THE UNIVERSITY OF ALBERTA

EFFECT OF ENERGY AND AMINO ACID LEVELS ON PROTEIN REQUIREMENTS OF POULTRY

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ABSTRACT

Experiments were conducted to study the effects of productive energy and amino acid levels of rations on the protein requirements of growing chicks and laying hens.

The results of the study with chicks indicated that the protein level of chick rations could be reduced below that usually recommended, without reducing rate of growth or feed efficiency, when the content of the most limiting amino acids was maintained at the same level as in the high protein rations. It was demonstrated that lysine was the most limiting essential amino acid for optimum growth of chicks fed rations containing meat meal as a protein supplement; methionine was not found to be limiting in this type of ration. The data obtained indicated that increasing the level of productive energy in the ration increased the requirement for lysine. Efficiency of feed conversion apparently was not affected by the productive energy level of the rations used.

An experiment with Single Comb White Leghorn pullets indicated that in rations which differed in productive energy content those containing 13.5 or 14.5 per cent of protein supported a higher rate of egg production than ones containing 12.5 per cent of protein. In addition, body weight loss was lower and average egg size was larger in the groups fed rations containing higher levels of protein. Average egg size was affected by the productive energy level

of the rations fed; the groups receiving low energy rations produced larger eggs than those fed high energy rations. The addition of amino acids to low protein rations tended to improve rate of egg production in the group fed the high energy ration but did not affect rate of production in the group fed the low energy ration. Blood plasma cholesterol level and hemoglobin regeneration time apparently were not affected by the levels of energy and protein in the rations used in the experiment.

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INTRODUCTION

The trend towards specialization in the poultry industry has resulted in the adoption of improved methods of production with emphasis being placed on increased efficiency of production. Since feed is a major item in a poultry production program, a great deal of attention has been given to nutritional factors affecting efficiency of feed use.

The most important single factor governing the efficiency of a "complete" poultry ration is its energy content. As energy level is increased, a proportional increase in efficiency of production generally occurs. This has resulted in progressive increases in the energy concentration of poultry rations during the past few years.

As energy level is increased and efficiency of production is improved, requirements for other nutrients in the ration are increased. In formulating rations, the procedure that has been followed has been to adjust the level of nutrients more or less in proportion to the energy content of the ration. The validity of this procedure is questionable since the nutrient interrelationships involved have not been fully established. Consequently, rations so devised may not represent the most efficient use of all nutrients.

In view of the possible importance of inter-relationships that may exist between constituents of a

ration, experiments were undertaken to study the effect of energy and amino acid levels on protein requirements of poultry.

REVIEW OF LITERATURE

A. History

In recent years many experiments have been conducted to study the effects of different dietary energy levels on the productive performance of poultry. Experiments in this area of poultry nutrition were stimulated by the studies of Fraps (1946) which indicated a wide variation in the energy level of poultry feed ingredients. The values reported showed that the more fibrous feedstuffs generally supplied a lower level of productive energy.

It had been noted previously that feeds of lower fibre content, and consequently higher productive energy concentration, were more effective in poultry rations than feeds of higher fibre content. Heuser *et al.* (1945) observed that the replacement of ingredients such as oats and wheat by-products by less fibrous ingredients such as corn and wheat yielded rations that supported better growth, egg production, and maintenance of body weight. Bird and Whitson (1946) concluded that fibre contributed by wheat by-products, oats, and alfalfa meal, while not affecting rate

of egg production, did exert a detrimental effect on efficiency of feed utilization.

In a short time, it was established that growth rate and efficiency of feed utilization of poultry are closely related to the productive energy content of the ration fed. Scott et al. (1947) reported that rations high in energy content promoted more rapid growth rate and better feed efficiency in chickens than those lower in energy. Panda and Combs (1950) noted that the crude fibre level of the ration influenced growth rate of chicks through its effect on the energy content of the ration. Subsequently, it was recognized that efficiency of production in poults (Dymsza et al., 1955) and laying hens (Hill et al., 1956) is also related to the productive energy level of the ration.

It soon became apparent that the productive energy content of a ration exerts a definite effect on food intake. Hill and Dansky (1954) observed that feed intake of chicks was primarily controlled by the productive energy content of the ration. Similarly, Peterson et al. (1954) noted that as the productive energy content of the ration decreased feed intake increased, within physiological limits, to satisfy the energy needs of the chick.

The indication of increased efficiency of production resulting from the use of higher levels of energy in poultry feeds focused attention on the relationships existing between

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energy concentration and the level of other nutrients in the ration. Numerous experiments relating to this aspect of poultry nutrition have been conducted with growing and laying birds. It is proposed to deal with the experiments relating to each in turn.

B. Effect of Energy Level of the Ration on Growth of Chicks

Growth rate, feed consumption, and feed efficiency

Following the studies (Fraps, 1946; Scott et al., 1947) which led to general recognition that level of productive energy in a ration exerted an effect on growth rate and feed efficiency a number of reports appeared which indicated that the principal effect of energy level of the ration was on efficiency of production, with lesser effects on rate of growth. Hill and Dansky (1954) found that rations ranging in productive energy content from 505 to 975 Calories per pound supported normal growth in chicks as measured by body weight; the chicks compensated for decreased energy level in the ration by increasing feed intake. Similarly, Peterson et al. (1954) and Williams and Grau (1956) noted that chicks were able to adjust feed intake on rations of varying energy level without affecting growth rate. Dymsza et al. (1955) observed that poults also were able to maintain weight gains by adjusting feed intake according to the productive energy level of the ration. Several reports with chicks (Leong et al., 1955; Donaldson et al., 1956;

and Leong *et al.*, 1959) and poults (Lockhart and Thayer, 1955; Dymsza *et al.*, 1955; and Ferguson *et al.*, 1956) indicated that the feed requirement per unit of gain decreased as the energy level of the ration was increased.

Protein requirements

The observation that feed intake and efficiency of feed use were influenced by the energy concentration of the ration led to studies of the relationships between productive energy concentration and protein requirements of poultry. Hill and Dansky (1950) found that growth rate of chicks fed a low protein-high energy ration could be increased by decreasing the energy content of the ration. Peterson *et al.* (1954) observed that, although reducing the energy content of low protein rations improved the growth rate of chicks, a similar energy reduction in rations containing optimum protein levels progressively reduced growth rate. Biely and March (1954) showed that improved feed efficiency accompanied an increased energy level only when the protein content of the ration was adequate. Sunde *et al.* (1956) noted that the growth rate of chicks on a high protein-low energy ration could be improved by increasing the energy content.

