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PROTEIN AND METHIONINE
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PROTEIN AND METHIONINE REQUIREMENTS FOR STARTING AND LAYING RING-NECKED PHEASANTS

By

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ABSTRACT

PROTEIN AND METHIONINE REQUIREMENTS
FOR STARTING AND LAYING RING-NECKED PHEASANTS

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Three experiments with starting and laying Ring-necked pheasants (Phasianus colchicus) in their first and second year of egg production were conducted to assess their protein and methionine or total sulfur amino acid (TSAA) requirements.

The experiment with four hundred and twenty starting pheasants from zero to four weeks of age was carried out in battery brooders (three replicates of ten birds per treatment). Fourteen isocaloric practical diets containing 24%, 26% and 28% protein and 0.36%, 0.40%, 0.44%, 0.48% and 0.51% of methionine within each protein level were formulated. The diet that contained the highest protein level (28%) and the lowest methionine level (0.36%) could not be formulated with the available ingredients.

The minimum optimum levels of methionine for each protein level were calculated based on final body weight. For the diet containing 24% protein the minimum optimum methionine level was 0.637% of the diet, which lay outside the region of experimentation and it should not be considered a reliable value without further research including higher levels of methionine. For 26% and 28%

protein diets the minimum optimum levels of methionine calculated were 0.463% and 0.475% of the diet, respectively. The requirements for TSAA were 0.893% and 0.935% for the same diets, respectively.

The overall body weight mean of birds fed 28% protein diet was significantly higher than those of birds fed 24% and 26% protein diets. Feed consumption, feed conversion and mortality were not significantly affected by methionine and/or protein levels used.

Two experiments with laying pheasants were conducted in a cage management system for 112 days and 84 days. Nine isocaloric practical diets containing 14%, 16% and 18% protein and 0.25%, 0.29% and 0.33% of methionine within each protein level were fed to one hundred and fourty-four birds (four replicates of four birds per treatment), in each trial.

Hen-day percent egg production, body weight change and mortality were not significantly affected by methionine and/or protein levels used in both experiments.

Egg weight was significantly affected by protein levels. Laying pheasants in their first year of egg production fed diets that contained 18% protein laid eggs significantly heavier than those birds fed either 14% or 16% protein diets. Pheasant hens in their second year of egg production fed 16% and 18% protein diets laid eggs significantly heavier than those birds fed 14% protein diets.

Feed consumption of laying pheasants in their first year of egg production was significantly affected by protein levels. Feed consumption of birds fed the 14% protein diet was not significantly different from those fed either 16% or 18% protein diets. However, birds fed the 18% protein diet had a significantly higher feed consumption than those fed the 16% protein diet.

The observations made in these studies suggest that: 1) Diets containing 26% protein and 0.463% of methionine (or 0.893% of TSAA) seem to be appropriate for raising pheasants for shooting preserves; 2) diets containing 28% protein and 0.475% of methionine (or 0.935% of TSAA) might be used when pheasants are intended for meat production; and 3) diets containing a minimum level of 16% protein and 0.33% of methionine (or 0.59% of TSAA) seem reasonable to be used with satisfactory results in egg production rate and egg weight of laying Ring-necked pheasants in their first and second year of egg production.

To the memory of my parents who had the foresight to guide me into higher education.

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I. INTRODUCTION

The Chinese Ring-Necked Pheasant (*Phasianus colchicus torquatus*) is considered an economically valuable natural resource in the state of Michigan and it is grown for several purposes. Fanciers raise them for studying unusual feather patterns. Some growers produce birds for meat although there is a limited market for game birds in this market. Other producers raise them for selling to shooting preserves.

During recent years, the wild population of Ring-necked pheasants in Michigan has suffered a tremendous decline and the demand for birds by private shooting preserves has increased. The game bird industry, not only in the state of Michigan, but all over the country, has experienced fast growth and more efficient methods of production have become necessary. Many state game agencies interested in the improvement of wildlife habitat and also on restocking programs have been supporting research projects with pheasants.

In 1972, the Department of Natural Resources (DNR), Lansing, and the Department of Poultry Science, Michigan State University, established a cooperative pheasant research project to study confinement rearing, intensive management, nutrition and breeding conditions for these birds (Flegal, 1979).

Studies on pheasant nutrition have been conducted since the 1930s. In these studies it has been demonstrated that most of the nutrient requirements for pheasants resemble those of the domestic chicken except for the protein requirement which resembles that for the turkey poult.

The National Research Council (NRC, 1977) guidelines in Nutrient Requirements of Poultry include the requirements for starting and growing pheasants. However, no recommendations for laying pheasants are made in this publication. The knowledge of specific nutrient requirements of pheasants is necessary for the formulation and use of more efficient rations.

The most common diets fed to poultry in the U.S.A. use a combination of corn-soybean meal with minerals and vitamins. The amino acid balance is of concern to nutritionists when a corn-soybean meal ration is used. Methionine and total sulfur amino acids (TSAA) are of considerable interest because they are the first limiting amino acid(s) in corn-soybean meal diets for chickens and turkeys. Methionine has various functions in the animal body and it has been demonstrated that it can be used in the chicken for at least three distinct functions, namely: 1) as an essential amino acid for protein synthesis,

2) as a precursor of cystine, and 3) as a methylating agent.

According to the background information collected, a study of the methionine (or TSAA) requirements for pheasants was easily justified. Because protein and methionine requirements are interrelated these two nutrients were evaluated simultaneously in a factorial design. In this work the following objectives were established: 1) Adding some information to the existing knowledge on the minimum/optimum levels of methionine (or TSAA) with various protein levels for starting pheasants from zero to four weeks of age, and 2) to study the influence of protein and methionine levels (or TSAA) on the performance of laying pheasants in their first and second year of egg production.

II. LITERATURE REVIEW

A. Requirements of protein, methionine and/or total sulfur amino acids (TSAA) for game birds

Publications concerning the protein requirement of game birds have appeared since the 1930s. Callenbach and Hiller (1933), Norris *et al*. (1936), Nestler *et al*. (1942), Scott and Reynolds (1949) and Baldini *et al*. (1950) reported that the usual corn-soybean oil meal practical diets for game birds must contain approximately 28% protein to support rapid growth. Most of these early studies were conducted without taking into consideration the amount of methionine, lysine or other critical amino acids in the rations.

Baldini *et al*. (1953a, 1953b) reported that the protein requirement of Bobwhite quail and turkey poults could be reduced to 20% protein and still promote results almost equivalent to a 28% protein diet when a practical corn-soybean oil meal diet was adequately supplemented with methionine and lysine. Based on the results by Baldini, Scott *et al*. (1954) indicated the desirability to investigate the methionine and lysine requirements of pheasants at various levels of protein. They also reported that the protein requirement of pheasant chicks was 28% for the first 2-3 weeks of age and may be reduced to 24% for the 3-5th week growth period.

Vohra (1973) reported that adequate growth could be obtained in Japanese quail and the chukar partridge when they were fed low-energy starter diets (2320 kcal ME/kg) that contained 16% protein well balanced in the essential amino acids. Vohra tended to minimize the importance of higher dietary protein levels for rapid early growth, because the birds tended to attain near maximum body weight on all treatments by the time they reached about twelve weeks of age.

Andrews et al. (1973) indicated that a level of 28% protein in diets of Bobwhite quail was required for maximum growth.

Woodard et al. (1976) reported that pheasants grow poorly during the first month of age on diets containing less than 20% protein.

Woodard et al. (1977) suggested that starter rations for pheasants must contain at least 24% protein until 8 weeks of age, but they did not mention the percentages of methionine and cystine.

Tuttle et al. (1953) reported an excellent growth of Bobwhite quail when they were fed diets that contained 26.5% protein supplemented with methionine or methionine hydroxy analog.

Scott *et al.* (1963), using corn-soybean meal practical diets, reported that the protein requirement of young Bobwhite quail and young Ring-necked pheasants was shown to be 26.5% when the diet was supplemented with 0.1% methionine hydroxy analog. The addition of 0.1% methionine hydroxy analog raised the total sulfur amino acid (TSAA) content in the diet to 3.46% of the protein. This indicated the TSAA requirements of pheasants and quail to be approximately the same as the requirement of young domestic chickens when expressed as percentage of the protein in the diet. The metabolizable energy content of this diet was 1370 Calories per pound (or 3014 kcal/kg) indicating that the metabolizable energy : protein ratio for optimum growth and efficiency in young pheasants and quail is approximately 52 calories of metabolizable energy for each one percent of protein in the diet.

Scott (1966) reported that studies on amino acid requirements of game birds indicated that they are similar to those of young turkey poults when expressed as percentage of the protein in the diet, and he considered that the approximate methionine and cystine requirements of young pheasants and quail are 2.0% and 1.5%, respectively, as a percentage of dietary protein.

Millar and Smith (1971) evaluated different levels of methionine and lysine in a 26% protein starter diet for Ring-necked pheasants during a five week's period and indicated that the methionine requirement of the pheasant chick appeared to be slightly higher than 0.66% of the diet, the highest level studied in that experiment, but the percentage of cystine in the diet was not mentioned.

Serafin (1977) conducted some experiments with purified and practical diets to examine the influence of protein level and to estimate the TSAA requirement of young Bobwhite quail. Results showed that Bobwhite quail require no more than 26% protein for maximum growth and efficiency of feed utilization when the total sulfur amino acids (TSAA) level of the diet was approximately 1.0%.

The National Research Council (1977) established the TSAA requirement for the starting pheasant as 1.0 percent of the diet and the protein level as 30 percent. In Appendix A, Table 1, the protein, methionine and TSAA requirements of starting chickens, turkeys and pheasants are listed according to the National Research Council (NRC, 1977).

B. Requirements of protein, methionine and/or total sulfur amino acids (TSAA) for chicks.

Grau and Almquist (1943) stated that the methionine plus cystine requirement of chicks is approximately 1.0 to 1.1% of the diet. The requirement may be met by varying proportions of these amino acids except that the minimum methionine level is approximately 0.5% to 0.6%.

McGinnis and Evans (1947) found that New Hampshire chicks fed soybean oil meal, as the only source of protein, grew very well. The diet contained 22.4% protein, 0.26% methionine and 0.46% cystine or 0.72% methionine plus cystine. Growth was not improved by the addition of methionine, cystine or methionine plus cystine to the diet. Evans and McGinnis (1948) found that all of the methionine and cystine in the diet fed by McGinnis and Evans (1947) was not utilized by the chicks, because they excreted part of these amino acids unchanged in the droppings. Only 84% of the methionine and 47% of the cystine consumed were available to the chick. The diet, therefore, contained 0.22% available methionine and 0.22% available cystine or 0.44% available methionine plus cystine determined by balance studies with chicks.

Grau and Kamei (1950) conducted a study of total sulfur amino acid requirements of White Leghorn chicks in relation to protein level and they stated that this requirement is proportional to protein intake ranging from 10 to 40%. The minimum methionine requirement at 20% protein was estimated as 0.50%. The cystine requirement at this level of methionine was estimated as 0.30%.

Milligan <u>et al.</u> (1951) fed a 21% protein all-vegetable diet, based on corn, alfalfa meal and soybean meal, which contained, by analysis, 0.32% methionine and 0.28% cystine, to Rhode Island Red chicks to six weeks of age, and they found that there was an improvement in growth when 0.10% methionine was added, but not with further additions. The methionine requirement of Rhode Island Red chicks was no higher than 0.42% to six weeks or a combined total of 0.70% of methionine and cystine.

Almquist (1952) stated that vitamin B_{12} is required by chicks and plays a specific part in the metabolism of methyl groups, such as the methyl group on methionine. The minimum methionine requirement should be estimated only under conditions of ample supplies of choline, cystine and vitamin B_{12}, so that the metabolic load on methionine is diminished. Almquist also stated that the requirement for methionine and other essential amino acids expressed as a percentage of the dietary protein appeared to decrease as the protein content of the diet increased. He reported a study with three varieties of chickens receiving diets well provided with vitamin B_{12}, choline, cystine and antibiotics and the methionine requirement at 20% and 30% protein levels was no more than 2.5% of the protein. In his review, Almquist stated that the methionine requirement of young chickens was 0.45% in a diet that contained 20% protein, or the total sulfur amino acid requirement was 0.80%.

Griminger et al. (1954) fed chicks a diet that contained soybean oil meal as the only source of protein and they obtained maximum growth when 0.14% cystine and 0.10% DL-methionine were added to the diet which contained 0.23% methionine. It was concluded that, in the presence of adequate cystine, the methionine requirement of the chick, until 28 days old, does not exceed 0.33% of the ration.

Williams et al. (1954) determined the amino acid requirement of chicks by carcass analysis and found that the methionine requirement was 0.22% of the diet and the methionine plus cystine requirement was 0.44%. They stated that these data were on the basis of utilized methionine. These results were the same as those obtained by Evans and McGinnis (1948).

Baldini and Rosenberg (1955), in studies with methionine deficient broiler diets which contained 20-22% protein, observed that the methionine requirement of chicks was more nearly related to the energy content of the diet than to the diet weight. They stated that the methionine requirement of the chick expressed as percent of the diet increases as the energy level of the diet increases. Based on this, they stated that perhaps differences in energy content of the diets used by the different investigators might be a part of the reason for variation in the available methionine and cystine requirements calculated.

Evans et al. (1956) reported that, depending upon the study used, the "available" methionine plus cystine requirement of growing chickens is between 0.22% and 0.46% with 0.15% to 0.42% as methionine. They also stated that one of the reasons for the wide differences in the reported methionine requirement of chicks might well be in the differences of availability of the methionine and cystine in the dietary constituents. They called "available" methionine or cystine the amount of methionine liberated from a feed by in vitro digestion with trypsin and erepsin. They observed that the percentage of methionine in soybean oil meal that was liberated by in vitro digestion with trypsin and erepsin was very nearly the same as the percentage utilized by chicks fed soybean oil meal as the only source of protein (Evans and McGinnis, 1948). They stated that by inference the same may be true for cystine.

Rosenberg and Baldini (1957) reported that the results obtained with isocaloric diets at different protein levels indicated that the energy content of the diet governs the methionine requirement. When sufficient energy is available from non-protein sources to permit full utilization of the protein for tissue synthesis and repair, methionine requirement, expressed as percent of diet, increases as protein level increases. In the absence of a sufficient amount of energy to permit the birds to make full use of the protein offered for growth purposes, increasing levels of dietary protein were not found to require corresponding amounts of methionine.

Leong et al. (1959), working with purified diets fed to growing chicks, came to the same conclusion as Rosenberg and Baldini (1957) that the requirement for methionine plus cystine, expressed as percent of the diet, increases as the protein level of the diet increases, and it also increases as the dietary energy increases, if protein is adequate.

Nelson et al. (1960) stated that the quantitative requirement of chicks for total sulfur amino acids was 3.51% ± 0.025% of the protein. This relationship was found to be constant for all protein and energy levels studied.

Klain et al. (1960) studied the amino acid requirement of the growing chick and the methionine requirement stated in the absence of L-cystine was 0.47% of the diet, while in the presence of 0.4% L-cystine the methionine requirement was 0.18% of the diet. Featherston and Stephenson (1960) found that a corn-soybean diet for broilers with or without added choline needed to have more than 0.89% methionine plus cystine. Quillin et al. (1961) stated that broilers receiving a diet with added choline needed 0.42% methionine. However, when choline was not added, the methionine requirement was 0.50% of the diet.

Dean and Scott (1965) re-examined the chick's requirements during the early stages of growth for each of 14 amino acids necessary for maximum chick growth during the second week post-hatching. They stated that the methionine and cystine requirements were 0.45% and 0.35%, respectively, when expressed as percentage of the diet.

Bornstein and Lipstein (1975) determined the amount of dietary protein that can be reduced in milo-soybean meal pratical-type starter broiler diets by satisfying the first two limiting amino-acids, namely methionine and lysine. The protein requirement of broilers between one and four weeks of age in these trials was about 22% and the total sulfur amino acid requirement was about 0.80% of the diet or 3.63% expressed as a percentage of the protein.

Woodham and Deans (1975) determined the amino acid requirements for broiler chickens between 14 and 28 days of age using an 18% protein diet consisting mainly of conventional ingredients. The methionine plus cystine requirement expressed as a percentage of the diet was 0.58% or 3.2% expressed as a percentage of the protein.

Pesti et al. (1979) stated the total sulfur amino acid requirement of chicks as 3.2% of protein which is in good agreement with those values reported by Woodham and Deans (1975) (3.2%), Graber et al. (1971) (3.3%) and somewhat above that determined by Boomgardt and Baker (1973) (3.05%) and lower than the estimates made by Nelson et al. (1960) (3.51%), NRC (1977) (4.04%) and Bornstein and Lipstein (1975) (3.63%).

C. Requirements of protein, methionine and/or total sulfur amino acids (TSAA) for turkey poults.

Kratzer et al. (1949) fed a ration that contained isolated soybean protein as the only source of protein (except 2% of condensed fish solubles) to Bronze poults. Approximately 0.5% methionine and 0.3% cystine were required for the optimum growth of the poults in a ration that contained 24% crude protein.

