partition equation in order to determine the relative efficiency of the rations fed these pullets.

It has been demonstrated that the body weight of the laying hen and the rate of egg production profoundly affect the amount of feed required per unit weight of egg. It was shown by Byerly (1941) that as the weight of the hen increased more feed was required for maintenance. Thus a five pound hen would require more feed per unit of egg weight than would be required by a three pound hen.

For the most part the feed consumed by a hen is used for maintenance and egg production. That quantity of the feed which is required for the maintenance of a hen of a given live weight remains more or less constant, no matter what the rate of egg production may be. The feed required for egg production, however increases as the rate of egg production increases. Thus, it has been demonstrated (Winter and Funk, 1947) that the energy requirement of a four to five pound hen include 0.145 to 0.227 pounds of feed daily for maintenance, and 0.078 to 0.1 pound for egg production, or a total of 0.223 to 0.327 pounds. Thus, when hens are producing at a high rate, the quantity of feed required per unit of egg weight is less than that of hens producing at a lower rate. The weight of the eggs produced per pound of feed increases as egg production increases.

In many of the studies of the effect of ration on egg production, the efficiency of production either has been ignored or has been calculated in terms of weight of eggs produced per unit weight of feed consumed, without regard to change in body weight or to maintenance requirement.

In order to compare efficiencies, it was necessary to have an adequate partition equation which would include the effect of the body weight of the hen, change in body weight, and average daily egg weight on the efficiency of feed utilization. The equation derived by Byerly (1941) was the equation used in order to determine the amount of feed necessary to feed a pullet of a certain weight, with a certain daily weight change and producing eggs of a certain average daily weight. This predicted feed consumption was compared to the actual feed consumption in order that the relative efficiency of the ration might be determined.

Relative efficiency

$$= \frac{100 \text{ X predicted feed consumption}}{\text{Observed feed consumption}}$$

Predicted rate of feed consumption

$F = 0.523\ W^{0.653} \pm 1.126\ \Delta W + 1.135\ E$ in which

F = predicted feed consumption in grams per hen per day

W = average hen weight in grams

ΔW = average daily weight change of hen in grams

E = grams of eggs produced per hen per day

RESULTS

Metabolism Trial

It was demonstrated (Table IV) that in the first three of the four collection periods there was a significant difference in energy utilization between the three high and the low-energy levels. There was no apparent difference (Figure 1), however in the utilization of the energy of the high-energy 2 NRC + NB, high-energy 2 NRC and the high-energy 1 NRC ration. The observations, therefore, indicated that the vitamin levels apparently had no effect upon energy utilization within a given energy level.

It was noted that there was a decrease in the energy utilization in pullets fed high-energy rations during the warm months of April and June. In contrast, the energy utilization of the pullets fed the low-energy requirement remained relatively constant. Since the energy requirement of the pullets, for maintenance would be greater during the cold weather months, it would be reasonable to assume that at this time the energy utilization would be higher than during the warmer months.

A significant difference of nitrogen utilization was demonstrated in two out of the four collection periods. Although there was little difference in nitrogen utilization, the pullets fed the high-energy 2 NRC + NB ration, with one exception, utilized nitrogen more efficiently than the pullets fed the other three rations. The pullets fed the high-energy 1 NRC ration used nitrogen less efficiently while

Table IV Level of significance and corresponding F values for adjusted mean values of energy, total nitrogen, calcium and phosphorus utilization

	Probability level Percent	F value	Probability level Percent	F value
Periods	November		December	
Total Nitrogen	88.0	2.19	50.0	0.51
Energy	99.8	7.05	98.5	4.27
Calcium	50.0	0.22	50.0	0.63
Phosphorus	50.0	0.95	80.0	1.72
Periods	April		June	
Total Nitrogen	54.5	0.94	83.5	1.84
Energy	99.7	6.38	50.5	0.82
Calcium	91.2	2.51	50.0	0.28
Phosphorus	91.3	2.56	50.0	-0.06