It soon was recognized that, in order to achieve optimum growth and feed efficiency, it was necessary to maintain a balance between energy concentration and protein

level of the ration. Combs and Romoser (1955) observed that the optimum Calorie-protein ratio (C/P ratio) in broiler rations was approximately 42 Calories of productive energy per pound for each per cent of crude protein in the ration. Guttridge (1957) reported that a C/P ratio of 44:1 supported maximum growth in chicks to 6 weeks of age, while a ratio of 56:1 was adequate at 8 weeks of age. The wider ratio reflects the lessening need of the older bird for protein. Vondell and Ringrose (1958) demonstrated that, in general, rate of growth increased as the C/P ratio increased until a ratio of approximately 45:1 was attained. Higher ratios did not promote a more rapid rate of growth. The results, together with those cited earlier, suggest that the optimum C/P ratio in chick starters lies within the range of 42 to 45:1.

The ratio of energy to protein in rations for poults has also been shown to affect growth rate and feed efficiency. Lockhart and Thayer (1955), Ferguson *et al.* (1956), and Atkinson *et al.* (1956) reported optimum Calorie-protein ratios for poults of 29, 29, and 27 to 30:1, respectively.

Amino acid requirements

While the importance of maintaining a balance between energy and protein in rations of varying energy content was quickly recognized, it soon became apparent

that the concept should be modified to include relationships that might exist between energy level and essential amino acid content of the ration. Several reports appeared which indicated that the requirements for individual amino acids are related to energy content of the ration. Baldini and Rosenberg (1955) showed that the methionine requirement of chicks, expressed as a percentage of the ration, increased as the productive energy level of the ration increased. A similar relationship between productive energy level and methionine requirements of poults was reported by Baldini et al. (1957). Schwartz et al. (1958) demonstrated that a linear relationship existed between dietary energy and lysine requirements of chicks. In contrast to the above observations, Grau and Kamei (1950), Almquist (1952), and Griminger et al. (1956) reported that the amino acid requirements of growing birds increased at a decreasing rate as the protein level of the ration was increased.

More recent studies have demonstrated that amino acid requirements are probably directly related to the productive energy content of the ration. Rosenberg and Baldini (1957) noted that the methionine requirement of the chick increased as the protein level increased when sufficient energy was available from non-protein sources; however, when sufficient energy was not available, increased levels of protein did not result in a corresponding increase in methionine requirement. Gordon et al. (1958) observed

that within any one set or combination of protein supplements "the proportion of energy content to protein content appears to fix amino acid requirements of broilers". Consequently, it might be concluded that the amino acid requirements of growing birds (as a percentage of the ration) are primarily dependent upon the energy content of the ration.

In view of the implication that energy level governs individual amino acid requirements, Calorie-protein ratios alone are of little value unless the amino acid composition of the protein is considered. Williams and Grau (1956) showed that increased feed intake, and as a result increased lysine intake, in response to reduced energy levels in the ration resulted in increased rate of growth by chicks fed a lysine deficient ration. Schwartz *et al.* (1958) found that increasing the productive energy level of chick rations resulted in improved growth rate of chicks fed rations containing high levels of lysine, but gave no response with rations deficient in lysine.

The observation that amino acid requirements may be directly related to the energy level of a ration gave rise to the theory that protein levels in growing rations might be reduced, provided that the essential amino acids were present in sufficient quantity to maintain an optimum ratio with productive energy. Gordon *et al.* (1957) reported that it was possible to lower the protein level of

a broiler ration from 25 to 21 per cent, without affecting growth rate, by supplementing the ration with methionine; the most limiting amino acid. Balloun and Phillips (1957) have shown a similar reduction may be made in rations for turkey poults. Thus, the growth promoting potential of a protein may be governed by the level of the most limiting amino acid in relation to the energy level of the ration.

C. Effect of Energy Level of the Ration on Egg Production

Rate of egg production

The effect of productive energy level of the ration on rate of egg production has been found to be quite variable. In some experiments it has been shown that higher energy levels in the ration may increase egg production. Heuser *et al.* (1945) and Hill *et al.* (1956) observed a higher rate of production from hens receiving high energy rations than from those receiving low energy rations. In other experiments, energy level of the ration has had little or no effect on rate of egg production. Miller *et al.* (1957) reported that feeding rations with productive energy values ranging from 640 to 1,075 Calories per pound did not affect rate of egg production. Similarly, Dymsza *et al.* (1954) and Robblee and Clandinin (1959) found no indication that varying the energy level of the ration affected the rate of egg production in turkey breeding hens.

Efficiency of egg production

The greatest effect of productive energy level in rations for laying hens is on efficiency of egg production. Bird and Whitson (1946) reported that efficiency of egg production was related inversely to the fibre content of the ration. Hill et al. (1956) found that gross efficiency of egg production was markedly influenced by energy level. As the productive energy level of the ration was increased, the feed requirement per dozen eggs was reduced. Other investigators (Hochreich et al., 1958; Price et al., 1957; Miller et al., 1957; MacIntyre and Aitken, 1957; and McDaniel et al., 1959) have also reported that efficiency of egg production is more or less proportional to the productive energy level of the ration.

Although the effect of energy level on efficiency of egg production may indicate that laying hens eat to meet their energy needs, exceptions to this generalization have been reported. Singsen et al. (1958) observed that heavy meat-type hens appeared to consume more energy than required when fed a high-energy ration. Hill (1959) reported an inability of laying hens to control their energy intake when they were changed abruptly from rations of moderate energy content to those of higher energy concentration. Robblee and Clandinin (1959) found that the average daily feed consumption of turkey breeders was little affected by the productive energy level of the ration.

Protein requirement for egg production

The protein requirement of laying hens has been found to vary widely. A number of workers (Reid *et al.*, 1951; Milton and Ingram, 1957; Hochreich *et al.*, 1958; and Denton and Lillie, 1959) have reported that levels of 15 to 18 per cent of protein in rations are required for optimum egg production. Other workers (Carpenter *et al.*, 1954; Heywang *et al.*, 1955; Miller *et al.*, 1957; Thornton *et al.*, 1957; Johnson and Fisher, 1959; Frank and Waibel, 1959; and MacIntyre and Aitken, 1959) have indicated that satisfactory production may be obtained with protein levels of 12 to 15 per cent.

Since energy level of the ration affects efficiency of production, it might be expected that protein requirements of laying birds would be influenced by energy concentration in much the same way as the protein requirements of growing birds are affected. Berg and Bearse (1957) observed that rate of lay on a high energy-high protein ration was the same as on a low energy-low protein ration, while a low level of protein was inadequate on a high energy ration. Thornton and Whittet (1959) reported that 11 per cent of protein in a ration was comparable to higher levels of protein when the dietary energy level was reduced. Frank and Waibel (1959) noted that optimum egg production was obtained on a series of high and low energy rations by 15 and 12.5 per cent of protein, respectively.