Almquist (1952) stated the methionine requirement for turkey poults as 0.45% of the diet or 0.75% total sulfur amino acids in a 24% protein diet.

Ferguson et al. (1957) fed Broad Breasted Bronze turkey poults diets that contained 24%, 26%, and 28% protein with two productive energy levels and further supplemented with 0.05% and0.10% DL-methionine. They found that the maximum growth was obtained in the group fed a 28% protein diet that contained 914 productive energy calories per pound supplemented with 0.05% methionine but they did not mention the total level of methionine in the diet. Feed efficiency was improved at each protein level when the diet was supplemented with methionine or the energy level was increased. Also, there was a significant linear response to increasing levels of protein from 24% to 28%. Waibel (1959) reported that supplementary methionine for turkey poults resulted in consistent growth responses with high-energy, isocaloric, 24% and 28% protein diets. Birds receiving the 28% protein gained weight more rapidly than those fed a 24% protein diet.

Fitzsimmons and Waibel (1962) reported that a 24% protein starter diet for young turkeys was deficient in methionine and marginal in lysine, while a 20% protein diet was deficient in both methionine and lysine which is supported by Baldini et al. (1954) and Fisher et al. (1956).

Potter et al. (1966) found no improvement by methionine supplementation of turkey starter diets that contained 25%, 29% and 33% protein.

Couch et al. (1969) fed computer-programmed least cost rations to turkey poults for the first three weeks and they found that the predicted methionine requirement was 0.50% and the total sulfur amino acids 0.85% of the diet.

Kelly (1970) using crystalline amino acid-corn starch purified diets reported the methionine requirement of young turkeys during the first two weeks of life as 0.50% of the diet, but he did not mention the level of cystine.

Kummero *et al.* (1971) conducted some experiments to determine lysine and total sulfur amino acid requirements of a commercial strain of Large White turkey poults from 0 to 3 weeks of age using natural feed ingredients and they predicted the optimum requirement of total sulfur amino acids as 0.282% per megacalorie per kg or 0.82% of the diet, for both sexes.

Investigations made by Warnick and Anderson (1973), Murillo and Jensen (1974, 1976), Potter and Shelton (1974, 1976a, 1976b) and Potter *et al.* (1977) indicated that the total sulfur amino acid requirement of young turkeys was in the range of 1.0% to 1.10% of the ration.

Atkinson *et al.* (1976b) stated that turkey poults receiving a diet containing 22.1% protein supplemented with 0.2% lysine and 0.3% methionine have an acceptable rate of growth. The basal diet contained 0.65% methionine plus cystine. They observed that rations much lower in protein than normally recommended may be utilized if the proper levels and balance of amino acids such as arginine, lysine and methionine are maintained.

Potter and Shelton (1979) conducted experiments to determine the methionine or total sulfur amino acid and the protein requirements of Medium White turkeys between zero to four weeks of age. They stated that the total sulfur amino acids in a practical type corn-soybean diet with 5% or less menhaden fish meal is the most limiting of the amino acids. They reported that the total sulfur amino acid requirement of Medium White turkeys to four weeks of age is approximately 1.10% of the diet or 3.8 mg TSAA per kilocalorie of metabolizable energy. The protein requirement during this period should be at least 27%, when diets contained sufficient total sulfur amino acids.

Behrends and Waibel (1980) investigated the total sulfur amino acid requirements of starting Large White male turkeys from 1 to 4 weeks of age using diets which contained soybean meal, faba beans and field peas. They stated that the total sulfur amino acid requirements with diets marginally deficient in cystine were 0.95% to 1.01% of the diet or 0.298% to 0.332%/therm ME/kg of feed. The minimum methionine requirement determined with excess dietary cystine was 0.46% of the diet.

D. Requirements of protein, methionine and/or TSAA for laying chickens and turkey breeder hens.

Titus (1955) and Harms (1966) indicated that methionine was the first limiting amino acid in a corn-soybean meal diet for laying chickens, but some researchers, Reid et al. (1951), Mehring et al. (1954), Heywang (1956), Britzman and Carlson (1964) and Sell (1964), did not find any beneficial results when they supplemented layer diets that contained 13% to 18% protein with methionine.

Scott (1960), based on calculated amino acid analysis and requirements, concluded that for egg production and tissue replacement, many high energy diets for laying chickens are limiting in methionine. Later, Scott (1962), demonstrated that both 15% and 17% protein diets were improved by methionine hydroxy analog additions.

Other researchers, Waldroup and Harms (1961), Yates and Schaible (1961), Bradley and Quisenberry (1961), Harms et al. (1962), Barton and Stephenson (1960) and Heywang et al. (1963), also reported improved egg production and/or feed efficiency from the addition of methionine or methionine hydroxy analogy to laying chicken diets containing from 11% to 16% protein.

Scott (1962) stated that the high protein intake of laying hens was correlated with the high content in egg proteins of certain essential amino acids, specifically methionine, lysine, isolecucine and valine. The average content of these amino acids, when considered as percent of the protein in the usual corn-soy laying diet is markedly lower than in egg proteins. However, he found that by adding 2.5% of fish meal to a corn-soybean meal diet the required amounts of critical and essential amino acids, except methionine, could be met with a protein level of 15.50%. To bring methionine to the required level, the addition of approximately one and a half pound of DL-methionine per ton was necessary. He concluded that the amino acid requirements could be met by increasing the amount of total protein in the diet, but this procedure may be more expensive and less satisfactory from a nutritional standpoint.

Biely and March (1964), while studying responses of laying birds to different amino acid balances and different levels of dietary protein, noticed that birds which received a diet that contained 16 percent protein consistently laid larger eggs than did those receiving a diet that contained 14 percent protein. Supplementation of the 14 percent protein diet with lysine and methionine increased egg weight markedly over that obtained with the unsupplemented 14 percent protein diet and to a greater extent than did supplementation with either lysine or methionine alone. They concluded that the amino acid balance of protein fed to laying chickens has a greater bearing upon egg size than does the level of protein, provided that protein intake is adequate for normal egg production.

Thornton et al. (1957), using four protein levels (11%, 13%, 15% and 17%) fed to Single Comb White Leghorns, found that the protein level within the range studied had no effect on egg production, feed efficiency or maintenance of body weight. Egg weight was reduced in a highly significant manner at the 11% protein level.

The addition of 0.2% of DL-methionine increased egg weight and reduced small egg incidence at all protein levels.

Heywang et al. (1955), Miller et al. (1956), Frank and Waibel (1959), Griminger and Fisher (1959) and Thornton et al. (1959) reported satisfactory egg production with protein levels of 13% to 15% for laying chickens. However, Reid et al. (1951), Quisenberry and Bradley (1962) and Denton and Lillie (1959) reported optimum performance of laying chickens with diets that had a protein level above 15 percent.

Milton and Ingram (1957) found that pullets produced as well on 14% as on 16% and 18% protein diets. However, slightly higher production was obtained from old laying chickens with 16% and 18% protein diets. Feed conversion was somewhat in favor of the 16% and 18% protein diets.

Gordon et al. (1962) indicated that increasing the protein level in laying diets from 11% to 19% resulted in a highly significant increase in egg production, egg weight and feed efficiency.

Quisenberry and Bradley (1962) found that egg production, egg weight and feed efficiency of laying chickens improved as dietary protein level was increased from 13% to 17%.

Deaton and Quisenberry (1965) reported that egg production of laying chickens receiving 17% or 14% protein in their diet was not significantly different. On the other hand, the hens that received a 17% protein diet laid significantly heavier eggs and had significantly better feed efficiency.

Harms et al. (1966) reported that New Hampshire pullets performed well on low levels of protein (11% and 13% protein). They concluded that breeder diets might contain more protein than is necessary for maximum performance.

Summers et al. (1967) reported that a level of 14% protein in a broiler breeder hen diet supported egg production that was not signicantly different from that of hens that received 16% or 18% protein diets. These workers, however, suggested a level of 16% protein for optimum performance.

Lillie and Denton (1967) compared dietary protein levels of 12%, 14% and 16%. They found that when the 12% protein level was fed, egg production was significantly lower than that obtained with the 14% protein level, but equivalent to that obtained with the 16% protein level. They stated that a 14% protein diet was adequate for egg production, but not for body weight maintenance or egg weights; at least 16% and 18% protein were required for these two traits, respectively.

Smith (1967) tested three diets with different protein levels (11%, 15% and 18%) in commercial laying pullets and found that egg production was not affected but egg weight and body weight responded to increased protein. Supplementation of the laying diets with methionine and lysine singly and in combination failed to improve any of the performance criteria, indicating that neither one of these amino acids was limiting. He also stated that the required amino acid pattern of the laying hen is not altered as a result of changing the dietary protein level.

Marret and Sunde (1967) conducted experiments with yearling hens. The basal diet contained 14% protein and it was supplemented with lysine and methionine. The overall results showed no differences among groups for egg production and feed efficiency.

Arscott and Bernier (1968) reported that the addition of 0.05% DL-methionine to the 12% protein diet of dwarf White Leghorn layers increased egg weight, but they did not mention the level of methionine in the diet. They also observed that egg weight in dwarfs increased with protein levels. The protein levels studied varied from 12% to 21%.

Santana and Quisenberry (1968) indicated that a diet that contained 16% protein was satisfactory for body weight gain and resulted in the highest egg production of laying chickens.

Gleaves et al. (1968) reported that as estimated dietary protein level was increased from 13% to 19% in the diet of laying chickens, there was an increase in observed body weight gain, egg production and egg weight.

Guenthner et al. (1972) reported that an increase of protein level from 13.9% to 18.3% in the laying chicken's diet did not affect feed intake or feed conversion. Although the rate of egg production tended to increase, it was not significantly altered.

Reid and Webber (1974) found that feeding laying chickens with a 14% protein that contained 0.55% TSAA, supported maximum egg production.

Thayer et al. (1974), working with hybrid laying hens, concluded that the protein consumption of 14 g/hen /day was adequate to support egg production and egg weight but did not produce body weight gains comparable to gains with higher protein intakes. A protein intake of 15 g/hen /day was recommended for practical conditions. Strict attention was given to amino acid balance pattern in this study.

Reid (1976) found that a 14.6% dietary protein was adequate to support an egg production rate of 77% at an average intake of 16.54 g/hen/day.

Ingram et al. (1951), working with laying hens, fed diets containing peanut meal as a source of protein supplemented with tryptophan and lysine to determine the methionine requirement. They stated that the methionine requirement of laying hens is not more than 0.38% of the diet and the combined methionine and cystine requirement is not more than 0.63%. They also observed that the amino acid composition of the egg and the hatchability were not affected by a methionine deficiency in the laying hen diet.

Leong and McGinnis (1952), using practical diets in which 75% of the total protein was supplied by Alaska pea meal, reported that for laying hens the methionine level required for supporting maximum egg production, body weight gain and egg size appeared to be approximately 0.28% of the diet in the presence of 0.25% cystine with a protein level of 15.4%.

Mehring, et al. (1954) found that the addition of 0.0847% DL-methionine and 9 mcb/lb vitamin B_{12}, singly and together to a corn-soybean diet fed to New Hampshire pullets, had no statistically significant effect on egg production, the quantity of feed required for the production of a dozen eggs or the gain in live weight. The basal diet used contained by calculation 16.83% protein and 0.25% to 0.31% methionine and 0.26% cystine.

Ingram and Little (1958), using a wheat-peanut meal basal diet supplemented with various levels of

DL-methionine, reported that the requirement of the laying hen for this amino acid was determined to be 0.25% of the diet. Levels of methionine as low as 0.225% supported egg production. However, egg size and body weight were not maintained.

Combs (1960) developed tables for calculating and determining the methionine requirement of layers according to body weight, egg production, gain or loss in body weight and temperature condition. He found the methionine requirement of the layers to vary from as low as 0.208% (4 lb hen, gaining no weight, in the winter, producing 27 g of eggs per hen per day and fed a diet containing 900 calories of productive energy) to as high as 0.341% (4 lb hen gaining 1.5 g of weight per day, producing 57 g eggs per hen day, during the summer and fed a diet containing 1050 calories of productive energy). This proposal suggested that methionine requirements could not be considered to be "static" but must be considered on the basis of a particular set of well-defined conditions.

Daghir <u>et al</u>. (1964), using an all-plant corn-soybean meal breeder chicken diet with 15.54% protein, which contained 0.28% methionine and 0.25% cystine, found that supplementation of this diet with 0.05%, 0,10% and 0.15% methionine hydroxy analog calcium (90%) had no statistically significant effect on egg production, body weight gain, mortality, egg weight and methionine content of eggs produced. The requirement for methionine and cystine found by these authors is in accord with that stated by Leong and McGinnis (1952).

Lepore (1965) established lines of chickens by selection for high and low body weight at three weeks of age on a normal and a methionine deficient diet to study the overall mechanism of growth rate inheritance. The methionine and protein requirements, as estimated under their dietary conditions of all four selected lines, were found to be similar. A methionine level of 0.39% of the diet was the requirement in an 18.4% protein diet.

Combs (1964) reported that in a methionine requirement assay for laying hens an intake of 295 mg of available methionine per hen per day was necessary for a maximum egg yield of 46.5 g per day.

Bray (1965) conducted a detailed study of the methionine requirement of young laying pullets and reported that not only total intake and dietary levels must be considered but the egg yield as well must be considered in order to achieve maximum results. A dietary level of 0.216% methionine microbiologically available in a 12% protein diet was adequate to support an egg yield of 40.58 g. A daily intake of 223.5 mg of methionine was required. This methionine requirement for egg production was lower than that reported by

Combs (1964), which was 295 mg/hen/day (chick assay). The difference of 5.9 g in daily egg output may explain why Combs found an intake requirement of 295 mg of available methionine compared to 223.5 mg of available methionine found in Bray's test. The level or the intake of protein was not given in Comb's test.

Holmes and Kramer (1965), working with laying pullets, found that the best egg production and feed efficiency were obtained when the diets (that contained soybean meal as the only source of protein) had a content of 0.31% of methionine, 0.28% cystine, 985.6 mg of choline and 6.6 microgram of vit B_{12} per kilogram of diet. The protein level of the diet was 16.4% and 2992 Calories of ME per kg.

Harms and Damron (1969) conducted several experiments to determine the methionine and sulfur amino acid requirements of commercial egg production type pullets as influenced by diet formulation. Data from this study indicated that the hen requires 250 mg to 280 mg of methionine daily provided she is supplied a total of 530 mg of sulfur amino acids. This requirement was met by a level of 0.268% methionine and 0.533% total sulfur amino acids in a diet that contained 2887 kcal of metabolizable energy per kg. However, this level of methionine did not support maximum performance when the diet contained lower levels of total sulfur amino acids.

Carlson and Guenthner (1969) worked with Single Comb White Leghorns to determine the requirement of methionine and lysine supplementation of typical corn-soybean diets. They stated that diets containing 14% protein needed methionine supplementation for maximum egg production. Diets with 16% protein without methionine supplementation were quite adequate under the conditions used. Also, they estimated that the methionine requirement per hen would be in excess of 300 mg/day during the first four months of production and between 289 and 328 mg/day/hen during later stages in the laying cycle.

Petersen <u>et al</u>. (1971) conducted several experiments with White Leghorn pullets and found that a 12% protein diet (13.3 g protein/day/hen) resulted in a rate of lay and egg weight significantly lower than did 14%, 16%, and 18% protein diets. Also, they stated that 14 g protein/hen/day and 260 mg methionine/hen/day resulted in egg production equal to that from 18 g daily protein intake. Egg weight increased with each increase in methionine at 14 g protein intake but did not equal that from the 18 g protein intake.

Jensen <u>et al</u>. (1974) stated that a level of methionine for laying hens between 290 and 300 mg/hen/day should be adequate to meet the requirements of laying chickens at different stages of the laying cycle. Also, they stated that a corn-soybean meal diet formulated to contain 16% protein is adequate in TSAA for obtaining optimum egg yield in laying chickens.

Picard (1975) concluded that the daily methionine requirement for semi-heavy laying hens was between 360 and 390 mg. The diets used were based on maize, wheat and soybean and the total protein was 13% and 15%.

Waldroup et al. (1976), using a prediction equation, found that the maximum daily needs for energy for normal breeder hen was 422 kcal ME/day with 380 mg/day of methionine.

Schutte and Weerden (1978), working with diets based on maize and soybean meal during 52 weeks of egg production, stated that 775 mg to 800 mg total sulfur amino acids of which 390 to 440 mg was methionine was the daily requirement per hen for a maximum egg production of 80 to 83 eggs per hundred hens daily. Diets with 13.8% protein supplemented with methionine and lysine supported egg production and feed utilization as effectively as a diet with 16.7% protein.

