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UTILIZATION OF DIETARY PHOSPHORUS BY POULTRY

by

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A THESIS

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Date Nov. 26, 1981.....

Dedicated to my husband

Agwonorobo Eruvbetine

with deep gratitude

ABSTRACT

The requirements for phosphorus in poultry rations are high and it is costly to supply the levels needed. As a result there has been interest in greater use of canola meal, which contains a high level of phosphorus (1.1%), in poultry rations. Consequently, a series of experiments was conducted with broiler chickens (0 to 4 weeks), starting turkeys (0 to 4 weeks) and laying hens to study the utilization of and requirement for phosphorus by these birds when they were fed different levels of canola meal. The parameters studied included level of mortality, rate of growth, feed conversion, incidence of leg disorders, thyroid size and bone ash levels in the starter chickens and turkeys. In the laying hens in addition to the above parameters rate of egg production, egg quality, egg shell quality and blood inorganic phosphorus levels in plasma were also measured.

For starting chickens it was observed that a level of 0.58% total dietary phosphorus was sufficient to meet their P requirements provided that at least 0.25% inorganic phosphorus was included in the ration. When canola meal was added to the ration and the amount of supplemental inorganic phosphorus was reduced to keep the total phosphorus constant, rate of growth and feed efficiency were not affected. When canola meal was autoclaved at 121°C for 15, 30 or 45 minutes it appeared that the availability of phytin phosphorus was increased thus allowing a decrease in added inorganic phosphorus without affecting performance.

Studies with starting turkeys indicated that the requirement for phosphorus may be lower than the 0.80% listed in the National Research Council publication on Nutrient Requirements of Poultry. A level of 0.67 to 0.73% in the diet was adequate for normal growth rate and feed efficiency. Higher levels of phosphorus resulted in increased bone ash values but an increase in incidence of leg disorders was also observed. Inclusion of 10, 15 or 20% canola meal in the rations fed resulted in performance similar to that observed on wheat-soybean meal type rations.

In the experiments with hens it was observed that laying pullets could be fed on much lower levels of phosphorus than that specified (0.50%) in the National Research Council publication on the Nutrient Requirements of Poultry, irrespective of the type of housing, without affecting productive performance. With hens raised on floors a minimum level of 0.40% phosphorus seemed to be adequate while in cages a level of 0.44% phosphorus was sufficient. The experiments also showed that the laying hen was capable of utilizing the phytin phosphorus in the diet when the level of phytin phosphorus was increased and the level of inorganic phosphorus was decreased through the inclusion of canola meal in the diet.

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I. INTRODUCTION

Phosphorus (P) is an essential nutrient for poultry. It is a major component of bone, and compounds containing P are involved in most metabolic reactions in the animal body. Because of its important role in nutrition it is accepted that P is required for growth, development, production and maintenance of all animals.

The dietary requirements for P by poultry are high. The publication "Nutrient Requirements of Poultry" (NAS-NRC, 1977) lists requirement levels of 0.70% for starting chickens (0 to 8 weeks), 0.40% for growing chickens (8 to 18 weeks). 0.50% for laying hens, 0.80% for starting turkeys (0 to 8 weeks), 0.60% for growing turkeys (8 weeks to market) and 0.60% for turkey breeders. In formulating rations for poultry, the contribution of P by the individual ingredients composing the ration are taken into account. Grains and plant protein supplements which usually make up the major portion of the ration, provide a part of the P requirement of the bird and the remainder is supplied by the animal protein feedstuffs and inorganic P supplements.

In meeting the requirement for P it is necessary to consider not only the total P content of the diet but also the availability of the P. The availability of P in the different feedstuffs may vary widely. Phosphorus derived from animal protein feedstuffs and inorganic P supplements is considered to be wholly available; however, P derived from grains and plant proteins is only partially available.

Approximately 70% of the P in grains and plant protein supplements is found in organic combination as phytin, which is considered to be largely unavailable to young growing chickens and turkeys but appears to be available to some extent to the laying hen.

The proportion of phytin P to total P in the diet may vary considerably. In practical-type poultry rations the amount of P supplied by grains and plant protein supplements usually varies from 0.35 to 0.40%. If it is assumed that 70% of the total P from plant products is phytin P then in most rations the level of phytin P varies from 0.25 to 0.28%. Thus the contribution of phytin P in different rations may range from as low as 40% to as high as 60% of the total P requirements.

Due to the high cost involved in the addition of inorganic P supplements to the ration, increasing interest has been extended to the large proportion of phytin P in the ration and the extent to which it can be utilized by poultry. In addition there has been some interest in the possibility of using rations containing canola meal (CM) as a source of P in poultry rations. Canola meal contains a very high level of P (1.1%) in comparison to soybean meal (0.65%). Since limited information was available on the extent to which the P in CM was utilized by poultry it seemed desirable that studies be conducted to assess its availability. Attempts have also been made to try to improve the availability of phytin P by poultry by varying the processing conditions applied to the feed.

The experiments reported herein were undertaken to determine the influence of processing on the availability of phytin P and to determine the levels of total and inorganic P required by growing chickens, turkeys and laying hens in rations containing varying levels of CM.

II. LITERATURE REVIEW

The role of P in nutrition is important as this element, in addition to being a major constituent of bone, is also an essential component of organic compounds involved in almost every aspect of metabolism. Hence P is one of the major minerals required in the diet of all animals. Since many of the ingredients used in rations for livestock and poultry contain less P than is required for normal growth and body functions, it is necessary to include supplemental sources of P in the rations fed.

A. General Introduction

1. Sources

Phosphorus in feeds occurs in two forms, one of which is organic and the other inorganic. Most of the P in organic combination is in the form of phytin which is the calcium and magnesium salt of inositol phosphoric acid (Plimmer and Page 1913). It is the principle form of P of all naturally occurring feed ingredients of plant origin (Nelson 1967).

The percentage of P occurring as phytate varies considerably in different plants. McCance and Widdowson (1935) and Giri (1938) reported that approximately 40 to 70% of P of cereal grains was present in the form of phytin. Common (1940) showed that the phytin P content of feedstuffs derived from processed oilseeds was one-half to two-thirds of the total P content. Mollgaard (1946) reported that 70 to 80% of the P in processed oilseeds and 65 to 85% of the P in cereal grains was phytate P. Other reports indicated that 81% of the total P in wheat bran was in phytate (Boutwell et al. 1946) and 30 to 60% of the P in seeds, grains and tubers was in phytate combination (Oke 1965).

The non-phytate content of plant feedstuffs ranges from 15 to 50% but the degree to which this is utilized has not been clearly established (Nelson 1967). In general, it is considered (NAS-NRC 1977) that 30% of a mixture of plant sources of P is utilized by young chicks or poults with considerably more of the phytin P utilized by older birds.

The inorganic sources of P generally added to rations to meet the nutrient requirements are considered to be highly available to the animal. Gillis et al.(1948) compared the availability of a number of common P supplements and pure phosphate compounds. It was reported that beta tricalcium phosphate and other reagent grades such as mono-, di- and tricalcium phosphate were highly available sources of P for chickens. Defluorinated rock phosphate was less available than the pure orthophosphate but was more available than pure metaphosphate and pyrophosphate. It was later confirmed (Gillis et al. 1954) that feed grade samples of tricalcium phosphate, defluorinated phosphate and domestic steamed bone meal were readily available sources of P. Other products of slightly lower availability were spent bone char, bone ash and imported bone meal of unknown history. Dilworth and Day (1964) observed that P sources with an availability of 79% or more when compared to sodium acid phosphate could be considered satisfactory sources of P.

The availability of P in different inorganic sources may vary considerably. The relative availability of P in rock phosphate is dependent on the source of the material. Availabilities ranging from 0 to 87% were reported by Gillis et al. (1954) for rock phosphates obtained from different locations and an availability of 0% for two imported rock phosphates by Wilcox et al.(1954). It was reported by both groups of workers that the P in colloidal phosphate was highly unavailable for young growing birds.

2. Requirements

The dietary requirements of P for poultry has always been closely associated with that of calcium. Early reports have emphasized the importance of the calcium-phosphorus ratio rather than the quantitative requirements for these minerals (Bethke et al. 1930; Hart et al. 1930). Wilgus (1931) reported that the P requirement of growing chicks was 0.50% or less in rations in which the calcium-phosphorus ratio varied from 1:1 to 2.2:1.

The inability of the chicken to utilize phytin P has resulted in requirements often being stated in terms of inorganic P or non-phytin P rather than total P requirements. Gillis et al. (1949) observed that chicks required approximately 0.40% of a readily available form of inorganic or non-phytin P when the diet contained a total of at least 0.60% of this element. O'Rourke et al. (1952) using purified diets, reported a requirement of 0.46% inorganic P for chicks up to 3 weeks of age with the requirements decreasing with increasing age of the bird. Almquist (1954) concluded that growing birds up to 4 weeks of age require 0.45% of a readily available P source. Temperton and Cassidy (1963a) working with light-type hybrid pullets observed that a level of 0.48% total P from plant feedstuffs containing 0.23% non-phytin P was sufficient for normal growth and calcification. In further studies Temperton et al. (1965a,b,c) confirmed that pullets (8 weeks old) could be raised up to the laying period on P derived from plant sources alone supplying a level of 0.45 or 0.46% total P.

As a result of the low availability of organic P, the requirements for total P in poultry rations are considerably higher than the requirements expressed in terms of available P. Requirement levels of 0.60 to 0.65% P for growing chicks have been reported (Lillie et al. 1964; Mehring and Titus 1964; Twinning et al. 1965; Waldroup et al. 1965). On the basis of these studies and taking into account the fact that approximately 30% of P in plant products is non-phytin P, a total requirement of 0.70% for chickens from 0 to 8 weeks has been listed (NAS-NRC 1971,1977).

The reported P requirement of laying hens has varied widely. Early work on the P requirements of laying hens indicated that 0.75% P was needed (Norris et al. 1934). Later, Miller and Bearse (1934) and Evans et al. (1944) suggested that 0.80% P in laying rations resulted in better productive performance than 0.60%. Gillis et al. (1954) reported that laying hens utilized phytin P approximately one-half as effectively as an inorganic source of P and recommended dietary levels of 0.50% inorganic or 'available ' P for egg production and maintenance of body weight and 0.60% for maintenance of optimum blood levels of P and prevention of decalcification of bone. O'Rourke et al. (1954) indicated that the requirements for P for laying pullets were considerably lower than had been proposed by previous investigators. They concluded that 0.30% total P was sufficient for normal egg production and hatchability. Pepper et al. (1959) reported that a level of 0.38% total P was sufficient to maintain a high rate of production for a 44-week laying period with hens kept in floor pens with litter.

The requirements of P appeared to be influenced by the type of housing provided. Singsen et al. (1962) observed that hens kept in laying cages required a higher level of dietary P than those kept in floor pens. The performance of birds fed 0.40, 0.50 or 0.60% P in floor pens were similar, probably due to coprophagy, but in laying cages the performance improved as the P level was increased. It was concluded that the P requirement of laying hens kept in cages was more than 0.45% (0.35% available) but not more than 0.60% (0.55% available). Hamilton and Sibbald (1977) indicated that under practical conditions 0.45% available P was adequate for laying hens. The requirements for total P for laying birds (NAS-NRC 1977)) has been lowered to 0.50% from a previous stated requirement of 0.60%. This change was based on the observation that "older birds have the ability to utilize most, if not all of the phytin or organic P in plant material".

B. Methods and techniques for determining phosphorus availability

Several techniques have been developed for determining P availability in various species of animals. In poultry, the most commonly used methods involve, balance studies, isotope trials, measurement of calcification, determination of blood levels and assessment of factors such as body weight, egg production and egg quality.

1. Balance studies

This method is simple and involves measuring the total intake of P and the output of P in feces. The difference, expressed as a percentage of intake, gives a measure of P retention. Common (1933, 1940) measured the excretion of phytin P by laying chickens using this technique and concluded that a large proportion of the phytin in feedstuffs was unhydrolysed by the chicken. Temperton and Cassidy (1963a,b) in balance studies with growing chickens found that they could retain a large proportion of phytin P in their body. The obvious disparity in the results gives rise to some question of the validity of this technique for measuring the utilization of phytin P.

In studies with pigs Tonroy et al. (1973) using the apparent digestibility technique observed that the apparent digestibility of dicalcium phosphate varied widely in two separate experiments. Pierce et al. (1977), also in studies with pigs on the availability of P in cereal grains, concluded that other factors besides digestibility values are necessary for assessing adequacy of dietary P.

Recently, Nwokolo et al. (1976) developed a method with chickens for measuring P availability in which a correction for endogenous P secretion was made. It was suggested that the method gave a closer estimate of the 'true' availability of P.

2. Isotope trials

The availability of radioactive P has allowed investigators to study the metabolism of phytate P more precisely. Singsen et al. (1950) fed labelled calcium phytate to turkey poults and found that ^{32}P appeared readily in various parts of the body. This showed that exchange reactions occurred between labelled phytate and other forms of P either in the intestine or after absorption. Keane et al. (1954) reported similar exchange reactions in studies in vitro. Gillis et al (1957) compared ^{32}P labelled phytate with ^{32}P labelled inorganic orthophosphate as a source of P for chickens and poults. They found that ^{32}P labelled calcium phytate was utilized one-tenth as effectively as inorganic orthophosphate ($Na_2H^{32}PO_4$) by chicks and even less efficiently by turkey poults. Ashton et al. (1960) using a source of labelled phytin in oats found that growing chickens utilized one-fifth of the phytate P from oats and did incorporate some of it into actively growing tissue. The disadvantages of this method were the difficulty of incorporating the label into the source of phytin in the plant and the fact that net retention of radioactive ^{32}P did not necessarily indicate the net utilization of the phytate.

3. Measurement of calcification

Since P is a major component of bone, biological studies involving calcification are an appropriate and practical way of assessing P availability. In such studies the ash content of bones serves as an indicator of the degree of calcification that has occurred. To estimate the extent of calcification, the ash content of the tibias, toes or occasionally femurs may be determined.

a. Tibia ash

The ash content of the tibia of chicks, expressed as a percentage of the fat free, dry bone has been used to estimate P availability. Initially, the procedure was used as a criterion for determining calcification in relation to vitamin D levels in the diet but the obvious interrelationship between calcium, P and vitamin D in calcification prompted the use of bone ash as a measure of availability of calcium and P as well (Bethke et al. 1930; Wilgus Jr. 1931; Evans et al. 1944). With the recognition of the limited utilization of phytin P by monogastric animals, researchers have used bone ash values to determine the extent of phytin utilization (Lowe et al. 1939; Kreiger et al. 1940; Kreiger and Steenbock 1940; Kreiger et al. 1941; McGinnis et al. 1944; Gillis et al. 1948; Miller and Joukovsky 1953).

Gillis et al. (1953) were the first to attempt to determine the biological value of different sources of inorganic phosphates used in poultry rations. A standard curve was developed by measuring tibia bone ash content of chicks fed graded levels of an inorganic source of phosphate (beta-tricalcium phosphate) assumed to be 100% available. The test ingredient was then compared to the standard curve and a value for biological availability was derived. Nelson and Walker (1964) and Dilworth and Day (1964) also used tibia ash values in evaluating the availability of P compounds and indicated that tibia ash was more accurate than body weight as a measure of dietary levels of P. Nelson (1967) observed that bone ash served as a sensitive and practical criterion for evaluating dietary P availability. Sullivan (1966) reported that a 4-week period of growth which gave optimum bone ash values was a more accurate and sensitive measure of P availability than the 10-day assay developed by Ammerman et al. (1960).

Pierce et al. (1977) in a study with pigs also used bone ash values for assessing phytate utilization. It was found that productive performance and skeletal development were of equal value in assessing adequacy of dietary P supply.

b. Toe ash

Due to the time involved in dissection and the lengthy fat extraction required for tibia ash determinations Baird and MacMillan (1942) suggested that the ash content of toes be used as a criterion of calcification and outlined a method for toe ash determination. Campbell et al. (1945) investigated the toe ash method as a means of measuring vitamin D status of chicks and found that there was no significant difference in the precision of the values obtained with toe ash and tibia ash methods. The procedure had the advantage that the bird could continue to live after removal of the toes; however, other variations like degree of calcification due to cornification of the pads and breaking of the claws may have affected the values obtained (Campbell et al. 1945).

Yoshida and Hoshii (1976) developed a simplified and rapid biological assay procedure which used slope ratio assay of the linear regression lines between added P and toe ash content. Hoshii and Yoshida (1977a) determined the availability of 25 ingredients by bioassay on toe ash content and found this method comparable to total carcass retention of P for measuring P availability (Hoshii and Yoshida 1977b).

4. Blood levels

The levels of inorganic P in blood plasma has been used to assess the availability of P in the diets of chickens. Gillis et al. (1953) determined inorganic P plasma levels in laying birds to find the amount of inorganic P required by the birds for normal growth and production. Later studies showed that inorganic P levels in plasma may be affected by dietary calcium. Arscott et al. (1962) and Reichmann and Connor (1977) observed a significant increase in plasma inorganic P when calcium was increased in the diet. This showed that plasma P was positively and linearly related to dietary calcium and also indicated that the absorption of P was dependent on the level of dietary calcium. Pierce et al. (1977) presented data supporting the observation that plasma inorganic P level was a good indicator of P adequacy in pigs only when they were fed adequate levels of calcium.

Miller et al. (1977) found that the level of inorganic P in serum was closely related to the egg laying cycle of the hen. This was confirmed later by Choi et al. (1979) who found that the excretion pattern of P was also closely related to the egg laying cycle of the hen. In evaluating P status of the laying hen using inorganic P levels in plasma it is important to select birds at the same stage of lay, usually immediately after oviposition (Miles et al. 1980).

5. Body weight

Rate of growth of chickens may be influenced by the level of P supplied. Consequently growth trials have been used to try to assess P requirements and availability.

In early studies on the utilization of P from legumes and cereals, Heuser et al. (1943) and Singsen and Mitchell (1944) found that body weight gain of growing chicks at 8 weeks of age did not accurately assess P availability. Consequently growth of birds at an even earlier age was used as a measure of P availability. Using 4-week body weight as a measure, Carver et al. (1946) and Gillis et al. (1948) were able to determine the P requirements of the growing chicken. When the total P in the diet was below requirements, organic plant P could be utilized to support body weight gains but was somewhat less available for bone calcification (Waldroup et al. 1965). While these results are in contrast to reports of many previous workers concerning plant P availability, it was stressed that calcium and vitamin D supplementation of the diets was designed to permit maximum usage of organic P as shown in earlier studies (Vanderpopuliere et al. 1961; Harms et al. 1962; Waldroup et al 1964a,b). In addition, the organic P remained in its natural form in the plant material rather than as the chemically isolated compound (Waldroup et al. 1965).

Growth curves, using body weight data, have been widely used as a means of assessing P availability. Wilcox et al. (1954) determined the availability of P in various phosphate sources by comparison with a standard growth curve obtained by feeding graded levels of a reference phosphate to growing birds and determining their corresponding body weights. Nelson (1967) criticized the use of growth curves in evaluating phosphates and stated that the method became inaccurate when the level of dietary P approached the chick's requirement for this element.

The possibility of using more than one criteria to assess P availability prompted Sullivan (1966) to compute a formula to determine the biological value of P and the relative biological value of different sources of P. Using the 3-response criteria body weight, percent bone ash and gain:feed ratios the following formulae were proposed:

$$\text{Phosphorous biological value} = \frac{\text{4-week body weight., grams}}{10} + \text{4-week \% bone-ash} + 10 \times \text{(4-week gain:feed)}$$

$$\text{Relative phosphorus biological value} = \frac{\text{Biological value of unknown source}}{\text{Biological value of reference standard}} \times 100$$

The relative biological values obtained using the above formula for feed sources included in a corn-soybean meal diet fed to young turkeys were 95.7 to 101% for commercial dicalcium phosphates, 82.6 to 98.2% for defluorinated rock phosphates and 91.2% for a raw rock phosphate source using monocalcium phosphate monohydrate as a reference standard.

6. **Egg production**

Since calcium and P metabolism are very closely related, it seems probable that in egg shell formation in which calcium is of major importance, P would also be involved. Common (1932) observed a retention of calcium and P preceding the egg laying period. After egg production commenced, a heavy voiding of P in the droppings occurred.

In later studies, Common (1934) found an increased phosphatase content of blood suggesting that calcium phosphate stored in the bones was being withdrawn. Since most of the calcium was utilized for egg shell formation the excess P was excreted.

Early studies on P requirements for the laying bird showed that a high level (0.80%) was required for egg production (Miller and Bearse 1934; Evans et al. 1944). Later studies (Gillis et al. 1953; Singsen et al. 1962) showed that the levels of P proposed earlier were higher than required; levels above 0.60% P were not found to be of any benefit in improving egg production. O'Rourke et al. (1952) demonstrated that as little as 0.30 P was sufficient to maintain egg production.