There is considerable evidence that the laying hen is not too sensitive to differences in the Calorie-protein ratio of the ration. Miller _et al._ (1957) showed that increasing the productive energy content of rations from 640 to 1,075 Calories per pound at protein levels varying from 12.5 to 20.9 per cent (C/P ratios varied from 31 to 86:1) did not affect egg production or body weight maintenance in White Leghorn pullets. Thornton _et al._ (1957) reported that feeding rations in which the C/P ratios varied from 50 to 86:1 had no effect on rate of egg production. A similar lack of response by the laying hen to varying C/P ratios has been observed by MacIntyre and Aitken (1957), Touchburn and Naber (1959), and McDaniel _et al._ (1959).

Some of the variability that has been found in studies of the relationship between energy and protein in rations for laying hens may have been caused by differences in the level of essential amino acids in the ration. Attempts have been made to study the effect of amino acid composition of laying rations on productive performance, but these have not been extended to include consideration of the relationship between essential amino acid content and energy level of the ration. Griminger and Fisher (1959) in studies with low protein rations noted that the addition of gelatin improved performance while the addition of glutamic acid decreased production rate. It was concluded that amino acid balance may be an important factor in laying hen nutrition at marginal intakes of protein. The

experimental results of Carpenter et al. (1954) also implied that amino acid balance may be an important factor in determining the protein requirement of laying hens. It was observed that although 14 per cent of protein from plant sources supported normal egg production, 11 per cent of plant protein was inadequate; normal egg production was obtained at the lower level of protein when a high quality protein source, such as fish meal, supplied 3 per cent of protein in the ration. Middendorf et al. (1959) found that egg production was significantly improved by methionine and lysine supplementation of a corn-soybean oil meal ration containing 10.9 per cent crude protein. Thornton et al. (1957) also indicated that corn-soybean oil meal rations may be deficient in methionine and, depending on the protein level, possibly lysine.

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Experiments were designed to study:

I. Effect of productive energy and amino acid levels of rations on the protein requirement of growing chicks.

II. Effect of productive energy and amino acid levels of rations on the protein requirement of laying hens.

I. Effect of Productive Energy and Amino Acid Levels of Rations on the Protein Requirement of Growing Chicks.

Status of the Problem

The use of amino acid supplements in poultry feed formulation may be of considerable practical importance. In experiments on the role of productive energy concentration of rations on growth rate of chicks, it has been shown that a balance should be maintained between the productive energy and protein levels in the ration if optimum results are to be achieved; however, recent evidence would seem to indicate that it is not necessarily the energy-protein ratio which is critical but rather the energy-amino acid ratio (see "Review of Literature"). It therefore would appear that amino acid requirements may be directly related to the productive energy level of the ration. Assuming that such a relationship exists, it should be possible:

(1) to improve the quality of rations by supplementing with the most limiting amino acids, and

(2) to achieve as good or better growth rate and feed efficiency on low protein rations properly supplemented with amino acids as on higher protein rations.

Since the amino acids most likely to be limiting in practical-type chick rations are lysine and methionine, it seemed advisable to study the effect of additions of these amino acids to chick rations varying in productive energy and protein content.

Experimental (General)

The experimental procedure used was the same in all trials reported. Day-old chicks of mixed sexes were housed in electrically heated batteries with raised screen floors. Feed and water were supplied *ad libitum*. The trials were terminated at the end of 28 days at which time individual weights and feed consumption were recorded.

The composition of the basal rations used in the experiment is shown in Table 1. Modification of the protein content of the experimental rations was made in such a manner that the productive energy, calcium, and phosphorus levels of the resulting rations were essentially unaffected. Basal rations 1 and 4 were formulated to contain similar levels of protein (20 per cent) and productive energy (860 Calories per pound). The Calorie-protein ratio of 43:1 was within the range considered optimum for the chick. The calculated levels of essential amino acids in these

Table 1. - Composition of basal rations

INGREDIENTS	BASAL RATION NUMBER							
	1	2	3	4	5	6	7	8
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
Ground yellow corn	5	5	5	5	5	5	5	5
Ground wheat	57.35	57.35	57.35	49.30	53.05	56.80	71.80	79.30
Ground oats	2	7	12	4	4	4		
Wheat bran				5	5	5		
Wheat shorts (brown)	6.5	6.5	6.5	15	15	15		
Dehyd. alfalfa meal (20% Protein)	2	2	2	2	2	2	2	2
Meat meal (50% Protein)				17	12	7	19	9
Soybean oil meal (50% Protein)	20	15	10					
Multiphos	4.5	4.5	4.5		1.25	2.50		2.50
Ground limestone	1	1	1	1	1	1	.50	.50
Iodized salt (0.01% KI)	.35	.35	.35	.35	.35	.35	.35	.35
Fish oil (2,250 A. 300 D/gm.)	.10	.10	.10	.10	.10	.10	.10	.10
Vitamins (1)	1	1	1	1	1	1	1	1
Manganese sulfate (0.5 lb./ton)	+	+	+	+	+	+	+	+
Zinc sulfate heptahydrate (2)	+	+	+	+	+	+	+	+
DL-methionine	.20	.20	.20	.25	.25	.25	.25	.25
Calculated Analysis								
Protein - % -	20	18	16	20	18	16	20	16
Productive Energy (Therms/100 lb.) (3)	86.9	86.8	86.6	86.6	86.1	85.6	97.0	95.9

(1) Vitamins and antibiotic were added at the following levels per pound of ration: Vitamin A palmitate 1,135 I.U., vitamin D_3 375 I.C.U., d-alpha-tocopheryl acetate 10 I.U., vitamin B_{12} 0.003 mg., choline chloride 227 mg., riboflavin 1.5 mg., calcium pantothenate 5 mg., niacin 10 mg., folic acid 0.5 mg., biotin 0.125 mg., and procaine penicillin G 2 mg.

(2) Zinc sulfate heptahydrate was added at a level equivalent to 30 p.p.m. of zinc.

(3) Productive energy levels were calculated using values reported by Fraps (1946) for all ingredients, except meat meal and soybean oil meal, which were based on values reported by Titus (1955).

rations (Block and Weiss, 1956) were sufficiently high to meet the requirements of the chick (National Research Council, 1954). Basal rations 2 and 3 were similar to Basal ration 1 while Basal rations 5 and 6 were similar to Basal ration 4, except that the protein levels were reduced in each case to 18 and 16 per cent, respectively. Basal rations 7 and 8 were similar to Basal rations 4 and 6, respectively, except that the productive energy level in the rations was increased by substituting wheat and meat meal for oats, wheat bran, and wheat shorts.