PERCENT UTILIZATION OF TOTAL ENERGY

TOTAL NITROGEN, CALCIUM AND PHOSPHORUS

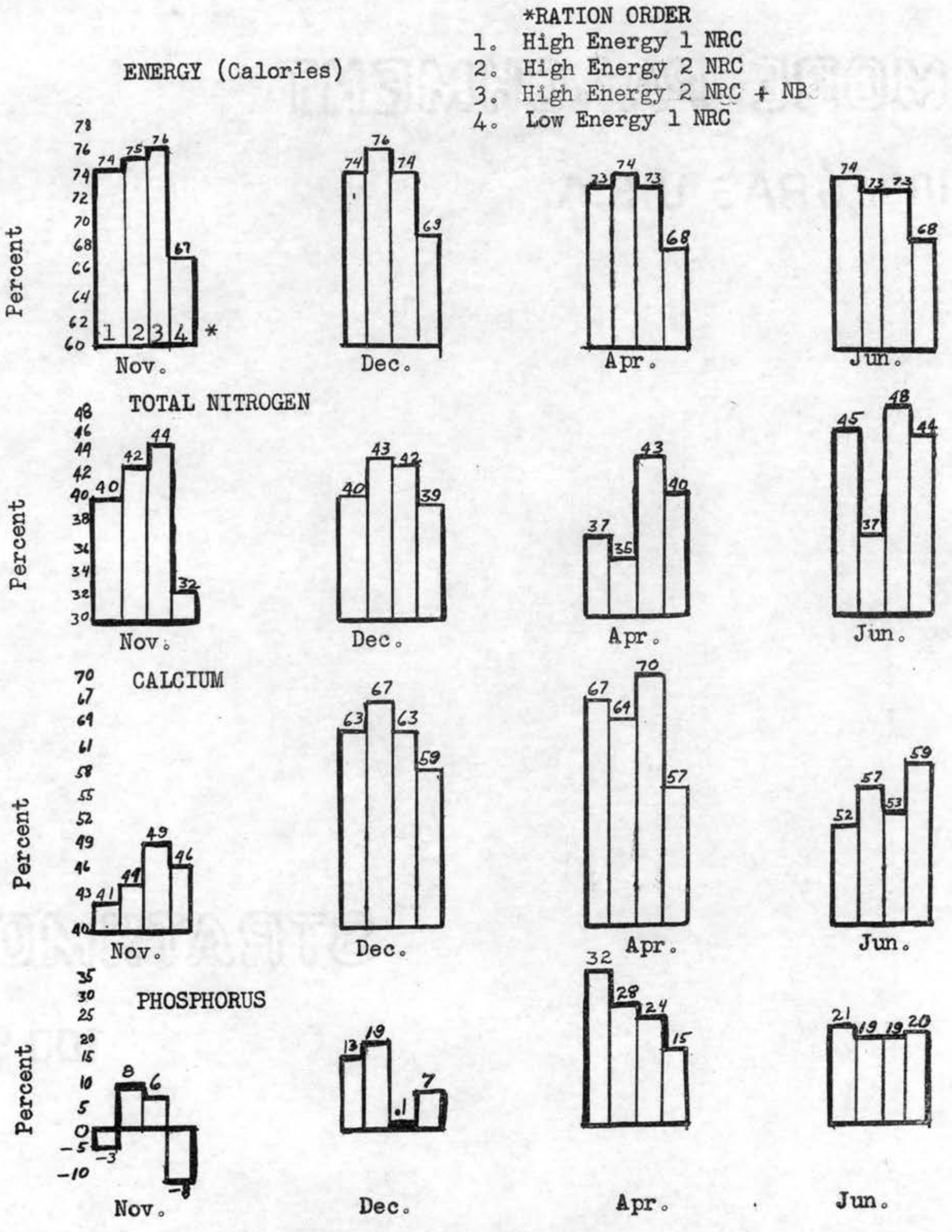


FIGURE 1. Effect of energy and vitamin levels on the percentage utilization of energy, protein, calcium and phosphorus