7. **Egg quality**

Since egg quality may be influenced by many dietary factors, efforts were made to relate P availability to various characteristics such as hatchability, egg shell quality, egg specific gravity, egg weight and egg interior quality.

a. Hatchability

Initial studies (Titus et al. 1937) indicated that the P content of the diet of the breeding hen had no effect on embryonic mortality. A later study (O'Rourke et al. 1954) however, demonstrated that at very low levels of P (0.20%), hatchability of eggs was decreased. This was later confirmed by other workers (Singsen et al. 1962; Waldroup et al. 1967) who observed that hatchability declined when the level of P was below 0.30%.

b. Egg shell and interior quality

Other egg quality traits such as shell quality, shell strength, shell thickness, specific gravity and Haugh units have also been used to try to determine the requirement of P for the laying bird. Miller and Bearse (1934) reported that egg shell quality was not influenced by the level of P in the diet and this was later confirmed by Evans et al. (1944); however, in both studies the level of P provided in the rations was higher than the current requirement levels (NAS-NRC 1977). Even when much lower levels of P (0.20 to 0.70%) were used, Singsen et al. (1962) observed that neither egg weight nor egg shell thickness was affected by any of the P levels used. Arscott et al. (1962) reported a decrease in egg specific gravity when dietary P levels were raised from 0.60% to 0.90%. This was supported by later studies showing that feeding diets with lower levels of P tends to produce thicker egg shells (Taylor 1965). Hunt and Chancey (1970) reported that egg shell quality tended to be inversely proportional to dietary P levels.

Other studies indicated that egg quality factors were not very sensitive to variations in dietary P. Hamilton and Sibbald (1977) observed that feeding diets containing 0.40 to 0.55% P had no significant effect on egg specific gravity, egg weight or incidence of blood spots. Similarly

Yannakopoulos and Morris (1979) reported that varying the non-phytate P level of the diet from 0.31 to 0.48% had no effect on egg shell thickness or shell deformation.

C. Methods of improving phosphorus availability

The presence of large amounts of P in plant feedstuffs in an organic form which is unavailable to the animal, coupled with the cost of inorganic P, has led to research to try to improve its utilization by animals. Methods used for this purpose have included variations in feed processing and enzyme hydrolysis.

1. The influence of feed processing on phosphorus availability

a. Autoclaving

The application of moist heat under high temperature and steam pressure was shown by Singsen (1948) to increase the availability of P in wheat bran. It was suggested that the application of heat may have resulted in the hydrolysis of phytin with release of inorganic P (O'Dell 1962; Lease 1966). This was later confirmed by Summers et al. (1967) who showed that the phytin P content of wheat by-products decreased as autoclaving time was increased. Kratzer et al. (1959) and Lease et al. (1960) suggested that autoclaving isolated soybean protein or sesame meal destroyed some of the phytic acid and thus reduced the chicks' or poults' need for zinc which may be chelated by phytic acid.

b. Steam pelleting

Attempts to improve the availability of P in feedstuffs by the use of steam pelleting has given variable results. Cisneros (1966) reported that steam pelleting a corn-soybean meal diet resulted in increased ash content of the bones of chicks fed a ration without added inorganic P. Summers et al. (1967) also observed that the P availability of wheat bran increased when the bran was pelleted separately in the whole ration. In later studies, however, Summers et al. (1968) showed that steam pelleting wheat bran and regrinding it lowered the availability of P. Bayley et al. (1968b) reported that when either corn or soybean meal (SBM) was pelleted separately before incorporation into a diet of low phosphorus content there was no improvement in growth rate or bone development. Similarly, no enhancement of P availability as a result of steam pelleting rations for laying hens was recorded by Pepper et al. (1969). Corley and Easter (1979) however, found that pelleting was of some benefit in improving P utilization in wheat bran and rice bran but Harrold et al. (1979) observed that steam pelleting was of no benefit in increasing the availability of P in barley. It was suggested (Bayley et al. 1968a) that the conflicting results obtained in the various studies were probably due to variations in pelleting conditions. It has been observed that the effects of steam pelleting can vary even when conditions are kept as uniform as possible (Pepper et al. 1969).

2. Enzyme hydrolysis

The enzyme phytase (myo-Inositol-hexakisphosphate 6-phosphohydrolase) catalyses the hydrolysis of phytic acid (myo-Inositol-hexaphosphoric acid) to inositol and orthophosphoric acid. Phytase is widely distributed in plant and animal tissue and in many species of fungi and bacteria. Suzuki et al. (1907) first isolated this enzyme from rice bran. Anderson (1915a,b) identified the enzyme in wheat bran. Patwardhan (1937) first reported its occurrence in the intestinal mucosa of rat. Steenbock et al. (1954) and Bitar and Reinhold (1972) reported the presence of the enzyme in the intestine of various species of animals. Peers (1953) recorded it to be one of the first enzymes found to liberate inorganic phosphate from organic P compounds.

For hydrolysis of phytin by phytase to take place, optimum conditions are needed. All feedstuffs may not contain the enzyme, but even if it is present, processing of the feed may destroy the enzyme (McCance and Widdowson 1944). Hill and Tyler (1954) discussed the optimum pH and temperature for both plant and intestinal phytase activity and indicated that unless optimum conditions are met, rate of hydrolysis is reduced. Nelson (1967) stated that feed ingredients cannot be considered to be a consistent source of phytase.

Since levels of phytase in feed ingredients and in the intestine may vary, other sources of the enzyme have been used to try to increase availablity of phytin P. Warden and

Schaible (1962) using a bacterial source of enzyme (lysed E.coli) found that it improved both growth and bone development when it was added to a ration for chickens. Shieh and Ware (1968) reported that a fungus (*Aspergillus ficuum NRRL* 3135) provided a large amount of active phytase that was effective in improving the availability of organic P. Nelson et al. (1968) used the same source of enzyme as Shieh and Ware (1968) to treat SBM in vitro. A marked improvement in growth and bone development was observed in chicks fed the treated meals. Later studies by Nelson et al. (1971) indicated that the feeding of the acetone dried enzyme preparation of *A.ficuum* was also beneficial in improving growth and bone development.

There are other indications that microbial sources of phytase may result in the hydrolysis of phytin. Reinhold (1975) reported that the use of yeast in the preparation of whole wheat bread was beneficial in destroying phytate thus preventing zinc deficiency in an Iranian population. He observed that one-third of the phytate in wheat was destroyed in 2 hours by adding yeast to the dough. Yen and Verum (1979a,b) observed that when 2% of a live yeast culture was included in rations for pigs it was of some benefit in improving P availability for finishing pigs but had no effect with growing pigs. Fardiaz and Markakis (1981) in studies conducted in vitro, fermented peanut presscake with various cultures of molds (*Neurospora sitophila*, *Rhizopus oligosporus* and a *Neurospora* strain isolated in

Indonesia) and found that the culture of *R. oligosporus* was most effective in destroying the phytic acid content. Fermentation of the peanut meal for 72 hours resulted in hydrolysis of 97% of the phytic acid that was present.

III. EXPERIMENTS AT THE UNIVERSITY OF ALBERTA

Experiments were conducted to study:

A. SECTION

Effects of processing canola meal on phosphorus utilization by broiler chickens

1. Influence of varying processing conditions.
2. Influence of processing and dietary phosphorus levels on the performance of broilers.

B. SECTION

Effects of dietary fibre and inorganic phosphorus levels on the performance of broiler chickens

C. SECTION

Utilization of dietary phosphorus by turkey poults

D. SECTION

Utilization of dietary phosphorus by laying hens

1. Influence of phosphorus levels in rations containing canola meal on performance of hens kept in floor pens.
2. Influence of phosphorus levels in rations containing canola meal on performance of hens kept in batteries.

A. SECTION: Effects of processing canola meal on phosphorus utilization by broiler chickens

1. Influence of varying processing conditions

Introduction

The conditions used in processing CM may influence the level and availability of nutrients. Consequently the processing conditions applied are designed to produce a product with optimum nutrient availability. In Canada the meals are most commonly processed by the prepress-solvent extraction method or by solvent extraction. The conditions maintained during the entire operation are responsible for the quality of the meal produced (Clandinin et al. 1978a).

Canola meal is a rich source of P and has a large proportion of its P in organic form as phytin (Finlayson 1977). There is therefore interest in the effects that processing conditions may have on P availability. With some feedstuffs, heat treatment in the presence of moisture has been found to cause some hydrolyis of the phytin present (Singsen 1948; O'Dell 1962; Lease 1966). Although the prepress-solvent extraction method involves pre-cooking of the crushed seed before fat extraction, hydrolysis of phytin does not seem to occur to any marked degree (Finlayson 1977). This suggests that the heat applied is not sufficiently high to bring about hydrolysis of phytin in the meal.

There is some evidence that the use of high temperatures during processing such as autoclaving or steam pelleting may be of some value in improving the availability of P in feedstuffs. Summers et al. (1967) showed that a decrease in the level of phytin P in wheat bran occurred as a result of autoclaving or steam pelleting. Bayley et al. (1968b) reported improved utilization of P from corn and SBM when the rations containing these ingredients were pelleted.

Since preliminary studies conducted indicated that the availability of P in CM may be improved by the application of heat, experiments were conducted to (i) study the influence of time and temperature used in autoclaving CM on utilization of P in the meal (ii) compare the effects of autoclaving and steam pelleting on phytin P utilization and (iii) compare laboratory prepared CM with commercial CM.

Materials and Methods

Experiment 1

Three hundred and eighty four, day-old broiler chickens (White Mountain ♂ x Hubbard ♀) were sexed and randomized into 24 groups with 8 male and 8 female birds each. Three groups were assigned to each of the ration treatments (Table III.1). The rations were formulated to be isocaloric and isonitrogenous and to contain equal amounts of total P. Canola meal was used at a level of 20% in all diets.

Table III.1 Composition of broiler rations (experiment 1)

	Ration Treatment							
	1	2	3	4	5	6	7	8
Ingredients(%):								
Ground wheat	62.55	51.35	51.35	51.35	51.35	51.35	51.35	51.35
Stabilised fat	1.00	4.60	4.60	4.60	4.60	4.60	4.60	4.60
Soybean meal (47.5% protein)	31.50	19.50	19.50	19.50	19.50	19.50	19.50	19.50
Canola meal (36% protein)	---	20.00	---	---	---	---	---	---
A-Canola meal (A)[1]	---	---	20.00	---	---	---	---	---
A-Canola meal (B)[2]	---	---	---	20.00	---	---	---	---
A-Canola meal (C)[3]	---	---	---	---	20.00	---	---	---
A-Canola meal (D)[4]	---	---	---	---	---	20.00	---	---
A-Canola meal (E)[5]	---	---	---	---	---	---	20.00	---
A-Canola meal (F)[6]	---	---	---	---	---	---	---	20.00
Ground limestone	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Biofos (18% Ca, 21% P)	0.60	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[7]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calculated analyses:								
Metabolizable energy (MJ/kg)	11.57	11.57	11.57	11.57	11.57	11.57	11.57	11.57
Protein (%)	23.09	23.13	23.13	23.13	23.13	23.13	23.13	23.13
Calcium (%)	0.99	1.01	1.01	1.01	1.01	1.01	1.01	1.01
Total phosphorus (%)	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Inorganic phosphorus (%)[8]	0.28	0.22	0.22	0.22	0.22	0.22	0.22	0.22
Chemical analyses:								
Protein (%)	23.41	22.99	22.76	23.05	22.98	22.90	23.61	23.22
Total phosphorus (%)	0.58	0.57	0.58	0.57	0.57	0.57	0.58	0.59

[1] Commercial candle CM autocalved at 110°C for 15 minutes
[2] Commercial Candle CM autoclaved at 110°C for 30 minutes
[3] Commercial Candle CM autoclaved at 110°C for 45 minutes
[4] Commercial Candle CM autoclaved at 121°C for 15 minutes
[5] Commercial Candle CM autoclaved at 121°C for 30 minutes
[6] Commercial Candle CM autoclaved at 121°C for 45 minutes
[7] Supplied the following per kilogram of ration: Manganese sulfate, 400 mg; zinc oxide, 100 mg; selenium, 0.1 mg; vitamin A, 6000 IU; vitamin D_3, 1200 ICU; vitamin E, 10 IU; menadione sodium bisulfate, 1 mg; riboflavin, 4 mg; calcium pantothenate, 5 mg; niacin, 20 mg; choline chloride, 60 mg; folic acid, 1 mg; DL-methionine, 750 mg; biotin, 200 mcg; vitamin B_{12}, 10 mcg; Amprol,(25% amprolium) 250 mg.
[8] Sum of added inorganic P and 30% of the total P supplied by all plant ingredients

Samples of CM used in the experiment were prepared by heating them in the autoclave (A-CM) at different temperatures for varying lengths of time. The meals were placed in trays to a depth of approximately 2.5 cm, covered with aluminium foil and autoclaved at a temperature of 110°C or 121°C for a period of 15, 30 or 45 minutes (Table III.1). At the end of the heating period the meals were cooled as quickly as possible and dried at room temperature.

The chickens were raised in electrically heated Petersime batteries with raised screen floors. Feed and water were supplied ad libitum. Continuous lighting was provided.

Records were kept on mortality, body weight, feed consumption and incidence of leg disorders. All birds that showed evidence of perosis (slipped tendons) or other abnormalities such as bowed legs or enlargement of the tibial-metatarsal joint were recorded as suffering from leg disorders.

The experiment was terminated after 28 days on treatment. At the end of the experiment four birds from each group were selected at random and killed by cervical dislocation. The thyroid glands were then removed and their weights recorded. The tibia and middle toe (between the second and third digital bones) from the left side of each chicken were also removed. The tibias were cleaned of adhering flesh by immersing them in boiling water for a few minutes. They were then crushed and fat extracted with ether, dried to constant weight at 100°C, ashed individually at 800°C for 1 hour and the percentages of ash calculated (AOAC 1980). Four toes from birds in each replicate were dried together to constant weight at 100°C and ashed at 600°C for 4 hours. Ash percentages for each treatment were then calculated (AOAC 1980).

The data were subjected to analyses of variance. Percentage data for mortality and leg disorders were transformed using arcsin transformation; however, for ease of interpretation means were expressed as percentages. Significance of differences were assessed by applying Student-Newman-Keuls' procedure (Steel and Torrie 1980) at the 0.05 level of probability.

Experiment 2

In this experiment, 420 day-old broiler chickens (White Mountain ♂ x Hubbard ♀) were sexed and randomized into 21 groups with 10 male and 10 female chickens in each group. Three groups were placed on each of the rations shown in Table III.2. The rations were formulated to be isocaloric and isonitrogenous and to contain equal amounts of total P. The levels of CM used were either 20 or 30%.

A sample of commercial meal produced by the prepress solvent method was used in the experiment. The meal was further processed either by autoclaving or steam pelleting. A portion of the meal was autoclaved in the same way as in Experiment 1 at 121°C for 45 minutes. Another portion of the

Table III 2 Composition of broiler rations (experiment 2)

	Ration Treatment						
	1	2	3	4	5	6	7
Ingredients(%):							
Ground wheat	62.55	51.35	51.35	51.35	45.95	45.95	45.95
Stabilised fat	1.00	4.60	4.60	4.60	6.40	6.40	6.40
Soybean meal (47.5% protein)	31.50	19.50	19.50	19.50	13.30	13.30	13.30
Canola meal (36% protein)	---	20.00	---	---	30.00	---	---
A-Canola meal[1]	---	---	20.00	---	---	30.00	---
SP-Canola meal[2]	---	---	---	20.00	---	---	30.00
Ground limestone	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Biofos (18% Ca,21% P)	0.60	0.20	0.20	0.20	---	---	---
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[3]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calculated analyses:							
Metabolizable energy (MJ/kg)	11.57	11.57	11.57	11.57	11.57	11.57	11.57
Protein (%)	23.09	23.13	23.13	23.13	23.07	23.07	23.07
Calcium (%)	0.99	1.01	1.01	1.01	1.02	1.02	1.02
Total phosphorus (%)	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Inorganic phosphorus (%)[4]	0.28	0.22	0.22	0.22	0.19	0.19	0.19
Chemical analyses:							
Protein (%)	23.22	23.26	23.41	22.92	22.81	23.60	23.81
Total phosphorus (%)	0.57	0.58	0.57	0.58	0.57	0.58	0.59

[1] Commercial Candle CM autoclaved at 121°C for 45 minutes
[2] Commercial Candle CM steam pelleted commercially
[3] As in Table III.1
[4] As in Table III.1

meal was steam pelleted (SP-CM) using a commercial-type pellet mill and reground. The exact temperature, time and pressure applied during steam pelleting could not be measured.

The chickens were raised under conditions similar to those used in Experiment 1 and records were kept on mortality, rate of growth, feed consumption and incidence of leg disorders. At the end of 28 days, four chickens from each group were killed and their thyroid glands were removed and weighed. Tibias and toes from the left side of the chicken were also removed and ash percentages determined as in Experiment 1.

The data were analysed statistically in the same way as in Experiment 1.

Experiment 3

In this experiment, 224 day-old male broiler chickens (White Mountain ♂ x Hubbard ♀) were randomly distributed into 14 groups of 16 birds each. Two groups were placed on each of the rations fed (Table III.3). The rations were formulated to be isocaloric and isonitrogenous and to contain equal amounts of total P. Canola meal was used at a level of 10% in all diets. The A-CM was prepared as in Experiment 1 using a temperature of 121°C for 45 minutes.

Canola cake was prepared in the laboratory by processing Candle rapeseed. The seed was ground in a Waring stainless steel blendor with dry ice to prevent caking. Fat

Table III.3 Composition of broiler rations (experiment 3)

	Ration Treatment						
	1	2	3	4	5	6	7
Ingredients(%):							
Ground Wheat	62.10	56.70	56.70	56.90	56.90	56.90	56.90
Stabilised fat	1.00	2.80	2.80	2.80	2.80	2.80	2.80
Soybean meal (47.5% protein)	31.95	25.75	25.75	25.75	25.75	25.75	25.75
Canola meal (36% protein)[1]	---	10.00	---	---	---	---	---
A-Canola meal (A)[2]	---	---	10.00	---	---	---	---
Canola meal (B)[3]	---	---	---	10.00	---	---	---
Canola meal (C)[4]	---	---	---	---	10.00	---	---
Canola meal (D)[5]	---	---	---	---	---	10.00	---
Canola meal (E)[6]	---	---	---	---	---	---	10.00
Ground limestone	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Biofos (18% Ca, 21% P)	0.60	0.40	0.40	0.20	0.20	0.20	0.20
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[7]	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calculated analyses:							
Metabolizable energy (MJ/kg)	11.57	11.57	11.57	11.57	11.57	11.57	11.57
Protein (%)	23.07	23.06	23.06	23.06	23.06	23.06	23.06
Calcium (%)	0.99	1.01	1.01	1.01	1.01	1.01	1.01
Total phosphorus (%)	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Inorganic phosphorus (%)[8]	0.28	0.25	0.25	0.25	0.25	0.25	0.25
Chemical analyses:							
Protein (%)	23.22	23.80	23.61	22.81	22.63	23.11	23.21
Total phosphorus (%)	0.57	0.58	0.58	0.57	0.57	0.57	0.57

[1] Commercial Candle CM
[2] Commercial Candle CM autoclaved at 121°C for 45 minutes
[3] Candle seed, ground and fat extracted
[4] Candle seed, ground, fat extracted and autoclaved
[5] Candle seed, ground, autoclaved and fat extracted
[6] Candle seed, autoclaved, ground and fat extracted
[7] As in Table III.1
[8] As in Table III.1

was extracted with a Soxhlet apparatus for a period of 24-36 hours using hexane as the solvent. Following fat extraction the cake was desolventized with a current of air at room temperature. Four laboratory prepared canola meals were produced by processing the cake in different ways. The processing sequences applied were as follows. The cake was (i) ground and fat extracted (ii) ground, fat extracted and autoclaved (iii) ground, autoclaved and fat extracted (iv) autoclaved, ground and fat extracted. Autoclaving was carried out at 121°C for 45 minutes.

The chickens were raised under conditions similar to those used in Experiment 1 and records were kept on mortality, rate of growth, feed consumption and incidence of leg disorders. At the end of 28 days, four chickens from each group were killed and their thyroid glands removed and weighed. The tibias and toes from the left side of the bird were removed and bone ash was determined in the same way as in Experiment 1.

The data were statistically analysed in the same way as in Experiment 1.

Results and Discussion

Experiment 1

The results obtained are summarized in Table III.4.