Trial 1

Object

To determine the effect of maintaining a constant level of lysine and methionine in rations with the same productive energy concentration but varying in protein content.

Experimental

One hundred and eighty White Plymouth Rock chicks were divided into 10 comparable groups of 18 birds each. The experimental procedure followed was the same as that outlined previously.

The treatments used are shown in Table 2. Lysine and methionine additions to the rations containing 18 and 16 per cent of protein increased the content of these amino acids to the same level as in the rations containing 20 per cent of protein.

Results and Discussion

A summary of the effect of treatment on average weight and efficiency of feed conversion of chicks at 28 days of age is presented in Table 2.

Table 2. - Effect of protein level and amino acid supplementation on average weight and efficiency of feed conversion of chicks

	TREATMENT				
GROUP NUMBER	BASAL RATION USED	LEVEL OF PROTEIN	AMINO ACID SUPPLEMENTATION	AVERAGE WEIGHT 28-DAYS	EFFICIENCY OF FEED CONVERSION
		%		gm.	gm.feed/ gm.gain
Soybean Oil Meal Series					
1	1	20	None	366	1.9
2	2	18	None	367	1.9
3	3	16	None	267	2.1
4	2	18	0.05% DL-methionine + 0.15% L-lysine	413	2.0
5	3	16	0.10% DL-methionine + 0.30% L-lysine	331	2.0
Meat Meal Series					
6	4	20	None	329	2.0
7	5	18	None	275	2.2
8	6	16	None	203	2.1
9	5	18	0.05% DL-methionine + 0.15% L-lysine	372	2.1
10	6	16	0.10% DL-methionine + 0.30% L-lysine	320	1.9

It is evident from the results obtained that the plane of growth of chicks fed rations in which soybean oil meal was used as the protein supplement was superior to that of chicks fed similar rations in which meat meal was used. Little difference in efficiency of feed conversion was noted.

In the meat meal series (Groups 6 - 8) each reduction in the level of protein in the ration resulted in a decline in rate of growth. In the soybean oil meal series (Groups 1 - 3) similar reductions in the level of protein in the rations resulted in a decrease in rate of growth only in the group receiving the ration containing 16 per cent of protein (Group 3).

The addition of lysine and methionine to the lower protein rations produced a marked improvement in rate of growth of chicks as compared to that of those fed the unsupplemented rations. The improvement was greatest in the groups fed rations which contained meat meal as the protein supplement. When rations containing 18 per cent of protein were supplemented with lysine and methionine the average weights of chicks fed the rations were considerably higher than the average weights of chicks fed rations containing 20 per cent of protein (Groups 4 and 9 vs. Groups 1 and 6, respectively). No explanation can be offered for the increased rate of growth noted since the addition of lysine and methionine presumably maintained the contents of these amino acids at the same levels as in the higher protein

rations. In the soybean oil meal series, the rate of growth of chicks fed the 16 per cent protein ration supplemented with lysine and methionine (Group 5) was slightly lower than that of chicks fed the ration containing 20 per cent of protein (Group 1). In the meat meal series the average weight of chicks fed the ration containing 16 per cent of protein supplemented with lysine and methionine (Group 10) was comparable to that of chicks fed the ration containing 20 per cent of protein (Group 6). Hence it would appear that with certain rations amino acid supplementation may permit a reduction in the level of protein required by the chick.

Summary

(1) Reductions in the level of protein in rations of the same productive energy concentration generally resulted in a decline in rate of growth of chicks.

(2) The addition of lysine and methionine to low protein rations resulted in a marked improvement in the average weight of chicks.

(3) In rations containing meat meal it was possible to reduce the level of protein without reducing rate of growth of chicks when the content of lysine and methionine was maintained at the same level as in the higher protein ration.

(4) Efficiency of feed conversion was not affected by the protein or amino acid levels used.

Trial 2

Object

The results of the previous experiment indicated that the addition of lysine and methionine to low protein rations containing meat meal resulted in growth comparable to that obtained on a higher protein ration. Since it has been reported by Kratzer and Davis (1959) that meat meal may also be deficient in tryptophan for the chick, a trial was undertaken to study the effect of adding lysine, methionine, and tryptophan, singly or in combination, to low protein rations containing meat meal as a source of protein supplement.

Experimental

One hundred and eighty White Plymouth Rock chicks were divided into 9 comparable groups of 20 birds each and fed the experimental rations. The experimental procedure followed was the same as outlined previously.

An outline of the basal rations used and the level of amino acid supplementation employed is shown in Table 3. The amino acid additions were made to provide levels equivalent to those in the ration containing 20 per cent of protein (Basal ration 4).

Results and Discussion

The effect of treatment used on average weight and efficiency of feed conversion at 28 days of age is presented in Table 3.

Table 3. - Effect of amino acid supplementation on growth rate and efficiency of feed conversion of chicks

GROUP NUMBER	TREATMENT: BASAL RATION USED	TREATMENT: LEVEL OF PROTEIN	TREATMENT: AMINO ACID SUPPLEMENTATION	AVERAGE WEIGHT 28-DAYS	EFFICIENCY OF FEED CONVERSION
		%		gm.	gm.feed/ gm.gain
11	4	20	None	243	2.2
12	6	16	None	165	2.4
13	6	16	0.30% L-lysine	216	2.3
14	6	16	0.025% L-tryptophan	156	2.4
15	6	16	0.10% DL-methionine	153	2.6
16	6	16	0.30% L-lysine + 0.025% L-tryptophan	222	1.9
17	6	16	0.30% L-lysine + 0.10% DL-methionine	205	2.3
18	6	16	0.025% L-tryptophan + 0.10% DL-methionine	162	2.4
19	6	16	0.30% L-lysine + 0.025% L-tryptophan + 0.10% DL-methionine	191	2.2

A low plane of growth and poor efficiency of feed conversion was noted in this trial. Although rate of growth was below normal, it was apparent that the addition of lysine was effective in improving rate of growth (Groups 13, 16, 17 and 19). The addition of lysine, either alone or in combination with other amino acids, did not result in growth rate equal to that of the ration containing 20 per cent of protein (Group 11). The addition of methionine and/or

tryptophan apparently had no effect on rate of growth or feed efficiency. It therefore appeared that lysine was the most limiting amino acid in low protein rations supplemented with meat meal. (It should be noted, however, that the basal rations used contained supplemental methionine since calculated amino acid levels had shown insufficient methionine to meet the requirement of the chick.)

Summary

(1) The addition of lysine to low protein rations containing meat meal resulted in an improvement in rate of growth of chicks fed these rations.

