THE EVALUATION OF SOME NIGERIAN LIMESTONE DEPOSITS AS SOURCES OF CALCIUM FOR POULTRY.

BY

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A Dissertation

Submitted to the Postgraduate School, Ahmadu Bello University, Zaria, in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Animal Science.

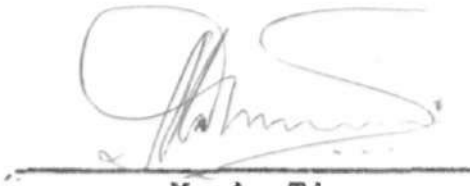
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September, 1989.

DIGITI

DECLARATION

I hereby declare that this thesis has been written by me and that it is a record of my own research work. It has not been presented before in any previous application for a higher degrees.



M. A. Tion
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CERTIFICATION

This thesis entitled "EVALUATION OF LIMESTONE SOURCES AS CALCIUM SOURCE FOR POULTRY" by MICHAEL ATSEMBE TION, meets the regulations governing the degree of DOCTOR OF PHILOSOPHY of Ahmadu Bello University, Zaria and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

THIS THESIS IS DEDICATED TO MY CHILDREN, TERZUNGWE, NGUVEREN AND DOOSUUL, WHO SUFFERED THE AGONY OF PARENTAL NEGLECT DURING THE LONG PERIOD IT TOOK TO COMPLETE THIS PROGRAMME.

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ABSTRACT

Four trials were carried out to evaluate six Nigerian limestone deposits as sources of calcium in poultry rations. The sources tested came from the Ashaka, Calabar, Jakura, Sokoto, Ukpila and Yandev deposits. Four feeding trials were conducted with broiler chickens, while one trial was carried out with laying hens. In the first feeding trial the limestone from the various sources were used to formulate six broiler starter diets with no consideration to variations in calcium contents. The diets were fed to broiler chicks from 0-4 weeks of age. The response parameters studied include growth rate, efficiency of feed utilization, fat free tibia weight, tibia ash weight, percent ash, bone length, ash calcium, plasma alkaline phosphatase, leg abnormalities and mortality rate. Results indicate no noticable deterimental effects of limestone sources on the response parameters.

The second feeding trial involved broiler finisher chicks (5-8 weeks) and utilized the same limestone sources also without consideration to the variations in their calcium contents. The same response parameters as for trial 1 were monitored. The results of this trial agreed with the findings of the previous study.

The third trial was a further study on the effects of limestone sources on the performance of broiler chicks 0-4 weeks of age (see trial 1). This time however, consideration was given to the calcium contents of

the various limestone sources as obtained by chemical analysis before incorperation into the diets. Also the amount of limestone in each diet was increased at the expense of the dicalcium phosphate in order to allow for wider calcium-phosphorus ratio in the diets. Results indicate that growth rate responded significantly ($P<0.05$) to dietary treatments but generally all the birds grew well. Moreover, on the basis of growth response all the limestone sources were not significantly different from each other and only the control group significantly excelled the Calabar, Sokoto and Yandev sources. All other parameters responded in a similar manner as in the previous trial (1 and 2).

Trial 4 was also a further study on the effect of limestone sources on the performance of broiler finisher chicks 5-8 weeks (see trial 2). This trial also utilized limestone sources that were chemically analysed for calcium and consideration was given to the various calcium contents before incorperation into broiler finisher diets. Results indicate that, the Calabar and Yandev limestone sources could be incriminated in the deaths that occured. This effect however needs further investigations. Although ash weights resulted in significant variations among treatment means, no incidence of leg abnormality due to calcium source was evident and the birds grew well to market weight.

For the layer study, limestone from various sources were incorperated into the diets with due consideration to their analysed calcium contents. Response parameters monitored include rate of egg production, egg weight, feed per dozen eggs, feed intake, shell thickness and change in body weight. Results did not show any significant effect of diets (limestone sources) on rate of egg production but egg weight was significantly influenced by diets. Generally, diets that gave numerically higher rate of egg production resulted in significantly ($P<0.05$) smaller egg weights.

The results of the solubility test indicated significant ($P<0.01$) variations among the sources tested. The Ukpila limestone source was more granular and gave the least solubility rate. The results of the bioavailability study indicated that Jakura source (marble) was more available and generally availability of the limestone sources ranged from 87.0 to 92.8 percent . For no apparent reason, there seems to be no direct relationship between the results of the solubility test and these of the bioavailability study.

From the results of these trials, it is concluded that all the limestone sources tested were suitable for inclusion into poultry rations for any stage of production.

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INTRODUCTION

The term, protein malnutrition, (which results in kwashiokor in children) is not new in developing countries; of which Nigeria is the most populous in black Africa. In this country it has often been said that the chicken has the greatest potential for bridging the protein deficiency gap (David West 1979; Ikpi and Akinwumi, 1979). This is because the chicken is highly prolific and has the highest feed conversion ratio among farm animals. To these important advantages must be added the fadt that poultry meat and egg are animal products that are unaffected by social, cultural and religious taboos militating against their consumption world-wide. The interest shown in poultry production in Nigeria in recent times is considerable. The Nigeria Agricultural and co-operative Bank Kaduna, 1981 annual report showed that, of the 191 on-going project being financed by that bank alone, at least fifty were in poultry production as compared to only three in swine production (Balogun and Chineme, 1981).

Since the advent of commercial poultry production, the quest into feedstuffs that would be ideal for poultry has been on the increase. Until redently in Nigeria, many of the feedstuffs used for livestock rations especially poultry were imported because local supplies were limited. Because of this factor costs of such conventional feedstuffs are very high. Animal nutritionists in this country have

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therefore been faced with the challenge of evaluating local ingredient sources for livestock rations, especially for monogastric animals. The need for this has become more acute in view of the ban on importation of many of the major feedstuffs for livestock rations. Studies on the evaluation of local sources of feedstuffs have been more concerned with sourcing for energy and protein feedstuffs. Indeed not much work has been carried out in the area of local sources for mineral nutrients.

One of the essential macro-minerals whose local sources can be investigated because of its abundance, is calcium. The sources in this country include limestone, oystershell and bone meal. Nigeria has several limestone deposits. The most important at the moment are at Nkalagu in Anambra State, Ashaka in Bauchi State, Ukpila in Bendel State, Yandev in Benue State, Calabar in Cross River State. Abaji in the Federal Capital Territory, Jakura (marble) in Kwara State, Ewekoro in Ogun State, and Sokoto in Sokoto State. While these deposits have generated the establishment of several cement and marbel plants, not much has been done to determine the quality of these deposits as sources of calcium in livestock diets and more especially in poultry diets (where calcium accounts for about 80 percent of the total mineral component of

the layer diets). To date, the sources of calcium used in livestock rations in Nigeria are mainly bone meal, oystershell and imported dicalcium phosphate. If the feed milling industry is to grow, the availability of bonemeal may be inadequate to meet the calcium needs of livestock. Besides, calcium from bone meal alone cannot balance the layer and possibly broiler diets because of its phosphorus content.