Mortality was not significantly affected by any of the treatments used. The body weights of the broiler chickens fed 20% CM or 20% CM autoclaved at 110°C for 15, 30 or 45

Table III.4 Performance of broiler chickens fed autoclaved canola meals (experiment 1)

Treatment	Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100 g body weight	Tibia ash[1] (%)	Toe ash[2] (%)
Soybean control[3]	4.2*a*	688*e*	1.61*a*	0*a*	10.2*a*	42.6*c*	22.3*d*
20% Canola meal	0*a*	540*b*	1.65*a*	18.8*a*	17.6*c*	34.0*a*	17.7*a*
20% A-Canola meal (A)[4]	6.3*a*	490*a*	1.79*b*	39.6*b*	18.3*c*	38.5*b*	18.8*ab*
20% A-Canola meal (B)	0*a*	603*c*	1.65*a*	6.3*a*	16.6*c*	40.0*bc*	20.2*bc*
20% A-Canola meal (C)	0*a*	610*cd*	1.64*a*	8.4*a*	16.3*c*	40.9*bc*	19.8*bc*
20% A-Canola meal (D)	2.1*a*	624*cd*	1.64*a*	8.4*a*	14.8*bc*	40.0*bc*	19.9*bc*
20% A-Canola meal (E)	0*a*	645*cde*	1.68*ab*	2.1*a*	14.3*bc*	40.1*bc*	19.9*bc*
20% A-Canola meal (F)	0*a*	661*de*	1.71*ab*	0*a*	11.3*ab*	41.6*c*	21.2*bcd*
SEM[5]	1.6	13	0.03	4.1	1.1	0.6	0.4

[1] Expressed on a fat free, dry matter basis
[2] Expressed on a dry matter basis
[3] Column values with the same letters are not significantly different ($P \leq 0.05$)
[4] Processing conditions shown in Table III.1
[5] Standard error of the mean

minutes were significantly lower than those of birds fed the SBM control ration. The inclusion of meal autoclaved at 121°C for 15 minutes also resulted in some decrease in rate of growth; however, when the meal was autoclaved at 121°C for 30 or 45 minutes, body weights at 4 weeks of age were not significantly different from those of the controls.

The results obtained suggest that the level of inorganic P present in the CM rations was insufficient to support normal body growth. Autoclaving the CM at 110°C for 15 minutes apparently did not improve the availability of phytin P but heating at 110°C for 30 or 45 minutes did achieve increased body weight, perhaps by increased hydrolysis of phytin which would then increase the levels of inorganic P in the ration. The growth rate observed when a temperature of 121°C was used for 30 or 45 minutes suggests that the higher temperature of autoclaving was probably more effective in increasing the release of inorganic P from phytin combination.

The treatments used in the experiment had little influence on efficiency of feed conversion. Only the inclusion of 20% A-CM autoclaved at 110°C for 15 minutes gave a feed conversion value that was significantly higher than that of the controls. Feed conversion values for all other treatments were similar to that of the controls.

The incidence of leg disorders in the experiment was high and may have been related in part to the levels of inorganic P in the rations. Occurrence of leg disorders was high in the groups fed CM or A-CM autoclaved at 110°C for 15 minutes but was greatly reduced when the meal was autoclaved at 110°C for 30 or 45 minutes or at 121°C for 15, 30 or 45 minutes. The incidence of leg disorders observed in the different treatments appeared to be related to the growth performance achieved; the higher the rate of growth the lower the incidence of leg disorders.

The inclusion of CM or A-CM in the ration resulted in some increase in thyroid size except in the groups fed A-CM autoclaved at 121°C for 45 minutes. The reason for the lack of enlargement in this treatment as compared to the controls is not known but might be due to denaturation of the intact glucosinolates in the meal when it was heated at a high temperature for an extended period of time.

The addition of 20% CM to the ration resulted in a significant reduction in levels of ash in the tibias and toes of the broilers. When the meal was autoclaved, bone ash values increased, with the levels tending to be higher with longer time of heating and increased temperature. The level of tibia and toe ash in the groups fed CM autoclaved at 121°C for 45 minutes was almost as high as that found in the control group. This suggests that the heat treatment was effective in increasing the availability of P for bone formation.

Although the calculated level of inorganic P in the rations with 20% CM or A-CM was only 0.22%, it appeared that autoclaving was effective in releasing some of the phytin-bound P. This was reflected in the increased body weight, reduced incidence of leg disorders and the high level of bone ash. It would seem however, that autoclaving at a lower temperature of 110°C for 15 minutes was insufficient to increase the availability of the phytin-bound P. Heating at a higher temperature was apparently more effective in increasing P utilization from CM.

Experiment 2

The results obtained (Table III.5) indicated that there was no significant effect of treatments on level of mortality. A higher rate of mortality was observed in this experiment than in Experiment 1. No reason for the increased mortality level was evident.

Body weight was influenced by the treatments used. Addition of 20% CM to the ration containing 0.22% inorganic P resulted in significant depression in rate of growth as compared to the controls. Inclusion of 20% A-CM or 20% SP-CM in the diet, however, resulted in 4-week body weights similar to the controls. Heat treatment of CM either by autoclaving or by steam pelleting seemed to be of some benefit in improving rate of growth when the level in the diet was 20%. When the level of inclusion was increased to 30% with CM, A-CM or SP-CM, body weights of the chickens were significantly lower than the controls. Although the rations with 30% CM, A-CM or SP-CM contained the same level

Table III.5 Performance of broiler chickens fed autoclaved and steam pelleted canola meals (experiment 2)

Treatment	Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100 g body weight)	Tibia ash[1] (%)	Toe ash[1] (%)
Soybean control[3]	3.3a	683b	1.89b	3.3a	7.6a	43.4c	23.2c
20% Canola meal	5.0a	601a	1.84a	3.3a	13.8c	35.9ab	17.0a
20% A-Canola meal	3.3a	691b	1.81a	3.3a	9.1ab	44.0c	22.1c
20% SP-Canola meal	5.0a	675b	1.82a	13.3ab	11.7bc	38.0b	19.3b
30% Canola meal	8.3a	599a	1.85a	23.3b	15.1d	34.2a	16.8a
30% A-Canola meal	3.3a	617a	1.82a	23.3b	10.6abc	36.9ab	19.0b
30% SP-Canola meal	6.7a	586a	1.85a	20.0b	11.9bc	34.8a	17.8a
SEM[4]	3.2	17	0.04	3.3	0.9	0.7	0.4

[1] Expressed on a fat free, dry matter basis
[2] Expressed on a dry matter basis
[3] Column values with the same letters are not significantly different (P≤0.05)
[4] Standard error of the mean

of total P in the rations as with a 20% level of addition, the calculated inorganic P content was only 0.19% as compared to 0.22% because of the high phytin P content of CM. When 30% CM was used no additional source of inorganic P was added to the ration to achieve a level of 0.58% total P. With this low level of inorganic P, heat treatment either by autoclaving or steam pelleting apparently did not release enough phytin P to promote increased rate of growth. This supports the observation of Gillis et al. (1948) who reported that chickens can utilize phytin P only in the presence of a substantial amount of inorganic P.

The treatments used had no influence on feed conversion values. Feed conversion values were higher in this experiment than those observed in Experiment 1.

Incidence of leg disorders was markedly affected by the level of CM and inorganic P in the ration. When 20% CM or 20% A-CM was added in the ration the incidence of leg disorders was similar to the controls; however, inclusion of 30% CM, A-CM or SP-CM in the diet resulted in a significant increase in the incidence of leg abnormalities. The combined effect of the lower level of inorganic P (0.19%) and a high level of CM (30%) in the ration was apparently responsible for the increased leg disorders. It has been previously noted (Clandinin et al. 1978b) that the use of very high levels of rapeseed meals resulted in increased incidence of perosis in broiler chickens at 4 and 8 weeks of age while Wilgus et al. (1937) showed the importance of P levels in normal leg formation.

Thyroid size was affected to some extent by the treatments used. The inclusion of 20% or 30% CM in the diet of chickens resulted in significantly larger thyroids than those of the controls. Heat treatment of CM reduced the degree of enlargement; thyroid size of the birds fed A-CM was similar to that of the controls. A similar increase in thyroid size as a result of inclusion of CM was reported by Clandinin et al. (1978b).

Calcification, as measured by the ash content of tibias and toes of the chickens was dependent on the ration fed. Feeding the chickens a diet with 20% or 30% CM resulted in tibia and toe ash values significantly lower than the controls. When CM was autoclaved before inclusion in the ration at a level of 20%, calcification, as indicated by tibia and toe ash percentages, was improved and was similar to the controls. When 30% A-CM was used a slight increase in tibia and toe ash was noted but the levels were significantly lower than the controls. Steam pelleting the CM was not effective in increasing tibia or toe ash levels. This suggests that calcification is dependent on the level of inorganic P in the diet. The 30% CM ration containing 0.19% inorganic P provided too little available P to allow normal calcification. From these results it would appear that autoclaving CM at 121°C for 45 minutes may have been effective in releasing some P from organic combination.

Experiment 3

The performance of chickens in experiment 3 are summarized in Table III.6.

The treatments used had no significant effect on mortality. Level of mortality was low in all groups throughout the experimental period.

Body weight was influenced by the treatments used. The body weights of birds fed rations containing 10% commercial CM, either autoclaved or non-autoclaved, were similar to the controls; however, the groups fed rations with 10% of laboratory prepared meals had body weights significantly lower than the controls. Since the level of total and inorganic P in the rations containing either commercial or laboratory prepared CM were the same, the reduction was not related to the P levels in the rations. Differences noted may have been related to the processing conditions used in preparing commercial meals as compared to those produced in the laboratory. Commercially prepared meals are cooked at temperatures ranging from approximately 86 to 95°C for an average of about 1.2 hours before fat extraction and are heated to a temperature of about 71 to 110°C during desolventization for approximately 50 minutes. Since extraction and desolventizing temperatures used in preparing meals in the laboratory were low, it would appear that the higher temperatures and longer periods of time used during pre-cooking and desolventizing the commercial meals may have been responsible for their better quality.

Table III.6 Performance of broiler chickens fed rations with autoclaved and laboratory prepared canola meals (experiment 3)

Treatment	Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg (%)	Thyroid size (mg/100 g body weight)	Tibia ash[1] (%)	Toe ash[2] (%)
Soybean control[3]	6.3a	720b	1.82a	3.1a	8.1a	43.6b	24.8c
10% Canola meal	0a	644ab	1.93a	3.1a	11.1b	40.7b	22.2bc
10% A-Canola meal (A)[4]	0a	708b	1.79a	3.1a	11.5b	43.9b	23.4bc
10% Canola meal (B)	0a	616a	1.81a	3.1a	11.0b	39.2b	20.1b
10% Canola meal (C)	3.1a	566a	1.96a	18.8a	11.6b	32.2a	15.9a
10% Canola meal (D)	3.1a	612a	1.96a	3.1a	10.5b	40.6b	20.1b
10% A-Canola meal (E)	3.1a	593a	1.90a	18.8a	10.1b	33.6a	16.3a
SEM[5]	1.4	20	0.03	3.5	0.6	1.4	0.8

[1] Expressed on a fat free, dry matter basis
[2] Expressed on a dry matter basis
[3] Column values with the same letters are not significantly different ($P \leq 0.05$)
[4] Processing conditions shown in Table III.3
[5] Standard error of the mean

The treatments used had no significant effect on feed conversion values which ranged from 1.79 to 1.96 for the different treatments used.

Incidence of leg disorders in the experiment was not affected by the different treatments used. Chickens fed two of the laboratory prepared meals, however, did show a higher incidence of leg disorders but they were not significantly different from the controls.

Thyroid size of the birds was influenced to a small extent by the presence of CM in the ration. The inclusion of commercial CM, A-CM or laboratory prepared CM in the diets of the chickens resulted in thyroid glands that were significantly larger than those of the controls.

Calcification as measured by tibia and toe ash percentages was affected by the different processing techniques used in the preparation of the meals. The commercially prepared CM or the A-CM, when included in the rations fed, resulted in tibia and toe ash values similar to the controls. When the ration contained laboratory prepared meals in which the cake was autoclaved prior to grinding, the tibia and toe ash values of the birds were markedly reduced. No reason for the reduction in calcification that occurred was apparent.

Summary

Three experiments were conducted with broiler chickens to study the influence of varying processing conditions of CM on P utilization. The following results were obtained:

1. The use of A-CM at a level of 20% for broiler chickens was of some benefit in improving rate of growth and calcification.
2. Autoclaving the meal at 121°C for 45 minutes appeared to be most effective in increasing P utilization.
3. Commercial steam pelleting of the meal was not of any benefit in improving calcification, although some increase in rate of growth occurred when 20% SP-CM was included in the ration.
4. Heat treatment of CM either by autoclaving or steam pelleting was of no advantage in improving rate of growth or calcification when the meal was included at a level of 30% in the diet.
5. Commercial CM was superior to laboratory prepared meals in promoting growth and bone calcification.

2. Influence of processing and dietary phosphorus levels on the performance of broilers

Introduction

The limited utilization of P from phytic acid or phytates in plant material has stimulated researchers to determine its availability from various plant sources (McGinnis et al. 1944; Waldroup et al. 1965). Generally 30 to 40% of the total P in plant feedstuffs has been found to be utilized (Taylor 1965). This agrees with the NAS-NRC (1977) statement that approximately 30% of P in plant materials is available to young growing birds. Since the availability of P is low, studies have been conducted to try to improve it. Among the methods used to increase utilization of P from the phytin in vegetable feedstuffs, as indicated in A.Section-1, autoclaving has shown some benefit.

Canola meal is a rich source of P containing approximately 1.1% of this element; however, 70 to 80% of the P occurs as the calcium and magnesium salt of phytic acid (Finlayson 1977) and is therefore presumably unavailable to poultry. Since CM contains a high level of phytin P it would be particularly desirable to maximize the biological utilization of this element. Consequently studies were undertaken to determine whether increased levels of inorganic P in the diet affected the utilization of phytin P in raw or autoclaved CM by growing chickens.

Materials and Methods

Experiment 1

Two hundred and fifty six, day-old (White Mountain ♂ x Hubbard ♀) broiler chickens were sexed and randomized into 16 groups with 8 male and 8 female chickens in each group. Two groups were placed on each of the ration treatments shown in Table III.7. The autoclaved CM was prepared by spreading the commercial CM on flat trays to a depth of approximately 2.5 cm and heating them in an autoclave at 110°C for 30 minutes. At the end of this time period the autoclaved meal (A-CM) was immediately spread out on sheets of paper, cooled and air-dried at room temperature.

The rations were formulated to be isocaloric and isonitrogenous and to contain equal amounts of total P but varying amounts of inorganic P. Variations in phytin P levels were affected by adding 10, 20 or 30% CM. Rations 1 and 5 which were the same formulation served as the controls in the experiment.

The chickens were raised in electrically heated Petersime batteries with raised screen floors. Feed and water were supplied ad libitum. Continuous lighting was provided.

Records were kept on mortality, body weight, feed consumption and incidence of leg disorders. All birds which had bowed legs, swellings at the tibial-metatarsal joint or

Table III.7 Composition of broiler rations (experiment 1)

	Ration Treatment							
	1	2	3	4	5	6	7	8
Ingredients(%):								
Ground wheat	62.10	56.70	51.30	45.90	62.10	56.70	51.30	45.90
Stabilised fat	1.00	2.80	4.60	6.40	1.00	2.80	4.60	6.40
Soybean meal(47.5% protein)	31.95	25.75	19.55	13.35	31.95	25.75	19.55	13.35
Canola meal (36% protein)	---	10.00	20.00	30.00	---	---	---	---
A-Canola meal[1]	---	---	---	---	---	10.00	20.00	30.00
Ground limestone	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Biofos (18%Ca,21%P)	0.60	0.40	0.20	---	0.60	0.40	0.20	---
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calculated analyses:								
Metabolizable energy (MJ/kg)	11.57	11.57	11.58	11.59	11.57	11.57	11.58	11.59
Protein (%)	23.08	23.07	23.05	23.04	23.08	23.07	23.05	23.04
Calcium (%)	0.99	1.01	1.00	0.99	0.99	1.01	1.00	0.99
Total phosphorus (%)	0.58	0.58	0.58	0.58	0.58	0.58	0.58	0.58
Inorganic phosphorus (%)[3]	0.28	0.25	0.22	0.19	0.28	0.25	0.22	0.19
Chemical analyses:								
Protein (%)	23.62	23.48	23.37	23.60	23.22	23.85	23.05	23.32
Total phosphorus (%)	0.57	0.58	0.57	0.57	0.58	0.59	0.58	0.57

[1] Commercial CM autoclaved at 110°C for 30 minutes
[2] Supplied the following levels per kilogram of ration: Manganese sulfate, 400 mg; zinc oxide, 100 mg; selenium, 0.1 mg; vitamin A, 6000 IU; vitamin D_3, 1200 ICU; vitamin E, 10 IU; menadione sodium bisulfate, 1 mg; riboflavin, 4 mg; calcium pantothenate, 5 mg; niacin, 20 mg; choline chloride, 60 mg; folic acid, 1 mg; DL-methionine, 500 mg; biotin, 200 mcg; vitamin B_{12}, 10 mcg; Amprol, (25% amprolium) 250 mg.
[3] Sum of added inorganic P and 30% of the total P derived from all plant ingredients

perosis (slipped tendons) were recorded as suffering from leg disorders.

The experiment was terminated after 28 days. At the end of the experiment, four birds from each group were selected at random and killed by cervical dislocation. The thyroid glands were then removed and their weights recorded. The left tibia and the middle toe (between the second and third digital bones) of each chicken were also removed. The tibias were removed from adhering flesh by placing them in boiling water for a few minutes, they were then crushed, fat extracted with ether, dried to constant weight at 100°C, ashed at 800°C for 1 hour and the percentages of ash calculated (AOAC 1980). Four toes from each group were pooled together, dried to constant weight at 100°C ashed at 600°C for 4 hours and their ash content calculated (AOAC 1980).

The data were subjected to analysis of variance. Percentage data for mortality and leg disorders were transformed using arcsin transformation (Steel and Torrie 1980); however, for ease of interpretation, means were expressed as percentages. Significance of differences were assessed by applying Students-Newman-Keuls' procedure (Steel and Torrie 1980) at the 0.05 level of probability.

Experiment 2

In this experiment 432 day-old (White Mountain ♂ x Hubbard ♀) male broiler chickens were randomly distributed into 27 groups of 16 birds each. Three groups were placed on each of the ration treatments (Table III.8). A 3 x 3 factorial design with 3 levels of CM and 3 levels of P was used in the experiment.

The A-CM was prepared as in Experiment 1 but using a temperature of 121°C for 45 minutes. The rations were formulated to be isocaloric and isonitrogenous. The control rations and those with 30% CM or 30% A-CM were formulated to contain 0.58, 0.63 and 0.68% total P. The increased levels of P were achieved by adding an inorganic P supplement. In order to maintain the same level of total P in the control ration containing SBM as in those containing 30% CM a higher level of inorganic P supplement had to be added to the control rations. As a consequence the levels of inorganic P in the control rations were higher than in those containing CM.

The chickens were raised under conditions similar to those used in Experiment 1 and records were kept on mortality, body weight, feed consumption and incidence of leg disorders. At the end of 28 days, four chickens from each replicate were killed and their thyroid glands, left tibia and middle toe were removed. The thyroid glands were weighed immediately and the tibias and toes were analyzed as in Experiment 1.

Data were analyzed using analysis of variance. Sources of variation were CM treatments (n=3), level of P (n=3) and CM treatment x phosphorus (n=9). Percentage data for

Table III.8 Composition of broiler rations (experiment 2)

	Ration Treatment								
	1	2	3	4	5	6	7	8	9
Ingredients(%):									
Ground wheat	62.55	62.40	62.25	45.95	45.80	45.65	45.95	45.80	45.65
Stabilised fat	1.00	1.00	1.00	6.40	6.40	6.40	6.40	6.40	6.40
Soybean meal (47.5% protein)	31.50	31.50	31.50	13.30	13.30	13.30	13.30	13.30	13.30
Canola meal (36% protein)	---	---	---	30.00	30.00	30.00	---	---	---
A-Canola meal[1]	---	---	---	---	---	---	30.00	30.00	30.00
Ground limestone	2.00	1.90	1.80	2.00	1.90	1.80	2.00	1.90	1.80
Biofos (18%Ca,21%P)	0.60	0.85	1.10	---	0.25	0.50	---	0.25	0.50
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calculated analyses:									
Metabolizable energy (MJ/kg)	11.57	11.55	11.53	11.59	11.57	11.55	11.59	11.57	11.55
Protein (%)	23.09	23.07	23.05	23.09	23.07	23.05	23.09	23.07	23.05
Calcium (%)	0.98	0.99	0.99	1.01	1.02	1.02	1.01	1.02	1.02
Total phosphorus (%)	0.58	0.63	0.68	0.58	0.63	0.68	0.58	0.63	0.68
Inorganic phosphorus (%)[3]	0.27	0.32	0.37	0.19	0.24	0.29	0.19	0.24	0.29
Chemical analyses:									
Protein (%)	23.27	22.95	23.04	23.11	22.82	22.76	22.85	23.23	23.09
Total phosphorus (%)	0.57	0.63	0.67	0.58	0.62	0.67	0.58	0.64	0.69

[1] Commercial canola meal autoclaved at 121°C for 45 minutes
[2] As in Table III.7 with an additional 250mg. DL-methionine
[3] As in Table III.7

mortality and leg disorders were transformed using arcsin transformation (Steel and Torrie 1980); however, for ease of interpretation, means were expressed as percentages. Means for significant sources of variation were compared using the Students-Newman-Keuls' procedure (Steel and Torrie 1980) at the 0.05 level of probability.