Harms and Wilson (1980) conducted experiments with Cobb-color-sexed broiler breeder hens to determine their protein and sulfur amino acid requirements. They found that a daily intake between 400 and 478 mg of methionine and 722 and 830 mg of total sulfur amino acids was necessary for maximum egg production with a 13.07% protein diet.

Jensen and McGinnis (1961), in experiments with large type turkey breeder hens, did not observe protein deficiency even though a ration containing as low as 10% protein was fed for a period of eleven weeks. They considered that the 15% protein level recommended by the NRC is more than adequate.

Bradley et al. (1969), working with Broad Breasted Bronze and Beltsville Small White turkey hens, suggested that Broad Breasted Bronze turkey hens require at least an 18% protein level and Beltsville Small White turkey hens require at least 15% for normal reproductive performance.

Minear et al. (1970) fed isocaloric diets that contained different protein levels (10%, 12%, 14%, and 16%) to Large White turkey breeder hens and measured mortality, egg production, feed efficiency, fertility and hatchability. They found no significant difference in reproductive performance when 14% or 16% protein diets were fed. Also, they stated that a diet that contained 10% protein could maintain reproductive performance comparable to a 16% protein diet. However, turkey breeder hens fed a 12% protein diet did not perform at the level of those fed 10%, 14%, or 16% protein.

Luther and Waldoup (1970) fed diets that contained 14.7% protein (0.45% total sulfur amino acids) and 16% protein (0.46% total sulfur amino acids) to Broad Breasted

Bronze turkey breeder hens. DL-methionine was added to diets up to 0.66% total sulfur amino acids. In comparison to a standard 18% protein breeder diet fed, there were no differences in reproductive performance of turkeys fed any of the experimental diets.

Jensen (1973), adding methionine to a wheat-dry pea (Pisum sativum) diet that contained 12% protein, failed to significantly improve the reproductive performance of Large White turkey breeder hens. The daily feed intake per hen fed the basal diet was estimated to be 337 mg methionine and 716 mg TSAA.

Atkinson et al. (1974, 1975), working with Beltsville Small White turkey breeder hens, stated that the total sulfur amino acid requirement of the turkey hen for optimum egg production is approximately 0.60% of the diet.

Atkinson et al. (1976a), using Beltsville Small White turkey hens, found that the addition of 0.05% methionine to a practical type turkey breeder diet which contained 0.50% methionine plus cystine improved production, feed efficiency and egg size significantly. The diet contained 18.26% protein and 2893 kcal ME/kg.

III. MATERIAL AND METHODS

Experiment I.

Eggs were obtained from breeders kept by the Department of Natural Resources (DNR) game bird farm at Mason, Michigan. All eggs were set in a Jamesway 252, single stage incubator in the Poultry Science section, M.S.U. The eggs were incubated for three weeks at 37.5°C and at 60% of relative humidity. After 21 days of incubation, the eggs were transferred to appropriate baskets and placed in hatching units where the temperature was approximately 37°C and at 70% relative humidity. On 8/16/78, the 26th day of incubation, the hatch was taken off. Chicks were individually weighed and divided into groups according to weight. Then, 42 groups of 10 birds with approximately the same weight were formed. Birds were banded and transported to brooding facilities at the Poultry Science Research and Teaching Center (PSRTC).

These 42 groups of birds were randomly distributed into four thermostatically heated battery brooders equipped with wire mesh floors (Petersime battery). The battery brooders were equipped with lights and also the rooms had incadescent light bulbs to provide better illumination. Birds were maintained on 24 hours light during the first two weeks and then 14 hours light and 10 hours dark until the end of the experiment. Lights were controlled by an electric clock. The temperature was approximately 37.8 - 35°C (100-98°F) for the first week, 35 - 32°C (95-90°F) for the second week and 29.4°C (85°F) for the third and fourth weeks.

Feed and water were provided ad libitum. Water was provided in small glass waterers, one for each replicate of 10 birds. Since the battery waterers were not divided to avoid birds of different treatments drinking from the same waterer, individual glass waterers were used during the whole experiment.

Practical mash-type diets that contained common feed ingredients were used in order that the results of this experiment could be applicable to the game bird industry. These diets were formulated to contain by calculation three different protein levels and each protein level was formulated to contain five methionine levels. All diets were computer formulated. Restrictions on methionine, cystine, lysine and tryptophan levels were employed.

The NRC (1977) states the protein requirement for growing pheasants from zero to four weeks as 30% and the methionine plus cystine as 1.0% of the diet, but it does not establish the methionine requirement alone. In this study, the protein levels studied were 24%, 26% and 28%. Within each level of protein, five methionine levels were used -- 0.36%, 0.40%, 0.44%, 0.48% and 0.51% of the diet. There was one exception, the diet that contained 28%

protein with methionine at 0.36% of diet could not be formulated with the available ingredients. Therefore, with those rations that contained 28% protein, only four methionine levels were considered. The levels of protein and methionine studied were lower than those values recommended by NRC (1977). Cystine was maintained constant at each level of protein. For cystine and tryptophan, since NRC (1977) does not state a requirement for growing pheasants, the levels used were according to those values recommended by Scott (1966) when expressed as percent of protein. All diets were maintained isocaloric at 1250 kcal/lb or 2750 kcal/kg of metabolizable energy. The composition and the calculated analysis of diets used are shown in Table 2, Appendix A. Nutrient values of feed ingredients used in calculating the composition of diets are shown in Appendix A, Table 4. Enough feed was mixed at one time so all replicates were fed with feed ingredients from the same source. The vitamin/mineral premix is also shown in Table 5, Appendix A.

Four hundred and twenty birds were used in this experiment. Each one of the 14 dietary treatments had three replicates and each replicate had 10 birds. The experiment had a duration of four weeks due to the vertical space limitations of the Petersime battery-brooders. Individual body weights were obtained at the beginning of the experiment and at 14 and 28 days of age. Data on feed consumption were acquired biweekly for each replicate or group of 10 birds. From these data, feed consumption per bird and feed conversion were calculated. Only the final data, at 28 days of age, were used for statistical analysis. Mortality was registered each day. When a bird died its weight was recorded and it was considered for calculating feed conversion. Birds that died during the trial were taken to the Michigan State University diagnostic laboratory where necropsies were performed.

Experiment II.

Ring-necked pheasant pullets obtained from DNR were raised on the floor at the Poultry Science Research and Teaching Center. At the age of 240 days, 144 birds were confined in a house with suspended single bird cages. The cages had the following dimensions: 20.3 x 35.6 x 30.6 cm which gave a space of 722.7 sq cm per bird. One inch (2.54 cm) mesh and 14 gauge wire was used to construct the cages. These cages had a 5.08 cm sloping wire floor and a 10.2 cm wide egg tray. Each cage was numbered and the egg tray was divided by a piece of wire according to cage number. Water was provided *ad libitum* from nipple type waterers. Feed was also provided *ad libitum* in plastic troughs. Each feeder was used by four hens; therefore, four adjacent cages of birds fed from a common feeder was used as the experimental unit.

The house had no window. It was lighted by six 75 watt incadescent light bulbs, uniformly spaced on the ceiling. The lights were controlled by an electric clock which was set to light the room 14 consecutive hours in each 24 hours. Therefore, 14 hours light and 10 hours dark (14 L : 10 D) was the lighting program used during the whole experiment. Attempts were made to maintain the room temperature in a range between 13-18.3° C (55.3-65° F) through manipulation of a temperature control device. A gas brooder canopy hanging approximately 30 cm from the ceiling near the center of the house supplied supplementary heating, since this experiment was conducted during cold weather.

After birds were moved to the cage house, one week of acclimation was allowed before the trial began. Birds that did not readily adapt to cage management were changed. Some birds killed themselves by repeatedly jumping and hitting the cage top. During this period all birds had the wings and tail feathers clipped and specks removed. The allocation of birds to each treatment was done randomly. Cages were arranged into four statistical blocks and the nine different diets were assigned at random into each of four blocks. There were four hens per replicate and four replicates per treatment.

Practical corn-soybean meal mash diets were formulated to contain by calculation three protein levels with three different levels of methionine and were fed to laying pheasants in their first year of egg production for 112 days or four 28-day periods, in winter-spring 1980. All diets were computer formulated. Restrictions on methionine, cystine, lysine and tryptophan levels were employed. NRC (1977) does not state any requirement for adult pheasants for these nutrients. The requirements stated for laying and breeding chickens as percentage of the diet were approximately followed in this study. Three protein levels, 14%, 16%, and 18%, with three methionine levels 0.25%, 0.29%, and 0.33% of the diet, were used. All diets were maintained isocaloric at 1250 kcal/lb or 2750 kcal/kg of metabolizable energy, approximately the level recommended by Scott (1966) for breeding Ring-necked pheasants.

Nutrient values of feed ingredients used in calculating the composition of diets are shown in Appendix A, Table 4. The calculated analysis and the composition of the diets used are shown in Table 3, Appendix A. The vitamin/mineral premix composition used is also presented in Table 5, Appendix A. Enough feed was mixed at one time to allow the birds to be fed with feed ingredients from the same source.

Egg production per bird was recorded daily. From these data, rate of egg production was calculated on a hen-day and hen-housed basis. For the first production period, the percentage of the production was calculated considering only 14 days since the egg production began approximately 14 days after the birds were lighted. Egg weight in grams ± .01 was measured by weighing all eggs laid on three consecutive days during the last week of each period.

Feed consumption per replicate of four birds was measured at 28-day intervals. From these data, grams of feed per bird per day was calculated. Birds were individually weighed at the beginning of the trial and at the end of each 28-day period. However, the change in body weight was calculated by the difference between the initial and final body weight of each surviving bird. Mortality was registered daily. Dead birds during the trial were taken to the Michigan State University diagnostic laboratory. Necropsies were performed to determine the cause of death.

Experiment III.

In this experiment recycled laying pheasants (in their second cycle of egg production) obtained from DNR were used. The experimental procedure, diets, management and data collection used were the same as described for Experiment II with the following exceptions: 1) the duration of the experiment was three consecutive periods of 28 days, or 84 days since the egg production declined rapidly, 2) these birds, when confined to the cage house, were in production and the egg production began to be registered seven days after the trial started. So, for the first period, rate of egg production was calculated on the basis of 21 days of production.

Statistical Procedures

Regression analysis and analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) were the statistical methods common to all the experiments.

A regression approach using a second order polynomial model for final body weight adjusted for initial weight was used in Experiment I, with growing birds. It was used to determine if the different methionine levels at each protein level had significant linear and/or quadratic effect(s) on final body weight. After a significant effect was detected a prediction equation $\hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_1^2$ in which $\hat{Y}$ = expected final body weight; $\hat{\beta}_0$ = constant; $\hat{\beta}_1$ = the linear coefficient or the regression coefficient of $\hat{Y}$ on x_1; x_1 = the methionine level; $\hat{\beta}_2$ = the quadratic coefficient or the regression coefficient of $\hat{Y}$ on x_1^2 was calculated for each of the three protein levels studied. As the individual final body weights were adjusted for initial weights, the prediction equations were accordingly adjusted:

$$\hat{Y} = \hat{\beta}_0 + \hat{\beta}_1 x_1 + \hat{\beta}_2 x_1^2 + \beta_3 \bar{x}_2$$

in which $\hat{\beta}_0$, $\hat{\beta}_1$, $\hat{\beta}_2$ and x_1 are as described above and $\hat{\beta}_3$ = the coefficient calculated for initial weight and $\bar{x}_2$ = the initial body weight average of birds for each treatment.

The optimum methionine level for each protein level was calculated using the following equation, according to Gill (1978) $x_s = -\hat{\beta}_1/2\hat{\beta}_2$, in which x_s = an estimate of the level of methionine at which maximum growth is expected to occur, $\hat{\beta}_1$ = the regression coefficient on x_1, $\hat{\beta}_2$ = the regression coefficient on x_1^2. The methionine levels as percentage of the diet 0.36, 0.40, 0.44, 0.48 and 0.51% were coded from the lowest of the highest level as 1, 2, 3, 4 and 5 for statistical analysis. After the optimum methionine levels were calculated, the values obtained were transformed into percentage of the diets.

For feed consumption, feed conversion and mortality analysis of variance (ANOVA) was used to indicate a significant difference among the different treatments.

With laying pheasants analysis of variance (ANOVA) was used in both experiments (Experiment II and Experiment III) to detect any significant effect of protein and methionine for each one of the variables studied: hen-day percent egg production, egg weight, feed consumption, change in final body weight and mortality. For further tests beyond the ANOVA for all experiments, Bonferroni t-statistic was used to determine the specific differences between means.

The square root of the mean square error divided by the number of observations per mean were used as the standard error of mean.

For each variable discussed with a statistical analysis, the ANOVA tables are provided in Appendices B to D. The 0.01 and 0.05 levels of probability provided the basis for all statements concerning statistically significant differences. All procedures used are described by Gill (1978).

IV. RESULTS AND DISCUSSION

Experiment I.

Body weight

The body weight means of young Ring-necked pheasants at four weeks of age feed diets with different protein and methionine levels are shown in Table 1.

The regression analysis at the 24% protein level (Table 1, Appendix B) showed a significant linear response ($P < 0.01$). As the methionine level increased the body weight means increased (Figure 1). Because a significant linear effect was detected a prediction equation to estimate the final body weight at this protein level was calculated (using values from Table 4, Appendix B): $\hat{Y} = 173.06156 + 10.27552x_1 + (-0.63694)x_1^2 + (0.710844 \times 22.93)$. $\hat{Y}$ denotes the estimated body weight, x_1 denotes the methionine level, and the last term is the adjustment for initial weight, in which the value of $\hat{\beta}_3$, coefficient for initial weight was multiplied by the initial body weight mean of pheasant chick for this treatment. Using the coefficients $\hat{\beta}_1$ and $\hat{\beta}_2$ calculated for this protein level, an estimate of the methionine level at which maximum body weight is expected to occur was found to be 0.637% of the diet, which lies outside of the range

Table 1. Final body weight (g) average of Ring-necked pheasants at four weeks of age fed different dietary levels of protein and methionine.[1]

Protein %	Methionine level (% of diet)					Overall mean ± SEM
	0.36	0.40	0.44	0.48	0.51	
24	198.61±6.12	206.10±607	220.52±7.41	214.68±5.59	226.41±6.33	213.21[a]±2.83
26	193.44±6.00	218.14±6.73	220.00±5.71	228.12±8.43	216.28±4.93	215.01[a]±2.78
28	-	202.93±6.13	233.79±5.72	237.55±6.69	227.64±6.06	225.45[b]±3.29

[1]Average of final body weight ± standard error of mean for birds completing the experiment.

[a,b]Means with different superscripts are considered significantly different ($P \leq 0.05$).

Note: The diet with 28% protein and 0.36% methionine could not be formulated.

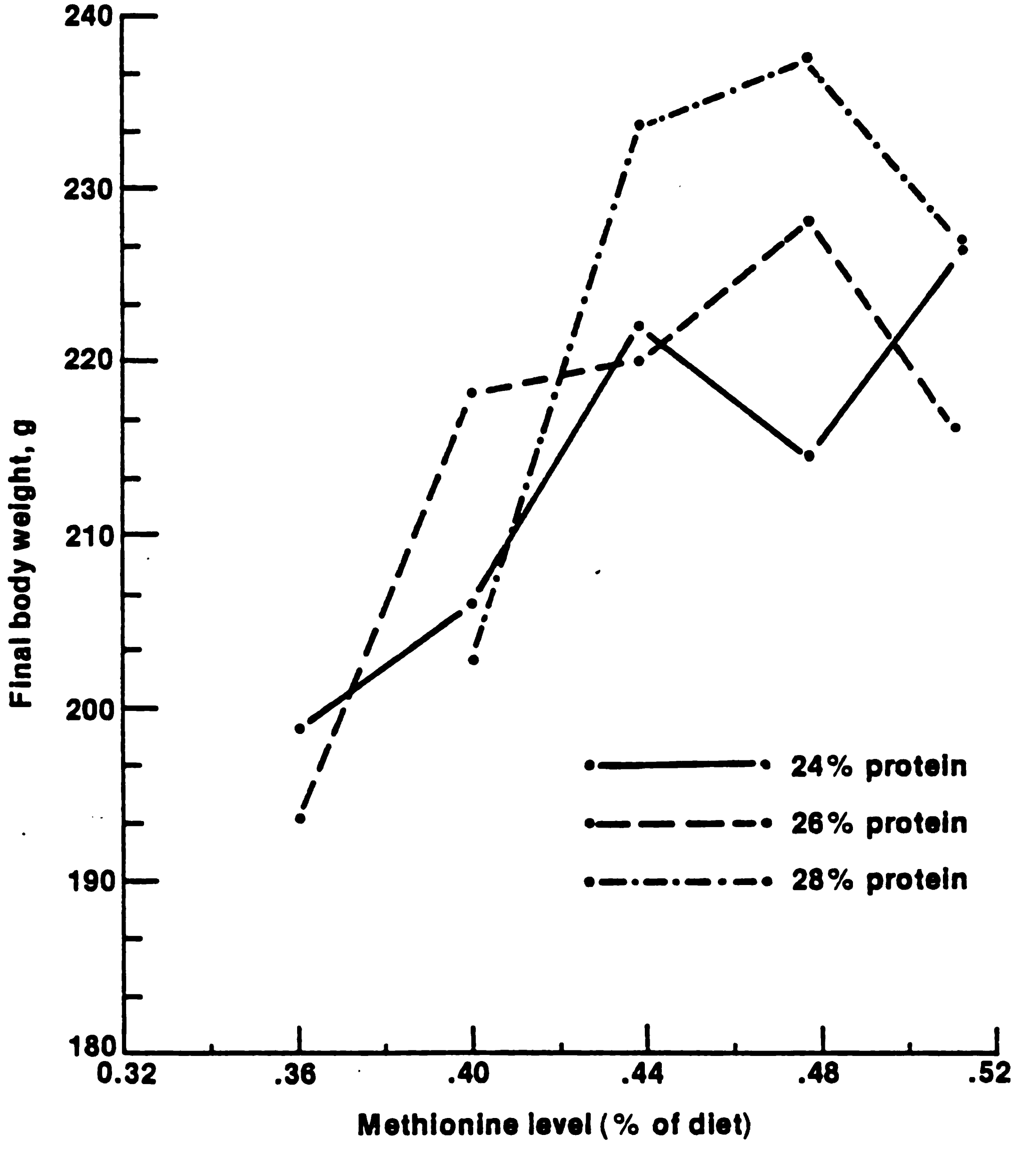

Figure 1. Effect of methionine in the diet on final body weight of Ring-necked pheasant at 4 weeks of age.

studied. According to Gill (1978), when the estimated optimum level lies outside the region of experimentation, the experimenter should not assume that the estimated optimum level is valid without additional experimentation in the region of the suggested optimum.