Oystershell availability is low especially in the hinterland with the result that the cost of this raw material as a source of calcium in other parts of the country is quite high to justify its use in a commercial operation. It also poses a problem to a small scale feed miller who is unlikely to possess the necessary machines for crushing the shells into desired particle sizes.

Dicalcium phosphate is imported, expensive and is usually used as a source of phosphorus. The calcium content at normal inclusion rates is often inadequate to meet the calcium needs in poultry diets.

While the sources of calcium mentioned above are good sources, the aforesaid reasons give credence to the call for investigation into alternative sources which will be cheaper and easier to procure locally. To boost poultry production in this country in an

effort to bridge the protein demand and supply gap, the cost of feed (which accounts for 55 - 75 percent of the total cost of poultry operation) must drop to an affordable range. The evaluation of this country's limestone deposits to establish the safe and good sources will certainly contribute to a reduction in feed prices, since these sources of calcium will be obtained locally and within easy reach of farmers. The need to evaluate these limestone sources is underscored by the spotty and unsubstantiated reports that inclusion of the limestone from some Nigeria deposits in poultry feeds had resulted in decreased productivity, and worse still, mortality of birds on some farms. These sort of reports suggest that some of the deposits may be associated with mineral elements (eg fluoride, selenium) that may be toxic even at low levels. Moreover, it has been pointed out (Iowa Limestone Company , 1974) that not all limestone sources are high enough in calcium content to be considered acceptable sources for inclusion in animal feeds. Limestone should contain a minimum of 38 percent calcium to be considered a good source. Many atimes the same product is expected as calcium carbonate and as such the limestone should contain about 95 percent calcium carbonate for inclusion in poultry feed.

The objectives of this study are to:

a) sample representative limestone deposits in the country

b) chemically analyse all the sample so procured

c) biologically assay all the samples using broilers and layers to determine which of the deposits may be safe as sources of calcium in poultry feeds.

LITERATURE REVIEW

CALCIUM AND ITS IMPORTANCE IN ANIMAL NUTRITION:

Calcium is a white silvery alkaline earth metal. It was discovered by Sir Humphrey Davy in 1808. It derives its name from calx, a latin word which means "lime", the oxide of the element. Calcium does not occur in a free state in nature but its compounds are extremely abundant. Although, not occuring native, calcium nevertheless enters into the composition of a considerable portion of the earth's crust where it forms about 3.5 percent of the total constituents and the fifth most abundant element in the earth's crust (Encyclopedia Britancia, 1973). About 99 percent of calcium in the animal's body is localised in the bones (Cromwell 1982, Scott, et al, 1982). Calcium in the bone exists in two forms; an amorphous or non-crystalline phase which is hydrated tricalcium phosphate, and a crystallized phase that resembles hydroxyapatite, a compound similar to the form in which calcium is found in nature (Irving, 1973). The remaining one percent body calcium which is found outside the bones is widely distributed throughout the organs and tissues with relatively large amounts found in the blood. The blood cells are almost devoid of calcium but the serum and plasma contain 9 to 12mg per dl in most species when not in reproductive activity (Simkiss, 1967).

Two forms of serum calcium have been identified. These are firstly, the non diffusable form which cannot pass through the pores of an organic membrane. It is made up of calcium - protein salts. Secondly, there is the diffusable form which can pass through the organic membrane. This form is composed of phosphate and bicarbonates and accounts for about 60 percent of serum calcium (Lloyd et al. 1978). About 50 percent of plasma calcium is in an ionized form and is physiologically active. Ionized calcium is generally assumed to be the fraction under hormonal control. The remaining 50 percent is in non-ionized form and physiologically inert. Of this form, 40 to 45 - percent is bound to protein and 5 - 10 percent is complexed with citrate, bicarbonate and phosphate.

Blood calcium originates from the diet but the level in serum is under the influence of parathyroid hormone. Parathyroid hormone maintains the minimal level of calcium in the serum by the mobilization of calcium from the bones, simultaneously stimulating the kidney to reserb more of the calcium and enhancing the absorption of calcium from the intestinal tract, (Lloyd et al. 1978). Calcium originating from the serum is important in the following functions:

- the process of blood coagulation,
- the maintenance of membrane intergrity and permeability,
- the transmission of nerve impulses
- efficient weight gain and feed utilization

- egg production and shell quality
- the activation of certain enzymes,
- as structural component of bone and
- the secretion of a number of hormones and hormone-releasing factors.

From all of the diverse functions ascribed to calcium, the importance of this nutrient to the well being of the animal will become apparent. It has already been pointed out that most of the calcium in the body is located in the skeletal system but serum calcium is the point of its origin. Skeletal system will be preferentially sacrificed to maintain serum calcium levels vital to the life of the animal and the effect of calcium deficiency or inbalance may first be shown as skeletal abberations (Pee, 1974). The calcium content of the animal body is estimated at 1.2 to 1.5 percent on fresh tissue basis, 3.5 to 4 percent on dry tissue basis and 26 to 30 percent on the ash basis (Georgievskii, 1982).

CALCIUM METABOLISM

Calcium absorption

The body of the hen contains approximately 20grams calcium while an egg contains 2grams. The amount of calcium secreted with a single egg amounts to about 10 percent of the total body calcium (Hurwitz 1973). This remarkable turn overe of calcium makes the chicken one of the largest users of calcium. It also emphasizes the importance of calcium absorption and control during egg laying process. This large loss of calcium must be met by equivalent absorption from dietary sources.

The presence of relatively large amounts of calcium in the faeces led to early suggestion that this was the path-way of calcium excretion. However, the calcium in the lumen of the small intestine is a combination of dietary calcium and endogeneus calcium secreted into the gut as a constitutent of digestive juice. Before the use of radiosotopic techniques in nutrition, the quantity of endogeneus calcium was difficult to aser ascertain, hence studies reported calcium absorption as a difference between intake and faecal excretion.