Experiment 3

In this experiment 540 day-old (White Mountain ♂ x Hubbard ♀) broiler chickens were sexed and randomized into 27 groups with 10 male and 10 female chickens in each. Three groups were placed on each of the ration treatments (Table III.9). The A-CM was prepared as in Experiment 1 using a temperature of 121°C for 45 minutes.

The rations were formulated to be isocaloric and isonitrogenous and to contain 0.54, 0.58 or 0.68% total P. Feed and water were supplied ad libitum.

The chickens were raised in floor pens 4.49 m x 1.85 m in size. Infrared brooders were used as a source of supplemental heat. The birds were given 16 hours of light daily.

Records were kept on mortality, rate of growth, feed consumption and incidence of leg disorders. At the end of 28 days, four chickens from each replicate were killed and their thyroid glands were removed and weighed. The tibia and middle toe from the left side of the chicken were also removed and prepared for ash determinations as in Experiment

Table III.9 Composition of broiler rations (experiment 3)

	Ration Treatment								
	1	2	3	4	5	6	7	8	9
Ingredients(%):									
Ground wheat	62.75	51.45	51.45	62.55	51.35	51.35	45.95	45.95	62.25
Stabilised fat	1.00	4.60	4.60	1.00	4.60	4.60	6.40	6.40	1.00
Soybean meal (47.5% protein)	31.50	19.50	19.50	31.50	19.50	19.50	13.30	13.30	31.50
Canola meal (36% protein)	---	20.00	---	---	20.00	---	30.00	---	---
A-Canola meal[1]	---	---	20.00	---	---	20.00	---	30.00	---
Ground limestone	2.00	2.10	2.10	2.00	2.00	2.00	2.00	2.00	1.80
Biofos (18%Ca,21%P)	0.40	---	---	0.60	0.20	0.20	---	---	1.10
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[2]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calculated analyses:									
Metabolizable energy (MJ/kg)	11.58	11.58	11.58	11.57	11.57	11.57	11.57	11.57	11.54
Protein (%)	23.11	23.14	23.14	23.09	23.13	23.13	23.07	23.07	23.04
Calcium (%)	1.02	1.01	1.01	0.99	1.01	1.01	1.02	1.02	1.00
Total phosphorus (%)	0.54	0.54	0.54	0.58	0.58	0.58	0.58	0.58	0.68
Inorganic phosphorus (%)[3]	0.24	0.18	0.18	0.28	0.22	0.22	0.19	0.19	0.38
Chemical analyses:									
Protein (%)	23.41	23.33	22.99	23.11	23.42	23.08	23.18	23.30	22.95
Total phosphorus (%)	0.53	0.53	0.54	0.57	0.58	0.57	0.59	0.58	0.67

[1] Commercial canola meal autoclaved at 121°C for 45 minutes
[2] As in Table III.7 with an additional 250mg. DL-methionine
[3] As in Table III.7

1.

Data were subjected to analyses of variance to test the effect of treatment on the traits measured. Pen means were used in the statistical analyses. Treatment sum of squares were partitioned into orthogonal and single degree of freedom comparisons to identify significant differences between level of dietary P, inclusion of CM and influence of autoclaving on the different biological traits measured (Steel and Torrie 1980). The data for mortality and incidence of leg disorders were transformed for statistical analysis using arcsin transformation (Steel and Torrie 1980); however, for ease of interpretation means were expressed as percentages.

Results and Discussion

Experiment 1

The results obtained are summarized in Table III.10 in which results from Rations 1 and 5 are combined. The level of CM in the ration affected incidence of mortality that occurred. Inclusion of 30% CM or A-CM resulted in a higher level of mortality than when lower levels of CM were used. Mortality in these treatments was mainly due to rickets that occurred after the birds had been on experiment for 2 weeks. This suggested that a deficiency of inorganic P may have been responsible for the inadequate growth and calcification. Mortality in all other groups was low with most occurring during the first two weeks of the experiment.

Table III.10 Influence of varying levels of canola meal on the performance of broiler chickens (experiment 1)

Treatment		Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100 g body weight)	Tibia ash[1] (%)	Toe ash[2] (%)
Soybean control[3,4]	(0.28)[5]	0a	568bc	1.84a	0a	7.8a	41.6c	23.3c
10% Canola meal	(0.25)	6.3a	554ab	1.93a	0a	10.9ab	39.9c	21.0c
20% Canola meal	(0.22)	3.1a	544ab	1.78a	3.2a	12.7bc	34.5b	19.3b
30% Canola meal	(0.19)	15.6b	467a	2.04a	56.3b	14.5c	31.3a	17.1a
10% A-Canola meal	(0.25)	0a	646c	1.74a	0a	10.6ab	41.1c	23.2c
20% A-Canola meal	(0.22)	0a	695d	1.70a	0a	10.5ab	40.2c	22.6c
30% A-Canola meal	(0.19)	34.4c	484a	2.11a	71.9b	14.2c	31.1a	16.3a
SEM[6]		1.8	22	0.09	5.8	0.7	0.5	0.4

[1] Expressed on a fat free, dry matter basis
[2] Expressed on a dry matter basis
[3] Column values with the same letters are not significantly different ($P \leq 0.05$)
[4] Values used in this row are averages of replicates in Ration 1 and 5 combined
[5] Numbers in parentheses indicate sum of added inorganic P and 30% of total P derived from all plant ingredients
[6] Standard error of the mean

Inclusion of 10% or 20% CM in the ration had no significant effect on body weight at 4 weeks of age; however, when 30% CM was used, body weight was markedly decreased. The addition of 10% or 20% A-CM resulted in increased rate of growth as compared to the chickens fed SBM control ration, but only in the case of the 20% A-CM was the difference significant. The growth response is an indication that when CM and SBM are used together there may be some complementary effect. When 30% A-CM was used body weight was significantly decreased and was similar to that obtained with 30% CM. The reduced growth at the 30% level probably was related to the low level of inorganic P in these diets.

The treatments used had no significant effect on feed conversion although the groups fed 10 and 20% A-CM did have lower feed conversion values.

Inclusion of 10 and 20% CM or A-CM did not affect the incidence of leg disorders; however, when 30% CM or A-CM was included in the ration, incidence of leg disorders was extremely high. This suggested that when 30% CM was used there was insufficient inorganic P present to allow satisfactory bone formation to occur.

As the level of CM in the diet was increased, an increase in thyroid size in relation to body weight occurred. The degree of enlargement, however, was small in comparison to the degree of enlargement observed when high glucosinolate rapeseed meals were used in broiler rations. Marangos et al. (1974) reported reduced enlargement of thyroid glands in broiler chickens as a result of feeding rations containing rapeseed meals with low oxazolidinethione content.

The ash content of the tibias and toes was not affected by the addition of either 10% CM or 10% A-CM to the ration. This suggests that the levels of total and inorganic P in these rations were sufficiently high to allow normal calcification of bone. Addition of 20 and 30% CM to the ration resulted in significant successive decreases in bone ash as the level of CM was increased. When 20% A-CM was included in the ration, tibia and toe ash values were significantly higher than those in the groups fed 20% CM. This confirms the results in A.Section-1 that autoclaving may have increased the availability of the phytin P in the meal. It also agrees with the observation of others that autoclaving feed ingredients resulted in more of the phytin P being made available for bone ash purposes (Singsen, 1948; O'Dell 1962; Lease 1966; Summers et al. 1967). Nelson (1967) in a review on P utilization stated that percentage bone ash was one of the most sensitive practical criteria for evaluating the availability of dietary P and was little affected by other variables that influence growth. The results of this study indicate that 0.25% inorganic P supplied in Ration 2 was adequate for normal calcification of the bones.

When 30% A-CM was used, the bone ash values were similar to those of the groups fed 30% CM. The decreased calcification that occurred when 30% CM or 30% A-CM was used may have been due to the high levels of phytin P and the low levels of inorganic P in these rations. Gillis et al. (1948) reported that the chicken can undoubtedly utilize phytin P to a limited extent only in the presence of a substantial amount of the more available P.

Experiment 2

The results obtained are summarized in Table III.11. Mean values for the CM treatments, the varying levels of total P and treatment x phosphorus interaction (average of 3 replicates) are included for each of the traits measured.

Level of mortality in the experiment was low and was not influenced by any of the treatments used.

The inclusion in the ration of either 30% CM or 30% A-CM resulted in significant decreases in body weight as compared to chicks fed the control ration containing SBM. This decrease was similar to that observed in Experiment 1. The level of total P in the ration also affected rate of gain. The body weight of chickens fed rations containing 0.58% total P was significantly lower than that of chickens fed 0.63 or 0.68% total P. It was evident that the chickens fed 30% CM or 30% A-CM with the lowest level of inorganic P (0.19%) had the lowest body weight. When the level of inorganic P in the ration was raised to 0.24 and 0.29%, body weights of chicks were similar to those of chicks fed the SBM control rations. From these results it appeared that the

Table III.11 Means for effect of canola meal treatment, phosphorus level and canola meal treatment x phosphorus level on the performance of broiler chickens (experiment 2)

		Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100 g body weight)	Tibia ash[1] (%)	Toe ash[2] (%)
CM Treatment								
Soybean control[3]		2.1a	779b	1.80a	0a	10.0a	45.3b	20.5b
30% CM		4.2a	705a	1.82a	5.6b	14.8b	40.6a	17.8a
30% A-CM		3.5a	692a	1.78a	8.3b	14.6b	40.7a	18.3a
SEM[4]		1.4	12	0.03	2.2	0.5	0.9	0.4
Phosphorus level								
0.58		4.2a	678a	1.77a	13.2b	14.0b	39.7a	17.3a
0.63		3.5a	747b	1.84a	0a	13.4ab	43.2b	19.1b
0.68		2.1a	751b	1.78a	0.2a	12.1a	43.7b	20.2b
SEM		1.4	12	0.03	2.2	0.5	0.9	0.4
CM Treatment x Phosphorus level								
Soybean	0.58 (0.27)[5]	0a	757bc	1.79a	0a	9.7a	45.7c	20.4b
	0.63 (0.32)	4.2a	778c	1.85a	0a	10.1a	46.3c	20.6b
	0.68 (0.37)	2.1a	801c	1.76a	0a	10.1a	43.9c	20.4b
30% CM	0.58 (0.19)	4.2a	666ab	1.78a	14.6b	16.5b	36.9a	15.8a
	0.63 (0.24)	4.2a	737bc	1.85a	0a	15.2b	41.7bc	18.0b
	0.68 (0.29)	4.2a	710bc	1.82a	0.7a	12.8ab	43.1c	19.7b
30% A-CM	0.58 (0.19)	8.4a	611a	1.73a	25.0b	15.9b	36.5a	15.7a
	0.63 (0.24)	2.1a	736bc	1.83a	0a	14.7b	41.5bc	18.7b
	0.68 (0.29)	0a	728bc	1.77a	0a	13.0ab	44.1c	20.5b
SEM		2.5	20	0.05	3.7	0.9	1.6	0.6
Significance								
CM Treatment		ns[6]	*[7]	ns	*	*	*	*
Phosphorus level		ns	*	ns	*	*	*	*
CM Treatment x Phosphorus level		ns	ns	ns	*	ns	ns	*

[1] Expressed on a fat free, dry matter basis
[2] Expressed on a dry matter basis
[3] Means within a block having the same letters within columns are not significantly different (P≤0.05)
[4] Standard error of the mean
[5] Numbers within parentheses indicate level of calculated inorganic P
[6] Non significant (P≤0.05)
[7] Significant (P≤0.05)

minimum requirement for inorganic P for broiler chickens up to 4 weeks of age was approximately 0.24%. This is in close agreement with the results of Temperton and Cassidy (1963b) who reported that a level of 0.23% non-phytin P in the rations for growing birds was adequate for normal growth. In this experiment autoclaving CM failed to give any improvement in body weight over the CM when included in the ration at the 30% level.

Feed conversion in all groups was similar. There were no significant differences attributable to CM treatment or to P levels in the rations.

No leg disorders were noted in the groups fed the control ration, but a significantly higher incidence was observed in the groups receiving either 30% CM or 30% A-CM. Examination of the data indicated that the level of P in the ration had a marked effect on occurrence of leg disorders. Practically all of the leg deformities that were recorded occurred in the groups fed the lowest level of total P; the incidence of leg disorders was highest in the groups receiving 0.58% P and was significantly different from those of chickens fed 0.63 and 0.68% P. Although the main effects produced significant changes in the incidence of leg disorders, significant interaction effects were also noted. This may indicate that appearance of leg disorders was influenced by the inclusion of CM as well as P level in the diet. None of the birds fed SBM showed leg disorders even at the lowest level of total P. The birds fed 30% CM or 30%

A-CM containing 0.19% inorganic P had a very high incidence of leg disorders which was significantly different from all other treatments. Addition of 0.05 or 0.10% inorganic P to these rations was effective in preventing leg disorders. This indicated that a level of 0.19% inorganic P in a broiler ration was insufficient for normal bone development.

Chickens fed a ration containing CM or A-CM showed enlarged thyroid glands resulting in thyroid to body weight ratios that were significantly higher than those fed the SBM control ration. The thyroid to body weight ratios were slightly higher in the groups fed 0.58% P than in those receiving 0.63 or 0.68% P level. This may have been a reflection of the lower body weights in the groups fed 0.58% P and probably was not related to the level of P in the diet.

The degree of calcification as indicated by tibia and toe ash values was lower in chicks fed rations containing 30% CM or A-CM as compared to that of chicks fed control rations. Average tibia and toe ash values were significantly higher in the control group than in the groups fed CM or A-CM.

The level of P in the rations fed influenced calcification. Higher levels of ash were found in tibias and toes of birds fed 0.63 and 0.68% P. With increasing levels of added inorganic P, corresponding increases in bone ash values were reported by Bayley et al. (1968b) and Summers et al. (1968). Although the main effects resulted in significant changes in bone ash values, there was a significant interaction in the case of the toe ash. This suggests that toe ash values may not be as sensitive to changing dietary P levels as tibia ash values.

In all cases the SBM controls showed the best calcification as indicated by the tibia and toe ash percentages. However, addition of inorganic P (0.05 or 0.10%) to the CM or A-CM ration did increase calcification. Bone ash values in these groups were similar to the controls.

Experiment 3

The results of the experiment are summarized in Table III.12. The mean square value of the performance data for the 9 treatments and the 8 different comparisons are given in Table III.13.

The level of mortality that occurred in the experiment was not affected by the levels of total P in the rations fed but was related to the levels of inorganic P. When no CM was included in the rations, with P levels ranging from 0.54 to 0.68%, no difference in mortality was observed; however, when the same level of total P was maintained but the level of inorganic P was reduced by the addition of CM a marked increase in mortality occurred. This was seen when 20% CM was added to a ration with 0.54% total P or when 30% CM was included in a ration with 0.58% total P. Since the levels of inorganic P in these diets were 0.18 and 0.19% respectively,

Table III.12 Influence of dietary phosphorus levels on the performance of broiler chickens (experiment 3)

Treatment	Phosphorus level[1] (%) Total	Phosphorus level[1] (%) Inorganic	Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100 g body weight)	Tibia ash[2] (%)	Toe ash[3] (%)
1. 0% CM	0.54	0.24	3.3	644	1.74	0	8.5	40.9	21.3
2. 20% CM	0.54	0.18	30.0	477	2.16	20.0	12.7	35.9	16.7
3. 20% A-CM	0.54	0.18	15.0	492	1.89	6.7	11.9	36.9	18.0
4. 0% CM	0.58	0.28	0	664	1.70	0	8.4	42.5	22.6
5. 20% CM	0.58	0.22	3.3	609	1.69	18.3	14.3	35.3	16.9
6. 20% A-CM	0.58	0.22	1.7	659	1.62	1.7	12.4	43.3	21.0
7. 30% CM	0.58	0.19	20.0	484	1.94	15.0	15.9	35.4	15.5
8. 30% A-CM	0.58	0.19	15.0	564	1.84	13.3	14.9	35.5	16.9
9. 0% CM	0.68	0.38	3.3	715	1.66	0	10.1	46.9	24.6
SEM[4]			4.0	29	0.07	5.7	0.9	0.8	0.5

[1] As shown in Table III.9
[2] Expressed on a fat free, dry matter basis
[3] Expressed on a dry matter basis
[4] Standard error of the mean

Table III.13 Mean square of the analysis of variance of the performance data (experiment 3)

Source of variation	df	Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100 g body weight)	Tibia ash (%)	Toe ash (%)
Treatments	8	264.6*	23819*	0.09*	223.8	22.0*	55.7*	29.4*
Level of Phosphorus								
1 vs 4	(1)	16.7	629	0	0	0.01	3.8	2.5
4 vs 9	(1)	16.7	3862	0	0	4.6	28.8*	6.1*
Influence of CM								
1 vs 2,3	(1)	612.5*	50846*	0.17*	355.6	30.3	39.6*	31.5*
4 vs 5,6	(1)	22.2	1768	0.01	234.7	49.8*	20.4*	23.4*
4 vs 7,8	(1)	612.5*	39260*	0.07*	401.3	99.2*	88.5*	80.9*
Influence of Autoclaving								
2 vs 3	(1)	204.2	340	0.10*	266.7	0.8	1.6	2.6
5 vs 6	(1)	0	3762	0.01	504.2*	5.4	95.9*	20.7*
7 vs 8	(1)	37.5	9448*	0.02	4.2	1.2	0	3.1
Error	18	49.0	2572	0.01	100.0	2.5	1.8	0.83

* Significant at the 0.05 level of probability

this indicated that a level of 0.19% inorganic P in the ration was not adequate. When the main effects were partitioned (Table III.13) significant increases in mortality were seen only when 20% CM was included in the ration with 0.58% total P.

Rate of growth was influenced by the level of inorganic P in the diet. When no CM was added to the diet, rate of growth was similar with dietary levels of 0.54, 0.58 or 0.68% total P. The corresponding levels of inorganic P in the rations were 0.24, 0.28 or 0.38%. When total P was maintained constant the level of inorganic P was reduced when CM was included in the ration because of the increased levels of organic P supplied. Thus the addition of 20% CM in a diet containing 0.54% total P and the addition of 30% CM to a diet with 0.58% total P resulted in a decrease in inorganic P and significant decreases in rate of growth as compared to chickens fed control rations containing 0.54 or 0.58% total P. It would appear that the low levels of inorganic P (0.18% in the 20% CM ration and 0.19% in the 30% CM ration) were insufficient to maintain optimum rate of growth. When 20% CM was used in a ration containing a level of 0.58% total P (0.22% inorganic P) growth was similar to the controls. The use of autoclaved CM resulted in a small increase in rate of growth in each treatment but only when 30% A-CM was added to the diet containing 0.58% total P was the increase significant.

Feed conversion values were not affected by the levels of total P or inclusion of CM in the rations fed except in the groups fed 20% CM or 20% A-CM in the ration containing 0.54% total P and 30% CM and 30% A-CM in the rations containing 0.58% total P. In these groups a significant depression in feed conversion as compared to the controls was noted even though the A-CM gave significantly better feed conversion than CM with rations containing 0.54% total P.

The incidence of leg disorders was closely related to the levels of inorganic P in the diet. When CM was added at either 20% or 30% level and the level of inorganic P was reduced, incidence of leg disorders was high. When A-CM was included at the 20% level incidence of leg disorders was markedly reduced; however, inclusion of 30% A-CM did not result in a reduction in the number of birds with leg disorders.

Thyroid enlargement occurred in all groups fed CM. The magnitude of enlargement was directly related to the level of CM or A-CM used. The size of the thyroids of the birds fed A-CM was similar to those of the birds fed CM.

Calcification was influenced by the level of inorganic P in the rations. This was evident in photographs of tibias from birds on the different treatments (Appendix 1). When the ration contained no CM the highest level of total P (0.68%) had the highest tibia and toe ash percentages. These were significantly higher than the bone ash values obtained when 0.58% total P was included in the ration. Addition of CM to the ration resulted in significantly lower bone ash values, because of the lower inorganic P levels in the rations. When CM was autoclaved the birds fed 20% A-CM in a ration containing 0.58% total P had bone ash values significantly higher than the values obtained when 20% CM was fed. This suggests that there may be some release of inorganic P from the organically-bound P in the meal during autoclaving. Similar results were obtained in Experiments 1 and 2.