When pheasant chicks were fed the ration that contained the 26% protein level, the regression analysis demonstrated that the methionine levels had both a linear and quadratic relationship with final body weight ($P < 0.01$) (Table 2, Appendix B).

This relationship can be observed in Figure 1. The prediction equation to estimate the final body weight at this protein level was : $\hat{Y} = 42.916183 + 32.463289x_1 + (-4.541548)x_1^2 + (5.445467 \times 22.89)$. $\hat{Y}$ = the estimated body weight; x_1 = the methionine level, the last term is the adjustment for initial weight, in which the $\hat{\beta}_3$ coefficient calculated for this protein level was multiplied by the initial body weight average of pheasant chicks at this protein level.

The optimum methionine level calculated for 26% protein level was 0.463% of the diet (or 1.78% of the protein). Because of the metabolic relationship of methionine to cystine, the level of cystine calculated for this protein level (0.43% of the diet) was added to the methionine requirement resulting in a total sulfur amino acid (TSAA) requirement of 0.893% of the diet (or 3.43% of dietary protein), assuming 100% biological availability.

As observed earlier, at 28% protein, both a linear and quadratic relationship between the methionine level and final body weight was significant ($P < 0.01$) (Table 3, Appendix B). These effects can also be observed in Figure 1. The prediction equation to estimate the final body weight of pheasant chicks at this protein level was: $\hat{Y} = 68.047950 + 78.517213x_1 + (-10.111522)x_1^2 + (0.825974 \times 23.12)$ in which $\hat{Y}$, x_1 and the last term means the same as explained previously. The optimum methionine level calculated for 28% protein level was 0.475% of the diet (or 1.70% of the protein). Cystine content calculated as percent of the diet for this protein level was 0.46%. When the cystine was added to the methionine requirement a TSAA requirement of 0.935% of the diet (or 3.34% of dietary protein) was determined. The values of $\hat{\beta}$ coefficients used in calculating the prediction equations and the optimum methionine levels for each protein level are given in Table 4, Appendix B, with its standard errors.

The values calculated in this experiment as requirement of methionine and TSAA for maximum growth are not a constant proportion of the level of dietary protein. These values, when expressed as percentage of dietary protein, decreased as protein level increased. This is in agreement with the findings of Grau and Kamei (1950) and Almquist (1952), who stated that the methionine or amino acid requirements of chicks expressed as percentage of the dietary protein decreased as the protein level increased. However, Nelson et al. (1960) stated that the quantitative requirement of chicks for TSAA was 3.51 ± 0.25 percent of the protein, and this relationship was found to be constant for all protein and energy levels studied.

The requirements of methionine and TSAA for starting Ring-necked pheasants from zero to four weeks of age as a percentage of the dietary protein found in this experiment were lower than those values recommended by Scott (1966) (3.5%) and closer to the values recommended by NRC (1977) 3.33%) for starting pheasants. Also, these values were lower than those values recommended by NRC (1977) for chicks (4.04%) and for turkey poults (3.75%).

The body weight means at each protein level were pooled together and the protein effect on final body weight was tested by using Bonferroni t-test. No significant difference was found between the body weight means of pheasant chicks fed the 24% and 26% protein diet, but both these means were significantly lower than the body weight mean of the pheasant chicks fed the 28% protein diet. This is in contrast to the finding of Woodard et al. (1977) who noticed that there was no significant difference in body weight of pheasant chicks fed either 24% or 28%

protein diets at four weeks of age. They did not mention the methionine or TSAA content of the diets used, but all their diets were supplemented with 0.45% DL-methionine. Norris et al. (1936) also found no significant difference in growth of pheasants fed 24%, 27% and 30% protein diets. They reported that the greatest growth at eight weeks of age was obtained when pheasant chicks were fed a 30% protein diet. However, Callenbach and Hiller (1933) reported that pheasant chicks fed a diet that contained 28% protein attained significantly better growth at 12 weeks of age than those fed a 24% protein diet. Scott et al. (1963) stated that a diet that contained 26.5% protein adequately supplemented with methionine hydroxy analog was sufficient for optimum growth and maximum efficiency of feed utilization in young Ring-necked pheasants. The TSAA content in that diet was 3.46% of the protein or 0.920% of the diet.

The final body weights of Ring-necked pheasants at four weeks of age obtained in this experiment were higher than those values reported by Woodard et al. (1977) and Stadelman et al. (1944), were similar to those values presented by Reynnells (1979) and Scott et al. (1954) and were a little lower than those obtained by Scott et al. (1963).

Currently pheasants are raised for shooting preserves and for meat production. Birds raised for release on shooting preserves do not need to attain a maximum body weight. However, birds raised for meat production should attain a maximum body weight as fast as possible. Depending on the purpose of the business, a poultry farmer can decide which level of protein is more economical to use. The three protein levels studied, when supplemented with an adequate amount of methionine, will give satisfactory results, although the 28% protein diet with 0.48% methionine (or 0.94% TSAA) resulted in a significantly higher final body weight than 24% and 26% protein diets. Considering this, when the goal is to produce meat, one would be advised to use diets with 28% protein level, since a rapid and maximum weight gain is desired to get more profits. However, when the pheasant chicks are raised for release, a 24% or 26% protein diet, sufficiently supplemented with methionine, will give satisfactory results, since lighter birds are acceptable. Therefore, a future study using higher methionine levels to determine the optimum methionine level for 24% protein diet is advised, since the calculated value determined in this experiment could not be considered a reliable value.

Feed Consumption

The average feed consumption in grams/bird/day for the four week period are shown in Table 2. These

Table 2. Feed consumption average (g/bird/day) of Ring-necked pheasants from zero to four weeks of age.[1,2,3]

Protein	Methionine level (% of diet)				
%	0.36	0.40	0.44	0.48	0.51
24	14.88[4]	15.74	15.66	15.98	16.13
26	14.00[4]	16.05	15.66	14.64	16.33
28	-	13.81	16.05	16.03	15.72

[1]Data are mean values of three replicates of ten birds each.

[2]There were no statistically significant differences at ($P \leq 0.05$).

[3]The pooled SEM was ± 0.90.

[4]These data were not used in statistical analysis.

Note: The diet with 28% protein and 0.36% methionine could not be formulated.

values of feed consumption were in a range from 13.81 to 16.33 g/bird/day with a SEM ± 0.90. The data when analyzed by using ANOVA (Table 5, Appendix B) showed no significant differences on feed consumption due to protein, methionine level and replication. This is in agreement with Woodard et al. (1977) who found no difference in feed consumption of pheasant chicks fed diets that contained 24% and 28% protein.

It was evident from these results that the methionine levels studied affected body weight but not feed consumption. Perhaps the lower methionine levels used were not enough to support maximal growth but did not affect feed intake. Chee (1978), working with young chicks, stated that a moderate deficiency in methionine had no effect on feed intake but prevented maximal growth.

The values of feed consumption obtained in this experiment were similar to those reported by Stadelman et al. (1944) for pheasant chicks at four weeks of age.

Feed Conversion

Average data on feed conversion of Ring-necked pheasants at four weeks of age (weight gain/feed consumed) for each treatment are shown in Table 3. No significant difference was detected for protein level, methionine level and replication (Table 6, Appendix B). However, there was a trend for improved feed conversion at each protein level as the methionine level increased.

Table 3. Feed conversion of Ring-necked pheasant from zero to four weeks of age.[1,2,3]

Protein %	Methionine level (% of diet) .36%	.40%	.44%	.48%	.51%	Overall Mean ± SEM
24	2.44[4]	2.44	2.32	2.36	2.21	2.33±0.06
26	2.33[4]	2.32	2.22	2.11	2.38	2.26±0.06
28	-	2.21	2.16	2.12	2.11	2.15±0.06

[1]Data are mean values of three replicates of ten birds each.

[2]There were no statistically significant differences at ($P \leq 0.05$).

[3]The pooled SEM was ± 0.13.

[4]These data were not used in statistical analysis.

Note: The diet with 28% protein and .36% methionine could not be formulated.

Pheasant chicks fed the 24% protein diet had the best feed conversion at 0.51% methionine level, the highest methionine level used. For pheasants fed the 26% and 28% protein diets the best feed conversion was shown when the methionine content was 0.48% of the diet. The same trend was observed for the protein effect. The feed efficiency improved as the protein level increased in the diet and the best feed efficiency was obtained by birds fed the 28% protein diet. However, the differences in feed efficiency of birds fed the three protein levels were not statistically significant. These values reported are similar to those obtained by Scott et al. (1963), but somewhat lower than those values reported by Stadelman et al. (1944). Perhaps the differences in energy content of the diets used in the experiments caused these differences.

Mortality

Mortality data, shown as the number of birds that died from the total thirty birds (three replicates of ten birds each) for each diet used, is presented in Table 4. A total of 30 birds (out of 420 birds) died during the experiment. There was no significant effect due to protein or methionine (Table 7, Appendix B). Approximately 60% of the total (18 birds) died during the first seven to ten days of the experiment, without apparent cause. Pheasant chicks appeared to be more

Table 4. Mortality of Ring-necked pheasants from zero to four weeks of age.[1,2]

Protein %	Methionine levels (% of diet)				
	0.36	0.40	0.44	0.48	0.51
24	2[3]	1	5	2	1
26	1[3]	2	1	5	2
28	-	2	1	3	2

[1]Number of dead birds out of 30 birds (three replicates of ten birds each).

[2]There were no significant differences at ($P \leq 0.05$).

[3]These data were not considered for statistical analysis.

Note: The diet with 28% protein and 0.36 methionine could not be formulated.

sensitive to environmental conditions early in life than domestic chicks. As the pheasant chicks are a wild species, when frightened their instinctive reaction is to try to fly. As they were raised in battery brooders, when this occurred, they beat themselves at the top of the battery killing themselves by repeatedly jumping. Although great care was taken to avoid this, 40% of the mortality (or 12 birds) killed themselves that way.

Woodard et al. (1977) stated that the incidence of mortality in Chinese Ring-necked pheasants from one to twenty weeks of age was influenced by the protein level, but in that study protein levels as low as 16% were used. The highest mortality occurred at 16% protein level (19.3%) when compared to 20% (9.1%) and 24% (4.5%) protein levels. His trial was conducted using floor pens. According to Reynnells (1979) pheasant chicks raised on the floor had lower mortality than those pheasants raised in battery brooders, although no signicant difference was registered.

Experiment II.

Hen-day percent egg production

Mean values of hen-day percent egg production of laying Ring-necked pheasants fed the different diets are shown in Table 5. Although no significant effect for protein and methionine was found when the data were analyzed by using ANOVA (Table 1, Appendix C) there

Table 5. Mean values of hen-day egg production (%) of laying Ring-necked pheasants fed three different levels of protein and methionine for 112 days.[1,2,3]

Protein %	Methionine level (% of diet)			Overall
	.25	.29	.33	mean ± SEM
14	60.89	60.76	63.67	61.77±1.70
16	62.38	63.72	65.24	63.78±1.70
18	58.98	68.26	68.22	65.15±1.70

[1]Means of 16 observations (four replicates of four birds each x four periods).

[2]There were no significant differences at ($P \leq 0.05$).

[3]The pooled SEM was ± 2.95.

was a trend to increase egg production as the methionine level increased at each protein level (Figure 2). The highest rate of egg production at 14% and 16% protein levels was obtained when the methionine level was 0.33% of the diet. The cystine content calculated for 14% and 16% protein diets was respectively, 0.23% and 0.26% of the diet. These values added to the methionine level made a TSAA content of 0.56% of the diet (or 4.00% of the protein) for the 14% protein diet and 0.59% of the diet (or 3.69% of the protein) for the 16% protein diet. For birds fed the 18% protein diet, 0.29% methionine level resulted in the highest rate of egg production which was the same rate obtained at 0.33% methionine level. The cystine content (0.30%) of the diet added to the methionine level resulted in a TSAA content of 0.59% of the diet (or 3.28% of the protein).

The methionine levels or TSAA that produced the highest egg production of laying pheasants when considered as percentage of the diet were higher than those values recommended for the laying chicken by NRC (1977), Harms and Damron (1969), Daghir et al. (1964), Bray (1965), Ingram and Little (1958) and Leong and McGinnis (1952). However, they were lower than those values recommended by Ingram et al. (1951) and Lepore (1965) and approximately the same as that recommended by Holmes and Kramer (1965).

The requirements of methionine or TSAA stated by NRC (1977) for turkey breeder hens are also lower than

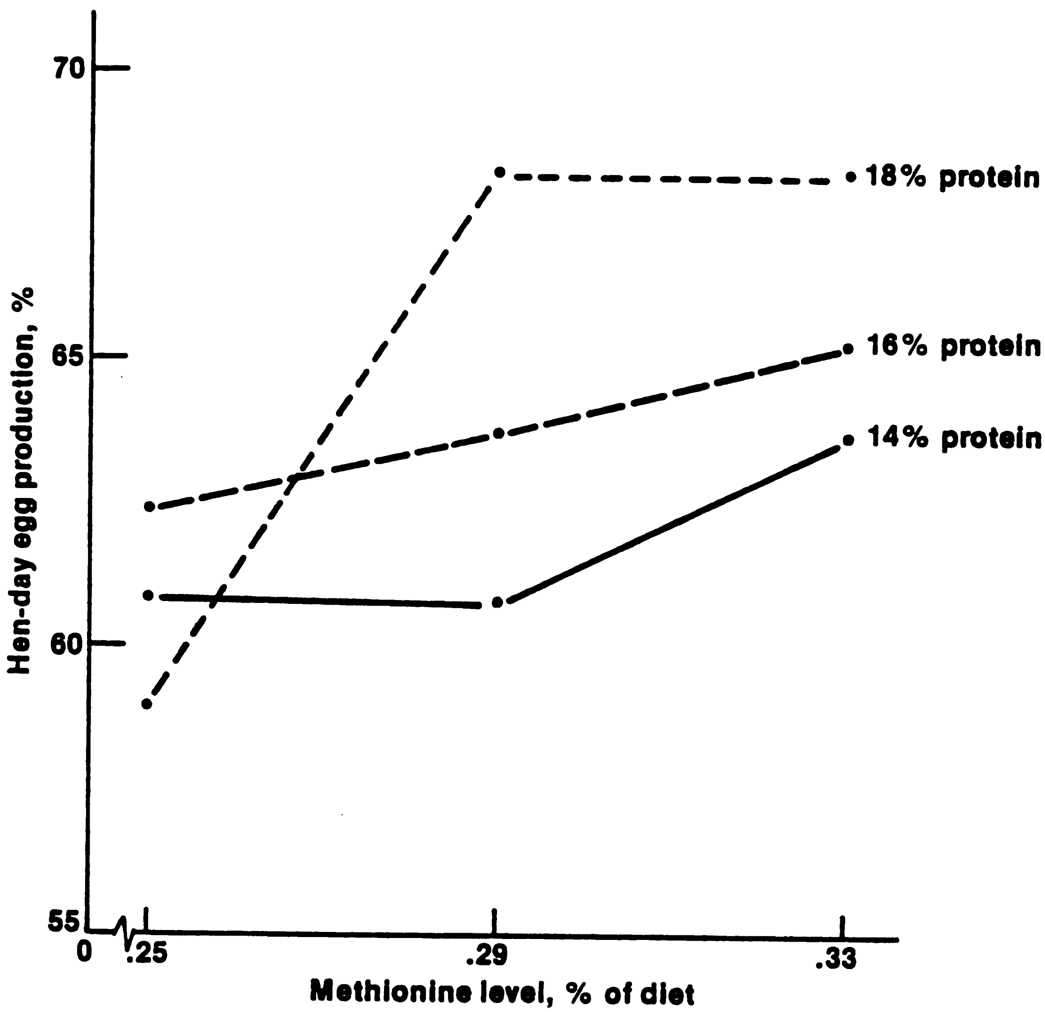

Figure 2. Effect of methionine level in diet on hen-day egg production at three different protein levels.

the values found in this experiment for laying pheasants. Atkinson et al. (1976a) stated that the requirement of TSAA of turkey breeder hens for optimum egg production, egg size and feed efficiency was 0.55% of the diet (or 3.01% of the protein). The protein content of the diet was 18.26%. The requirement of TSAA for the turkey breeder hen was also lower than the value of TSAA required for laying pheasants for an optimum rate of egg production in this experiment.