(2) The addition of tryptophan and/or methionine, whether alone or in combination with lysine, had no apparent effect on rate of growth of chicks fed the low protein ration.

(3) It appeared that lysine was the most limiting amino acid in low protein rations containing meat meal as the protein supplement.

Trial 3

Object

The results of Trial 2 supported the observations of March *et al.* (1950) and Patrick (1953) that lysine was the principal amino acid deficiency in rations containing meat meal as a source of protein. Since the energy level of the ration may be important in determining the dietary requirement of an amino acid, a study was initiated to ascertain the effect of productive energy and protein level on the lysine requirements of chicks fed rations supplemented with meat meal.

Experimental

Two hundred and forty crossbred New Hampshire x White Plymouth Rock chicks were divided into 12 comparable groups of 20 chicks each and fed the experimental rations. The experimental procedures followed were the same as those used in the previous trials.

The basal rations used (Basal rations 4, 6, 7, and 8) supplied 2 levels of protein (20 and 16 per cent) and 2 levels of productive energy (860 and 970 Calories per pound). Lysine was added to the basal rations at levels of 0, 0.2, and 0.4 per cent. The highest level of added lysine was calculated to supply the chicks' requirement on the high energy-low protein ration. An outline of the treatments used is shown in Table 4.

Results and Discussion

The effect of ration treatment on average weight and efficiency of feed conversion is summarized in Table 4.

Table 4. - Effect of lysine supplementation on rate of growth and efficiency of feed conversion of chicks

GROUP NUMBER	TREATMENT: BASAL RATION USED	TREATMENT: DESCRIPTION OF THE RATION	AVERAGE WEIGHT 28-DAYS	EFFICIENCY OF FEED CONVERSION
			gm.	gm.feed/ gm.gain
Low Energy Series				
20	4	20% Protein	344	2.0
21	4	20% Protein + 0.2% L-lysine	408	1.8
22	4	20% Protein + 0.4% L-lysine	390	1.8
23	6	16% Protein	225	2.4
24	6	16% Protein + 0.2% L-lysine	351	2.0
25	6	16% Protein + 0.4% L-lysine	371	1.9
High Energy Series				
26	7	20% Protein	307	2.0
27	7	20% Protein + 0.2% L-lysine	379	1.7
28	7	20% Protein + 0.4% L-lysine	375	1.7
29	8	16% Protein	173	2.4
30	8	16% Protein + 0.2% L-lysine	308	2.0
31	8	16% Protein + 0.4% L-lysine	348	2.0

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It is interesting to note that additions of lysine resulted in increased rate of growth of chicks. The greatest increase in weight gains occurred on the low protein rations; particularly at the higher level of productive energy (Groups 30 and 31 vs. Group 29). Although a level of 0.2 per cent of added lysine resulted in optimum growth of chicks fed the "20% protein" rations (Groups 21 and 27) a level of 0.4 per cent of added lysine was required for those fed the "16% protein" rations (Groups 25 and 31). The highest level of supplemental lysine produced the greatest percentage increase in rate of growth on the high energy-low protein ration (Group 31 vs. Group 29).

The results of the study indicate that lysine was limiting for growth of chicks fed rations containing meat meal as the protein supplement and that the deficiency was more critical in the high energy-low protein rations. The growth data support the observations of Baldini and Rosenberg (1955) that amino acid requirement, expressed as a percentage of the ration, increases as the productive energy level of the ration increases.

The feed conversion data indicated that feed efficiency was not affected by the productive energy content of the ration. Efficiency of feed conversion appeared to be inversely related to the rate of growth obtained; consequently, additions of lysine improved feed efficiency through their effect on rate of growth of the chicks.

Summary

(1) Additions of lysine to rations containing meat meal as the protein supplement resulted in a marked increase in the rate of growth of chicks fed the rations.

(2) A level of 0.2 per cent added lysine resulted in optimum rate of growth of chicks fed high protein rations while a level of 0.4 per cent added lysine was required for optimum growth of chicks fed the low protein rations.

(3) Efficiency of feed conversion was not affected by the energy level of the ration; differences which occurred were closely related to the rate of growth obtained.

Trial 4

Object

Since the basal rations used in the previous trials contained added methionine, the present study was initiated to determine whether supplementary methionine was required and, if so, the most effective levels for chicks fed rations supplemented with meat meal and containing varying levels of productive energy and protein.

Experimental

Two hundred and forty crossbred New Hampshire x White Plymouth Rock chicks were divided into 12 comparable groups of 20 chicks each and fed the experimental rations. The experimental procedures followed were the same as those used in the previous trials.

The basal rations used (Basal rations 4, 6, 7, and 8) were modified by removal of methionine from the rations. A level of 0.4 per cent of L-lysine monohydrochloride was added to these basal rations to supply the chicks' requirement for lysine. The rations supplied 2 levels of productive energy (860 and 970 Calories per pound) and 2 levels of protein (16 and 20 per cent). Methionine was added to the modified basal rations at levels of 0, 0.2, and 0.4 per cent. An outline of the treatments used is shown in Table 5.

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Results and Discussion

The effects of treatment used on average weight and efficiency of feed conversion at 4 weeks of age are shown in Table 5.

Table 5. - Effect of methionine supplementation on average weight and efficiency of feed conversion of chicks

GROUP NUMBER	TREATMENT: BASAL RATION USED	TREATMENT: DESCRIPTION OF THE RATION	AVERAGE WEIGHT 28-DAYS	EFFICIENCY OF FEED CONVERSION
			gm.	gm.feed/ gm.gain
		Low Energy Series		
32	4	20% Protein	359	1.9
33	4	20% Protein + 0.2% DL-methionine	361	1.8
34	4	20% Protein + 0.4% DL-methionine	367	1.9
35	6	16% Protein	302	2.1
36	6	16% Protein + 0.2% DL-methionine	315	2.0
37	6	16% Protein + 0.4% DL-methionine	307	2.0
		High Energy Series		
38	7	20% Protein	343	1.8
39	7	20% Protein + 0.2% DL-methionine	332	1.7
40	7	20% Protein + 0.4% DL-methionine	340	1.7
41	8	16% Protein	293	2.0
42	8	16% Protein + 0.2% DL-methionine	300	1.9
43	8	16% Protein + 0.4% DL-methionine	296	1.9

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[illegible]	[illegible]	[illegible]	[illegible]

The addition of methionine to the basal rations did not affect the average weight of chicks at 4 weeks of age. Since the calculated levels of methionine in the basal rations were well below the chicks' requirement (National Research Council, 1954) in all cases, it may be concluded that either the indicated requirements for methionine are too high or the average composition values used in calculating levels of methionine in the ration were too low. A recent report (Klain *et al.*, 1960) gives some indication that the methionine requirement of chicks may be lower than has been reported previously.