Summary

Three experiments were conducted with broiler chickens to determine the dietary inorganic P levels required in rations containing raw and autoclaved CM. The following results were obtained:

1. A minimum dietary level of 0.24% or 0.25% inorganic P was required for normal growth and calcification.
2. When the level of inorganic P was reduced to 0.18 or 0.19% in diets containing 20 or 30% CM with 0.54 or 0.58% total P respectively, growth rate and calcification were reduced.
3. Inclusion of 20% A-CM in diets resulted in improved rate of growth and calcification as compared to the inclusion of 20% CM in diets containing 0.58% total P (0.22% inorganic P). When 30% CM or A-CM was included in diets containing 0.58% total P (0.19% inorganic) growth rate and

calcification were reduced.

4. Addition of 0.05 or 0.10% inorganic P to rations containing 30% CM or 30% A-CM to raise the inorganic P level to 0.24% or 0.29% respectively, resulted in growth rate and calcification similar to those observed with chickens fed SBM control rations.

B. SECTION: Effects of dietary fibre and inorganic phosphorus levels on the performance of broiler chickens

Introduction

The chicken does not have the ability to utilize dietary fibre as a source of energy; consequently in formulating poultry diets it is usual practise to keep the level of fibre low. When a feedstuff such as CM is added to the ration there is an increase in the level of fibre in the diet as well as an increase in its phytin P content. A high level of fibre in the diet is of some concern because it has been shown in some instances to interfere with the utilization of minerals. Nwokolo and Bragg (1977) reported a significant negative correlation between the crude fibre content of various oilseed meals and the availabilty of P; a large reduction in the availability of P in rapeseed meal which is higher in fibre content than in SBM was observed. A reduction in the availability of minerals may be brought about by binding of the minerals to the fibre matrix (Ismail-Beigi et al. 1977) or by binding to phytic acid (Nelson et al. 1968).

Since there appears to be some relationship between fibre content of the diet and the availability of P, experiments were conducted to study i) the influence of added fibre or fibre supplied by CM on P availability in rations for broiler chickens and ii) the level of total and inorganic P required in diets containing CM for broiler chickens.

Materials and Methods

Experiment 1

In this experiment 270 day-old (White Mountain ♂ x Hubbard ♀) male broiler chickens were distributed at random into 18 groups of 15 birds each. Three groups were placed on each of the experimental rations (Table III.14). All rations were formulated to be isocaloric and isonitrogenous and to contain equal amounts of total P. The fibre levels in the rations of 2.82, 4.56 and 5.43% were attained by adding alphafloc, a non-nutritive cellulose, or by the addition of 20 or 30% CM. The chickens were raised in electrically heated Petersime batteries with raised screen floors. Feed and water were supplied ad libitum. Continuous lighting was provided.

Records were kept on mortality, body weight, feed consumption and incidence of leg disorders. All birds which had bowed legs, swellings at the tibial-metatarsal joint or perosis (slipped tendons) were recorded as suffering from leg disorders.

The experiment was terminated after 28 days. At the end of the experiment, four birds from each group were selected at random and killed by cervical dislocation. The thyroid glands were then removed and their weights recorded. The left tibia and the middle toe (between the second and third

Table III.14 Composition of broiler rations (experiment 1)

	Ration Treatment					
	1	2	3	4	5	6
Ingredients(%):						
Ground wheat	62.35	58.00	55.60	51.25	49.25	45.75
Stabilised fat	1.00	2.50	3.30	4.60	5.30	6.40
Alphafloc	---	1.80	2.70	---	0.90	---
Soybean meal (47.5% protein)	31.50	32.55	33.25	19.40	19.80	13.30
Canola meal (36% protein)	---	---	---	20.00	20.00	30.00
Ground limestone	2.00	2.00	2.00	2.00	2.00	2.00
Biofos (18% Ca,21% P)	0.70	0.70	0.70	0.30	0.30	0.10
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[1]	2.00	2.00	2.00	2.00	2.00	2.00
DL- Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Calculated analyses:						
Metabolizable energy (MJ/kg)	11.51	11.52	11.53	11.53	11.51	11.54
Protein (%)	23.07	23.01	23.02	23.08	23.02	23.08
Fibre (%)	2.82	4.56	5.43	4.56	5.43	5.43
Total Phosphorus (%)	0.60	0.60	0.60	0.60	0.60	0.60
Inorganic phosphorus (%)[2]	0.30	0.30	0.30	0.24	0.24	0.24
Chemical analyses:						
Protein (%)	22.95	22.88	23.13	23.22	23.50	23.42
Total phosphorus (%)	0.60	0.59	0.60	0.60	0.61	0.61

[1] Supplied the following levels per kilogram ration: Manganese sulfate, 400 mg; zinc oxide, 100 mg; selenium; 0.1 mg; vitamin A, 6000 IU; vitamin D_3,1200 ICU; vitamin E,10 IU; menadione sodium bisulfate,1 mg; riboflavin,4 mg; calcium pantothenate, 5 mg; niacin, 20 mg; choline chloride, 60 mg; folic acid, 1 mg; DL-methionine, 500 mg; biotin, 200 mcg; vitamin B_{12}, 10 mcg; Amprol,(25% amprolium) 250 mg.

[2] Sum of added inorganic P and 30% of the total P derived from plant ingredients

digital bones) of each chicken were also removed. The tibias were removed from adhering flesh by placing them in boiling water for a few minutes, they were then fat extracted with ether for 24 hours, dried to constant weight at 100°C ashed at 800°C for 1 hour and the percentages of ash calculated (AOAC 1980). Four toes from each group were pooled together, dried to constant weight at 100°C, ashed at 600°C for 4 hours and ash percentages calculated (AOAC 1980).

The data were subjected to analysis of variance. Percentage data for mortality and leg disorders were transformed using arcsin transformation (Steel and Torrie 1980); however, for ease of interpretation, means were expressed as percentages. Significance of difference was assessed by applying Students-Newman-Keuls' procedure (Steel and Torrie 1980) at the 0.05 level of probability.

Experiment 2

In this experiment 384 day-old male broiler chickens (White Mountain ♂ x Hubbard ♀) were randomized into 24 groups with 16 birds in each. Three groups were assigned to each of the ration treatments (Table III.15). The rations were formulated with either 0 or 20% CM and designed to be isocaloric and isonitrogenous and to contain levels of total P ranging from 0.60 to 0.76%. Levels of inorganic P in the rations varied from 0.24 to 0.40%. The influence of varying fibre levels on performance was assessed by adding alphafloc to two diets (Rations 2 and 4) to bring the level up to that

Table III.15 Composition of broiler rations (experiment 2)

	Ration Treatment							
	1	2	3	4	5	6	7	8
Ingredients(%):								
Ground wheat	62.[illegible]	58.00	61.85	57.30	51.45	50.95	50.75	50.35
Stabilised fat	1.00	2.50	1.00	2.50	4.60	4.60	4.60	4.60
Alphafloc	---	1.80	---	1.80	---	---	---	---
Soybean meal (47.5% protein)	31.30	32.55	31.50	32.75	19.20	19.40	19.40	19.50
Canola meal (36% protein)	---	---	---	---	20.00	20.00	20.00	20.00
Ground limestone	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Biofos (18% Ca, 21% P)	0.70	0.70	1.20	1.20	0.30	0.60	0.80	1.10
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
DL-Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Calculated analyses:								
Metabolizable energy MJ/kg	11.60	11.61	11.54	11.54	11.60	11.57	11.54	11.51
Protein (%)	23.00	23.00	23.00	23.00	23.01	23.04	23.02	23.01
Fibre (%)	2.82	4.56	2.86	4.56	4.56	4.56	4.56	4.56
Calcium (%)	1.00	1.00	1.04	1.04	1.01	1.05	1.05	1.10
Total phosphorus (%)	0.60	0.60	0.70	0.70	0.60	0.66	0.70	0.76
Inorganic phosphorus (%)[2]	0.30	0.30	0.40	0.40	0.24	0.30	0.34	0.40
Chemical analyses:								
Protein (%)	23.66	23.21	22.95	23.32	23.70	23.51	23.60	22.80
Total phosphorus (%)	0.60	0.60	0.71	0.71	0.59	0.66	0.69	0.77

[1] Supplied the following per kilogram of ration: Manganese sulfate, 400 mg; zinc oxide, 100 mg; selenium, 0.1 mg; vitamin A, 600 IU; vitamin D_3, 1200 ICU; vitamin E, 10 IU; menadione sodium bisulfate; 1 mg; riboflavin, 4 mg; calcium pantothenate, 5 mg; niacin, 20 mg; choline chloride, 60 mg; folic acid, 1 mg; DL-methionine, 500 mg; biotin, 200 mcg; vitamin B_{12}, 10 mcg; Amprol (25% amprolium) 250 mg.

[2] As in Table III.14

present in the diets containing 20% CM.

The chickens were raised under conditions similar to those used in Experiment 1 and records were kept on mortality, body weight, feed consumption and incidence of leg disorders. At the end of 28 days, four chickens from each replicate were killed and their thyroid glands, left tibia and middle toe were removed. The thyroid glands were weighed immediately and the tibias and toes were analyzed as in Experiment 1.

The data were statistically analyzed as in Experiment 1.

Results and Discussion

Experiment 1

The results obtained are summarized in Table III.16. Mortality was not affected significantly by any of the treatments although the ration containing 30% CM did show a higher level of mortality.

Body weight was not affected when the level of fibre in the diet was increased by the addition of alphafloc. There were no significant differences among the groups fed rations with 2.82, 4.56 and 5.43% fibre. The inclusion of 20% CM either with or without additional alphafloc resulted in some reduction in body weight as compared to the controls but the differences were not significant. Inclusion of 30% CM to the ration however, did result in a significant reduction in growth rate as compared to the controls and the other

Table III.16 Performance of broiler chickens fed varying levels of fibre (experiment 1)

Treatment	[illegible])	Fibre (%)	Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100g body weight)	Tibia ash[2] (%)	Toe ash[3] (%)
0% CM[4]	0.30	2.82	0a	737bc	1.76ab	2.2a	8.3a	44.0c	21.2c
0% CM	0.30	4.56	2.2a	745bc	1.71ab	0a	8.2a	43.6c	21.2c
0% CM	0.30	5.43	2.2a	784c	1.65a	2.2a	7.1a	43.3c	21.7c
20% CM	0.24	4.57	4.4a	687b	1.86b	11.1ab	13.4b	41.2bc	19.9b
20% CM	0.24	5.43	2.2a	685b	1.82ab	11.1ab	15.2bc	39.2b	19.2b
30% CM	0.21	5.43	8.9a	568a	1.80ab	24.2b	16.6c	32.9a	15.4a
SEM[5]			2.6	22	0.04	4.6	0.8	0.7	0.3

[1] Sum of added inorganic P and 30% of total P derived from plant ingredients
[2] Expressed on a fat free, dry matter basis
[3] Expressed on a dry matter basis
[4] Means within a column with the same letters are not significantly different ($P \leq 0.05$)
[5] Standard error of the mean

treatments. Since the level of fibre in the ration was the same as when alphafloc was added to the control ration or to the ration containing 20% CM it does not appear that a higher fibre level per se exerted a growth depressing effect. It is possible however, that the type of fibre found in the CM was different from the alphafloc used as a substitute for fibre and thus may act differently. Thompson and Weber (1981) studied the effect of dietary fibre sources on chickens and found that some fibre sources were beneficial to growth of chickens while others were not. This was confirmed by Camrie and Clydesdale (1981) in studies in vitro in which variations in processing conditions were found to affect reactions of different fibre sources.

Feed conversion was not affected by the dietary fibre level. Even the highest levels of dietary fibre gave feed conversion similar to the control. This would indicate that the adjustments in level of added fat to keep the rations isocaloric were satisfactory.

Leg problems seemed to be associated with the inclusion of CM rather than the fibre level in the diet. All groups fed rations containing CM showed some increase in incidence of leg disorders; however, only the groups fed rations containing 30% CM had a significantly higher incidence of leg disorders than the controls. This was probably due to lower levels of inorganic P in the diet.

Thyroid size did not appear to be influenced by the level of fibre in the diet but was affected by the inclusion of CM. The groups fed the control ration or those with alphafloc but no CM showed no increase in thyroid size. The thyroids of the chickens fed rations containing 20% CM or 30% CM were enlarged with the maximum enlargement occurring in the groups fed 30% CM.

Calcification was not influenced by the level of fibre in the diet. The rations containing no CM but with 2.82, 4.56 or 5.43% fibre had similar levels of bone ash. Calcification, however, was affected by the addition of CM in the diet. With the exception of the groups fed 20% CM with no added fibre the bone ash values of the groups fed rations containing CM were significantly lower than the controls. The groups fed 20% CM with or without added fibre showed significantly higher tibia and toe ash values than those of chickens fed the ration containing 30% CM. From these results it is apparent that the decreased level of inorganic P in the ration containing 30% CM was responsible for the reduced calcification that occurred.

Experiment 2

The results of the experiment are summarized in Table III.17.

Mortality was low throughout the experimental period and was not influenced by any of the treatments.

Rate of growth was similar in all groups. A minimum level of 0.60% total P and 0.24% inorganic P supplied in a ration containing 20% CM appeared to be adequate for normal

Table III.17 Performance of broiler chickens fed varying levels of inorganic and total phosphorus (experiment 2)

Treatment	Phosphorus level (%) Total	Inorganic	Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100g body weight)	Tibia ash[1] (%)	Toe ash[1] (%)
0% CM[3]	0.60	0.30	2.1a	716a	1.73a	0a	8.3a	42.3b	21.2b
0% CM	0.60	0.30	2.1a	722a	1.75a	0a	8.9a	42.3b	20.8b
0% CM	0.70	0.40	2.1a	700a	1.83a	6.3a	9.3a	44.4c	22.5b
0% CM	0.70	0.40	2.1a	693a	1.86a	2.1a	8.5a	44.5c	22.0b
20% CM	0.60	0.24	2.1a	673a	1.74a	8.3a	15.9b	35.1a	17.4a
20% CM	0.66	0.30	4.2a	711a	1.81a	0a	14.3b	42.5b	21.2b
20% CM	0.70	0.34	2.1a	700a	1.80a	0a	14.3b	44.4c	21.1b
20% CM	0.76	0.40	2.1a	697a	1.88a	2.1a	14.2b	46.2d	22.9b
SEM[4]			2.4	15	.05	3.8	1.02	0.5	0.6

[1] Expressed on a fat free, dry matter basis
[2] Expressed on a dry matter basis
[3] Column values with the same letters are not significantly
[4] Standard error of the mean (P≤ 0.05)

growth. Higher levels of total P (0.66 to 0.76%) or increased levels of inorganic P (0.30 to 0.40%) in the other treatments did not result in any further increase in rate of gain.

Feed conversion was not affected by the treatments used. There were no significant differences in the feed conversion ratios in any of the groups.

Incidence of leg disorders was not significantly affected by any of the treatments although the birds fed rations with 0.24% inorganic P showed a higher incidence of leg disorders as compared to the other birds. This suggested that the level of inorganic P supplied in this ration may have been borderline for normal bone formation.

Enlargement of the thyroid glands was related to the presence of CM in the diet. All chickens fed diets containing CM had thyroid glands which were significantly larger than those fed rations without CM.

Calcification of the tibias and toes was influenced by the level of inorganic P in the diet. A level of 0.24% inorganic P in the diet resulted in significantly lower tibia and toe ash values than when higher levels of inorganic P were used. This indicated that 0.24% inorganic P was too low for normal calcification. A level of 0.30% inorganic P supplied in diets containing SBM, with or without added fibre, or in the diet containing 20% CM gave tibia and toe ash values that were similar to each other but higher than when 0.24% inorganic P was used. When the level of inorganic P was increased to 0.34% in a diet containing 20% CM tibia ash values were higher than when 0.30% inorganic P was present and similar to those observed when 0.40% inorganic P was supplied in rations containing SBM, with or without added fibre. The highest tibia ash values were found in the treatments which received the rations containing 20% CM with a total P content of 0.76%. Since this ration (Ration 8) contained the same level of fibre and inorganic P as Ration 4 it would appear that the fibre content did not interfere with P utilization.

The influence of treatment on levels of toe ash were less than that observed with tibia ash values. Only the groups fed Ration 5 containing 0.24% inorganic P had toe ash values that were significantly lower than those in all other treatments. It therefore appeared that toe ash values were not as sensitive to changes in dietary levels of total and inorganic P as tibia ash values.

Summary

Two experiments were conducted with broiler chickens to study the influence of added fibre or fibre supplied by CM, and the levels of total and inorganic P required in diets containing CM. The following results were obtained:

1. Increasing the dietary fibre level of a ration by adding CM had no influence on body weight or calcification.
2. When 30% CM was added to diets in which the total P content of 0.60% was maintained constant, the reduction in

inorganic P content that occurred was apparently responsible for depressed growth rate and reduced bone ash values.

3. A dietary level of 0.60% total P and 0.24% inorganic P was adequate for normal growth of the bird but a level of 0.66% total P and 0.30% inorganic P was required for optimum calcification.

C. SECTION: Utilization of dietary phosphorus by turkey poults

Introduction

The requirement for P by turkey poults is higher than that of broiler chickens. The publication "Nutrient Requirements of Poultry" (NAS-NRC 1977) lists a requirement of 0.80% total P for starting turkeys (0 to 8 weeks) as compared to 0.70% for broiler chicken (0 to 8 weeks).

The ability of poults to utilize phytin P is limited (Singsen et al. 1950; Gillis et al. 1957). Several researchers have therefore reported P requirements in terms of available P rather than total P. Almquist (1954) concluded that the available P requirement of the young poult is approximately 0.60%. Sullivan (1966) reported adequate growth and normal calcification in turkey poults (4 weeks) when corn-soy diets containing 0.48% available P were fed.

Canola meal, which contains a high level of P when included in turkey rations increases the total P levels in the diets as compared to rations containing SBM. The extent to which the phytin P in the CM is utilized by growing turkeys is not known. Consequently an experiment was conducted to determine the level of inorganic (available) P required in rations for turkey poults containing CM.

Materials and Methods

Two hundred and fifty six, day-old Broad-Breasted White turkeys were randomly distributed into 16 groups. Two groups were placed on each of the experimental rations (Table III.18). The rations were formulated to be isocaloric and isonitrogenous and to contain varying levels of total P. The different levels of total P were achieved by the addition of 10, 15 or 20% CM and 0.70 or 1.70% of an inorganic P supplement (Biofos).

The turkeys were raised in electrically heated Petersime batteries with raised screen floors. Feed and water were supplied ad libitum. Continuous lighting was provided.

Records were kept on mortality, body weight, feed consumption and incidence of leg disorders. All birds which showed evidence of perosis (slipped tendons) or other abnormalities such as bowed legs, hock disorders or enlargement of the tibial-metatarsal joint were recorded as suffering from leg disorders.

The experiment was terminated after 28 days. At the end of the experiment three birds from each group were selected at random and killed by cervical dislocation. The thyroid glands were then removed and their weights recorded. The tibia and middle toe (between the second and third digital bones) from the left side of each poult were also removed. The tibias and toes were cleaned, dried and ashed in the same way as in the previous experiments with broilers

Table III.18 Composition of turkey starter rations

	Ration Treatment							
	1	2	3	4	5	6	7	8
Ingredients(%):								
Ground wheat	46.20	40.90	38.25	35.60	47.20	41.90	39.25	36.60
Stabilised fat	1.00	2.70	3.55	4.40	1.00	2.70	3.55	4.40
Alfalfa meal	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Soybean meal(47.5% protein)	46.00	39.60	36.40	33.20	46.00	39.60	36.40	33.20
Canola meal(36% protein)	---	10.00	15.00	20.00	---	10.00	15.00	20.00
Ground limestone	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Biofos (18% Ca,21% P)	1.70	1.70	1.70	1.70	0.70	0.70	0.70	0.70
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Microingredients[1]	1.75	1.75	1.75	1.75	1.75	1.75	1.75	1.75
Calculated analyses:								
Metabolizable energy (MJ/kg)	11.10	11.10	11.00	11.00	11.20	11.20	11.10	11.10
Protein (%)	28.02	28.00	27.98	27.93	28.15	28.13	28.11	28.06
Calcium (%)	1.23	1.24	1.24	1.24	1.13	1.13	1.12	1.12
Total phosphorus (%)	0.84	0.88	0.91	0.94	0.63	0.67	0.70	0.73
Inorganic P (%)[2]	0.53	0.53	0.54	0.54	0.31	0.32	0.32	0.33
Chemical analyses:								
Protein (%)	27.99	28.90	27.71	27.66	28.23	28.00	28.61	28.42
Total phosphorus	0.82	0.86	0.88	0.91	0.62	0.66	0.70	0.74

[1]Supplied the following per kilogram of ration: Manganese sulfate, 400 mg; zinc oxide, 100 mg; vitamin A, 12,000 IU; vitamin D_3, 1800 ICU; vitamin E, 10 IU; menadione sodium bisulfate, 1 mg; riboflavin, 4 mg; calcium pantothenate, 5 mg; niacin, 20 mg; choline chloride, 60 mg; folic acid, 1 mg; vitamin B_{12}, 10 mcg; DL-methionine, 1 g; biotin, 200 mcg; selenium 0.1 mg; Amprol(25% amprolium) 250 mg; NF-180 100 mg.