When the protein levels were considered, the same trend was observed within each methionine level (Figure 3). The egg production rate increased at equivalent methionine levels as the protein level increased. However, at the highest protein level fed (18%), the lowest level of methionine (0.25%) resulted in a decrease in egg production rate. This indicates that perhaps at this protein level methionine became a marginal amino acid. Although no significant difference in egg production was found between the different protein levels used, the results showed an increase in egg production rate when the protein level increased. This concept is supported by the findings of some researchers who worked with laying chickens.

Reid et al. (1951) found that laying chickens fed an 18% protein diet had a higher but not significantly

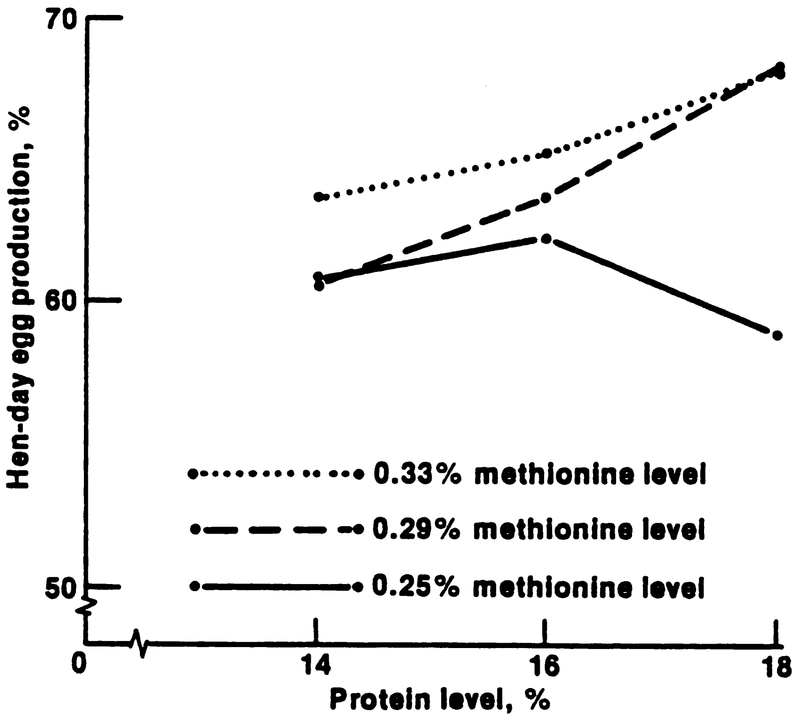

Figure 3. Effect of protein level in diet on hen-day egg production at three different methionine levels.

improved egg production when compared to laying chickens fed either a 13% or 15% protein diet. Guenthner et al. (1972) reported an increase in egg production rate when the protein level in the diet of laying chickens increased from 13.9% to 18.3%, although no significant difference was detected. On the other hand, Quisenberry and Bradley (1962), Gordon et al. (1962) and Gleaves et al. (1968) reported a significant effect on egg production, egg weight and feed efficiency of laying chickens when the protein level in the diet increased gradually from 13% to 19%. Miller et al. (1956), however, found that neither egg production nor body weight maintenance of laying chickens was affected by increasing the protein level from 12% through 21%. Similarly, Smith (1967), Summers et al. (1967) and Thornton et al. (1957) found that egg production of laying chickens was not affected when diets with protein levels which ranged from 11% to 19% were fed. Milton and Ingram (1957) stated that laying chickens fed a 14% protein diet produced as well as those fed 16% and 18% protein diets. Minear et al. (1970) reported no signficant difference in reproduction performance of Large White turkey breeder hens fed diets with 14% and 16% protein levels. Luther and Waldroup (1970) found no differences in reproductive performance of Broad Breasted Bronze turkey breeder hens when fed 14.7% and 16% protein diets which contained 0.66% of TSAA.

Based on these results, it can be stated that although no significant difference in egg production rate was found when laying pheasants were fed diets that contained 14%, 16% and 18% protein levels, the birds fed the higher protein level produced at a higher numerical rate. Perhaps the number of replications used in this experiment was not enough to support a significant difference in egg production among the diets used.

The highest rate of hen-day egg production (68.26%) was obtained by birds fed the 18% protein diet which contained 0.29% methionine (or 0.59% of TSAA). This rate of egg production was 3.02% and 4.60% higher than the egg production rate of those birds fed 16% and 14% protein diets, respectively, with 0.33% methionine. Although these differences in egg production rate were not significantly different, it is important to notice that in some cases an increase of 3.02% or 4.60% in egg production can economically compensate an increase in the protein level of the diet. The size of the business and the cost of the ingredients used in making the feed should be taken into consideration to make the decision on what protein level should be used.

Period analysis of the data showed that there was a statistically significant difference ($P < 0.001$) for percent hen-day egg production due to period (Table 6). The lowest egg production rate was obtained during the

Table 6. Mean and standard error of hen-day percent egg production, egg weight and feed consumption of laying Ring-necked pheasants for four periods of experimentation.

Period	Hen-day percent egg production[1]	Egg weight[1] (g)	Feed consumption[1] (g/bird/day)
1	$42.91^{a} \pm 1.96$	$27.55^{a} \pm 0.20$	$57.72^{a} \pm 0.73$
2	$76.72^{c} \pm 1.96$	$28.77^{b} \pm 0.20$	$67.32^{b} \pm 0.73$
3	$71.41^{c} \pm 1.96$	$29.01^{b} \pm 0.20$	$68.64^{b} \pm 0.73$
4	$63.24^{b} \pm 1.96$	$29.39^{b} \pm 0.20$	$68.37^{b} \pm 0.73$

[1]Data are means of the nine treatment combinations when pooled together by period for hen-day percent egg production, egg weight and feed consumption.

[a,b,c]Means within a column with different superscripts are significantly different at ($P \leq 0.05$).

first period followed by a sharp increase during the second period and then a decrease for both the third and fourth periods. This tendency was observed in all experimental groups (Table 2, Appendix C) and it was due to a normal change in production by birds in general as the production cycle progresses. The hen-day percent egg production values obtained in this experiment were similar to those values reported by Carpenter (1980) and Reynnells (1979) for Ring-necked laying pheasants and higher than that value reported by Smith et al. (1968).

Egg weight

The egg weight means for all treatment combinations are summarized in Table 7. After these data were analyzed using ANOVA (Table 3, Appendix C), a significant effect for protein level ($P < 0.001$) was found but none for methionine. Also, the interaction, protein and methionine, was significant ($P < 0.004$) indicating that the protein levels used did not produce the same difference in responses at each methionine level. Based on this, the egg weight means for the different protein levels were compared within each methionine level. At the lowest methionine level (0.25% of the diet) the egg weight response did not follow the same trend when compared to 0.29 and 0.33 percent methionine level.

Table 7. Means of egg weight (g) of laying Ring-necked pheasants fed three different levels of protein and methionine in the diet for 112 days.[1,2]

Protein %	Methionine level (% of diet)			Overall mean ± SEM
	.25	.29	.33	
14	28.88^{ab}	27.52^{a}	28.16^{a}	$28.19^{a} \pm 0.17$
16	27.89^{a}	28.82^{b}	28.49^{a}	$28.40^{a} \pm 0.17$
18	29.79^{b}	29.50^{b}	29.08^{a}	$29.46^{b} \pm 0.17$

[1]Mean weight of eggs produced by 16 birds (four replicates of four birds) through four 28-day periods.

[2]The pooled SEM was ± 0.30

[a,b]Means in the same column with different superscripts are significantly different at ($P \leq 0.05$).

At the 0.25% methionine level the mean egg weight of birds fed the 16% protein diet was significantly ($P < 0.05$) lower than that produced by birds fed the 18% protein diet. However, no significant difference was found when the egg weight means of birds fed the 16% and 18% protein diets were compared to that of birds fed the 14% protein diet.

At 0.29% methionine level there was an increase in egg weight as the protein level increased. The egg weight of birds fed a 14% protein diet was significantly ($P < 0.05$) lower than weight of those laid by laying pheasants fed the 16% and 18% protein diets. However, no significant difference was found between egg weight means of birds fed a 16% or 18% protein diet.

At 0.33% methionine level the egg weight means numerically increased as the protein level increased, but these differences were not statistically significant.

According to the results obtained it can be stated that a level of 0.25% methionine was enough to obtain an optimum egg weight at 14% and 18% protein levels. However, for birds fed the 16% protein level, 0.25% was not enough methionine to support optimum egg size, since the birds fed the 16% protein diet laid significantly smaller eggs than those fed the 18% protein diet. Based on this, a higher methionine level (0.29% of the diet) should be advised for the 16% protein level. Although the difference was statistically significant, it was small and may be attributable to the lower feed intake at this protein level and/or to the hen size (Table 8, Appendix C, and Table 8) rather than to the methionine level. The body weight averages of the different experimental groups were approximately the same for all groups at the beginning of the experiment, but the range of individual body weights was not the same for all groups at the end of the trial. This could cause some influence in egg weight because of the postive correlation between hen size and egg size as shown by Graham, cited by Thornton et al. (1957), and Funk (1935).

When the cystine contents calculated as percentage of the diet were added to the methionine requirements, a TSAA of 0.50%, 0.55% and 0.53% of the diet or 3.57%, 3.44% and 2.94% of the dietary protein were the best levels for 14%, 16% and 18% protein of laying pheasants diets for an optimum egg weight.

These results indicate that the methionine or TSAA requirements of laying pheasants for an optimum egg weight at each protein level were lower than those found for maximum rate of production. This finding is not in agreement with that reported by Harms and Damron (1969) and Bray (1965) for laying chickens. They found that TSAA requirement for maximum egg weight was slightly higher than that for maximum rate of egg production.

When the overall egg weight means for the different protein levels were compared, there was a tendency to increase egg weight as protein level increased. Birds fed the diet that contained 18% protein level produced a significantly heavier egg weight than those fed either 14% or 16% protein diets. No significant differences was found between egg weight of birds fed 14% and 16% protein diets. This result is supported by the findings of Deaton and Quisenberry (1965), Smith (1967), Biely and March (1964) and Thornton et al. (1957) for laying chickens. These authors reported that differences in protein levels of from 11% to 19% did not affect or slightly affected egg production but did affect egg weight. They reported that birds fed a higher protein diet laid significantly heavier eggs than those fed lower protein levels.

Period caused a significant effect ($P < 0.001$) on egg weight. Table 6 shows that egg weight increased with time. This effect was expected and it was due to a normal change associated with the increasing age of the laying pheasants. These birds were approximately eight months old at the beginning of the trial. Period one showed the lowest egg weight mean, which was significantly different ($P < 0.05$) from the following three periods, confirming the lack of total physical development. The differences shown between periods two, three and four were not significant. Table 4, Appendix C, shows the egg weight mean for each treatment combination through the four periods. The egg weight means found in this study ranged from 26.20 to 31.22 g with a SEM ± 0.61. This range is a little wider than those reported by Reynnells (1979), Hinkson et al. (1970), Greeley (1962), Woodard et al. (1978) and Labisky et al. (1969), but similar to those values reported by Breitenbach et al. (1963). These differences may be due to the fact that the birds used in this experiment were of different age and size than the birds in the other trials reported.

Feed consumption

The feed consumption (grams/bird/day) as average values of four replicates of four birds each through four 28-day periods for each treatment combination is shown in Table 8. These data, when analyzed (Table 5, Appendix C) showed that feed consumption was significantly affected by protein ($P < 0.007$) and period ($P < 0.001$). Methionine effect was nearly significant ($P < 0.058$). The mean values for each protein level and methionine level were calculated (Table 9) and Bonferroni t-test was used to compare means. The feed consumption of birds fed 14% protein diet was not significantly different from those fed either 16% or 18% protein diets. However, birds fed the 18% protein diet had a significantly higher feed consumption than those fed the 16% protein diet.

Table 8. Feed consumption (g/bird/day) of laying Ring-necked pheasants fed diets with three different protein and methionine levels for 112 days.[1,2]

Protein	Methionine level (% of diet)		
%	.25	.29	.33
14	65.78	65.07	65.03
16	62.90	65.40	64.37
18	64.24	68.63	68.36

[1]Data are means of 16 observations (four replicates x four periods).

[2]The pooled SEM was ±1.10.

Table 9. Protein and methionine effect on feed consumption (g/bird/day) of laying Ring-necked pheasants for 112 days.[1,2]

Protein level (%)			Methionine level (% of diet)		
14	16	18	.25	.29	.33
65.29[ab]	64.22[a]	67.07[b]	64.30[a]	66.30[a]	65.92[a]

[1]Data are means of all protein or methionine values pooled together.

[2]The pooled SEM was ± 0.64.

[a,b]Means with different superscripts are significantly different ($P < 0.05$).

When the methionine effect on feed consumption was tested, no significant differences were detected between the means representing feed consumption for each methionine level; although, birds fed the diet that contained the lower methionine level (0.25%) showed a lower numerical feed consumption when compared to those fed 0.29% and 0.33% methionine levels.

Harms and Damron (1969) reported that laying chickens fed diets that contained a methionine level as low as o.188% of the diet had a slightly reduced feed intake. However, no differences in feed intake were found between diets that contained higher methionine levels (0.228%, 0.268%, 0.301% and 0.348%). This finding supports the results obtained in this study with laying pheasants. The methionine levels ranging from 0.25% to 0.33% of the diet did not significantly affect feed consumption.

Most of the research done with laying chickens shows a better feed efficiency when the protein level in the diet is increased, but the researchers usually did not mention the feed consumption. Only Guenthner et al. (1972) reported that feed intake and feed conversion of laying chickens were not affected when the protein level in the diet increased from 13.95 to 18.3%, in contrast to the results obtained in this study.

The means and standard error of feed consumption for the different periods are shown in Table 6. The only significant difference found was for period one when compared with each of the other three periods. These averages paralleled the egg weight pattern (Table 6) and they were related to the normal change associated with the physical development of the pheasant hens and the increased egg production. Feed consumption averages of the different diets through the four 28-day periods ranged from 54.2 to 72.64 $\pm$ 2.20 grams/bird/day (Table 6, Appendix C). These values are similar to those found by Breitenbach et al. (1963) and Reynnells (1979). On the other hand values of feed consumption reported by Hinkson et al. (1970) ranged from 96 to 111 g/bird/day, which were higher than those values found in this study. This difference may have been due to the fact that these authors reared the birds on the floor and included the males in determining the average feed consumption.

Body Weight

The body weight change as an average for each group of laying pheasants fed diets with three different protein and methionine levels during the 112 days of the experiment is shown in Table 10. The change in body weight as measured by the difference in weight at the beginning and end of the experimental period, adjusted for initial weight, showed no significant differences within protein and methionine levels studied. However,

Table 10. Means of body weight gain (g/bird) of laying Ring-necked pheasants fed diets with three protein and methionine levels for 112 days.[1,2]

Protein %	Methionine level (% of diet) .25	.29	.33	Overall mean ± SEM
14	119.64	127.82	115.00	120.82±15.99
16	100.31	169.06	126.67	132.01±15.82
18	159.68	180.00	146.33	162.00±15.99
Overall mean	129.54	158.96	129.33	
± SEM	±15.99	±15.82	±15.99	

[1]Data are means of live birds at the end of the experiment.

[2]There were no significant differences at ($P \leq 0.05$).

when the protein effect was considered, the overall mean showed a higher weight gain as the protein level increased. This observation is supported by the findings of Harms _et al_. (1962, 1969), Gleaves _et al_. (1968) and Smith (1967) who reported that an increase in protein level resulted in larger body weight gains of laying chickens.

The laying pheasants showed a gain in weight during the trial in all treatments. This was expected due to a normal change in the physical development, since these birds were about eight months old at the beginning of the experiment.