Liu et al. (1940) reported on calcium absorption (intake less faecal excretion) in young animals, that up to 80 percent lumenal calcium is absorbed. Chicks (Omdahl and DeLuca, 1973), laying hens (Hurwitz and Bar, 1969a, and other animals (Nicolaysen et al., 1953)

adapt to low calcium containing diets by increasing their ability to absorb calcium from the intestine. Schachter and Rosen (1959) used calcium - 45 on everted gut sac and observed that calcium passed through the mucosal membrane against a concentration gradient indicating an active transport mechanism. Schachter et al. (1960) later demonstrated that the absorption of calcium was greater from the duodenum and jejunum than from the other distal parts of the small intestine. However, Schuette and Linkswiler (1984) later pointed out that the duodenum exhibited the greatest transport capacity per unit length and that, in vivo calcium absorption depends on the length of intestinal segment, the length of time the feed is present in that part of the segment and the calcium concentration. Hurwitz and Bar (1965, 1966) stated that although the absorption of calcium 45 is greatest in the duodenum than the jejunum or ileum, the jejunum plays an important role physiologically since the element passes rapidly through the duodenum, spending relatively more time in the middle and lower region of the intestine.

Kaupp and Ivey (1923) reported that the digestive process of laying hen is very rapid; with fast passage of feed through the digestive system and most of the calcium being absorbed in the upper part of the small

intestine. Hurwitz and Bar (1968) found that the concentration and activity of calcium in ultrafilterates made from the intestinal contents of laying hens was dependent upon dietary calcium level and source.
In experiments to investigate the mechanism for calcium absorption in the laying hen, Hurwitz and Bar (1968) measured the activity, concentration and lumenal electrochemical potential difference (ECPD) of calcium in different parts of the intestine. Hurwitz and Bar (1968) reported that the ECPD (defined as position where calcium was greater in lumen of the intestine than blood) was unfavourable for absorption of calcium in the lower ileum and colon as compared to the duodenum and jejunum, suggesting that there was no net calcium absorption from the distal segments as already reported. Further investigations were made by Hurwitz and Bar (1969b) in chickens and hens into the relationship between the calcium ECPD, calcium concentration and calcium-binding protein of the mucosal cells. When calcium concentration in the diet varied from 0.6 to 3.9 percent, the electrochemical potential difference in the hens was always positive in the duodenum and jejunum and calcium was absorbed down the gradient. In an <u>in vivo</u> study of calcium transport in the laying hen intestine, Bar and Hurwitz, (1969) suggested a simple diffusion as a mechanism of calcium transport into the intestinal mucosa.

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In the chick, the electrochemical potential difference was positive with calcium concentrations of 0.71 and 1.11 percent but negative with a concentration of 0.31 percent. Hence at the two higher concentrations, absorption was against the gradient. In the three week old chick, Hurwitz and Bar (1970) found that most calcium was absorbed in the upper ileum.

Hurwitz et al. (1973) found that calcium absorption was higher along the small intestine during shell formation than when no shell was being formed, except for the lower ileum. They concluded that two regulatory mechanisms operate during calcium absorption. The action of one regulating mechanism in the duodenum responds to relatively long term changes in calcium demands, while the other along the small intestine responds to the short term changes in the calcium demand. Roland et al. (1973) reported after two experiments that the total amount, rate of passage and absorption of feed and calcium in the digestive system was 48 percent of the ingesta and 61 percent calcium in the digestive system between 8pm to 6am.

Calcium retention

In an attempt to estbalish why the hen excretes heavy amounts of phosphorus during ovipositien period, Tyler (1946a) collected excreta from laying birds at

8 hourly interval and was able to show the heavy phosphorus excretion appeared about the time of oviposition, as a result of mobilization of calcium phosphate from the bone in which calcium used up and phosphorus excreted. Tyler (1946a) also observed that there was a significantly lower excretion of calcium and carbonate in the period between 12 midnight and 8.00am but this period was not associated with shell formation. He suggested that, the absence of calcium in the fecal sample during this time represented the last portion of the previous meal which had already lost much of its calcium and carbonate by leaching in the crop and gizzard. Definite evidence of this was obtained by Tyler (1946b) when he examined the changes in feed material as it moved along the alimentary tract of the fowl. In the non-laying hen, Tyler (1946a) observed a marked decrease in the solubility of the calcium in the digestive tract, particularly from the anterior to the posterior part of the small intestine, this decrease corresponds with a rise in pH but this was not so noticable in the laying hens.

Knowles *et al.* (1935) reported that in the excreta of non-laying pullets fed a ration containing adequate amounts of calcium, the calcium not bound to carbonate was combined as dicalcium phosphate. Wilwock (1970)

reviewed the literature on the rate of absorption and retention of calcium by the laying hen in relation to age of the bird. From this review, he fixed the value of maximum absorption of calcium at 70 to 80 percent of intake and retention at about 10 percent less. He then planned experiments to cover the laying season for hens of different ages and productive capacities. Birds were assigned to low, medium or high calcium diets. Calcium was supplied to provided 0.5, 1.0 and 2.0 percent of the feed. Retention was estimated from the ratio of calcium-47 to chromium651 in feed and droppings when the excretion of chromium-51 was completed. He found in the first test that before the onset of lay 17 weeks old hens given 0.5, 1.0 and 2.0 percent calcium retained 41.3, 28.6 and 18.2 percent calcium which were equivalent to 0.17, 0.27 and 0.35g calcium respectively. Before and at the onset of laying, hens given 2, 3 or 4 percent calcium in the ration retained 74.20, 59.6 and 55.4 percent calcium which was equivalent to 1.43, 1.80 and 2.10 gram calcium respectively. In the tenth month of lay, calcium retention had fallen to 64.3, 41.7 and 27.2 percent calcium which was equivalent to 0.95, 1.05 and 0.78 gram calcium respectively. In the shell of eggs laid up to 8 hours after starting to eat labelled feed, less than 10 percent of shell calcium was

labelled. However, secretion of labelled calcium in the eggshell increased with time. Eggs that were laid 25 to 32 hours after eating the labelled feed had 61 (1.00g), 63.8 (1.14g) and 69.5 (1.15g) percents labelled calcium for young hens. The older hens gave 32.9 (0.54g), 36.0 (0.66g) or 30.2 (0.58g) percents labelled calcium in eggs.