[2] Sum of added inorganic P and 30% of total P derived from all plant products

(A.Section-1). Three toes from each treatment were used in the pooled samples.

The data were analyzed statistically using the same procedure as in previous experiments described in A.Section-1.

Results and Discussion

The results obtained are summarized in Table III.19.

Mortality was low throughout the experimental period and was not affected by any of the treatments used.

Rate of growth was not significantly affected by any of the rations used. Although an increased rate of growth was observed in the groups fed rations containing the highest level of total P (0.91%) and inorganic P (0.54%) all other groups had similar body weights.

There were no significant differences in feed conversion in the different treatments. Feed-gain ratios ranged from 1.60 to 1.68 on the different rations.

Incidence of leg disorders was not significantly affected by any of the ration treatments. However, leg abnormalities were observed only in the four treatments with highest levels of inorganic P (Rations 1-4). This suggests that care should be taken to avoid too high a level of inorganic P in diet formulations since high levels may possibly be detrimental to the normal leg development of the bird.

Table III.19 Performance of turkey poults fed different levels of total and inorganic phosphorus

Treatment	Phosphorus level (%) Total	Inorganic	Mortality (%)	Body weight (g)	Feed conversion (feed/gain)	Incidence of leg disorders (%)	Thyroid size (mg/100g body weight)	Tibia ash[1] (%)	Toe ash[1] (%)
0% CM[3]	0.84	0.52	0a	625a	1.68a	4.5a	7.6a	44.5c	23.3a
10% CM	0.88	0.53	0a	624a	1.60a	2.3a	9.2a	46.2cd	24.4b
15% CM	0.91	0.54	0a	611a	1.65a	6.8a	8.4a	45.8cd	22.5a
20% CM	0.94	0.55	0a	673a	1.68a	2.3a	9.0a	47.0d	24.8b
0% CM	0.63	0.31	0a	620a	1.67a	0a	6.5a	38.3a	20.8a
10% CM	0.67	0.32	2.3a	600a	1.60a	0a	7.0a	41.4b	21.0a
15% CM	0.70	0.33	0a[4]	606a	1.62a	0a	13.2a	42.4b	21.1a
20% CM	0.73	0.34	2.3a	614a	1.65a	0a	11.7a	42.6b	21.7a
SEM[4]			1.1	31	0.05	3.1	1.4	0.5	0.5

[1] Expressed on a fat, free dry matter basis
[2] Expressed on a dry matter basis
[3] Column values with the same letters are not significantly different ($P \leq 0.05$)
[4] Standard error of the mean

Thyroid size was not influenced by the presence of CM in the diet. The birds fed rations containing CM showed very little thyroid gland enlargement as compared to those fed the SBM control ration. No significant differences were noted among the treatments. From this experiment it would appear that the turkey poult is not as sensitive as the broiler chicken to the presence of low levels of glucosinolates in CM.

Calcification as indicated by bone ash values was significantly influenced by the level of total and inorganic P in the diet. Significantly higher tibia ash values were seen in all groups fed the rations containing the higher levels of inorganic P (Rations 1-4) as compared to those fed rations containing the lower levels of inorganic P (Rations 5-8). This showed that the level of inorganic P in the diet directly affects the percentage of ash in the bones. Wilcox et al. (1955) noted an increase in tibia ash levels with increasing levels of added P. Although lower levels of 0.32 to 0.34% inorganic P in the diet did produce reduced ash percentages as compared to the higher inorganic P levels of 0.52 to 0.55%, the levels of bone ash still appeared to be sufficiently high to be considered optimum for normal bone formation.

When the level of inorganic P in the diet was low, the degree of calcification was affected by the level of total P supplied. There was a significant increase in tibia ash level when total P was increased from 0.63% (Ration 5) to

.67% (Ration 6) by the addition of 10% CM. Increasing the levels to 0.70% or 0.73% total P in the ration by adding 15 or 20% CM (Rations 7 and 8) did not result in a significant increase in tibia ash above that obtained with 0.67% total P.

As was the case with broilers (A.Section-2) toe ash values did not seem to be as sensitive to changes in dietary levels of total or inorganic P as tibia ash values. Although all groups fed rations containing the higher levels of inorganic P had higher toe ash percentages than those fed rations with a lower level of inorganic P, differences only reached significance in groups receiving 0.88% and 0.94% total P (Rations 2 and 4).

Summary

An experiment was conducted to determine the level of inorganic and total P required by starting turkey poults fed with rations containing CM. The following results were obtained:

1. A dietary P level of 0.63% total P (0.31% inorganic P) was adequate for normal growth but P levels of 0.67 to 0.73% (0.32 or 0.33% inorganic P) were required for normal bone calcification.

2. The use of total P levels of 0.84 to 0.94% (0.53 to 0.54% inorganic P) resulted in increased bone ash values but incidence of leg disorders was increased in comparison to poults fed the diets containing 0.67 to 0.73% total P.

D. SECTION: Utilization of dietary phosphorus by laying hens

1. Influence of phosphorus levels in rations containing canola meal on performance of hens kept in floor pens

Introduction

The presence of high levels of organic P compounds in plant products has prompted many researchers to study the extent to which these compounds (phytin) can be utilized by laying hens. Gillis et al. (1953) indicated that laying hens utilized phytin P one-half as effectively as an inorganic P source. Waldroup et al.(1967), however, showed that organic plant P did not support egg production or hatchability but aided in preventing embryonic rickets.

Several studies have been conducted to determine the P requirement of the laying hen. Early reports Norris et al. (1934) and Miller and Bearse (1934) recorded optimum egg production at P levels of 0.75% and 0.80% respectively. Later studies, O'Rourke et al. (1955) and Pepper et al. (1959) observed that normal egg production could be maintained at lower levels of 0.43% and 0.38% total P respectively. These workers also concluded that the P derived from an "all vegetable" ration was sufficient for adequate egg production. On the basis of these studies and others (Singsen et al. 1962; Crowley et al. 1963; Salman et al. 1969) a requirement of 0.60% total P was indicated (NAS-NRC 1971). This was based on the assumption that 30% of the P of plant products was inorganic and may be considered to be part of the available P required. In a later publication (NAS-NRC 1977) the requirement was reduced to 0.50% total P. The reduction was based on the evidence that " the older bird has the ability to use most, if not all, of the phytin or organic phosphorus in plant products ". This was supported by the reports of Pepper et al. (1959) and Salman et al. (1969) who studied the availability of plant P in rations for laying hens. It was concluded that P derived from plant products alone, without supplementation of inorganic P, was adequate to meet the P requirements of the laying hen.

Although the laying hen may have the ability to use most of the phytin P in plant products, there is no information available on the availability of P from CM. Canola meal is a rich source of P containing approximately 1.1% of this element. This compares to a level of approximately 0.65% in SBM. There is some question as to the comparative availability of P in CM and SBM. In studies with growing birds, Nwokolo et al. (1976) reported that the P in CM was less available than that in SBM. Consequently it seemed desirable that information be obtained on the utilization of P from CM by the laying hen. The experiment reported herein was designed to study the utilization of P by laying hens fed rations of varying P content in which the increased levels of P were supplied by the use of increased levels of CM or by the inclusion of an inorganic P supplement.

Materials and Methods

Five hundred and sixty Shaver Starcross pullets, 22 weeks of age, were divided into 16 comparable groups of 35 birds each. The birds were kept in floor pens (1.8 m x 4.8 m) at a density of one bird per 0.25 square meters. Two pens were placed on each of the experimental rations (Table III.20). Rations 1 and 5 which were the same formulation served as the controls in the experiment. The rations were formulated to be isocaloric and isonitrogenous and to contain 0.40, 0.44, 0.48 or 0.50% total P. The level of P was increased by the addition of 10, 20 or 25% CM or by the addition of 0.20, 0.40 or 0.50% of an inorganic P supplement (Biofos). Feed, water and insoluble grit were fed ad libitum. Fourteen hours of light were provided daily. The experiment was terminated after a production period of 46 weeks.

Records were kept of daily egg production, average egg weight (from one days collection each week), feed consumption and mortality. Birds that died were sent to the Provincial Veterinary Laboratory, Edmonton for autopsy in order to ascertain the cause of death. At 4-week intervals, specific gravity and Haugh unit values were determined on 15 eggs from each replicate. Egg fracture force and deformation

Table III.20 Composition of rations for laying hens kept in floor pens

	Ration Treatment							
	1	2	3	4	5	6	7	8
Ingredients(%):								
Ground wheat	71.65	65.65	59.65	56.65	71.65	71.55	71.45	71.40
Alfalfa meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Stabilised fat	---	2.00	4.00	5.00	---	---	---	---
Soybean meal (47.5% protein)	15.00	9.00	3.00	---	15.00	15.00	15.00	15.00
Canola meal (36% protein)	---	10.00	20.00	25.00	---	---	---	---
Ground limestone	9.00	9.00	9.00	9.00	9.00	8.90	8.80	8.75
Biofos (18%Ca, 21%P)	---	---	---	---	---	0.20	0.40	0.50
Iodized salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Micronutrients[1]	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Calculated analyses:								
Metabolizable energy (MJ/kg)	10.96	10.95	10.91	10.90	10.96	10.96	10.95	10.94
Protein (%)	16.86	16.86	16.86	16.86	16.86	16.85	16.84	16.83
Calcium (%)	3.53	3.56	3.60	3.62	3.53	3.50	3.50	3.51
Total phosphorus (%)	0.40	0.44	0.48	0.50	0.40	0.44	0.48	0.50
Inorganic phosphorus (%)[2]	0.13	0.15	0.16	0.17	0.13	0.19	0.24	0.27
Chemical analyses:								
Protein (%)	16.96	16.69	16.81	16.92	16.58	16.66	16.96	16.89
Total phosphorus (%)	0.39	0.44	0.49	0.50	0.39	0.43	0.48	0.49

[1] Supplied the following per kilogram of ration: vitamin A, 6,000 IU, vitamin D_3, 1,200 ICU; riboflavin, 3 mg; calcium pantothenate, 6 mg; niacin, 15 mg and vitamin B_{12}, 7.5 mcg; manganese sulfate, 400 mg; zinc oxide, 100 mg; vitamin E, 11 IU; choline chloride, 100 mg; biotin, 100 mcg; DL-methionine, 500 mg

[2] Sum of added inorganic P and 30% of the total P derived from plant ingredients

measurements were carried out on 15 eggs from each replicate at the end of 6, 12, 23 and 40 weeks of lay using an electronic apparatus (Appendix 2) referred to as the 'Ottawa Texture Measuring System' (Voisey 1971; Voisey et al. 1977). Blood samples were taken from four birds in each replicate at the end of 12, 24 and 36 weeks of the experiment for determination of the inorganic P content of the plasma (Goldenberg and Fernandez 1966).

At the end of the experimental period, four birds from each replicate were selected at random and killed by cervical dislocation. The thyroid glands from each bird were removed and weighed. The tibia bone and middle toe (between the second and third digital bones) were removed from the left side of the chickens. Each tibia was cleaned of adhering tissue by immersing in boiling water and then crushed. They wer fat extracted with ether, dried to constant weight at 100°C and ashed at 800°C for 1 hour. The ash content of the bones was then calculated (AOAC 1980). Four toes from birds from each replicate were dried together to constant weight at 100°C and ashed at 600°C for 4 hours. Ash percentages for each treatment were then calculated (AOAC 1980).

Average values obtained for replicates were subjected to analysis of variance. Percentage data for mortality were transformed using arcsin transformation (Steel and Torrie 1980); however, for ease of interpretation, means were expressed as percentages. Significance of differences were assessed by applying Students-Newman-Keuls' test (Steel and Torrie 1980) at the 0.05 level of probability.

Results and Discussion

The results of the experiment are summarized in Tables III.21 and III.22 with the results from Rations 1 and 5 being combined.

Mortality was not significantly affected by any of the treatments employed. Deaths that occurred were mainly due to a mild outbreak of coccidiosis, hemorrhagic liver syndrome and avian leucosis. In general, the level of mortality encountered was low.

There were no significant differences in either hen-housed production or hen-day production as a result of the various treatments. Although the rations which contained 20 and 25% CM had the lowest rate of production they were not significantly different from the controls. The high levels of phytin (0.29 to 0.33%) in the rations containing CM were not detrimental to egg production. Gillis et al. (1953) also observed that egg production of hens fed a diet containing 0.47% total P with 0.32% phytin P was similar to those fed a diet with 0.49% P containing only 0.10% phytin P.

Feed conversion ratios were similar in all treatments. This indicated that there was no additional consumption of feed to meet the P requirements. This agrees with the observations of Davidson and Boyne (1970) and Ademosun and

Table III.21 Performance of laying hens fed varying levels of phosphorus

Measurements	Level of CM							SEM[1]
	0	10	20	25	0	0	0	
	Phosphorus level (%)							
	0.40[2]	0.44	0.48	0.50	0.44	0.48	0.50	
Mortality (%)[3]	7.2	7.2	12.8	11.5	5.8	10.0	7.2	2.3
Hen-housed production (%)	73.6	76.6	69.8	71.5	74.8	76.9	78.7	2.9
Hen-day production (%)	80.3	78.8	73.2	74.9	79.5	80.8	80.9	2.3
Feed/dozen eggs (kg)	1.93	1.89	1.94	1.88	1.93	1.90	1.85	0.06
Initial body weight (kg)	1.58	1.60	1.59	1.60	1.57	1.59	1.58	0.02
Final body weight (kg)	1.88	1.87	1.86	1.87	1.83	1.91	1.85	0.03
Thyroid size (mg/100 g body weight)	8.1a	13.4b	20.5c	22.4c	7.4a	7.6a	6.7a	0.9
Blood inorganic phosphorus levels (mg/100 ml plasma)	4.03	4.26	4.02	4.20	4.49	4.59	4.45	0.23
Tibia ash (%)[4]	57.4	60.1	59.4	56.4	57.3	59.8	57.4	0.7
Toe ash (%)[5]	27.6	28.1	27.9	28.2	27.2	28.2	28.3	0.4

[1] Standard error of the mean
[2] Values used in this column are averages of replicates in Rations 1 and 5 combined
[3] Row values with the same letters or no letters are not significantly different (P≤ 0.05)
[4] Expressed on a fat free, dry matter basis
[5] Expressed on a dry matter basis

Kalango (1973) that P levels of the diet did not affect intake. It also agrees with the observation of Summers et al. (1976) who found that feed intake was depressed only when the level of available P in the diet was reduced to 0.15%. Reichmann and Connor (1977) also reported no effect of P level on feed conversion values.

The levels of P in the diets had no influence on body weight gain during the experiment. The final body weights were similar in all treatments. Pepper et al. (1959) observed little difference in body weights as a result of varying P levels in the diets.

Thyroid size of the birds was related to the supplemental level of CM and was not influenced by the level of P in the diet. The birds fed the rations without added CM showed no enlargement of the thyroids but as the level of CM in the diet was increased there was a corresponding increase in thyroid size. The degree of enlargement was similar to that previously reported (Thomas et al. 1978) in hens fed rations containing CM (cult. Tower).

There were no significant differences in inorganic P levels in plasma as a result of feeding varying levels of P to laying birds. Levels observed ranged from 4.02 to 4.59 mg/100ml. of plasma. Since the range in total P levels in the experimental rations used was relatively narrow (0.4 to 0.5%) it was unlikely that any difference in blood plasma inorganic P would be observed. Reichmann and Connor (1977) similarly found no difference in inorganic P levels in plasma when the diets contained from 0.45 to 0.80% P, but higher levels than this in the diet produced marked increases in plasma inorganic P levels.

The degree of calcification in the bones was apparently not affected by the levels or type of P used in the experiment. There were no differences in percentage of ash in the tibias and toes of the laying birds as a result of the varying levels of P. The birds fed rations containing no supplemental inorganic P (Rations 1-5) had ash levels in the tibias and toes similar to those observed in the hens fed rations containing an added source of inorganic P. This indicated that bone ash was not depleted even when all of the P in the rations fed was of plant origin. This is in agreement with previous reports (Pepper et al. 1959; Waldroup et al. 1965; Salman et al. 1969) which indicated that feedstuffs of plant origin provide an adequate source of P for the laying hen.

Egg quality traits as measured by Haugh unit values, egg specific gravity, egg fracture force, deformation and egg weights were not affected by the level of P in the diet. Haugh unit values were fairly constant in all groups ranging from 69.2 to 72.3 and were not affected by the high levels of CM in the diet. Shell quality as assessed by specific gravity, fracture force and deformation was uniform throughout the experimental period. Average values for specific gravity were 1.083 or 1.084 in all the treatment groups. Average fracture force of eggs ranged from 3.40 to

Table III.22 Performance of laying hens fed varying levels of phosphorus as measured by egg quality

Measurements	Level of CM							SEM[1]
	0	10	20	25	0	0	0	
	Phosphorus level (%)							
	0.40[2]	0.44	0.48	0.50	0.44	0.48	0.50	
Egg weight (g)	61.5	62.0	60.9	60.5	61.2	61.7	61.5	0.5
Haugh units	71.8	71.9	71.4	69.2	71.5	72.3	72.0	0.6
Specific gravity	1.084	1.084	1.083	1.084	1.084	1.084	1.084	0
Fracture force (kg)	3.46	3.46	3.41	3.40	3.50	3.45	3.44	0.04
Deformation (um)	72.0	73.0	72.5	74.0	71.5	73.5	74.5	0.8

[1] Standard error of the mean
[2] Values used in this column are averages of replicates in Rations 1 and 5 combined

3.50 kg. Voisey and Hunt (1976) observed that fracture force would be the best indicator of shell strength. There was very little variation in average deformation values of eggs (72 to 74.5 um) between the various ration treatments. There was also little difference in egg weights (60.5 to 62.0 g) as a result of the different treatments. The lack of any significant effect on egg quality traits agrees with the observation of others. Pepper et al. (1959) observed no response in egg specific gravity by increasing the level of P from 0.37 to 0.47%. Hamilton and Sibbald (1977) also showed that feeding diets containing 0.40 to 0.55% P had no effect on specific gravity, egg weight or Haugh units. Yannakopoulos and Morris (1979) similarly showed that dietary P had no effect on shell thickness, deformation or specific gravity of the eggs.

Summary

An experiment was conducted to determine dietary P requirements of laying hens kept in floor pens. The birds were fed rations of varying P content in which the levels were altered by adding either CM or an inorganic P supplement. The following results were obtained:

1. A level of 0.40% P with all of the P derived from plant sources appeared to meet the dietary requirements of the laying hen.
2. Increasing the dietary level of P to 0.44, 0.48 or 0.50% through the addition of CM or an inorganic P supplement had

no effect on mortality, rate of growth, feed conversion, final body weight, blood P levels or calcification as measured by tibia or toe ash content.

3. Inclusion of CM in the laying rations resulted in increased thyroid size which was directly related to the level of CM used.

4. The treatments used had no effect on egg quality traits as measured by egg weight, Haugh unit values, specific gravity, fracture force or deformation.

It may therefore be concluded that for laying hens kept in floor pens the requirement for P is not in excess of 0.40%.

2. Influence of phosphorus levels in rations containing canola meal on performance of hens kept in batteries

Introduction

The results of the previous experiment indicated that a dietary level of 0.40% P derived from plant sources appeared to meet the requirements of the laying hen. Since this level was well below the listed requirements for P by laying hens (NAS-NRC 1977) but agreed with the levels observed by O'Rourke et al. (1955) and Pepper et al. (1959), it seemed possible that the apparently low requirement for P may have been a result of keeping the birds in floor pens rather than in cages. It has been reported that hens raised in cages have a higher P requirement than hens raised on floors (Harms et al. 1961; Marr et al. 1961). Singsen et al. (1962) suggested that hens raised on litter gain access to P through coprophagy thus lowering their apparent P requirements.

The following study was undertaken to determine the minimum level of P required by laying birds kept in batteries and fed diets in which no P was derived from animal protein sources.

Materials and Methods

One hundred and twenty eight Shaver Starcross pullets fed diets with adequate amounts of P were divided after 29 weeks of lay into eight comparable groups of 16 birds each. The birds were placed in laying cages with raised wire floors; two birds were placed in each cage (0.3 m x 0.4 m).

Two groups were assigned to each of the experimental rations (Table III.23). The rations were formulated to be isocaloric and isonitrogenous and to contain 0.32, 0.38, 0.44 or 0.50% total P. The level of total P was increased by the addition of 10 or 20% CM or 20% CM plus 0.30% of an inorganic P supplement (Biofos). Feed and water were supplied ad libitum. Fourteen hours of light were provided daily. The experiment was terminated after 19 weeks.