Initial body weights were significantly different ($P < 0.001$) (Table 7, Appendix C). Although the experimental groups formed were approximately of the same weight (Table 8, Appendix C) the range of the individual body weights (900 g to 1500 g) was not the same for all groups. As the data were analyzed using individual weights, a significant effect was detected for initial weight.

Mortality

The mortality that occurred in this experiment is shown in Table 11. A total of five birds died during the whole experiment (112 days). There was no significant effect due to protein, methionine or period (Table 9, Appendix C). The causes of death were prolapse of oviduct

Table 11. Mortality of laying Ring-necked pheasants on diets with three different levels of methionine and protein for 112 days.[1]

Protein	Methionine level (% of diet)		
%	.25	.29	.33
14	2	0	0
16	0	0	1
18	0	1	1

[1]Number of dead birds out of 16 birds (four replicates of four birds each).

(2 birds), repeatedly jumping against the top of cage (2 birds), unknown (1 bird).

Experiment III.

Hen-day percent egg production

Hen day percent egg production means during the three 28-day periods can be seen in Table 12. Protein and methionine levels used did not significantly affect the rate of egg production (Table 1, Appendix D), although there was a strong significance ($P < 0.033$) for protein-methionine interaction. The methionine effect at each protein level was tested, comparing the mean values for the different methionine levels within each protein level, using Bonferroni t-statistics. The methionine levels used did not significantly affect egg production rate of birds fed diets that contained 14% and 16% protein level. However, the highest egg production rate at 14% protein level was obtained at 0.29% methionine. The cystine content calculated for this diet was 0.23%. This, when added to the methionine content, made a TSAA content of 0.52% of the diet (or 3.71% of the protein). For diets that contained 16% protein the highest egg production rate was obtained at 0.33% methionine level. The cystine value calculated for this diet (0.26%) when added to the methionine content made a TSAA content of 0.59% of the diet (or 3.69% of the protein). For birds

Table 12. Mean values of hen-day egg production (%) of laying Ring-necked pheasants fed three different levels of protein and methionine for 84 days.[1,2]

Protein %	Methionine level (% of diet) .25	.29	.33
14	45.50^{a}	55.41^{a}	44.59^{a}
16	48.79^{a}	50.09^{a}	57.35^{a}
18	59.61^{b}	41.61^{a}	52.92^{ab}

[1]Means of 12 observations (four replicates of four birds x three periods).

[2]The pooled SEM was ± 4.85.

[a,b]Means with different superscripts in the same row are significantly different at ($P \leq 0.05$).

fed the 18% protein diet the methionine levels used significantly affected the egg production rate. The highest egg production at 18% protein diet was obtained when the methionine content was 0.25% of the diet. This production rate was significantly higher than the egg production rate of birds fed the diet that contained 0.29% methionine. However, no significant difference in the egg production rate was found between birds fed diets that contained either 0.25% or 0.33% methionine. Also, no significant difference was found between the egg production rate of laying pheasants fed 0.29% and o.33% methionine. Therefore, for 18% protein a level of 0.25% methionine or 0.53% of TSAA of the diet (or 2.94% of the protein) resulted in the highest egg production.

The best levels of methionine or TSAA for maximum rate of egg production at each protein level obtained in this experiment were different from those obtained in Experiment II, with laying pheasants in their first year of egg production. In this trial, with pheasants in the second year of production the optimum methionine (or TSAA) levels found for diets that contained 14% and 18% protein were lower than the levels found in Experiment II. However, for 16% protein diet, the level of methionine required for maximum egg production was the same in both experiments.

When the protein effect was tested within each methionine level, the responses were not consistent and different trends were observed at each methionine level (Figure 4). When laying pheasants were fed diets that contained 0.25% methionine, the egg production rate increased as the protein level increased. However, when diets that contained 0.29% methionine were fed to the laying pheasants, the egg production rate decreased as the protein level increased. A different response was obtained when laying pheasants were fed diets that contained 0.33% methionine level. In this case, the egg production rate increased as the protein content in the diet increased from 14% to 16% and then decreased at 18% protein. No significant difference for egg production rate was found when the means for the different protein levels were compared within each methionine level.

According to this experiment the highest rate of egg production was obtained from laying pheasants fed the 18% protein diet that contained 0.25% methionine or 0.53% of TSAA (2.94% of the protein). Production of these birds was 2.26% and 4.20% higher than the egg production rate of hens fed 16% protein with 0.33% methionine and 14% protein with 0.29% methionine, respectively. Statistically, these differences were not significantly different. Based on this, either one of these

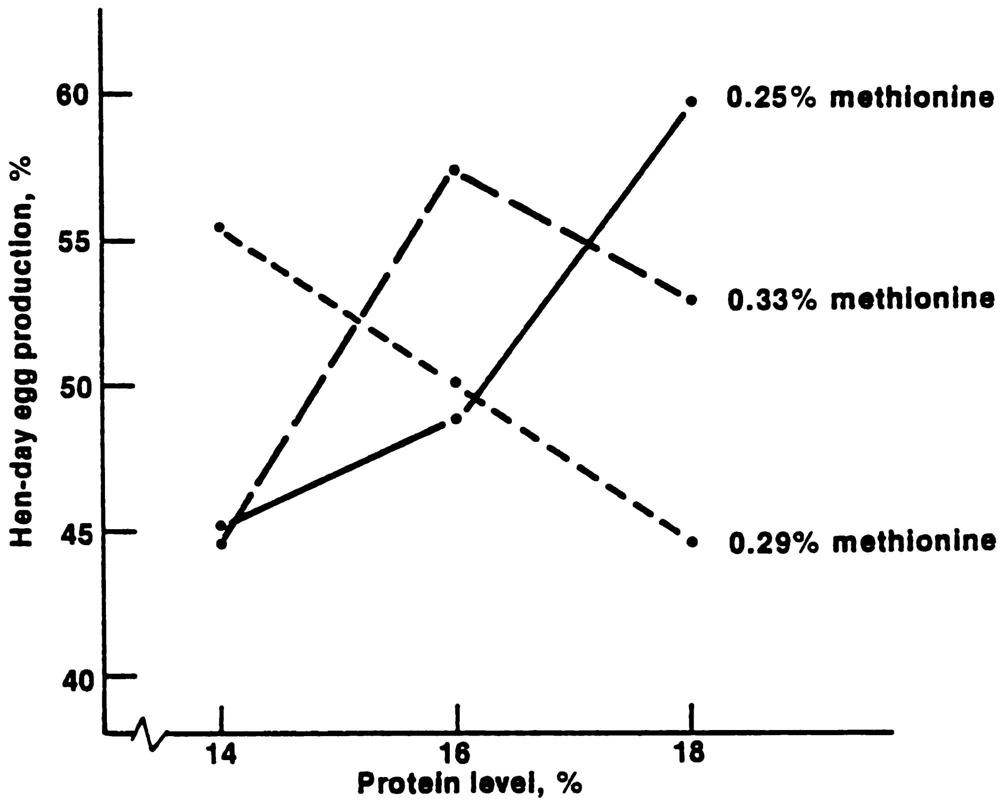

Figure 4. Effect of protein level in diet on hen-day egg egg production at three different methionine levels.

levels of protein with the adequate amount of methionine can be used for laying pheasants. However, a higher rate of egg production was obtained with higher protein (18%) diet. These results agree with those obtained in Experiment II. In both experiments, diets which contained the highest protein level resulted in the highest egg production, although no statistically significant difference was found between the diets used.

For period there was also a significant effect ($P < 0.001$) on egg production rate, which was considered normal. Table 13 shows the period effect on hen-day percent egg production. As these birds were in production when the experiment began, the first period showed a higher rate of egg production and then a decrease for the second and third periods. The only significant difference found was for the third period which was significantly lower when compared to the other two egg production periods. The same tendency was observed in all treatment combinations (Table 2, Appendix D).

Laying pheasants in their second production cycle had a lower percent hen-day egg production than those young laying pheasants used in Experiment II. This finding is not in agreement with Labisky et al. (1969) who found that laying pheasants showed greater egg laying capacity as two year olds than they did as yearlings. However, Smith et al. (1968) reported that laying pheasants in

Table 13. Mean and standard error of hen-day percent egg production and feed consumption of laying Ring-necked pheasants for three periods of experimentation.

Period	Hen-day percent egg production[1]	Feed consumption[1] (g/bird/day)
1	$61.36^{a} \pm 2.85$	$56.05^{a} \pm 1.37$
2	$51.57^{a} \pm 2.85$	$64.08^{b} \pm 1.47$
3	$39.02^{b} \pm 2.85$	$63.31^{b} \pm 1.37$

[1]Data are means of the nine treatment combinations when pooled together by period for hen-day percent egg production and feed consumption.

[a,b]Means within a column with different superscripts are significantly different at ($P \leq 0.05$).

their first year of production had a higher egg production rate than two year old laying pheasants, which is in agreement with the results obtained in these experiments.

Egg Weight

Table 14 shows a summary of egg weight means of laying pheasants in their second year of production fed nine different diets during 84 days of production. When the data for egg weight was analysed by using analysis of variance (Table 3, Appendix D), protein effect was significant ($P < 0.001$) and protein-methionine interaction also showed a significant effect ($P < 0.001$). As the interaction of protein-methionine was significant, the means for the different protein levels were compared at each methionine level. The protein levels did not produce the same differences in responses at each methionine level.

When the methionine level in the diets was 0.25%, the egg weight mean of birds fed 14% protein was significantly lower than the egg weight mean of birds fed 16%, but not significantly different from egg weight of birds fed 18% protein. Also, no significant difference was detected between egg weight mean of birds fed either 16% or 18% protein diets.

At 0.29% methionine, no significant difference was found between egg weight means of laying pheasants fed

Table 14. Mean and standard error of egg weight (g) of laying Ring-necked pheasants fed three different levels of protein and methionine in the diet for 84 days.[1]

Protein %	Methionine level (% of diet)			Overall mean ± SEM
	.25	.29	.33	
14	$28.24^{a} \pm .55$	$29.24^{a} \pm .55$	$29.50^{a} \pm .60$	$28.93^{a} \pm 0.33$
16	$31.19^{b} \pm .60$	$29.71^{a} \pm .55$	$31.69^{b} \pm .58$	$30.86^{b} \pm 0.33$
18	$29.49^{ab} \pm .58$	$33.04^{b} \pm .60$	$30.95^{ab} \pm .64$	$31.16^{b} \pm 0.35$

[1]Mean weight of eggs produced by 16 birds (four replicates of four birds) through three 28-day periods.

[a,b]Means in the same column with different superscripts are significantly different at ($P \leq 0.05$).

either 14% or 16% protein diet. However, the egg weight mean of birds fed the 18% protein diet was significantly heavier than that of those produced by birds fed the 14% and 16% protein diets. At 0.33% methionine level, the birds fed the 14% protein diet laid significantly smaller eggs than did those fed the 16% protein but not significantly smaller than did the birds fed the 18% protein diet. No significant difference was found between the egg weight mean of birds fed either 16% or 18% protein diets.

Similar to the results obtained in Experiment II, there was a significant effect for protein level and also for protein-methionine interaction. However, when the means of egg weight for each protein level were compared within each methionine level the responses obtained were different. This indicated that young laying pheasants and two-year-old laying pheasants behaved in a different way to the protein and methionine levels used.

According to the results obtained when the protein levels in the diets were 14% and 16%, a methionine content of 0.33% of the diet was required for an optimum egg weight. However, in a diet that contained 18% protein, a level of 0.29% methionine was sufficient for an optimum egg weight. When the cystine content calculated for each diet was added to the methionine values the TSAA content was 0.56%, 0.59% and 0.59% of the diet or 4.00%, 3.69% and 3.28 % of dietary protein for 14%, 16% and 18% protein diets, respectively. These values of methionine or TSAA found for an optimum egg weight were higher than those found for a maximum rate of egg production. This is in contrast to the results shown in Experiment II, but is in agreement with the findings of Harms and Damron (1969) and Bray (1965) for laying chickens.

The methionine or TSAA requirements for an optimum egg weight were higher for laying pheasants in their second year of production than were those for the laying pheasants in their first year of lay.

When the overall egg weight means for the different levels of protein were compared, the same tendency was observed in both experiments. The egg weight mean increased as the protein level in the diets increased. However, in trial III laying pheasants fed the 14% protein diet laid significantly smaller eggs than did those fed either 16% or 18% protein diets. The egg weight means of laying pheasants fed either 16% or 18% protein diet were not significantly different.

In Experiment III, the egg weight means ranged from 28.7 g to 33.43 g (Table 4, Appendix D). This range was wider than that reported by Labisky <u>et al</u>. (1969) for two-year-old laying pheasants. Perhaps, this was caused by the differences in body weight of laying pheasants used in both experiments. Also this range was wider than that range of weight means of eggs laid by the laying pheasants in their first year of production in Experiment II.

Feed Consumption

Feed consumption averages (g/bird/day) for the different diets are shown in Table 15. Protein and methionine levels used did not significantly affect feed consumption. The only significant effect ($P < 0.001$) found was for period (Table 5, Appendix D). Table 13 shows the average feed consumption by period. Period one had a significantly lower average feed consumption compared to the other two periods. No significant difference in feed consumption was detected between periods two and three.

Feed consumption averages did not parallel the rate of egg production and the highest rate of egg production was obtained in period one when these birds had the lowest feed consumption. Perhaps,this lower feed consumption in period one can be explained by the stress caused in changing these birds from a floor management system to a cage management system.

Table 15. Feed consumption averages (g/bird/day) of laying Ring-necked pheasants fed diets with three protein and methionine levels for 84 days.[1,2,3]

Protein	Methionine level (% of diet)			Overall
%	.25	.29	.33	mean ± SEM
14	63.49	64.25	60.47	62.74±1.37
16	60.00	58.61	64.46	61.02±1.37
18	59.90	58.01	61.17	59.69±1.37

[1]Data are means of 12 observations (four replicates x three periods).

[2]The pooled SEM was ± 2.38

[3]There were no statistically significant differences at ($P \leq 0.05$).

The results obtained for feed consumption in this experiment showed some differences when compared to the Experiment II, with the laying pheasants in their first year of lay. In general, the feed consumption averages for these old laying pheasants were lower than the feed consumption averages of the young pheasant hens, in Experiment II (Tables 9 and 15).

Protein and methionine did not affect feed consumption in these old laying pheasants, but protein did affect feed consumption in the young pheasant hens. Although protein levels did not significantly affect feed consumption, a tendency to decrease feed consumption as protein level increased was observed when the overall means were compared. Birds at the highest protein level (18%) showed the lowest feed consumption average. In contrast to this, the young laying pheasants, in Experiment II, had the highest feed consumption at this highest protein level.

Feed consumption averages of the different treatment combinations through the three 28-day periods ranged from 51.50 to 67.88±4.11 grams/bird/day (Table 6, Appendix D). These values were lower than those presented by Breitenbach et al. (1963) and Reynnells (1979), and similar to the values reported by Adams et al. (1967).

The lower feed consumption by these pheasant hens was perhaps the main cause of a lower peak and more rapid decline in the egg production rate and losses in body weight that occurred in Experiment III. Breitenbach *et al*. (1963) reported that laying pheasants in a limited feed intake (45 g/bird/day) had a pronounced ability to maintain egg size while the egg number was markedly decreased when compared with those pheasant hens fed *ad libitum*.

Body Weight

The body weight change as an average for each experimental diet, during the 84 days of the experiment, is shown in Table 16. The change in body weight was measured by the difference in weight at the beginning and end of the experimental period. These old pheasant hens (in contrast with the young pheasant hens, in Experiment II) lost weight during the experimental period when fed any of the diets used.

Data of individual body weight changes (adjusted for initial weight) were anlayzed (Table 7, Appendix D) and no significant effect for protein and methionine was found. Only initial body weight showed a significant effect ($P < 0.001$). This can be explained by the range of variation of individual body weights, which was not the same for all experimental groups. The individual

Table 16. Means of body weight loss (g/bird) of laying Ring-necked pheasants fed diets with three protein and methionine levels for 84 days.[1,2]

Protein	Methionine level (% of diet)			Overall
%	.25	.29	.33	mean ± SEM
14	77.81	65.36	124.38	89.18±20.19
16	147.85	66.66	65.33	93.28±20.64
18	77.00	101.56	105.94	94.83±19.97
Overall mean	100.89	77.86	98.55	
± SEM	20.42	20.42	19.98	

[1]Data are means of live birds at the end of the experiment.

[2]There were no significant differences at ($P \leq 0.05$).

bird weights ranged from 1000 g to 1750 g, although the body weight means of each experimental group had a narrow range of variation from 1293.33 to 1435.71 g (Table 8, Appendix D). These birds (as they were two years old) had a heavier body weight than those of Experiment II.

Protein and methionine did not significantly affect the weight loss. However, the overall mean of weight loss for the different protein levels showed an increase in weight loss as the protein level increased. Perhaps, this fact was related to feed consumption, since it was observed (Table 15) that as protein level in the diet increased, feed consumption decreased. Laying pheasants fed the 18% protein diet had the lowest feed consumption and also they had the highest **loss** of weight.