The effect of protein intake on calcium (and phosphorus) metabolism in broilers was studied by Floresell (1973). He noted that with the 23 percent protein diet calcium retention was 35.59 percent of intake and with 11.5 percent protein diet, retention of calcium was 11.81 percent. Bronsen et al. (1967) reported from the study of calcium retention in birds that young pullets retained 24.9 percent (98gm out of 239mg) calcium from its 36 hour passage through the gut. Six months old laying pullets ate 1.66grams calcium in 13 hours and retained 1.20g or 72.5 percent of intake. Hens that were one year old ate 1.94 gram and retained 1.3g or 66 percent of intake. Two months after hens had ceased to lay, they ate less calcium (1.4g) and retained only 31.9 percent of intake or less than half as much calcium as when they were laying. When laying hens were studied at shorter intervals, it was shown that while an eggshell was being calcified, retention of dietary calcium was almost 80 percent of intake but

immediately after the egg was deposited and for about 7 hours afterwards, it fell to 20 percent only to rise again when the next shell began to form. At about the same time, Itoh (1967) was also studying calcium metabolism in laying hens with respect to the relationship between eggshell formation and calcium retention by an indicator method. He reported that during shell formation, the calcium content of excreta was 2.79 ± 0.18 percent and did not differ between night and day. When no shell formation was taking place, the faecal output of calcium was 4.95 ± 0.46 percent and was high during the day than at night. Retention rate of calcium was about 70 percent during shell formation and about half that value when no shell formation was taking place.

Lennard and Roland (1981) reported that hen increased their calcium retention when fed high calcium diet followed by a low calcium diet. Instead of retaining 1.87 gram calcium ad did the control birds fed 3.5 percent calcium, birds fed a diet containing 6.4 percent calcium and followed the next day by a diet containing 0.4% calcium retained 3.25 gram calcium representing an increase of 74 percent in calcium retention over control. When calcium excretion in the urine before and during shell formation was studied in White Leghorn hens producing thick and thin shelled eggs, urine samples were collected at different periods after oviposition and Bust et al. (1980) reported that calcium in urine decrease in both

shell lines from 44mg per 100ml to 5 to 6mg per 100ml during shell formation. When no shell was being produced, hens of both lines excreted calcium in the urine at 34 to 53mg per 100ml throughout the 20 hour observation period. This was indicative of increased utilization and probably renal reabsorption of calcium during shell formation.

FACTORS INFLUENCING CALCIUM ABSORPTION AND RETENTION

Effect of dietary fats and oils

Fats are added to poultry diets as a source of energy, to improve palatability, reduce dustiness and in certain cases, a so-called extra caloric effect is observed (Cullen et al., 1962; Mateos and Sell, 1980). Biely and March (1967) reported that 12 percent saturated fat added to the diet did not increase calcium requirement of the chick. Lipstein and Bornstein (1968) found that neither type nor level of supplemental oil had any effect on bone calcification of the chick. Pepper et al. (1955) however, reported that 5 to 10 percent animal fat increased the requirement for calcium by the chick. Whitehead et al. (1971) used maize oil at varying percentages in the diet of chicks and found that the presence of any type of fat at or above the 5 percent level decreased calcium retention. Retention decreased with increasing dietary fat concentration and increased with increasing absorbability of fat. The

efficiency of fat utilization is dependent on their component fatty acids and saturated fatty acids are less efficiently utilized than are the unsaturated fatty acids (Scott et al., 1982).

The deterimental effects of fat on mineral metabolism specially calcium, magnesium and zinc have further been demonstrated by Whitehead et al. (1972) Hakanssen (1975b) and Atteh et al. (1983). Atteh et al. (1983) and Hakanssen (1975b) pointed out that this deterimental effect is due in part to the formation of insoluble soaps between fatty acids and minerals during digestion which renders both the fatty acid and these minerals unavailable for absorption. Hakanssen (1975a) recommended that when high levels of fat are introduced in poultry ration, calcium and magnesium levels should be increased. Edward et al. (1960) studied the interaction between energy, protein and fat content of ration on calcium utilization by the chicks. They reported that with the lower energy values, 20mg calcium per gram body weight gain was required to give maximum bone ash, but for the highest energy value, the corresponding value was increased to 26mg calcium per gram body weight gain.

Laying hens were fed 1.0 to 7.0 percent acidulated soyabean soapstock at 3.0 to 4.5 percent calcium by Hurwitz et al. (1969). They reported that egg production and size were not significantly affected by the level of fat or calcium in the diet. This in effect means that calcium from the dietary source was well utilized even at the highest fat level of 7.0 percent. Earlier, Lipstein

and Bernstein (1968) concluded that the presence of fat as free fatty acids had no effect on calcium utilization in the chick. Naber et al. (1964) had also shown that fat may infact be used to improve calcium utilization when higher calcium levels are fed.

Effect of age of bird

Calcium absorption and retention are also dependent on age. Wilwock (1970) showed that generally speaking, as the hen ages, calcium retention decreases. Pjescak (1972) studied the metabolism of calcium in chicks of different age groups, and reported that chickens one month old absorbed 24 percent more calcium than those that were 3 months of age. While studying some factors that affect the rate of calcium absorption in the fowl in vivo, Than Sav Ling et al. (1972) noted that laying hens absorbed significantly more calcium at 20 weeks than at 40 weeks and more at 40 weeks than at 60 weeks; and that females absorbed significantly more calcium than males. Petnkheva (1972) found that, while the intake of calcium in 12 months old birds was high, absorption and retention were not similarly high as compared to those that were only 8 months of age. Young hens are better able to absorb dietary calcium than older birds (Wassermann and Taylor, 1968) and the level of dietary calcium can influence calcium

absorption and retention (Hamilton and Cipera 1981). However, Roland (1980) suggested that the failure in calcium metabolism that occured as the hen ages may indeed be the cause of poor shell quality in older hens. He further pointed out that results from other studies suggest that the ability of aged hens to absorb calcium (and metabolize skeletal calcium in the process of shell calcification) was just as good as that of the young hen.

Effect of breed, sex and strain

In two experiments (Cheshmedzhiev and Krsteva, 1984), broiler chicks were fed to appetite. They reported that, calcium utilization decreased from 76.30 percent at 7 day old to 54.71 percent at 56 day old in the purelines and from 70.85 percent to 55.53 percent in the hybrid line during the same period. In the female chicken, it decreased from 68.29 to 46.33 percent in the pure line and 64.29 to 49.13 percent in the hybrid lines respectively during the same period. Calcium utilization was significantly greater in the male than in the female chicken and in the hybrid than in the pure breed respectively.

Effect of Vitamin D

The dominant and most easily demonstrable effect of vitamin D is the enhancement of the intestinal absorption of calcium (Wassermann, 1977). Over the years,

a considerable amount of effort has been given to understanding the mechanism by which vitamin D exerts its effect on the intestine. The demonstration by Schachter and Rosein in (1959) that the everted gut preparation can actively transport calcium gave substantial impetus to this field. With everted gut preparation, it was clearly shown that the vitamin D status of the animal from which the intestine was derived significantly affected the capacity of that intestinal preparation to transport calcium at a rate of about 5 times greater than that of preparation from a Vitamin D- deficient animal.