Records were kept of daily egg production, average egg weight (from one days collection each week), feed consumption and mortality. Birds that died were sent to the Veterinary Laboratory, Edmonton for autopsy in order to ascertain the cause of death.

At the end of the first three weeks on experiment and subsequently each four weeks, specific gravity and Haugh units were determined on all eggs produced in one day from each replicate. Egg fracture force and deformation measurements were carried out on 10 eggs from each replicate at the end of 7, 15 and 19 weeks on experiment using an electronic apparatus (Appendix 2) referred to as the 'Ottawa Texture Measuring System'. Blood samples were taken from three birds in each replicate at the end of the experiment for determination of the inorganic P content of plasma (Goldenberg and Fernandez 1966).

Table III.23 Composition of rations for laying hens kept in batteries

	Ration Treatment			
	1	2	3	4
Ingredients(%):				
Corn	30.00	30.00	30.00	30.00
Ground wheat	25.15	25.15	25.15	24.95
Stabilised fat	3.00	4.30	5.60	5.60
Cerelose	6.50	3.70	0.90	0.90
Alphafloc	2.00	1.00	---	---
Soybean meal (47.5% protein)	20.00	12.50	5.00	5.00
Canola meal (36% protein)	---	10.00	20.00	20.00
Alfalfa meal	2.00	2.00	2.00	2.00
Ground limestone	9.00	9.00	9.00	8.90
Biofos (18% Ca,21% P)	---	---	---	0.30
Iodized salt	0.35	0.35	0.35	0.35
Microingredients[1]	2.00	2.00	2.00	2.00
Calculated analyses:				
Metabolizable energy (MJ/kg)	11.55	11.55	11.55	11.54
Protein (%)	15.81	15.85	15.89	15.87
Calcium (%)	3.53	3.57	3.61	3.61
Total phosphorus (%)	0.32	0.38	0.44	0.50
Inorganic phosphorus (%)[2]	0.11	0.13	0.15	0.21
Chemical analyses:				
Protein (%)	15.99	16.10	15.90	16.08
Total phosphorus (%)	0.32	0.38	0.45	0.51

[1] Supplied the following per kilogram of ration: vitamin A, 8,000 IU; vitamin D_3, 1200 ICU; riboflavin, 4 mg; calcium pantothenate, 6 mg; niacin, 15 mg; vitamin B_{12}, 10 mcg; choline chloride, 100 mg; zinc oxide, 100 mg; manganese sulfate, 400 mg; vitamin E, 5 IU; biotin, 100 mcg; DL-Methionine, 500 mg.

[2] Sum of added inorganic P and 30% of total P derived from plant ingredients

At the end of the experiment, four birds from each treatment were killed, their thyroids removed and weighed, and the tibias and toes from the left side of the bird were removed for determination of bone ash in the same way as in the previous experiment.

Average values obtained for replicates were subjected to analysis of variance. Significance of differences were assessed by applying Students-Newman-Keuls' test (Steel and Torrie 1980) at the 0.05 level of probability. In the case of data for egg production, feed conversion ratios, egg weights, Haugh units, specific gravity, fracture force and deformation, average values for the treatments in each period were statistically analyzed separately.

Results and Discussion

The results of the experiment are summarized in Tables III.24, III.25, III.26 and III.27.

Egg production on either a hen-housed or a hen-day basis (Table III.24) was high in all the treatments with no significant differences during the first 11 weeks of the experiment. During the period from 11 to 19 weeks the hens fed the ration containing 0.32% P exhibited a marked drop in egg production which was significantly lower than that of the hens fed the higher levels of total P (Fig III.1). Waldroup et al. (1967) observed a similar drop in production of laying hens fed a corn-soy diet containing 0.34% P after a period of 28 days. The shorter time lapse before a drop in

Table III.24 Effect of dietary levels of phosphorus on egg production and feed efficiency

Measurements	Period	Total P levels (%) 0.32	0.38	0.44	0.50	SEM[1]
Hen-housed production (%)						
	1	84.9a[2]	82.5a	85.7a	83.5a	2.5
	2	81.7a	79.3a	75.1a	79.7a	5.9
	3	81.6a	79.3a	76.9a	75.7a	3.2
	4	44.5a	70.8b	73.8b	73.1b	3.6
	5	13.6a	69.0b	70.9b	66.5b	3.6
	Total	61.3a	76.2b	76.5b	75.7b	1.8
Hen-day production (%)						
	1	84.9a	82.5a	85.7a	83.5a	2.5
	2	81.7a	80.5a	75.6a	75.1a	5.7
	3	81.6a	81.8a	76.9a	75.7a	3.4
	4	44.6a	73.4b	73.9b	73.1b	4.7
	5	14.4a	71.3b	73.3b	66.5b	3.4
	Total	61.5a	77.9b	77.0b	75.7b	1.8
Feed/dozen eggs						
	1	1.68a	1.71a	1.61a	1.63a	0.07
	2	1.68a	1.68a	1.75a	1.63a	0.12
	3	1.76a	1.70a	1.83a	1.76a	0.12
	4	2.46a	1.87a	1.82a	1.79a	0.22
	5	6.82b	1.80a	1.66a	1.69a	0.21
	Total	2.88b	1.75a	1.70a	1.70a	0.08

[1] Standard error of the mean
[2] Row values with the same letters are not significantly different ($P \leq 0.05$)

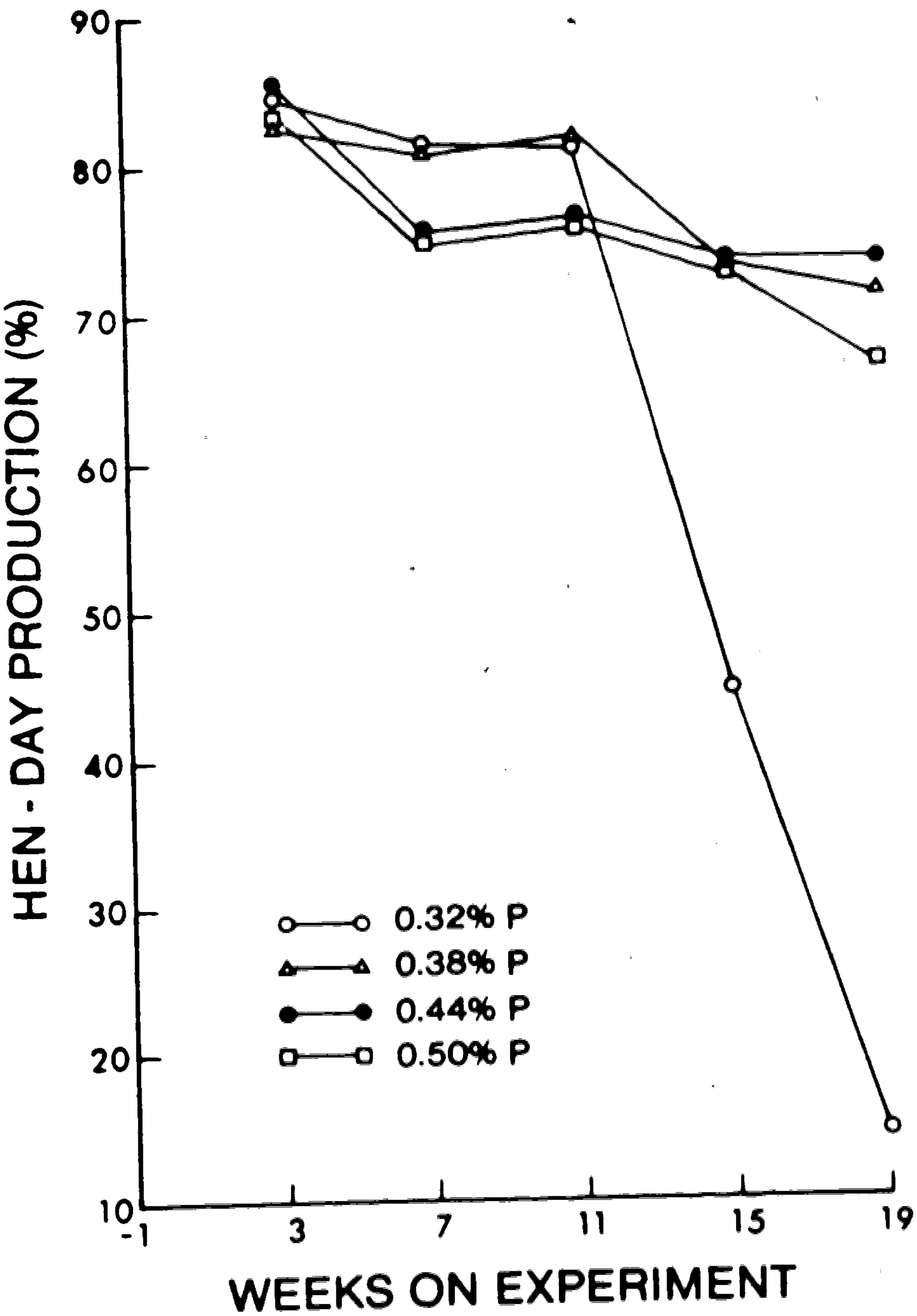

Fig. III.1 Influence of varying levels of phosphorus on hen-day production

production occurred may have been due to their using younger birds (24 weeks) just beginning their egg laying cycle. When 0.38, 0.44 or 0.50% total P was present in the ration, egg production was similar for the different treatments even in the last 2 periods. Singsen et al. (1962) reported that birds kept in wire cages had a requirement of more than 0.40% total P for optimum egg production while Crowley et al. (1963) observed that a level of 0.38% total P was adequate to support a high rate of egg production (65 to 70%) over a 44 week-period. Thus the results indicate that the extra P supplied through the inclusion of 10% or 20% CM was well utilized by laying hens.

The efficiency of feed conversion was not affected by the levels of P supplied until late in the experiment after rate of production had declined in the groups fed the lowest level of P (Fig III.2). No significant differences in feed conversion were noted until the end of 15 weeks on experiment when the birds fed the low P diet showed a significantly higher feed conversion as compared to the other groups.

Egg quality traits (Table III.25) as measured by egg weights, Haugh units and specific gravity were only affected to a small degree by the different treatments. Egg weights were not significantly affected by the different levels of P in the diet but during the period from 15 to 19 weeks hens receiving low P diets showed significantly reduced egg size.

Table III.25 Effect of dietary levels of phosphorus on egg quality

		Total P level (%)				
Measurements	Period	0.32	0.38	0.44	0.50	SEM[1]
Egg weight (g)						
	1	61.7a[2]	64.6a	62.6a	62.2a	1.4
	2	62.6a	65.6a	62.8a	63.4a	1.0
	3	62.9a	64.5a	64.2a	63.5a	1.4
	4	61.3a	66.0b	65.5b	64.3b	0.6
	5	57.6a	65.0b	63.6b	64.8b	0.8
	Total	61.2a	65.2a	63.3a	63.7a	1.4
Haugh units						
	1	84.7b	81.2ab	79.5a	81.7ab	0.9
	2	74.9a	70.6a	73.2a	72.7a	1.6
	3	81.7d	76.7b	78.3c	74.3a	0
	4	N.A.[3]	76.7a	78.3a	77.6a	2.3
	5	84.8b	76.1a	77.9a	74.6a	1.4
	Total	82.2b	76.7a	77.6a	76.4a	0.6
Specific gravity						
	1	1.084a	1.083a	1.085a	1.082a	0
	2	1.083a	1.080a	1.081a	1.079a	0
	3	1.082a	1.080a	1.080a	1.079a	0
	4	N.A.[3]	1.080a	1.083a	1.082a	0
	5	1.089a	1.082a	1.082a	1.081a	0
	Total	1.084a	1.081a	1.082a	1.080a	0

[1] Standard error of the mean
[2] Row values with the same letters are not significantly different ($P \leq 0.05$)
[3] Eggs were too few for adequate measurements

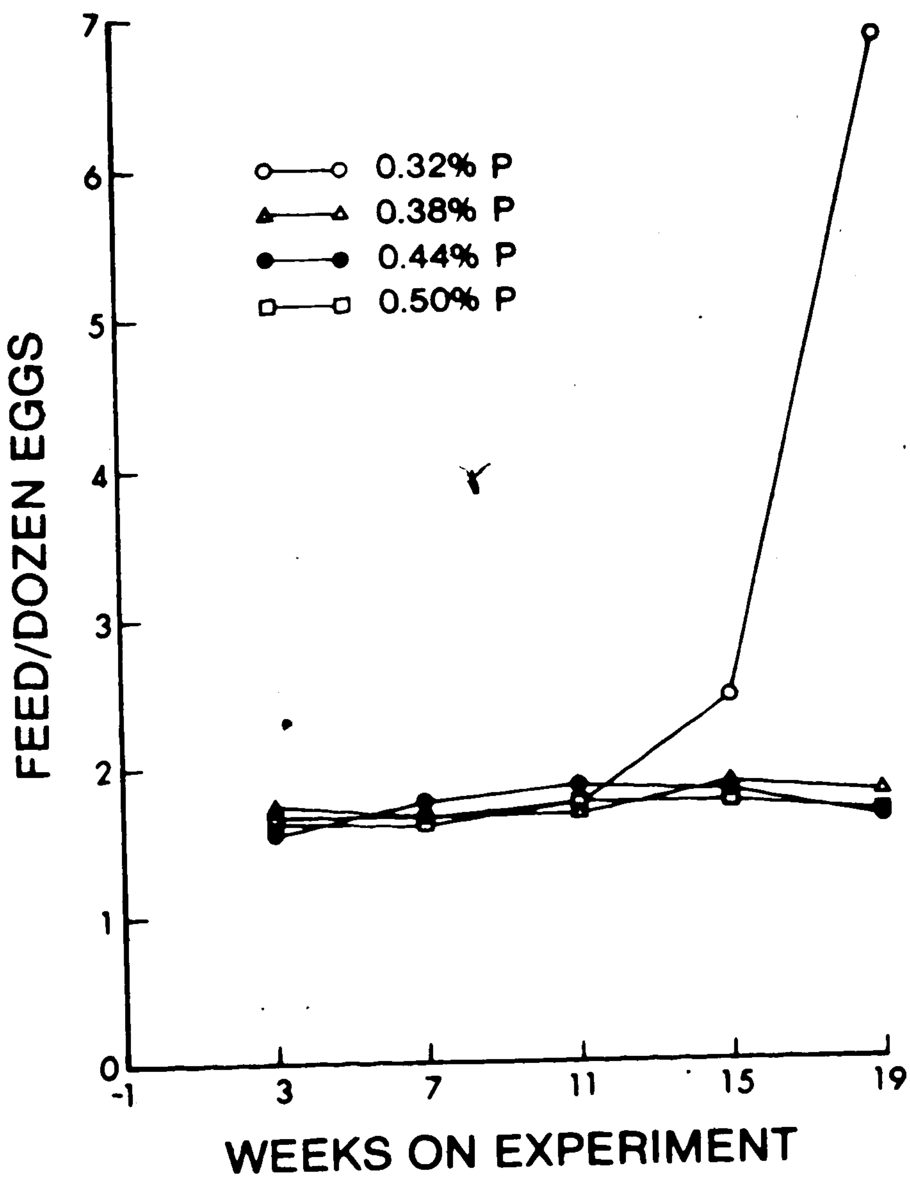

Fig. III.2 Influence of varying levels of phosphorus on feed conversion

Haugh unit values of the eggs were influenced by the level of P in the diet. Throughout the experiment in all periods the eggs from the hens fed 0.32% P recorded the highest Haugh unit values although significant differences were noted only in the third and fifth periods. In the fourth period the inadequate number of eggs from the hens given the low P diet prevented any measurements for Haugh units being taken. The better quality of eggs noted in the low P diet may be due to the fewer number of eggs being produced.

Specific gravity of eggs was not signifcantly affected by any of the treatments. Slightly higher specific gravity of eggs from hens fed the low P diet may have been a result of the decreased rate of production. Harms et al. (1961) similarly reported no response in egg specific gravity as a result of increasing P levels from 0.35 to 0.80%.

Other egg quality traits (Table III.26) such as fracture force and deformation were not significantly affected by the different levels of P fed. The eggs from the birds fed 0.32% P did show a somewhat lower deformation indicating better shell quality as compared to eggs from the other groups.

Mortality (Table III.27) was not significantly affected by the level of dietary P. The two birds that died in the groups fed diets containing 0.32% P suffered from trauma to the toes with extensive hemorrhage and fracture of the phalanges. The other two deaths that occurred were due to

Table III.26 Effect of dietary phosphorus on the shell quality of eggs

Measurements	Period	Total P level (%)				
		0.32	0.38	0.44	0.50	SEM[1]
Fracture force (kg)						
	1	3.42bc[2]	3.15a	3.59c	3.35b	0.05
	2	2.93a	2.87a	3.08a	3.03a	0.14
	3	3.17a	2.71a	2.82a	2.87a	0.10
	Total	3.17a	2.91a	3.16a	3.08a	0.08
Deformation (um)						
	1	66.8a	75.8a	69.0a	68.0a	3.6
	2	65.8a	75.4a	66.0a	73.0a	1.9
	3	69.6a	75.8a	76.5a	72.2a	3.9
	Total	67.4a	75.6a	70.5a	71.4a	2.0

[1] Standard error of the mean
[2] Row values with the same letters are not significantly different (P≤ 0.05)

Table III.27 Performance of laying hens fed varying levels of total phosphorus

Measurements	Total P levels (%)				
	0.32	0.38	0.44	0.50	SEM[1]
Mortality (%)[2,3]	6.3a	3.2a	3.2a	0a	3.8
Initial body weight (g)	1692.0a	1710.0a	1688.0 a	1677.0a	23.0
Final body weight (g)	1619.0a	1784.0a	1786.0 a	1745.0b	30.0
Thyroid size (mg/100g body weight)	13.9a	18.7ab	21.9b	25.3b	1.9
Plasma inorganic P (mg/100ml plasma)	3.63a	3.32a	4.07a	4.61a	0.31
Tibia ash (%)[4]	60.7ab	58.8a	60.7ab	62.2b	0.6
Toe ash (%)[5]	28.7a	27.3a	28.7a	29.3a	0.5

[1] Standard error of the mean
[2] Row values with the same letters are not significantly different ($P \leq 0.05$)
[3] All traits with the exception of mortality and toe ash values are based on individual bird data
[4] Expressed on a fat free, dry matter basis
[5] Expressed on a dry matter basis

hemorrhagic liver syndrome.

Body weight was significantly reduced in the hens fed 0.32% P. Although the initial body weight was similar in all the groups at the end of the experimental period there was a significant loss of weight in the hens fed diets with 0.32% P. The hens fed diets containing 0.38,0.44 or 0.50% P had improved body weights and were similar to each other. These results indicate that the birds fed 0.32% P did not have sufficient P for maintenance of body weight.

Plasma inorganic P level did not show any significant response to dietary P level. When the dietary P level was 0.32% the plasma inorganic P levels of the birds were comparable to those fed the higher P levels. Crowley et al. (1963) observed that not more than 0.265% P was required for maintenance of normal blood P levels. Although the birds fed 0.50% total P had the highest level of inorganic P in plasma it was not significantly different from the others. Similar results were noted in a previous experiment conducted with laying hens raised in floor pens.

Tibia ash values were affected by the treatments used. A significant difference in tibia ash was noted only between the groups fed 0.38% P and 0.50% P. This showed that a level of 0.38% P was not adequate for calcification when the bird was in an active state of production. The hens fed 0.32% P did not show any significant reduction in tibia ash values probably because production of eggs was reduced and therefore withdrawal of minerals from the bones for egg shell formation was reduced. When the level of P in the diet was increased to 0.44% P by the addition of 20% CM calcification appeared to be adequate. The addition of 0.06% extra P in the form of an inorganic P supplement did not result in any significant increase in bone ash values. This result is similar to that of Salman et al. (1969) who observed that plant P was as available for bone ash as a mixture of plant P and inorganic P.

Toe ash values did not show any significant differences between the different treatments. The hens fed 0.38% P or 0.50% P did show the lowest and highest values for toe ash as in the case of tibia ash but the difference was small.

Summary

An experiment was conducted with laying hens raised in batteries to determine the level of dietary P required when CM was included in the ration. The following results were obtained:

1. A dietary level of 0.32% P supplied by plant ingredients was inadequate for laying hens raised in batteries.
2. When the level of P was increased to 0.38% by the inclusion of 10% CM a high level of egg production was obtained but calcification of the tibias was lower than when a higher level of dietary P was used.
3. When the level of P was increased to 0.44% by the inclusion of 20% CM optimum production of good quality eggs and adequate calcification of the bones was observed.

Inclusion of 0.06% extra inorganic P in the diet containing 20% CM had no effect on egg production, egg quality, or bone ash values.

The results obtained in the experiment indicated that the P requirement of hens kept in batteries was 0.44%.