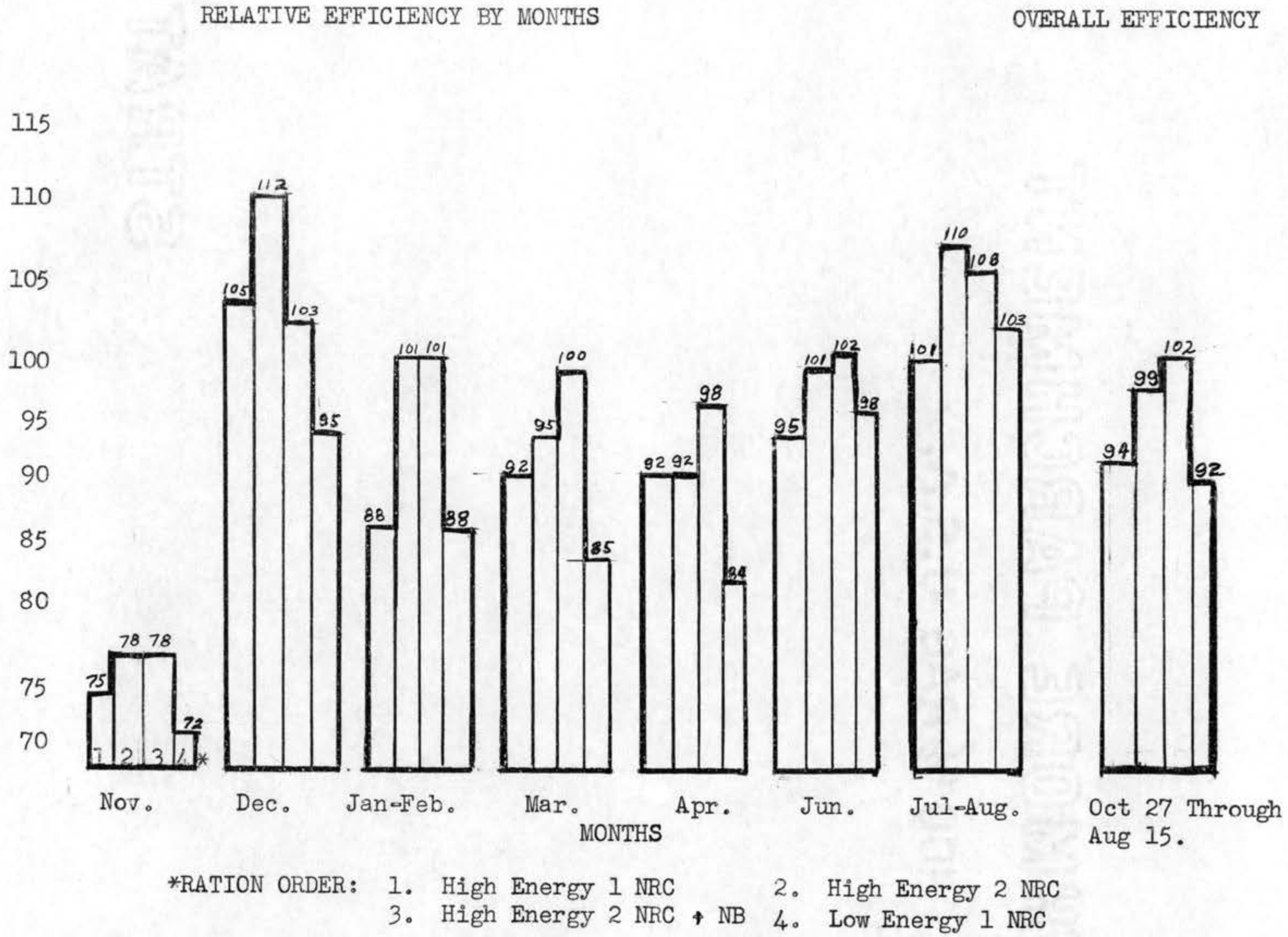
the high-energy 2 NRC ration and the low energy 1 NRC ration utilized nitrogen the least efficiently. It is not known just why the nitrogen utilization was so low when the high-energy 2 NRC ration was fed. It was noted during the first two months that the pullets fed the two high-energy 2 NRC rations demonstrated no differences in nitrogen utilization. However, in April the efficiency of nitrogen utilization with the high-enerhy 2 NRC ration had dropped sharply while the other three rations remained relatively constant. In June it was noted that the utilization of nitrogen with the high-energy 2 NRC ration increased slightly, while the remaining three rations increased their utilization of nitrogen rather sharply. It seems evident from these observations that there was something abnormal about the utilization of nitrogen during April and June. Nitrogen utilization of the pullets fed the low-energy 1 NRC ration increased progressively during the nine and a half month period.

The results of the calcium analyses showed there was a significant difference in the utilization of calcium between the high and low energy rations during the April collection period. The pullets fed the high-energy 2 NRC + NB ration were found to utilize calcium most efficiently during this period. Although no other significant differences were obtained in the utilization of calcium, it seemed to be most efficiently utilized by the pullets fed the high-energy 2 NRC rations. When the percent utilization of calcium for the four collection periods were averaged, it was shown that the pullets fed these two 2 NRC high-energy rations utilized an average of 59 and 58 percent calcium, respectively. The pullets fed the high-energy 1 NRC and the low-energy 1 NRC rations utilized an average of 56 and 54 percent calcium, respectively. This indicated that the difference in vitamin levels had some effect on calcium utilization. However, as these differences were neither significant nor consistent, no conclusions could be drawn from these results.

Although it was shown that there was a significant difference in phosphorus utilization between the pullets fed the high-energy rations and those fed the low-energy rations during the April collection period, there seemed to be little correlation between vitamin levels and utilization in the other collection periods. There seemed to be some indication however, that the pullets fed the high-energy rations utilized phosphorus more efficiently than those fed the low-energy ration. Since these results were neither consistent nor significant from period to period, no conclusion could be drawn.