In addition to stimulating active transport of intestinal calcium, vitamin D can also increase the passive diffusion of this cation across the epithelial cell layer (Harrison and Harrison, 1969; Wassermann and Tayler, 1968). This dual effect of vitamin D might represent distinct mechanisms or manifestation of the same fundamental change in the intestinal mucosa. Harrison and Harrison (1969) suggested that the primary effect of vitamin D was to increase the passive permeability of the intestine to calcium and secondarily, the active process.

The hypothesis that metabolite of vitamin D_3 enhances the synthesis of a protein (Calcium-binding protein, CaBP) which in turn enhances the absorption

of calcium from the intestinal mucosal cells was enuciated from the pioneering studies by Wassermann and Taylor (1966). They demonstratedt that the protein was not found in the mucosa of rachitic chicks but peared after vitamin D_3 treatment, suggesting that it was induced by this vitamin. Although the exact relationship of vitamin D_3 to calcium absorption is not yet completely understood, it is well established that vitamin D_3 must be metabolized to a more active form to produce its well known physiological effects (Neville and DeLuca, 1966). The metabolite fraction acts more rapidly than the parent vitamin D in initiating calcium absorption and in metabolizing calcium from bones (Blunt et al. 1968). Blunt et al. (1968) discovered that it was a metabolite of vitamin D_3 identified as 25 - hydroxy vitamin D_3 that was responsible for carrying out the function of vitamin D. It soon became apparent that the 25-hydroxy vitamin D_3 does not act directly on the target tissue but must be metabolized further by the kidney. The metabolite was later idenitified as 1, 25-dihydroxy vitamin D_3 (Holick et al., 1971). The 1, 25-dihydroxy vitamin D_3 acts more rapidly than 25-hydroxy vitamin D_3 in the initiation of calcium absorption and in the metabolization of calcium from the bone (Tanaka and DeLuca, 1971) representing the most potent form of vitamin D known at the present time to influence calcium metabolism.

Couch et al. (1947) reported that the level of calcium in the diet influence the amount of vitamin D requirement of the chick. The effect of cholecalciferol on bone formation and serum calcium, phosphate and magnesium was studied by Ramp et al. (1974). They reported that body weight of chicks deprived of vitamin D was significantly reduced after 3 weeks, whereas these chicks with higher vitamin D intake was only slightly reduced at 4 weeks. They further reported that serum calcium of deprived chicks was significantly lower after one week and fell progressively thereafter, whereas serum calcium of chicks with high vitamin D intake remained normal throughout the 4-week period. The effect of vitamin D deficient diet may not become apparent in a short time of about three weeks if the diet is high in calcium (Newotarski 1943), after this period, the vitamin D effect can progressively become evident. This was demonstrated by Newotarski (1943) using bone ash which did not show any significant difference from the control in a short term but decreased significantly at 10 weeks of age. This decrease was not observed in the presence of vitamin D.

In trying to establish the calcium, phosphorus and vitamin D requirements of chickens, Summers and Co-workers (1970) noted a linear improvement in feed utilization with increase of vitamin D_3 in the diet. In another

study, Summers et al. (1970) reported that higher levels of vitamin D_3 gave a better chick performance when calcium level was 0.8 percent but when the calcium level was increased to 1.0 percent, lower levels of vitamin D_3 were needed for better performance. Garlich and Wyatt (1971) studied the effects of increased vitamin D_3 on calcium retention and shell calcification. They found that extra vitamin D_3 did not alter the calcium content of shell nor did the amount of vitamin D_3 affect calcium retention or balance.

Effect of Alkaline Phosphatase enzyme

A review of literature by Njoku (1980) indicated that alkaline phosphates (orthophospheric monoester phosphorylase) enzyme is widely distributed in nature. The metabolic functions are not yet precisely understood but its activities however, have been demonstrated in a number of living organisms and tissues. In the tissues of vertebrates, alkaline phosphatase actively has been demonstrated in the intestine (Redwell, 1979), the liver (Redwell, 1979), the kidney (Metzok, 1950) and bone (Hays and Swenson, 1977). Alkaline phosphatase is reported by Hays and Swenson (1977) to catalyse the hydrolysis of various phosphate esters under an alklaine pH. It was observed that this enzyme was present in

calcifying areas and Robinson (1923) incorporated the essentiality of this enzyme into a scheme of calcification. Wassermann (1977) submitted that apart from calcium-binding protein, two other vitamin D- responsive proteins are the phosphatases, i.e. intestinal alkaline phosphatase and the calcium adenosine triphosphatase. Wassermann (1977) showed evidence to demonstrate that each of these is increased in concentration in mucosal tissue after vitamin D is given to a vitamin D deficient animal. According to this author, studies with alkaline phosphatase, particularly with regards to its relation to calcium absorption has tended to relegate this enzyme to a secondary role, since alkaline phosphtase activity after vitamin D treatment increases only some hours after calcium absorption has been stimulated. He suggested that alkaline phosphtase may be more concerned with phosphatase absorption than that of calcium.

Groth and Frey (1966) showed that vitamin D-deficiency will cause a rise in alkaline phosphatase. They fed a vitamin D deficient diet to chickens and reported a rise in the level of the enzyme and fall in serum calcium (and phosphorus) levels. The changes is alkaline phosphatase activity of the plasma which occur during growth and at the onset of lay, have been known for many years. Alkaline phosphatase level in the serum of laying hens is higher than that of cocks (Commons, 1936a) but lower in the adult hen than the young chicken

(Tanaka and Wilcox, 1960). Peterson and Parrish (1939) showed that blood plasma phosphtases varied in non-laying hens but attributed this to excessive handling and bleeding. Laying birds showed higher values when egg-shell formation was proceeding actively. They showed that phosphatase values tended to be paralleled by inorganic phosphatase values and may be correlated with bone removal. Peterson and Parrish (1939) for plasma were greater than for whole blood, but that this was not so with bitds of 16 months of age. They also reported that young birds had much higher plasma alkaline phosphatase values than older birds.

Gutowska and associates (1943) found that the phosphatase activity in the plasma of laying hens seemed to be related to their productivity but not clearly to their shell strength. Engh and Wilcox (1971) suggested that alkaline phosphatase activity was not directly related to egg production since strains slected for egg production had a great variation in isozyme frequency. When the dietary level of calcium is increased, the alkaline phosphatase, activity of the serum is decreased (Hurwitz and Griminger, 1961);

Dumanovsky, 1962; Paul and Snetsinger, 1969). A positive correlation exists between the activity of plasma alkaline phosphatase and egg production (Wilcox et al., 1963), but they did not find a consistent correlation between the enzyme activity in the blood and shell quality. Common (1934) noted that alkaline phosphatase activity reflected shell calcification. Good producers laying strong shelled eggs had higher plasma alkaline phosphatase levels than poor producers laying poor quality shelled eggs (Gutowska et al., 1943).