IV. GENERAL DISCUSSION

Although the dietary requirement of starting and growing chickens for P is usually expressed in terms of total P in the feed, the experiments conducted indicated that requirements expressed in terms of the inorganic P content of the ration might be more appropriate. In the experiments, optimum growth and calcification were more closely related to the levels of inorganic P rather than to total P levels in the diets. This suggested that there was very little utilization by growing chickens of the phytin P in CM for growth and calcification.

The experiments reported herein demonstrated that the available P in the diet was utilized first for growth and then for calcification. In rations in which the total P content was maintained constant and the level of phytin P was increased by successive increases in the level of CM, calcification was first affected and then growth rate was impaired. The reduced availability of phytin P was clearly evident when rations containing 30% CM, without any inorganic P supplement were used. Waldroup et al. (1965) similarly observed that while rations containing a high level of organic P supported growth it was less available for bone calcification.

It was apparent from these studies that young growing chickens need a supply of readily available inorganic P for optimum performance. This is in agreement with Gillis et al. (1948) who reported that chickens can utilize phytin P to a limited extent only in the presence of a substantial amount of available P.

Although commercial CM is subjected to heat treatment during processing, additional heat treatment by autoclaving seemed to be of some benefit in improving P utilization. The exact mechanism involved in improved P utilization is unknown but it is possible that the application of moist heat under high temperature and pressure may result in some hydrolysis of the phytin-bound P (O'Dell 1962; Lease 1966). A longer period of heating at a higher temperature apparently resulted in better utilization of dietary P than when a shorter period of heating at a lower temperature was used. Although heat was applied during steam pelleting, the diets containing pelleted CM did not perform as well as those containing autoclaved CM. This suggests that the shorter time and lower temperature used in pelleting as compared to autoclaving was not sufficient to cause extensive hydrolysis of phytin.

It has been suggested that high fibre levels in the diet may reduce the availability of P for growing chickens (Nwokolo and Bragg 1977). Since CM has a higher fibre content than SBM the inclusion of CM in rations results in increased fibre content. It is of interest, however, that in these experiments it appeared that the level of fibre had no effect on utilization of P. When a non-nutritive fibre source (alphafloc) was added to the diet to achieve the same levels of fibre as when 20 or 30% CM was included in the ration, there was no effect on rate of growth and bone calcification as compared to a control ration without CM.

Consideration of the results of the studies suggest that it may be possible to formulate rations with less P than stated requirements and still achieve optimum performance. The publication Nutrient Requirements of Poultry (NAS-NRC 1977) lists a requirement of 0.70% total P for starting chickens. From the experiments conducted it was apparent that optimum performance was achieved with 0.58% total P provided that at least 0.25% inorganic P was included in the ration. Since all experiments indicated that a minimum level of inorganic P was necessary it would appear logical that requirements should include inorganic P content as well as total P levels.

The experiments with turkey poults demonstrated that, as with broilers, satisfactory growth and feed conversion could be achieved with relatively low levels of total and inorganic P in the ration. In order to achieve maximum levels of bone ash, however, higher dietary levels of total and inorganic P were necessary. Since the higher levels of inorganic P resulted in greater incidence of leg disorders, further experiments are needed to determine whether high bone ash values are desirable.

The experiments conducted with laying hens indicated that the dietary requirements for total P was lower than that recommended in Nutrient Requirements of Poultry. A requirement level of 0.40% P for hens kept in floor pens and of 0.44% P for hens kept in batteries represents a significant reduction in comparison to the requirement of 0.50% P listed (NAS-NRC 1977).

The low requirement levels observed indicated that the laying hen has the ability to utilize the phytin P in the diet. Even in diets composed principally of plant products and without an inorganic P supplement the laying hen was able to utilize the phytin P to meet its dietary needs.

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APPENDICES

Appendix 1: Posterior and lateral views of left tibias of chickens fed different dietary inorganic phosphorus levels

The influence of dietary P on growth and calcification of the bones is shown in figure A. The photographs show two views of typical left tibias of chickens fed rations with varying levels of total and inorganic P and different levels of raw and autoclaved CM (Table III.9).

The first three tibias (Rations 4, 9 and 1) were from birds fed SBM control rations and containing 0.58, 0.68 or 0.54% total P and 0.29, 0.38 and 0.24% inorganic P respectively. The bones showed normal length and calcification.

The next three tibias (Rations 6,8 and 5) from broilers fed 20% A-CM, 30% A-CM and 20% CM; each contained 0.58% total P and 0.22, 0.19 and 0.22% inorganic P respectively. The length of the bones was close to normal but calcification was reduced as indicated by the darker color of the bones and twisting at the proximal end. There was less deformation of the tibia from the ration containing 20% A-CM than from the other two rations.

The last three tibias (Rations 3, 7 and 2) were from broilers fed rations containing 20% A-CM, 30% CM and 20% CM respectively. The total P level in Rations 3 and 2 were

0.54% and in Ration 7 was 0.58%. The inorganic P levels in the rations were 0.18 and 0.19% respectively. The length of the bones was shortened and calcification was reduced. It was observed that calcification and bone formation was closer to normal on Ration 3 containing 20% A-CM as compared to Ration 2 containing 20% CM.

Enlargements of the photographs are shown in Figures B, C, and D.

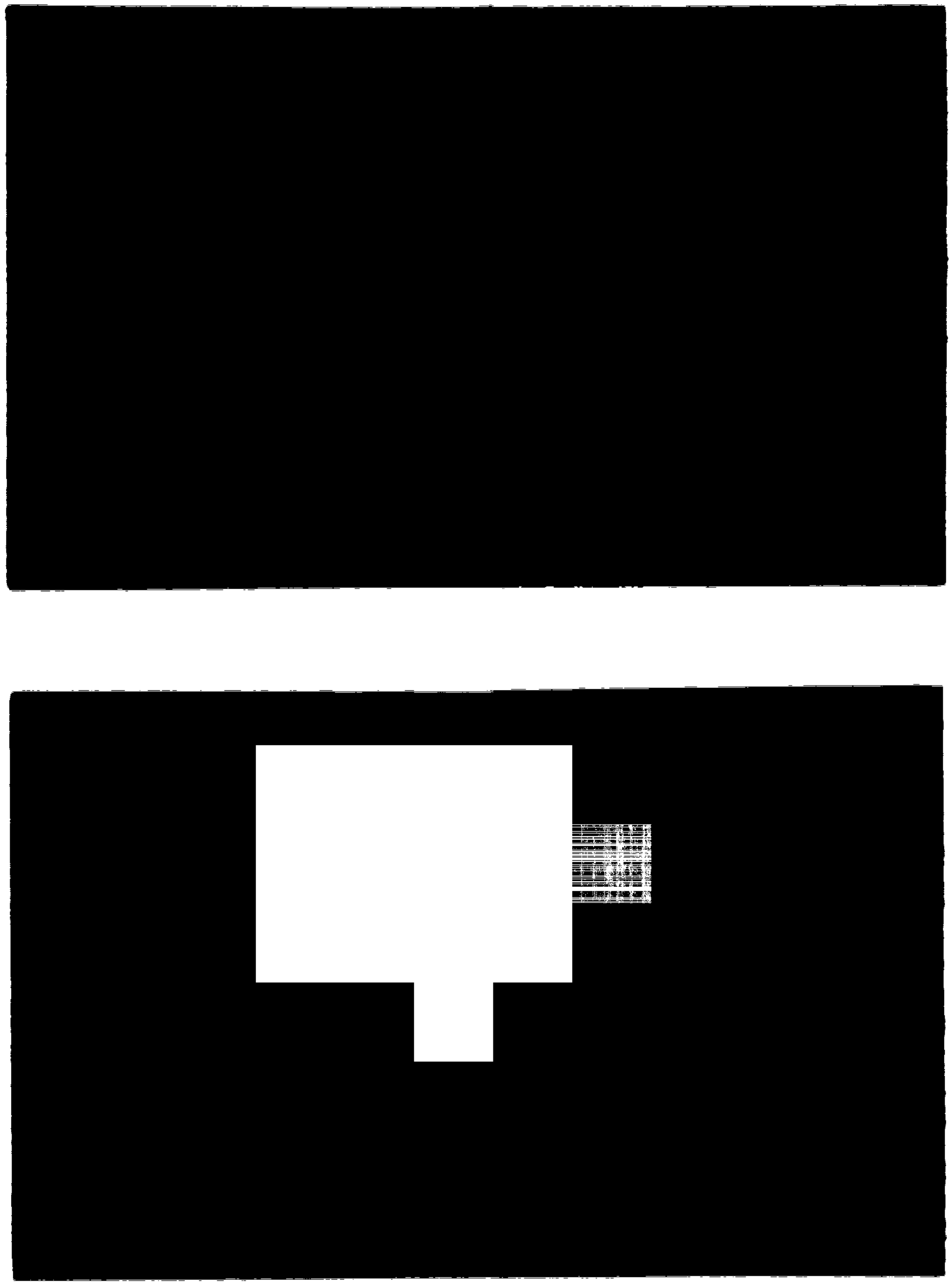


Fig A- Posterior and lateral views of left tibias of chickens fed rations with different levels of inorganic and total phosphorus

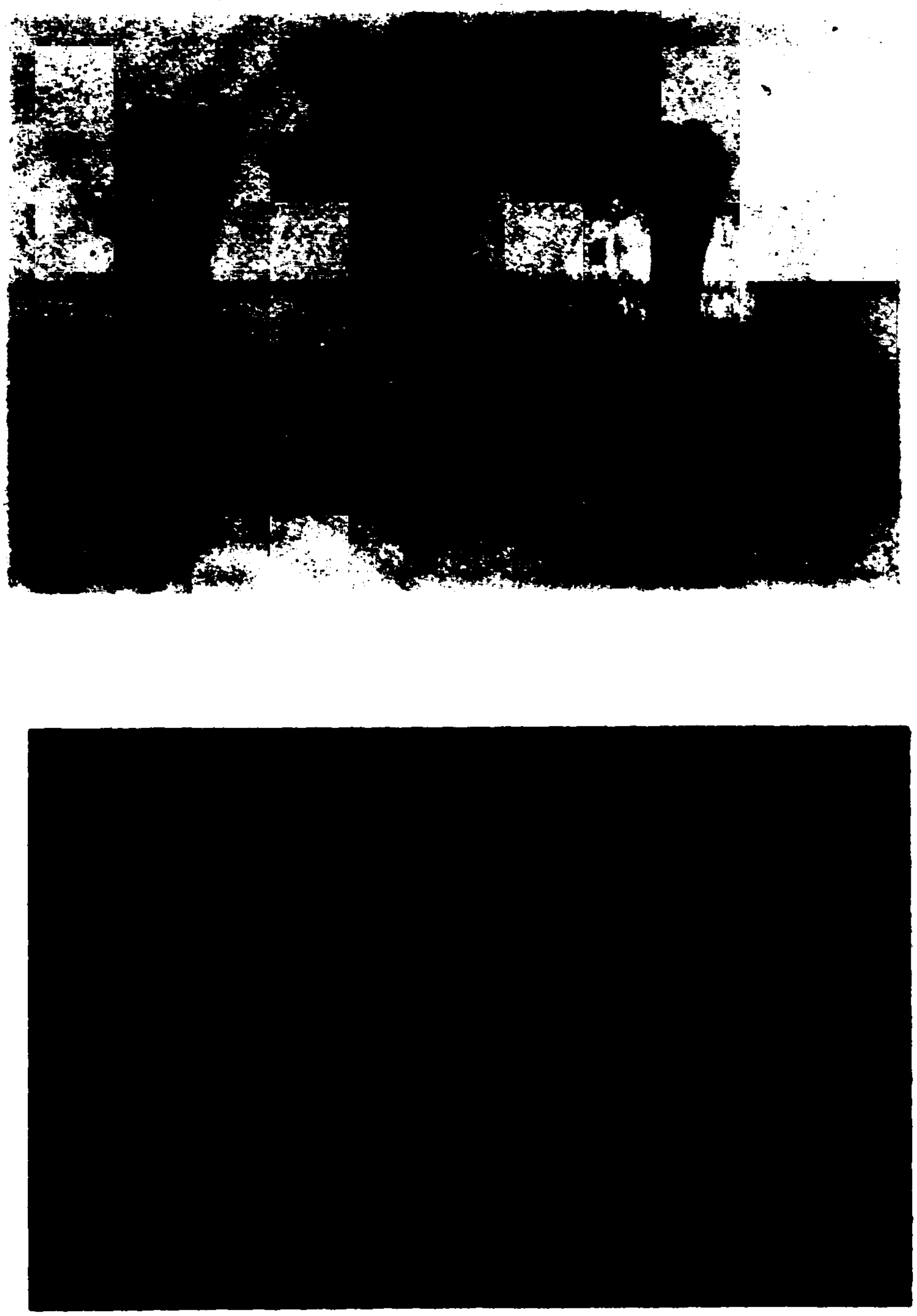
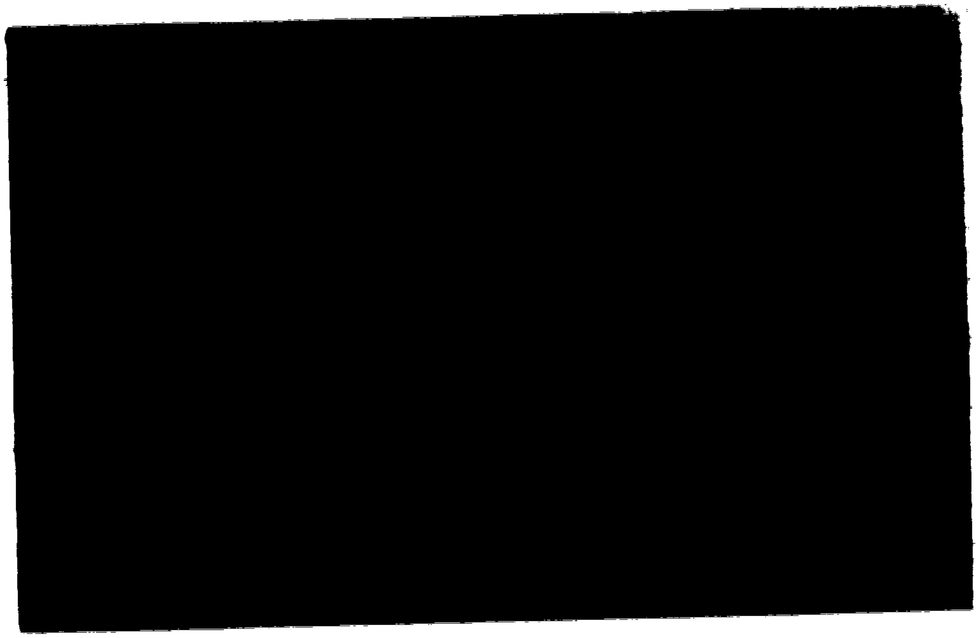


Fig B- Posterior and lateral views of left tibias of chickens fed Rations 4, 9 and 1



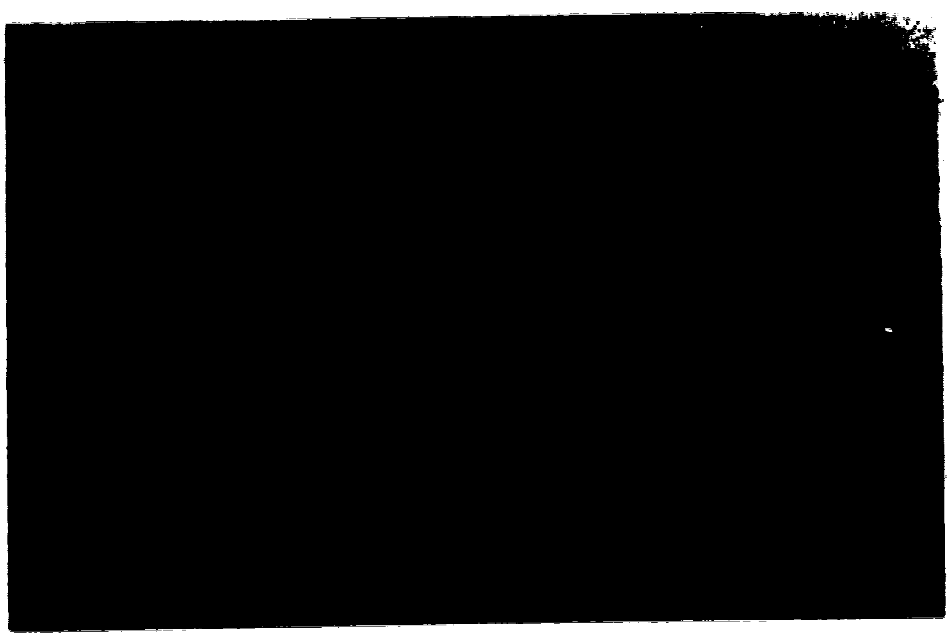


Fig C- Posterior and lateral views of left tibias of chickens fed Rations 6, 8 and 5

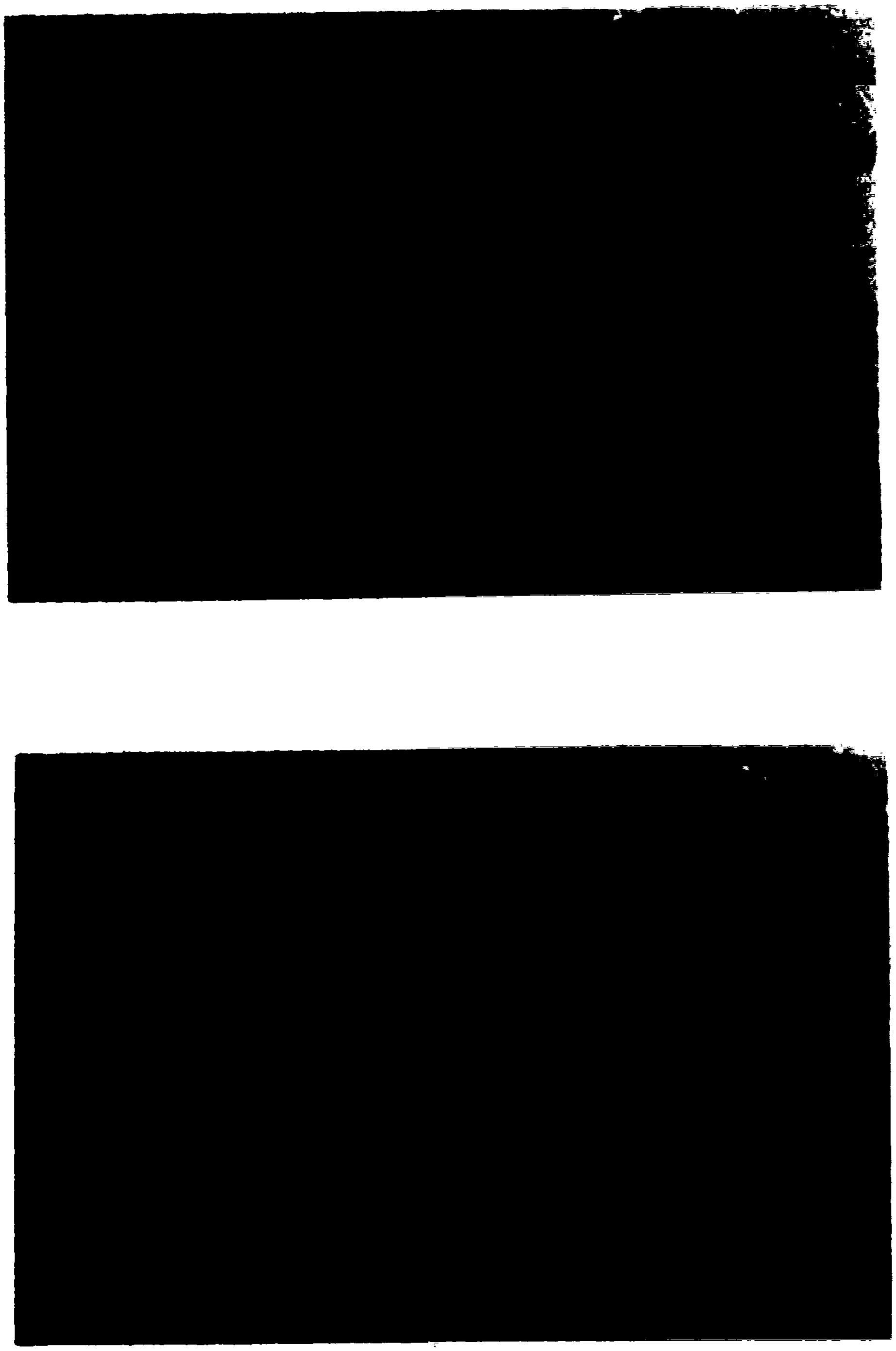


Fig D- Posterior and lateral views of left tibias of chickens fed Rations 3, 7 and 2

Appendix 2: Egg shell quality testing machine