When the overall means for methionine levels were considered, a marked trend was not observed. However, birds fed diets that contained 0.25% methionine had the highest loss of weight and birds fed the diet that contained 0.29% methionine had the lowest loss of weight, but no significant difference was detected. Labisky et al. (1969) reported that two-year-old pheasant hens lost between 2 and 2.5 grams of body weight per egg laid or approximately 232 grams during the laying season (120 days).

Mortality

Table 17 shows the mortality of laying Ring-necked pheasants through 84 days of the experiment. A total of seven birds died during the trial. There was no significant effect of protein, methionine and period (Table 9, Appendix D). All birds dead were autopsied at the Michigan State University Diagnostic Clinic. The deaths which occurred were not related to the diets used.

Table 17. Mortality of laying Ring-necked pheasants on diets with three different levels of protein and methionine for 84 days.[1]

Protein %	Methionine level (% of diet)		
	.25	.29	.33
14	0	2	0
16	2	1	1
18	1	0	0

[1]Number of dead birds out of 16 birds (four replicates of four birds each).

V. SUMMARY

Starting Pheasants

A study was conducted to determine the minimum optimum level of methionine or total sulfur amino acids (TSAA) in the diet for pheasants from zero to four weeks of age. Because protein and methionine requirements are interrelated these two nutrients were evaluated simultaneously in a factorial experimental design.

Fourteen isocaloric practical diets containing 24% 26% and 28% protein and 0.36, 0.40, 0.44, 0.48 and 0.51% methionine within each level of protein were used. The diet with 28% protein and 0.36% methionine could not be formulated with the ingredients available for this experiment.

From this study the following observations were made:

- Final body weight of Ring-necked pheasants at four weeks of age was influenced by methionine (or TSAA) at all protein levels studied. When the protein content in the diet was 24%, there was a significant linear relationship between methionine level and final body weight. As methionine level in the diet increased the final body weight increased. In this study, a 0.637%

methionine requirement was calculated as the optimum minimum methionine level which could not be considered a reliable value, because it was outside of the region of methionine values tested in this experiment. Therefore, further growth studies using higher methionine levels to determine the optimum methionine level for 24% protein is advised.

- In diets that contained 26% and 28% protein, a significant linear and quadratic relationship between methionine levels and final body weight was observed. The methionine requirements calculated were 0.463% and 0.475% of the diet or 1.78% and 1.70% of the dietary protein for 26% and 28% protein levels, respectively. The TSAA requirements were 0.893% and 0.935% of the diet or 3.43% and 3.34% of dietary protein for 26% and 28% protein levels, respectively.

- The requirements of methionine or TSAA when expressed as percentage of the dietary protein decreased as protein level increased.

- The overall body weight for birds fed the 28% protein diets was significantly higher than those for birds fed the 24% and 26% protein diets.

- The feed consumption, feed conversion and mortality were not significantly affected by the methionine and/or protein levels used in this study. However, there was a trend for improved feed conversion as methionine level increased within each protein level. The same trend was observed when protein level increased.

- According to these results a diet of 26% protein containing 0.463% of methionine (or 0.893% of TSAA) seems to be appropriate for raising pheasants for shooting preserves. A diet containing 28% protein with 0.475% of methionine (or 0.935% of TSAA) might be used when pheasants are intended for meat production.

Adult Pheasants

Two experiments with adult laying pheasants were conducted to study the influence of dietary protein and methionine or TSAA on egg production, egg weight, feed consumption, body weight change and mortality of the birds.

One experiment was carried out with pheasants in their first year of egg production for 112 (four 28-day periods). The other experiment was carried out with pheasants in their second year of egg production for 84 days (three 28-day periods). Both experiments were conducted with birds in individual cages.

Nine isocaloric practical diets containing 14%, 16% and 18% protein and 0.25%, 0.29% and 0.33% methionine for each level of protein were used.

From these studies the following observations were made:

- Hen-day percent egg production for birds in both the first and second year of egg production was not significantly affected by protein and/or methionine level in the diet. Nevertheless, a slight increase in egg production rate with increased protein and/or methionine was detected for the birds in their first year of production.

- Egg weight was significantly affected by protein level. Laying pheasants in their first year of egg production fed diets that contained 18% protein laid eggs significantly heavier than those fed either 14% or 16% protein diets. Birds in their second year of production fed 16% and 18% protein diets laid eggs significantly heavier than those fed a 14% protein diet.

- Feed consumption of laying pheasants in their first year of egg production was significantly affected by protein level. The feed consumption of birds fed 14% protein diet was not significantly different from those fed either 16% or 18% protein diets. However, birds fed the 18% protein diet had a significantly higher feed consumption than those fed the 16% protein level.

- Mortality and body weight change in birds in their first and second year egg production were not significantly affected by protein and/or methionine levels used. However, it could be observed that birds in their first year of production gained weight throughout the period of experimentation. On the other hand, birds in their second year of production lost weight during the same period.

- The observations made in these studies suggest that diets containing a minimum level of 16% protein and 0.33% methionine (or 0.59% of TSAA) seem reasonable to be used with satisfactory results in egg production rate and egg weight of laying Ring-necked pheasants in their first and second year of egg production.

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APPENDIX A

Table 1. National Research Council requirement recommendations for protein, methionine and TSAA.[1]

Chicken	Starting (0-3 weeks)	Laying hen
Protein	23	15
Methionine	0.50	0.27
TSAA	0.93	0.50
Turkey	**Starting (0-4 weeks)**	**Breeding hen**
Protein	28	14
Methionine	0.53	0.20
TSAA	1.05	0.40
Pheasants	**Starting**	**Laying hen**
Protein	30	-
Methionine	-	-
TSAA	1.0	-

[1]Values expressed as percentage of the diet.

Table 2. Starter diet composition (g/Kg) and calculated analysis.

	Treatment				
Ingredients	1	2	3	4	5
Corn	527.00	527.00	528.00	529.00	529.00
Soybean meal, 49%	278.00	277.00	276.00	275.00	275.00
Wheat bran	101.00	101.00	101.00	101.00	100.00
Feather meal	20.00	20.00	20.00	20.00	20.00
Methionine, DL	0.29	0.68	1.07	1.45	1.84
Limestone	10.00	10.00	10.00	10.00	10.00
Meat and bone meal	50.00	50.00	50.00	50.00	50.00
Dicalcium	6.00	6.00	6.00	6.00	6.00
Salt	2.74	2.74	2.75	2.75	2.75
Vit/Mineral	4.97	5.58	5.18	4.80	5.41
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated Analysis					
Crude protein*	24.00/1.92	24.00/1.92	24.00/1.92	24.00/1.92	24.00/1.92
Methionine (% of diet)	0.36	0.40	0.44	0.48	0.51
(% of ME/lb)	0.029	0.032	0.035	0.038	0.041
(% of protein)	1.50	1.67	1.83	1.96	2.12
Cystine (% of diet)	0.40	0.39	0.39	0.40	0.39
TSAA (% of diet)	0.76	0.79	0.83	0.88	0.90
(% of ME/lb)	0.061	0.063	0.066	0.070	0.072
(% of protein)	3.17	3.29	3.46	3.62	3.75
Lysine (% of diet)	1.20	1.20	1.20	1.19	1.19
Tryptophan (% of diet)	0.31	0.31	0.31	0.30	0.30
Metabolizable Energy					
kcal/lb	1250.00	1250.00	1250.00	1250.00	1250.00
kcal/kg	2750.00	2750.00	2750.00	2750.00	2750.00
Crude fiber (% of diet)	3.50	3.50	3.50	3.50	3.50
Total fat (% of diet)	3.00	3.00	3.00	3.00	3.00
Calcium (% of diet)	1.10	1.10	1.10	1.10	1.10
Phosphorus (avail.)(% of diet)	0.50	0.50	0.50	0.50	0.50

*As pct of ration/pct of ME.

Table 2 (cont'd.).

Ingredients	Treatment 6	7	8	9	10
Corn	496.00	497.00	497.00	498.00	499.00
Soybean meal, 49%	332.00	331.00	330.00	329.00	329.00
Wheat bran	79.00	78.00	78.00	78.00	78.00
Feather meal	20.00	20.00	20.00	20.00	20.00
Methionine, DL	-	0.38	0.76	1.15	1.54
Limestone	10.00	10.00	10.00	10.00	10.00
Meat and bone meal	50.00	50.00	50.00	50.00	50.00
Dicalcium	5.00	5.00	5.00	6.00	6.00
Salt	2.81	2.81	2.81	2.81	2.82
Vit/Mineral	5.19	5.81	6.43	5.04	3.64
Total	1000.00	1000.00	1000.00	1000.00	1000.00
Calculated Analysis					
Crude Protein*	26.00/2.08	26.00/2.08	26.00/2.08	26.00/2.08	26.00/2.08
Methionine (% of diet)	0.36	0.40	0.44	0.48	0.51
(% of ME/lb)	0.029	0.032	0.035	0.038	0.041
(% of protein)	1.38	1.54	1.69	1.85	1.96
Cystine (% of diet)	0.43	0.42	0.42	0.43	0.42
TSAA (% of diet)	0.79	0.82	0.86	0.91	0.93
(% of ME/lb)	0.063	0.066	0.069	0.073	0.074
(% of protein)	3.04	3.15	3.31	3.50	3.58
Lysine (% of diet)	1.35	1.35	1.35	1.35	1.35
Tryptophan (% of diet)	0.34	0.34	0.34	0.33	0.33
Metabolizable Energy					
kcal/lb	1250.00	1250.00	1250.00	1250.00	1250.00
kcal/kg	2750.00	2750.00	2750.00	2750.00	2750.00
Crude fiber (% of diet)	3.40	3.40	3.40	3.40	3.40
Fat (% of diet)	2.90	2.90	2.90	2.90	2.90
Calcium (% of diet)	1.10	1.10	1.10	1.10	1.10
Phosphorus (avail.)(% of diet)	0.50	0.50	0.50	0.50	0.50

*As pct of ration/pct of ME.

Table 2 (cont'd.).

	Treatment			
Ingredients	11	12	13	14
Corn	466.00	467.00	467.00	468.00
Soybean meal, 49%	385.00	384.00	383.00	382.00
Wheat bran	56.00	55.00	55.00	55.00
Feather meal	20.00	20.00	20.00	20.00
Methionine, DL	0.07	0.46	0.85	1.24
Limestone	10.00	10.00	10.00	10.00
Meat and bone meal	50.00	50.00	50.00	50.00
Dicalcium	5.00	5.00	5.00	5.00
Salt	2.88	2.88	2.88	2.88
Vit/Mineral	5.05	5.66	6.27	5.88
Total	1000.00	1000.00	1000.00	1000.00
Calculated Analysis				
Crude protein*	28.00/2.24	28.00/2.24	28.00/2.24	28.00/2.24
Methionine (% of diet)	0.40	0.44	0.48	0.51
(% of ME/lb)	0.032	0.035	0.038	0.041
(% of protein)	1.43	1.57	1.68	1.82
Cystine (% of diet)	0.45	0.45	0.46	0.45
TSAA (% of diet)	0.85	0.89	0.94	0.96
(% of ME/lb)	0.068	0.071	0.075	0.077
(% of protein)	3.03	3.18	3.36	3.43
Lysine (% of diet)	1.50	1.50	1.50	1.49
Tryptophan (% of diet)	0.37	0.37	0.37	0.36
Metabolizable Energy				
kcal/lb	1250.00	1250.00	1250.00	1250.00
kcal/kg	2750.00	2750.00	2750.00	2750.00
Crude fiber (% of diet)	3.20	3.20	3.20	3.20
Fat (% of diet)	2.70	2.70	2.70	2.70
Calcium (% of diet)	1.10	1.10	1.10	1.10
Phosphorus (avail.)(% of diet)	0.50	0.50	0.50	0.50

*As pct of ration/pct of ME.

Table 3. Breeder diet composition (g/Kg) and calculated analysis.

	Treatment		
Ingredients	1	2	3
Corn	685.90	680.60	681.10
Soybean meal, 49%	101.80	50.40	49.60
Alfalfa meal, 17%	-	27.50	32.00
Blood meal	4.20	12.50	12.80
Feather meal	15.80	7.50	7.20
Meat and bone meal	2.50	50.00	50.00
Wheat bran	85.60	86.40	81.90
Methionine, DL	0.40	0.80	1.20
Limestone	63.40	57.00	56.80
Dicalcium	30.60	18.30	18.30
Salt	4.80	4.00	4.10
Premix	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00
Calculated Analysis			
Crude protein*	14.15/1.12	14.45/1.12	14.44/1.12
Methionine (% of diet)	0.25	0.29	0.33
(% of ME/lb)	0.020	0.023	0.026
(% of protein)	1.77	2.01	2.28
Cystine (% of diet)	0.25	0.23	0.23
TSAA (% of diet)	0.50	0.52	0.56
(% of ME/lb)	0.040	0.041	0.045
(% of protein)	3.53	3.60	3.88
Lysine (% of diet)	0.59	0.59	0.59
(% of protein)	4.17	4.08	4.08
Tryptophan (% of diet)	0.17	0.16	0.16
(% of protein)	1.20	1.11	1.11
Metabolizable Energy			
kcal/lb	1250.00	1250.00	1250.00
kcal/kg	2750.00	2750.00	2750.00
Crude fiber (% of diet)	3.06	4.09	3.76
Total fat (% of diet)	3.02	3.54	3.48
Calcium (% of diet)	3.13	3.13	3.11
Phosphorus (avail.)(% of diet)	0.69	0.69	0.68

*As pct of diet/pct of ME.

Table 3 (cont'd.).

Ingredients	Treatment 4	Treatment 5	Treatment 6
Corn	660.70	660.70	660.80
Soybean meal, 49%	87.70	87.70	87.60
Alfalfa meal, 17%	99.80	99.40	99.00
Blood meal	0.50	1.20	1.90
Feather meal	19.50	18.80	18.10
Meat and bone meal	50.00	50.00	50.00
Methionine, DL	0.10	0.50	0.90
Limestone	55.20	55.20	55.20
Dicalcium	17.30	17.30	17.30
Salt	4.20	4.20	4.20
Premix	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00
Calculated Analysis			
Crude protein*	16.01/1.28	16.05/1.28	16.06/1.28
Methionine (% of diet)	0.25	0.29	0.33
(% of ME/lb)	0.020	0.023	0.026
(% of protein)	1.56	1.81	2.05
Cystine (% of diet)	0.26	0.26	0.26
TSAA (% of diet)	0.51	0.55	0.59
(% of ME/lb)	0.041	0.044	0.047
(% of protein)	3.18	3.43	3.67
Lysine (% of diet)	0.67	0.68	0.68
(% of protein)	4.18	4.24	4.23
Trypotophan (% of diet)	0.19	0.19	0.19
(% of protein)	1.19	1.18	1.18
Metabolizable Energy			
kcal/lb	1250.00	1250.00	1250.00
kcal/kg	2750.00	2750.00	2750.00
Crude fiber (% of diet)	4.60	4.23	4.58
Total fat (% of diet)	3.30	3.31	3.30
Calcium (% of diet)	3.12	3.12	3.12
Phosphorus (avail.)(% of diet)	0.69	0.69	0.69

*As pct of diet/pct of ME.

Table 3 (cont'd.).

Ingredients	Treatment 7	8	9
Corn	599.00	630.30	631.10
Soybean meal, 49%	128.90	143.80	142.50
Alfalfa meal, 17%	-	45.60	53.70
Feather meal	20.00	20.00	20.00
Meat and bone meal	50.00	50.00	50.00
Wheat bran	-	27.00	19.30
Wheat st. middlings	115.20	-	-
Methionine, DL	-	0.30	0.60
Limestone	57.90	56.70	56.50
Dicalcium	17.50	17.20	17.20
Salt	3.80	4.10	4.10
Premix	5.00	5.00	5.00
Total	1000.00	1000.00	1000.00
Calculated Analysis			
Crude protien*	17.75/1.42	18.00/1.44	18.00/1.44
Methionine (% of diet)	0.25	0.29	0.33
(% of ME/lb)	0.020	0.023	0.026
(% of protein)	1.41	1.61	1.83
Cystine (% of diet)	0.28	0.30	0.29
TSAA (% of diet)	0.53	0.59	0.62
(% of ME/lb)	0.042	0.047	0.049
(% of protein)	2.98	3.28	3.44
Lysine (% of diet)	0.79	0.81	0.81
(% of protein)	4.45	4.50	4.50
Tryptophan (% of diet)	0.20	0.21	0.21
(% of protein)	1.13	1.17	1.17
Metabolizable Energy			
kcal/lb	1250.00	1250.00	1250.00
kcal/lb	2750.00	2750.00	2750.00
Crude fiber (% of diet)	2.93	3.65	3.76
Total fat (% of diet)	3.36	3.19	3.18
Calcium (% of diet)	3.12	3.12	3.13
Phosphorus (avail.) (% of diet)	0.69	0.69	0.69

*As pct of diet/pct of ME.