Common (1934) found that the levels of alkaline phosphatase in cockerels and sexually immature hens were comparable while the levels in the laying and molting hens were higher. He pointed out that shell formation is mediated by carbonic anhdrase, and that only one-half of the total calcium need for shell calcification comes from dietary source, the remaining amount of calcium needed for this purpose is met through the skeletal tissue by way of the action of alkaline phosphatase. This may explain the observed higher alkaline phosphatase levels just before the onset of lay. The studies of Gutowska et al. (1943) showed that values for bone alkaline phosphatase of good and poor producing hens when compared with those of plasma alkaline phosphatase level were lower and not

the same for good and poor producers, but the plasma alkaline phosphatase levels were comparable in groups laying good and poor quality shell respectively. They suggested that since the diet was adequate in calcium there was no demineralization of the bone.

Bell (1960) found that non-laying hens and cocks showed plasma alkaline phosphatase activity of equal magnitude and that younger birds have a higher level of this enzyme activity than the adult birds. He suggested that the temporary increase in plasma phosphatase in laying hens probably was a reflection of a stimulation of osteoblastic recalcification process necessisitated by the drain on skeletal calcium required for eggshell formation.

Reichmann and Connor (1977) studied the effects of different dietary concentrations of calcium and phosphorus on production and some aspects of metabolism in the laying hen. They reported among other findings that as the level of dietary calcium increased, plasma calcium also increased significantly with a resultant decrease in alkaline phosphatase level. They concluded that alkaline phosphatase level was one of the suitable calcium status of the animal.

Garlich et al. (1984) reported that serum alkaline phosphatase activity was highest in immature non-laying hens than in the mature pullets or layers. They found

no consistent trend in the mature hens. The enzyme was significantly correlated with serum calcium at 55 weeks of age. They concluded that, their result was suggestive of the consequence of inadequate calcium intake during hot weather. Meluzzi et al. (1986) concluded that plasma alkaline phosphatase level was highest before laying and during molting states.

Effect of calcium - binding proteins (CaBP)

The mucosal calcium-binding protein was first reported by Wassermann and Taylor (1966, 1968). They found that the enzyme appeared in vitamin D deficient animals in response to the administration of vitamin D, suggesting that it was induced by the vitamin.

Accumulated evidence strongly suggests that calcium-binding protein (CaBP) plays an important role in calcium absorption (Wasserman and Taylor 1968; Taylor and Wasserman, 1969). Its capacity to bind calcium and form a complex with it, suggested that CaBP was related to the movement of calcium through the mucosa of the intestine. This finding was collaborated by the report of Ebel et al. (1969) and Wasserman (1974) who reported the appearance of CaBP in intestinal homogenates at the same time that an increase in calcium absorption due to vitamin D was observed. Wasserman (1977) and Hurwitz (1973) found that CaBP was higher in the laying hens than in non-laying hens. Hurwitz and Bar (1969a) related this

increase in the calcium-binding protein in the chick to the need of the chick to absorb calcium more actively due to the drop in the gradient of calcium from the intestinal lumen to the blood. Hurwitz (1973) also found that CaBP in maturing pullets was increased markedly but to a lower level than was found in older laying hens. The author failed to induce an increase in CaBP in pullets on a low calcium diet as in chick. Hurwitz (1973) concluded that in general terms, the appearance of CaBP correlated well with the known facts of changes in absorption of calcium during maturation and early egg laying.

Vitamin D-dependent CaBP occur in other tissues besides the intestine (Wassermen, 1977) and has been located in the kidney of several species and in the shell gland of laying hens. These tissues (intestine, kidney and shell gland) are those across which considerable amounts of calcium are translocated.

Influence of Bile and Bile Salts

Weblind and Holds (1965) have shown that calcium absorption is increased by bile and bile salts in both rachitic and vitamin D treated chicks. They suggested that bile may have three separate actions.

- by acting directly on the mechanism for absorption of soluble calcium
- by increasing vitamin D absorption and hence indirectly affecting the calcium mechanism
- by increasing the availability of calcium from sparing calcium phosphates.

Influence of pH

The effect of pH on calcium absorption and retention was demonstrated by Hesse and Brandt (1935). Using rats, they showed that the absorption of calcium depended on the pH of the gastrointestinal content and that water solubility of calcium preparation had no marked effect. With young growing rats on an acid diet, the addition of calcium citrate glucophosphate supplement caused an increase absorption and retention of calcium but with an alkaline diet, the reverse effect occured. Than Sau Ling and Jalaludin (1972) failed to demonstrate a significant effect of pH on calcium absorption when they studied some of the factors affecting the rate of calcium absorption in the fowl. Tyler (1946a) observed a marked decrease in the solubility of calcium in the tract of non-laying hens and this decrease corresponded to a rise in the pH of the intestinal content.

CALCIUM IN BODY TISSUES

Calcium in the bone

In addition to being the structural frame work of the body, bones also serve as the calcium and indeed mineral reservoir of the body. 99 percent of calcium in the animal body is in the bones and calcium constitutes about one third of the weight of fat-free dried bone (Scott et al., 1982). Despite the seeming

performance of the mineral deposits, bone is constantly being formed and resorbed more rapidly during early development but at a declining rate during adult life. In the many experiments concerncing loss or gain of minerals from the body, the changes in percentages of ash in dry fat free bones have been interpreted as indicating changes in the calcium (and phosphorus) status of the body (Duckworth and Hill, 1953). Many researchers are of the view that a decline in ash percentage is a good evidence that part of the bone salts has been leached from the original bone, and earlier histological methods gave results that supported this view.

McLean and Bloom (1941) used sections of uncalcified bone instead of the usual decalcified material and found that, when minerals were lost from the bone, the matrix and the mineral matter were removed simultaneously. The finding was later confirmed (Bloom et al. 1941) and this led to the general thesis that the mechanism by which mineral matter is lost from the skeleton is one of resorption and not demineralization i.e. when minerals are lost from the skeleton, bone weight and ash weight should be recuced but not ash percentage. However, Morgan et al. (1942), Warnock and Duckworth (1944), and Ellinger et al. (1952), all found reduction of the percentage of ash in dry fat free bone when loss of

mineral had occured. They each pointed out however that ash weight gave a more satisfactory result.