Efficiency of feed conversion tended to be improved by the addition of 0.2 per cent of methionine to the basal rations. Increasing the level of supplementation to 0.4 per cent of methionine resulted in no further improvement in efficiency.

The use of the higher energy rations resulted in a lower rate of growth than when lower energy rations were used; however, there was no indication that methionine requirement was, in any way, related to the level of productive energy in the ration. It is evident from the results of the experiment that methionine is not a limiting amino acid in practical-type rations in which meat meal is used as the protein supplement.

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Summary

(1) The methionine level of rations containing meat meal as the protein supplement did not limit the rate of growth of chicks fed the rations.

(2) The addition of 0.2 per cent of methionine resulted in some improvement in efficiency of feed conversion.

(3) The levels of productive energy in the rations apparently had no effect on the methionine requirement of chicks.

II. Effect of Productive Energy and Amino Acid Levels of Rations on the Protein Requirement of Laying Hens.

Status of the Problem

A level of 15 per cent of protein in rations for laying hens has been recommended (National Research Council, 1954); however, satisfactory results have been reported with lower levels of protein in laying rations. The recommendation was based, for the most part, on experiments involving rations in which corn was the principal grain. Since rations containing corn tend to be higher in productive energy content than those in which wheat, oats, and barley are used, and since it has been indicated that protein requirement may be directly related to energy content of the ration, it would be of interest to ascertain minimum protein requirements for egg production with rations containing the principal Canadian grains. In addition, the possibility that amino acid levels may be important in determining protein requirements has been suggested. Since information on the effects of essential amino acid levels, in relation to energy concentration of the ration, on the protein requirements of laying hens was lacking, it seemed desirable to determine whether this relationship was of importance. Consequently, an experiment was initiated to study the effect of energy and amino acid levels on the protein requirements of laying hens fed rations containing wheat, oats, and barley as the grain components.

Experimental

Two hundred and forty Single Comb White Leghorn pullets were randomly distributed into 8 groups of 30 birds each and fed the experimental rations. The birds were housed (2 birds per cage) in laying batteries with raised screen floors. Feed, water, oyster shell, and insoluble grit were supplied *ad libitum*.

The rations used are shown in Table 6. The rations were formulated to contain 3 levels of protein (12.5, 13.5, and 14.5 per cent) and 2 levels of productive energy (760 and 900 Calories per pound of ration). In addition the ration containing 12.5 per cent of protein at each energy level was supplemented with lysine, methionine, and leucine (Rations 4 and 8). It was considered that these amino acids were the ones most likely to be limiting in the rations used.

Records were kept on mortality, egg production, feed consumption, body weight, and average egg weight for each of the groups. Date of mortality was noted; egg production for each cage (i.e. for each 2 birds) was recorded daily; feed consumption for each group was determined at 28-day intervals; the birds were weighed individually at the commencement of the experiment and at 28-day intervals thereafter; and average egg weight was obtained by weighing all eggs produced by each group during the 12th, 16th, 21st, and 24th week of the experiment.

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Table 6. - Composition of rations

INGREDIENTS	RATION NUMBER							
	1	2	3	4	5	6	7	8
	lb.	lb.	lb.	lb.	lb.	lb.	lb.	lb.
Ground wheat	38	38	38	38	83	83	83	83
Ground oats	25	25	25	25				
Ground barley	10	10	10	10				
Wheat bran	10	10	10	10				
Wheat shorts (brown)	6	3		6	6	3		6
Dehyd. alfalfa meal (20% Protein)	3	3	3	3	3	3	3	3
Soybean oil meal (50% Protein)		3	6			3	6	
Ground limestone	4	4	4	4	4	4	4	4
Multiphos	2.25	2.25	2.25	2.25	2.25	2.25	2.25	2.25
Iodized salt (0.01% KI)	.50	.50	.50	.50	.50	.50	.50	.50
Fish oil (2,250 A. 300 D/gm.)	.25	.25	.25	.25	.25	.25	.25	.25
Vitamin mix (1)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Manganese sulfate (28% Mn) (0.5 lb./ton)	+	+	+	+	+	+	+	+
L-Lysine monohydrochloride				.16				.16
DL-Methionine				.10				.10
L-Leucine				.30				.30
Calculated Analysis								
Protein - % -	12.5	13.5	14.5	13.0	12.6	13.6	14.6	13.1
Productive Energy (Therms/100 lb.) (2)	76	76	76	76	90	90	90	90

(1) Vitamins were added at the following levels per pound of ration: vitamin A palmitate 1,135 I.U., vitamin D_3 750 I.C.U., d-alpha-tocopheryl acetate 10 I.U., riboflavin 1.5 mg., niacin 7.5 mg., calcium pantothenate 4.5 mg., and folic acid 0.5 mg.

(2) Fraps (1946).

At the completion of the experimental period the hens were maintained on the same rations and determinations were made on blood plasma cholesterol levels and rate of hemoglobin regeneration. Plasma cholesterol levels were determined by the method of Zlatkis et al. (1953) on 10 birds from each treatment. The effect of energy and protein levels on hemoglobin regeneration was assessed by determining oxyhemoglobin levels on 6 birds from each treatment at intervals of 0, 1, 2, 4, 7, and 11 days following injection with 5 mg. of phenylhydrazine per pound of body weight (Nichol et al., 1949). The level of phenylhydrazine necessary to induce a severe anemia was determined by preliminary tests.

Results and Discussion

The effects of treatment on mortality, rate of egg production, efficiency of feed utilization, body weight changes, and average egg weight are presented in Table 7.

The results of the experiment indicated that rate of production was affected by the treatments used. The higher levels of protein in the rations resulted in an increased rate of production in both the low energy and high energy series. Rate of production on the low energy-low protein ration (Group 1) was considerably higher than on the high energy-low protein ration (Group 5). Addition of amino acids to the low energy-low protein ration (Group 4) had no effect; however, there was some indication that a

Table 7. - Effect of treatment on mortality, rate of production, feed efficiency, body weight, and average egg weight

GROUP NUMBER	TREATMENT		MORTALITY	RATE OF EGG PRODUCTION		FEED PER DOZEN EGGS	BODY WEIGHT CHANGES	AVERAGE EGG WEIGHT
	RATION NUMBER	DESCRIPTION OF RATION		HEN-HOUSED	HEN-DAY			
			%	%	%	lb.	%	oz./doz.
Low Energy Series (760 Cal./lb.)								
1	1	12.5% Protein	3.3	56.9	57.0	6.2	-12.4	25.0
2	2	13.5% Protein	3.3	58.2	59.5	5.8	-14.6	25.1
3	3	14.5% Protein	0	68.5	68.5	5.1	- 7.0	25.6
4	4	12.5% Protein + Amino Acids	0	52.6	52.6	6.2	-12.0	25.6
High Energy Series (900 Cal./lb.)								
5	5	12.6% Protein	3.3	45.3	46.0	6.6	-21.4	23.7
6	6	13.6% Protein	6.6	56.2	57.2	5.8	-11.6	24.2
7	7	14.6% Protein	0	57.8	57.8	5.6	- 7.8	24.9
8	8	12.6% Protein + Amino Acids	0	51.2	51.2	6.4	- 9.6	24.6

similar addition to the high energy-low protein ration (Group 8) was beneficial.