Table 4. Composition values of feed ingredients used to calculate nutrient content of diets.

Ingredients	ME (kcal/1b)	CP (%)	Met (%)	Met+Cys (%)	Lys (%)	Try (%)	CF (%)	Fat (%)	Ca (%)	Phos. (Avail.,%)	Na (%)
Alfalfa meal, 17%	516	17.0	.25	.50	.72	.40	24.3	2.5	1.30	.23	.09
Blood meal	1300	80.0	.65	1.65	4.50	.72	1.0	1.0	.28	.28	.32
Corn, normal	1560	8.8	.18	.34	.28	.09	2.7	3.8	.02	.09	.02
Feather meal	1035	85.0	.35	2.35	1.05	.40	-	-	.20	.75	.70
Limestone	-	-	-	-	-	-	-	-	38.00	-	-
Meat and bone meal	1010	50.0	.53	1.10	2.40	.30	2.5	10.0	10.00	5.00	.73
Methionine, DL	-	98.0	98.00	98.00	-	-	-	-	-	-	-
Phosphorus, Dicalcium	-	-	-	-	-	-	-	-	21.00	18.50	.10
Salt	-	-	-	-	-	-	-	-	-	-	38.00
Soybean meal, 49%	1100	49.0	.70	1.46	3.14	.74	3.0	.5	.20	.24	.05
Wheat bran	510	15.0	.11	.41	.40	.30	10.5	4.0	.14	.12	.20
Wheat standard middlings	890	17.0	.16	.36	.65	.21	7.0	4.5	.14	.12	.20

Source: Computer formulation of poultry and game birds rations, 1975 Cooperative Extension Service, Purdue University, West Layfayette Indiana.

Table 5. Vitamin-Trace Mineral Premixes for Pheasants.

Ingredients	Per kg Premix	
	Starter	Breeder
Vitamin A	1,320,000	1,760,000
Vitamin D	330,000	440,000
Riboflavin, mg	880	1,540
Pantothenic acid, mg	1,760	2,640
Niacin, mg	4,400	5,280
Choline chloride, mg	88,000	88,000
Vitamin B_{12}, mg	2.2	2.64
Vitamin E, I.U.	660	1,100
Menadione sodium bisulfite, mg	330	330
Manganese, mg	11,880	11,880
Iodine, mg	220	220
Copper, mg	440	440
Cobalt, mg	44	44
Zinc, mg	5,500	5,500
Iron, mg	3,960	3,960

APPENDIX B

Table 1. Regression analysis for body weight of Ring-Necked pheasants at four weeks of age fed the 24% protein level.

Source of variance	df	Sum of squares	Mean square	F statistic
Methionine				
Linear	1	11830.3068 - 258.0825	11572.2243	11.0463**
Quadratic	1	11988.0718 - 11830.3068	157.7650	.1506
Initial weight	1	258.0825	258.0825	.2463
Residual	135	141427.45342	1047.61077	

** $P < 0.01$

Table 2. Regression analysis for body weight of Ring-Necked pheasants at four weeks of age fed the 26% protein level.

Source of variance	df	Sum of squares	Mean square	F statistic
Methionine				
Linear	1	23884.94297 - 16341.24708	7543.6959	7.0166**
Quadratic	1	31905.43265 - 23884.94297	8020.4897	7.4600**
Initial weight	1	16341.24708	16341.24708	15.1995**
Residual	135	145140.53857	1075.1151	

** $P < 0.01$

Table 3. Regression analysis for body weight of Ring-Necked pheasants at four weeks of age fed the 28% protein level.

Source of variance	df	Sum of squares	Mean square	F statistic
Methionine				
Linear	1	8912.7051 - 522.5789	8390.1262	7.9359**
Quadratic	1	20298.66928 - 8912.7051	11385.9642	10.7696**
Initial weight	1	522.57890	522.5789	.4943
Residual	108	114181.00929	1057.2316	

** $P < 0.01$

Table 4. β Values and standard errors calculated for each protein level.

	Protein levels %					
	24		26		28	
Variables	β values	std. error	β values	std. error	β values	std. error
Initial weight (β_3)	.710844	1.346773	5.445468	1.437403	.825974	1.650289
Methionine (β_1)	10.275533	10.037226	32.463289	10.169541	78.517213	21.740392
Met. squared (β_2)	-.636944	1.641331	-4.541548	1.662765	-10.111522	3.081177
Constant (β_0)	173.061560	33.502149	42.916183	35.189038	68.047950	50.296007

Note: These data were used to calculate the prediction equations and to determine the optimum methionine at each protein level.

Table 5. ANOVA for feed consumption (Experiment I)

Source of Variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Methionine	3.595	3	1.198	.490	.696
Protein	1.360	2	.680	.278	.762
Replication	1.718	2	.859	.351	.711
Met. vs. Prot.	12.057	6	2.009	.821	.575
Met. vs. Rep.	14.594	6	2.432	.993	.472
Prot. vs. Rep.	4.097	4	1.024	.418	.792
Residual	29.380	12	2.448		
Total	66.801	35			

Table 6. ANOVA for feed conversion (Experiment I).

Source of Variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Methionine	.078	3	.026	.539	.664
Protein	.205	2	.102	2.113	.164
Replication	.027	2	.014	.282	.759
Met. vs. Prot.	.146	6	.024	.504	.794
Met. vs. Rep.	.180	6	.030	.620	.712
Prot. vs. Rep.	.119	4	.030	.613	.661
Residual	.581	12	.048		
Total	1.336	35			

Table 7. ANOVA for mortality (Experiment I).

Source of variance	Sum of square	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Methionine	1.861	3	.620	.957	.444
Protein	.167	2	.083	.129	.881
Replication	.167	2	.083	.129	.881
Met. vs. Prot.	5.389	6	.898	1.386	.296
Met. vs. Rep.	4.722	6	.787	1.214	.363
Prot. vs. Rep.	4.667	4	1.167	1.800	.194
Residual	7.778	12	.648		
Total	24.750	35			

APPENDIX C

Table 1. ANOVA for hen-day egg production (Experiment II).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Protein	277.602	2	138.801	0.997	0.372
Methionine	623.582	2	311.791	2.239	0.111
Period	23812.491	3	7937.497	57.005	0.001
Prot. vs. Met.	442.752	4	110.688	0.795	0.531
Prot.vs. Per.	476.998	6	79.500	0.571	0.753
Met. vs. Per.	235.371	6	39.228	0.282	0.945
Residual	16709.048	120	139.242		
Total	42577.844	143			

Table 2. Means of the hen-day egg production (%) of laying Ring-necked pheasants through 112 days (Experiment II).[1,2]

Period	Protein %	Methionine (% of diet) .25	.29	.33
1	14	37.67	41.52	37.05
2	14	72.62	76.78	79.46
3	14	69.56	72.09	72.76
4	14	63.70	52.67	65.40
1	16	46.43	50.00	44.53
2	16	75.12	75.44	76.86
3	16	68.97	69.86	71.13
4	16	58.03	59.59	68.45
1	18	34.82	44.17	50.00
2	18	69.64	79.24	84.37
3	18	68.75	77.30	72.24
4	18	62.72	72.32	66.29

[1]Data are means of 4 individual observations (4 replicates of 4 birds each).

[2]The pooled SEM was ± 5.90.

Table 3. ANOVA for egg weight (Experiment II).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Protein	44.315	2	22.157	15.008	0.001
Methionine	2.198	2	1.099	0.744	0.477
Period	68.277	3	22.759	15.415	0.001
Prot. vs. Met.	23.901	4	5.975	4.047	0.004
Prot. vs. Per.	5.226	6	.871	0.590	0.738
Met. vs. Per.	6.276	6	1.046	0.709	0.643
Residual	177.168	120	1.476		
Total	327.361	143			

Table 4. Means of egg weight (g) of laying Ring-necked pheasants through 112 days (Experiment II).[1,2]

Period	Protein %	Methionine (% of diet) .25	.29	.33
1	14	27.99	26.20	27.84
2	14	29.01	27.53	28.68
3	14	29.16	27.74	28.08
4	14	29.36	28.61	28.06
1	16	27.13	26.97	27.69
2	16	27.98	28.86	28.67
3	16	28.07	29.12	28.57
4	16	28.37	30.32	29.02
1	18	28.24	28.28	27.63
2	18	29.26	29.75	29.28
3	18	30.44	30.02	29.92
4	18	31.22	29.95	29.57

[1]Data are means of egg weights produced by 16 birds (4 replicates of 4 birds). each).

[2]The pooled SEM was ± 0.607.

Table 5. ANOVA for feed consumption (Experiment II).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F Statistic	Approx. sig. prob. of F statistic
Protein	199.375	2	99.688	5.128	0.007
Methionine	113.473	2	56.736	2.919	0.058
Period	2919.195	3	973.065	50.058	0.001
Prot. vs. Met.	137.942	4	34.485	1.774	0.138
Prot. vs. Per.	44.802	6	7.467	0.384	0.888
Met. vs. Per.	32.437	6	5.406	0.278	0.946
Residual	2332.628	120	19.439		
Total	5779.851	143			

Table 6. Feed consumption (g/bird/day) of laying Ring-necked pheasants through 112 days (Experiment II).[1,2]

Period	Protein %	Methionine (% of diet) .25	.29	.33
1	14	54.25	58.56	58.01
2	14	66.93	67.28	67.25
3	14	70.89	67.66	67.80
4	14	71.05	66.80	67.06
1	16	57.27	58.95	55.38
2	16	63.60	65.73	65.81
3	16	64.83	68.57	67.65
4	16	65.89	68.37	68.59
1	18	55.78	59.82	61.97
2	18	66.64	71.35	71.32
3	18	67.82	72.64	69.88
4	18	66.68	70.71	70.28

[1]Data are means of 4 individual observations (4 replicates of 4 birds each).

[2]The pooled SEM was ± 2.20.

Table 7. ANOVA for body weight change (Experiment II).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Initial weight	459009.263	1	459009.263	39.036	0.001
Protein	41582.132	2	20791.066	1.768	0.175
Methionine	33729.160	2	16864.580	1.434	0.242
Prot. vs. Met.	39179.657	4	9794.914	0.833	0.507
Residual	1516876.868	129	11758.735		
Total	2065835.612	138			

Table 8. Body weight averages (g) of laying Ring-necked pheasants fed diets with different methionine and protein levels through 112 days (Experiment II).

Protein %	Methionine (% of diet)					
	.25		.29		.33	
	Initial weight[1]	Final weight[2]	Initial weight[1]	Final weight[2]	Initial weight[1]	Final weight[2]
14	1051.78	1171.43	1065.62	1193.44	1040.62	1155.62
16	1050.00	1150.31	1085.94	1255.00	1009.33	1136.00
18	1054.69	1214.37	1009.33	1189.33	1090.00	1236.33

[1]Data are means of 16 birds (4 replicates of 4 birds each).

[2]Data are means of live bird weight at the end of the experiment.

Table 9. ANOVA for mortality (Experiment II).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Protein	.014	2	0.007	0.199	0.820
Methionine	.014	2	0.007	0.199	0.820
Period	.075	3	0.025	0.731	0.536
Prot. vs. Met.	.236	4	0.059	1.694	0.156
Prot. vs. Per.	.153	6	0.025	0.731	0.626
Met. vs. Per.	.153	6	0.025	0.731	0.626
Residual	4.181	120	0.035		

APPENDIX D

Table 1. ANOVA for hen-day percent egg production (Experiment III).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Protein	253.131	2	126.566	0.431	0.651
Methionine	138.504	2	69.252	0.236	0.790
Period	9047.431	2	4523.715	15.408	0.001
Prot. vs. Met.	3236.811	4	809.203	2.756	0.033
Prot. vs. Per.	64.509	4	16.127	0.055	0.994
Met. vs. Per.	133.238	4	33.309	0.113	0.977
Residual	26130.440	89	293.600		
Total	39004.063	107			

Table 2. Mean values of the hen-day egg production (%) of laying Ring-necked pheasants through 84 days (Experiment III).[1,2]

Period	Protein %	Methionine (% of diet) .25	.29	.33
1	14	56.82	65.38	54.54
2	14	44.20	60.71	47.54
3	14	35.49	40.13	31.70
1	16	60.40	61.93	66.48
2	16	46.83	51.34	57.81
3	16	39.13	37.00	47.76
1	18	68.27	55.40	63.07
2	18	61.98	40.40	53.35
3	18	48.59	29.02	42.34

[1]Data are means of 4 individual observations (4 replicates of 4 birds each).

[2]The pooled SEM was ± 8.57.

Table 3. ANOVA for egg weight (Experiment III).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Protein	89.630	2	44.815	12.205	0.001
Methionine	22.085	2	11.043	3.007	0.056
Period	6.783	2	3.392	.924	0.401
Prot. vs. Met.	78.376	4	19.594	5.336	0.001
Prot. vs. Per.	4.221	4	1.053	.287	0.886
Met. vs. Per.	2.789	4	0.697	.190	0.943
Residual	286.406	78	3.672		
Total	484.282	96			

Table 4. Mean and standard error of the egg weight of laying Ring-necked pheasants through 84 days (Experiment III).[1]

Period	Protein %	Methionine (% of diet) .25	.29	.33
1	14	27.79 ± 0.96	29.25 ± 0.96	29.91 ± 0.96
2	14	28.07 ± 0.96	29.52 ± 0.96	29.67 ± 1.11
3	14	28.86 ± 0.96	28.96 ± 0.96	28.92 ± 1.11
1	16	31.06 ± 0.96	29.06 ± 0.96	31.36 ± 0.96
2	16	31.22 ± 0.96	29.67 ± 0.96	31.83 ± 0.96
3	16	31.28 ± 1.35	30.39 ± 0.96	31.88 ± 1.11
1	18	29.34 ± 0.96	32.32 ± 0.96	29.80 ± 1.11
2	18	29.02 ± 0.96	33.36 ± 0.96	31.49 ± 1.11
3	18	30.10 ± 1.11	33.43 ± 1.35	31.57 ± 1.11

[1]Data are means of egg weights produced by 16 birds (4 replicates of 4 birds each).

Table 5. ANOVA for feed consumption (Experiment III).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Protein	169.063	2	84.531	1.248	0.292
Methionine	54.728	2	27.364	0.404	0.669
Period	1413.337	2	706.668	10.431	0.001
Prot. vs. Met.	326.142	4	81.536	1.204	0.315
Prot. vs. Per.	30.625	4	7.656	0.113	0.978
Met. vs. Per.	66.713	4	16.678	0.246	0.911
Residual	6029.445	89	67.747		
Total	8090.053	107			

Table 6. Feed consumption (g/bird/day) of laying Ring-necked pheasants through 84 days (Experiment III).[1,2]

Period	Protein %	Methionine (% of diet) .25	.29	.33
1	14	58.01	58.03	55.97
2	14	65.44	66.87	63.54
3	14	67.01	67.85	61.90
1	16	53.83	56.39	59.64
2	16	62.34	60.27	67.88
3	16	63.84	59.17	65.87
1	18	51.50	53.48	57.58
2	18	66.42	61.56	62.41
3	18	61.63	58.99	63.52

[1]Data are means of 4 individual observations (4 replicates of 4 birds each).

[2]The pooled SEM was ± 4.11.

Table 7. ANOVA for change in body weight (Experiment III).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Initial weight	402552.511	1	402552.511	21.460	0.001
Protein	1913.539	2	956.769	0.051	0.950
Methionine	931.623	2	465.811	0.025	0.975
Prot. vs. Met.	39190.345	4	9797.586	0.522	0.719
Residual	382292.147	127	18758.206		

Table 8. Body weight averages (g) of laying Ring-necked pheasants fed diets with different protein and methionine levels through 84 days (Experiment III).

	Methionine (% of diet)					
	.25		.29		.33	
Protein %	Initial weight[1]	Final weight[2]	Initial weight[1]	Final weight[2]	Initial weight[1]	Final weight[2]
14	1370.31	1292.50	1330.36	1265.00	1396.88	1272.50
16	1435.71	1287.66	1293.33	1226.67	1376.33	1311.00
18	1323.67	1246.67	1370.31	1268.75	1377.19	1271.25

[1]Data are means of 16 birds (4 replicates of 4 birds each).

[2]Data are means of live bird weight at the end of the experiment.

Table 9. ANOVA for mortality (Experiment III).

Source of variance	Sum of squares	Degs. of freedom	Mean square	F statistic	Approx. sig. prob. of F statistic
Protein	0.130	2	0.065	1.010	0.368
Methionine	0.074	2	0.037	0.577	0.564
Period	0.019	2	0.009	0.144	0.866
Prot. vs. Met.	0.259	4	0.065	1.010	0.407
Prot. vs. Per.	0.148	4	0.037	0.577	0.680
Met. vs. Per.	0.204	4	0.051	0.793	0.533
Residual	5.713	89	0.064		
Total	6.546	107			