Duckworth and Hill (1953) concluded that ash percentage and ash weight together are better criteria for assessing changes in the mineral status of the adult skeletom than either alone.

Several methods of evaluating the mineral status of bone are employed. Using a modified Allo-Kramer Shear press to measure the breaking strength of tibia of 4 week old chicks, Roland et al. (1967) and Njoku (1980) reported that breaking strength was highly correlated with tibia ash. They both concluded that the breaking strength is a good measure of dietary calcium (and phosphorus) status in tibia ash. In another study, Roland et al. (1974) not only confirmed the results of their earlier study but also reported the correlation that existed between body weight and tibia ash. However, the later study was carried out using chickens that were 10 months in lay while the former utilized 4 weeks old chicks. The effect of calcium (and phosphorus) on performance of layers in Nigeria as measured by bone ash, calcium (and phosphorus) retention was studied by Ademosun and Kalango (1973). They found that both tibia ash and calcium content in the ash increased with increasing dietary calcium levels

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(2.0 to 4.25 percent). Birds on deep litter had significantly higher tibia calcium than those in cages. Tibia ash and calcium content of ash increased rapidly in the first 8 weeks in production and thereafter, remained fairly constant.

Animal bones are reported to contain approximately 36 percent calcium and this value, varies little among bones and among groups (Taylor et al., 1960; Behman et al., 1962 and Field et al., 1974). Therefore one bone may be selected for analysis as being representative of the skeleton as a whole.

The data presented in Table 1 show the marginal variation among bones. Calcium concentrations in the bones may however by decreased by dietary manipulations (Norris et al., 1972), and the productive state of the animal. When a calcium source is removed from laying hens during the period of moult or at the time of sale, Bell (1985) points out that the hen continues to lay eggs and must immediately take calcium from her bones in order to maintain her shell as long as possible. This results is fragile bones, increasing the chances of breakage in handling or moving. Bell (1985) observed that without oystershell, removal of feed for 10 days resulted in an 18.7 percent loss in ash and an 18.5 percent loss in calcium in the femur. The hens receiving oystershell had lost only 8.2 percent ash and 5.2 percent calcium respectively.

Table 1 : Calcium concentrations in the bones of chicks

Body weight in kg	Number of animals	Bone state	Bone type	% Calcium concentration	Reference
1.0	5	Ash	Femur	36.7	Taylor et al., 1960
1.0	5	Ash	Tibia	36.9	Taylor et al., 1960
0.2	10	Ash	Femur	34.6	Rucker et al. 1969
-	12	Ash	Ribs	37.2	Field et al., 1974
-	12	Ash	Femur	39.1	Field et al., 1974
2	10	Dry fat	Free Tibia	21.8	Norris et al., 1972
2	13	Wet	Femur	14.5	Ve et al., 1978

Calcium in the Medullary Bone

The formration of a large quantity of endosteal bone in pigeons was decribed by Kyes and Potter (1934). In birds, medullary bone seems to be formed at the expense of other parts of the skeleton. Hurwitz (1973) observed medullary bone in the point of lay pullets fed a calcium deficient diet and concluded that calcium deposited in medullary bone is derived from structural bone. He demonstrated by the use of calcium - 45, that even under sufficient calcium intake, some of the calcium used in the medullary bone formation was derived from neighbouring structural bones. Landauer et al. (1941) however found no evidence that the medullary bone was formed at the expense of other parts of the skeleton. The deposition of the medullary bone starts at about 10-14 days before a pullet comes into lay. This period corresponds with the time when the bird starts to secret extra sex hormones and to retain extra calcium and phosphorus (Hurwitz, 1973). Storage of calcium in the skeleton appears to have occured through two routes; firstly, the formation of medullary bone which is usually absent in immature pullets. This route represents at least 30-40 percent of the storage (Hurwitz, 1973). Secondly, through increase in calcification of the structural bone. Simkiss (1967) had also shown by means of radio isotopes that the

retained minerals are infact deposited in the medullary bones. Zondek (1936) observed that the sites for medullary bone are the long bones particularly the femur and tibia but not the humerus. The cycle of changes through which medullary bone passes shows that it is formed primarily as a storage site for minerals (Bloom et al., 1941), though there may be incidental strenghtening of the whole bone (Gardner, 1940).

Calcium in the blood

Deobald et al. (1938) reported that as a pullet approached the laying stage, there was a rise in blood calcium level from 7-9mg per 100g whole blood to 12-24mg per 100g. This rise was shown to start 20-25 days before the first egg is layed and it reached a peak in about 7 days and then fell again. Bloom et al. (1941) associated this rise in blood calcium level in pullets about to lay to the formation of medullary bone.

In an experiment designed to answer several questions regarding calcium metabolism during the pre-laying period, Hurwitz (1973) reported among other findings, that plasma calcium increased from around 10mg percent, (typical of non-laying hens and mammals) to over 20mg percent (typical of laying hens). He found no significant difference in this parameter among the various dietary calcium levels (4 percent and 1.3 percent) used in the

study. When low calcium diets were fed to laying hens, Deobald and co-workers (1938) found that eggs were laid when blood calcium was low or falling. When an adequate calcium diet was given following a low calcium diet, there was a big rise in blood calcium after a delay of a few days followed by a fall and the commencement of a regular laying. When calcium source was removed, laying continued for about 10 days with shells becoming progressively thinner and the blood calcium fell to 7-9mg per 100ml. They also reported that there was no correlation between shell thickness and blood calcium levels, and found that high producing hens showed little fluctuation in blood calcium level.

Deobald et al. (1938a) removed the oviduct from a number of pullets 6 to 8 weeks old. They reported that when laying commenced, there was no differences in blood calcium level between experimental and control groups, and the typical prelaying rise and fall in blood calcium was observed in each group. Earlier, Deobald and associates (1936) had found that the blood calcium level of the laying pullet does not show any marked variation during an entire laying cycle, but Common (1936) found a wide variation in the calcium content of the blood in individual hens during a four month study period. Russell et al. (1930) attributed the rise in

blood calcium level as the pullet comes into lay to an associated increase in ova size. Heller et al. (1934) concluded that half of the increase in blood calcium is present as a protein bound form and the other half as an unknown absorbable form of calcium, possibly colloidal calcium phosphate. Taylor and Russell (1935) found that diffusable calcium of the serum was 5.4 and 5.3mg per 100ml of serum for the laying and non-laying hens respectively. The non-diffusable calcium in the serum rose from a value of 6.4mg per 100ml of serum for non-laying hens to a value of 16.1mg per 100ml for the same birds when in lay. The effect of source and particle size of calcium carbonate on shell quality was studied by Roland and Harms (1973), who reported among other findings that, the substitution of hen or pullet-sized limestone or oystershell for two-thirds of the finely ground limestone increased serum calcium in the Summer but not in the Fall. Mitcku et al. (1971) confirmed the influence of calcium source on serum calcium in the Summer when they reported that fossile shell did result in the highest plasma calcium values. However, a similar experiment was not conducted in the Fall as in the previous case reported. Scott et al. (1971) reported that plasma calcium of hens fed calcium diet after 8 months was 28.1 and 27.1mg per 100ml at 4am and 4pm

respectively on limestone diet and 32.9 and 29.5ml per 100ml respectively during the same period on oystershell diets.