The effect of treatment on the rate of decline of egg production during the experimental period was assessed by calculating the individual regression coefficients for each group and conducting an analysis of errors of estimate from the average regression within the groups (Snedecor, 1946). The results of this analysis, presented in Table 8, show that the individual group regressions differed significantly.

Table 8. - Analysis of errors of estimate from average regression within groups

SOURCE OF VARIATION	D.F.	ERRORS OF ESTIMATE SUMS OF SQUARES	ERRORS OF ESTIMATE MEAN SQUARE	F
Deviation from average (error) regression within lots	87	1,058.71		
Deviation from individual lot regressions	80	679.11	8.489	
Difference among lot regressions	7	376.60	54.228	6.39**

** Significant at the 1 per cent level.

The regression equations are plotted for the 8 groups in Fig. 1. The group fed the low energy-high protein ration (Group 3) showed the least decline in rate of egg production

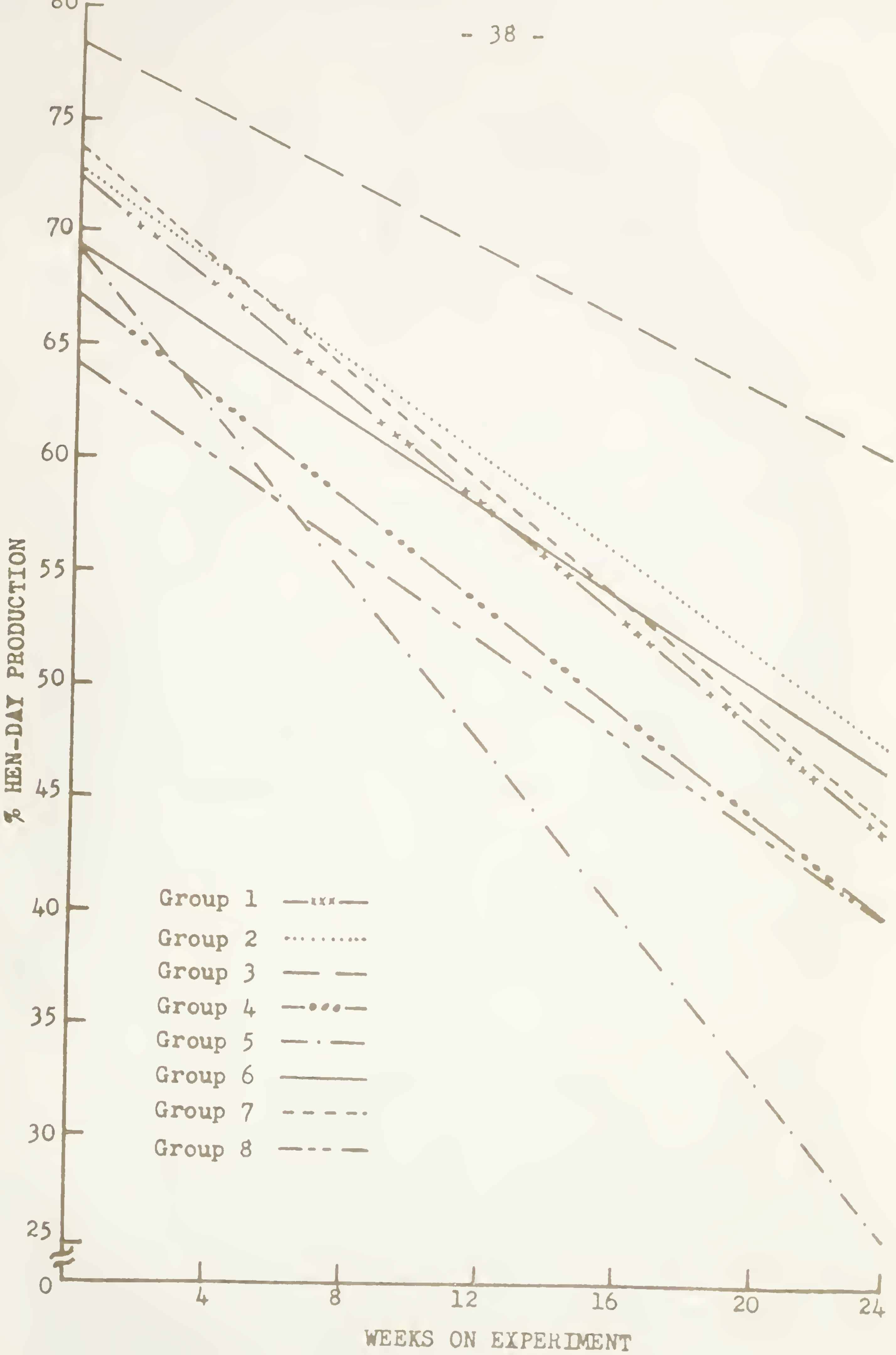

Figure 1. - Regression of Percentage Hen-Day Production on Time in Weeks

while the group fed the high energy-low protein ration (Group 5) showed the greatest decline. The regression lines for the other groups were closely grouped between the two extremes. The regressions also indicated that the addition of amino acids may have lessened the rate of decline in egg production on the high energy-low protein ration (Group 8 vs. Group 5). The results are in agreement with the conclusions of Griminger and Fisher (1959) that amino acid balance may be an important factor in the nutrition of the laying hen at marginal levels of protein intake.

The efficiency of feed conversion apparently was not affected by the productive energy level of the rations fed. Differences which occurred were more closely related to rate of production than to energy concentration of the ration. No explanation can be offered for the failure of energy level to affect the efficiency of production. The results, however, agree with the observation that a high degree of association exists between rate of production and feed required per unit of eggs produced (Miller and Quisenberry, 1959).

The data on average egg weights indicated that energy level of the ration affected egg size. Average egg weights were lower in all groups receiving the high energy rations than in those fed the low energy rations. The groups fed rations with higher levels of protein or rations to which amino acids were added showed higher average egg size than those fed the low protein rations.