The Byerly Partition Method

The relative efficiency values calculated by the use of the Byerly (1941) partition equation as described by Hill (1956) are shown graphically in Figure 2. In general, the most efficient utilization was obtained by pullets fed the high-energy 2 NRC ration and the pullets fed the high-energy 2 NRC + NB ration. Although there seemed to be essentially no difference in the relative efficiency of the pullets fed these two high-energy 2 NRC rations, it is evident that during the months of March, April and June the relative efficiency of the pullets fed the high-energy 2 NRC + NB ration remained approximately the same as during the January-February period. However, it was noted that the relative efficiency of the pullets fed the high-energy 2 NRC ration was decreased noticeably during March and April. This indicated that the pullets fed the high-energy 2 NRC + NB ration maintained its efficiency of utilization for longer periods of time than the pullets fed



*RATION ORDER:
1. High Energy 1 NRC
2. High Energy 2 NRC
3. High Energy 2 NRC + NB
4. Low Energy 1 NRC

Fig. 2. The relationship of relative efficiency of rations for hens to the energy and vitamin levels.

the high-energy 2 NRC ration.

Although there was little difference in the efficiency of feed utilization between the pullets fed the low-energy 1 NRC ration and the pullets fed the high-energy 1 NRC ration, it was demonstrated that from December through April the pullets fed the low-energy 1 NRC ration utilized their feed less efficiently than the pullets fed the other three rations. However, during the summer the relative efficiency of the pullets fed the low-energy ration improved and surpassed the relative efficiency of the high-energy 1 NRC ration. Thus it would seem that the high-energy rations were more efficiently utilized during the cooler months than during the warm summer months.

The overall relative efficiency of the entire experiment demonstrated that there seemed to be little difference between the low-energy 1 NRC ration and the high-energy 1 NRC ration.

The addition of two times the National Research Council's allowances of riboflavin, pantothenic acid, niacin and folic acid to the high-energy layer rations greatly increased the pullets' efficiency of feed utilization. The high-energy 2 NRC + NB ration was further improved by the use of proteins with a better balanced amino acid content, the addition of two percent fat, and by increasing the levels of available calcium and phosphorus.

CONCLUSIONS

1. The relative efficiency value as calculated by the use of the Byerly (1941) partition equation demonstrated that the most efficient utilization of the three treatments was obtained with the pullets fed the high-energy 2 NRC + NB ration. It was also indicated that the pullets fed the high-energy 2 NRC + NB ration maintained their efficiency of utilization for a longer period of time than those pullets fed the high-energy 2 NRC ration.
2. It was demonstrated that the pullets fed the low-energy 1 NRC ration improved in efficiency of feed utilization over the pullets fed the high-energy 1 NRC ration during the summer. Thus it would seem that the high-energy rations were more efficiently utilized during the cooler months than during the warm summer months.
3. It was evident from these data that the relative efficiency of a ration was increased by the addition of vitamins at levels of about twice the National Research Council's allowances. The vitamins for which increased levels were used were niacin, riboflavin, pantothenic acid and folic acid. The improvement in the efficiency of feed utilization by the pullets fed the high-energy 2 NRC + NB ration as compared to those fed the high-energy 2 NRC ration was due to the 2 percent of added fat, a better balance of amino acids, and an increase of the levels of available cal-

cium and phosphorus.

4. Vitamin levels apparently had no effect upon energy utilization, within a given energy level.
5. It was demonstrated also that energy was utilized more economically during the cooler months than during the warmer months.
6. Although there was only a significant difference in nitrogen utilization in two out of the four collection periods, it was demonstrated that the pullets fed the high-energy 2 NRC + NB ration, with one exception, utilized nitrogen more efficiently than the other three rations.
7. The difference in nitrogen utilization apparently was not affected by vitamin levels, as the pullets fed the high-energy 1 NRC ration retained only slightly less nitrogen than the pullets fed the high-energy 2 NRC + NB ration. It was demonstrated also that the pullets fed the high-energy 2 NRC ration retained considerably less nitrogen than the pullets fed the other high-energy rations.
8. The energy level did seem to improve the nitrogen utilization, as the average nitrogen utilization of the pullets fed the high-energy rations was higher than the average nitrogen utilization of those pullets fed the low-energy ration. The improvement in nitrogen utilization of the pullets fed the high-energy 2 NRC + NB ration over those fed the other high-energy rations was probably due to an increase in the energy level, and an increase in the quality of the protein used.
9. It was noted that calcium seemed to be more efficiently utilized by the pullets fed the high-energy 2 NRC + NB ration and the

high-energy 2 NRC ration. However, no conclusions could be drawn as the results were neither consistent nor significant from period to period.

10. Although no definite conclusions could be drawn, there did seem to be some indication that the pullets fed the high-energy rations utilized phosphorus more efficiently than did those fed the low-energy ration. These results, however, were neither significant nor consistent and showed little if any correlation between vitamin levels and efficiency of feed utilization from period to period.

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