Average body weight was influenced by the treatment used. Although all groups showed body weight losses during the experimental period, higher levels of protein or amino acid supplementation tended to lessen the loss in body weight. The results lend support to the suggestion by MacIntyre and Aitken (1957) that changes in body weight may serve as a good indication of the nutritional status of the bird.

The effect of treatment on blood plasma cholesterol levels is shown in Table 9. While considerable variability

Table 9. - Average blood plasma cholesterol level

GROUP NUMBER	TREATMENT	AVERAGE PLASMA CHOLESTEROL LEVEL
		mg. %
	Low Energy Series (760 Cal./lb.)	
1	12.5% Protein	209
2	13.5% Protein	191
3	14.5% Protein	188
4	12.5% Protein + Amino Acids	187
	High Energy Series (900 Cal./lb.)	
5	12.6% Protein	234
6	13.6% Protein	232
7	14.6% Protein	182
8	12.6% Protein + Amino Acids	190

was noted in blood plasma cholesterol levels within and between groups, an analysis of variance indicated that there was no significant difference in the average plasma cholesterol levels of the various groups. Kokatnur et al. (1958) observed that serum cholesterol level in adult Single Comb White Leghorn males seemed to show an inverse linear relationship with the absolute intake of protein.

The effect of treatment on observed hemoglobin regeneration time is presented in Table 10. The results

Table 10. - Effect of treatment on rate of hemoglobin regeneration following injection of phenylhydrazine

GROUP NUMBER	TREATMENT	OXYHEMOGLOBIN LEVELS DAYS AFTER PHENYLHYDRAZINE INJECTION					
		0	1	2	4	7	11
		mg./100 ml. blood					
	Low Energy Series						
1	12.5% Protein	7.9	5.1	4.1	5.1	7.1	8.4
2	13.5% Protein	7.2	5.4	3.4	4.6	6.4	8.8
3	14.5% Protein	6.2	4.8	3.5	4.7	6.4	8.4
	High Energy Series						
5	12.6% Protein	6.1	5.1	4.0	5.3	6.3	8.5
6	13.6% Protein	8.0	5.4	4.1	4.7	7.0	8.5
7	14.6% Protein	7.8	4.0	3.6	4.8	6.8	9.1

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obtained indicated that neither the protein nor the energy levels studied had any effect on hemoglobin regeneration time following a phenylhydrazine induced anemia.

Summary

(1) The higher levels of protein resulted in improved egg production of laying hens fed rations containing low and high productive energy contents.

(2) Amino acid supplementation had no effect on rate of egg production of hens fed a low energy-low protein ration; their addition appeared to improve egg production of hens fed a high energy-low protein ration.

(3) Efficiency of feed conversion apparently was not affected by the energy level of the rations fed.

(4) Average body weight declined in all groups during the experimental period; however, higher protein levels or amino acid supplementation tended to reduce body weight losses.

(5) Average egg weight was heavier in the groups fed low energy rations than in those fed high energy rations; higher levels of protein or amino acid supplementation also tended to increase egg size.

(6) Blood plasma cholesterol level and hemoglobin regeneration time were not affected by the energy or protein levels of the rations used.

GENERAL SUMMARY

Two experiments, designed to study the effect of productive energy and amino acid levels of rations on the protein requirements of growing chicks and laying hens, were conducted. A summary of the results obtained is presented below:

I. Effect of Productive Energy and Amino Acid Levels of Rations on the Protein Requirement of Growing Chicks.

Four trials were conducted to study the effect of productive energy and amino acid content on the level of protein required in rations for chicks. In the first trial chicks were fed rations containing the same productive energy levels but varying in protein content from 16 to 20 per cent. The effect of maintaining a constant level of lysine and methionine by adding these amino acids to the low protein rations was studied. It was observed that reducing the protein level generally resulted in a decline in rate of growth of chicks fed rations in which meat meal or soybean oil meal was used as the protein supplement; however, additions of lysine and methionine resulted in a marked improvement in growth rate of chicks fed the low protein rations. Reductions were made in the protein content of rations supplemented with meat meal without affecting rate of growth of chicks when the lysine and methionine contents were maintained at levels equivalent to those in the high

protein rations. No effects of treatments used on efficiency of feed conversion were noted.

The second trial was undertaken to study the effect of supplementing low protein rations containing meat meal with lysine, methionine, and/or tryptophan. It was observed that the additions of lysine improved the rate of growth of chicks fed the supplemented rations while the addition of methionine and/or tryptophan, singly or in combination with lysine, had no effect on growth rate.

In the third trial the effect of productive energy concentration and protein level on the lysine requirement of chicks fed rations containing meat meal as a source of protein supplement was investigated. The results indicated that the addition of lysine improved the rate of growth of chicks fed the rations. A level of 0.2 per cent of lysine resulted in optimum rate of growth of chicks fed the high protein rations while a level of 0.4 per cent of lysine was required for greatest weight gains on the low protein rations. The highest level of supplemental lysine (0.4 per cent) produced the greatest improvement in rate of growth on the high energy-low protein ration.

The fourth trial in the chick experiment was designed to determine whether supplementary methionine was required by chicks fed rations containing meat meal and varying in productive energy and protein content. The results indicated that methionine was not a limiting amino

acid for growth of chicks with the basal rations used. Addition of 0.2 per cent of methionine, however, tended to improve the efficiency of feed conversion.

The results of the experiment indicated that the protein level in chick rations may be reduced without affecting rate of growth provided the levels of the most limiting amino acids are maintained constant by their addition to the ration. The studies also demonstrated that lysine was the most limiting amino acid for optimum growth of chicks fed rations containing meat meal as a protein supplement. Methionine was not a limiting amino acid in this type of ration.

II. Effect of Productive Energy and Amino Acid Levels of Rations on the Protein Requirement of Laying Hens.

The experiment was designed to study the effect of productive energy and amino acid levels of rations on the minimum requirement for protein by the laying hen. The results obtained indicated that in rations of high or low productive energy content those containing 13.5 or 14.5 per cent of protein generally supported a higher rate of egg production in Single Comb White Leghorn pullets than ones containing 12.5 per cent of protein. In addition, body weight loss was lower and average egg size was larger in the groups fed rations containing the higher levels of protein. Average egg size was affected by the productive energy level of the rations fed; the groups receiving the

low energy rations consistently produced larger eggs than those fed the high energy rations. The addition of amino acids to low protein rations appeared to improve rate of egg production in the group fed the high energy ration but did not affect rate of production in the group fed the low energy ration. Blood plasma cholesterol levels and hemoglobin regeneration time were apparently unaffected by the levels of productive energy and protein in the rations used in the experiment.

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