Kim (1971) reported that hens given oystershell had 8.9 to 17 percent higher plasma calcium over those given limestone diets. He concluded that this difference in plasma level between oystershell and limestone sources may be due in part, to the constant "metering" of calcium from the gizzard into the blood, and recommended that limestone now commonly employed in layer ration as a calcium source be reduced to one-third and the other two-thirds be replaced by hen size oystershell.

The effect of protein level on plasma calcium level was demonstrated by Man and Toperova (1981). They found that during the first phase of egg production, the pullets fed the high protein diet (19.5 percent) had higher concentration of calcium (and phosphorus) fractions in blood serum than the control. During the second phase also, the high protein group showed a greater concentration of total and protein bound calcium (and phosphorus) fractions in the serum.

EFFECT OF HORMONES IN MAINTAINING BLOOD CALCIUM LEVEL:

The Parathyroid Hormone

Although the nutrient - calcium, is usually associated with the skeleton and eggshell formation, it is well

knwon that the calcium ion concentration in the blood and extracellular fluid is important in many critical body activities. The maintenance of a constant calcium environment in these body fluids is the responsibility of parathyroid hormone (PTH) secreted by the parathyroid gland. The parathyroids were first adequately described in 1860 by Sandstorm. It was not until 1909 however, that MacCallum and Voegtlin related the parathyroid to calcium metabolism by alleviating the symptoms of a parathyroid-ectomized animal with the administration of parathyroid hormone:

- raises the serum calcium and lowers the serum phosphorus,
- increases urinary excretion of phosphate but decreases excretion of calcium,
- removes calcium from the bones particularly if the dietary intake of calcium is inadequate,
- increases serum alkaline phosphatase level if changes in bone have been produced,
- activates vitamin D in renal tissue by increasing the rate of conversion of 25-hydroxycholecalciferol to 1, 25-dihydroxy cholecalciferol.

In the kidney PTH affects renal tubular reabsorption of calcium and reabsorption or excretion of phosphates. There is also evidence that parathyroid hormone increases the rate of absorption of calcium from the intestine, an

effect that is comparatively minor and more likely a result of its renal action to increased production of 1, 25-dihydroxy D_3 (Grodsky, 1979). The actions of the parathyroid hormone on bone and kidney are independent processes. Galliard (1959) demonstrated the effect of parathyroid hormone on bone reserption by placing a fragment of bone adjacent to a parathyroid gland and they reported that after several weeks, a dissolution of bone adjacent to the graft resulted while a proliferation on the side of the bone opposite the graft was seen. The mechanism by which the parathyroid hormone stimulates bone reserption is not known but Grodsky (1979) speculated that the hormone increases the amounts of both citric and lactic acids in the tissue and both of these acids could act to make bone soluble. However, Grodsky (1979) argued that the amount of acid produced appears insignificant to explain the degree of bone reserption observed. Another hypothesis posulated was that the parathyroid hromone may act to stimulate protein synthesis in the osteoclasts, which in turn effect reserption of bone. This view was supported by the observation by Grodsky (1979) that inhibition of RNA synthesis by dactinomycin blocks the activity of the hormone <u>in vivo</u>. He however further contradicted this hypothesis by saying that, the rapid acute effect of parathyroid hormone on bone

resorption is not inhibited by dactinomycin indicating that at least, part of the activity is independent of RNA and possibly of protein synthesis as well.

Changes in blood calcium in the fowl following the administration of crude extract parathyroid hormone have not been consistent, Collip (1931) was unable to demonstrate any effect of the hormone on the blood calcium of non-laying hens. Later MacOwen (1932) reported similar findings with moulting hens when he injected parathyroid hormone extract as large as 1ml. When up to 10.5ml of the hormone were administered into pullets however, the serum calcium level was raised 15 to 19 hours post administration. Knowles et al. (1935) found an increase in blood calcium in immature pullets and non laying hens when 3ml parathyroid hormone extract was injected but failed to demonstrate the same effect in cocks following similar administration. It then appears from the findings of Knowles and Co-workers (1935) with pullets that the one millilitre (1ml) administered by Collip (1931) and MacOwen (1932) was not large enough to cause any noticable physiological change. Altmann (1938) injected from 2 to 4mls of parathyroid extract intramuscularly over a 2 day period into immature

pullets and reported an increase of 47.17 percent in blood calcium.

Urist (1967) found that during the reproductive cycle, females of the avian species metabolize large amounts of calcium and deposit large amounts of apatite in intramedullary bone and consequently develop hyperplastic and hypertrophied parathyroid glands. Secondary hyper-parathyriodism developed when the amount of calcium in the diet was low. Hyperthyroidism. either endogenous or from injections of parathyroid hormone extracts, produced resorption of endosteal bone and not mobilization of the deposits of intra-medullary bone that normally stores calcium for shell calcification. He pointed out that, the specificity of the site of action of the hormone suggests that, the process of mobilization of minerals from intramedullary bone and fluctuations in the level of calcium in the blood are attributable not to the action of the parathyroid hormone but indirectly to cyclical changes in the output of oestrogen.

Candlish and Taylor (1970) reported that the metabolization of skeletal calcium in laying hens by parathyroid extract is extremely repaid. They suggested that this mechanism is capable of providing calcium for eggshell formation within a short time after release of the hormone. Candlish and co-worker (1970) further

pointed out that the effect of parathyroid extract on the excretion of calcium by the kidney of the fowl is equally rapid. Mueller et al. (1973) studied the response of laying hens fed diets containing either 2.26 percent or 5 percent calcium during both shell formation and when no egg was in the shell gland. Parathyroid hormone injection significantly decreased plasma calcium and phosphate within five minutes. This was followed by an increase in the concentration of both ions until a maximum was reached after 20 to 30 minutes. After one hour, plasma level of both ions in pullets fed 2.26 percent calcium diet were again practically normal. The maximum plasma calcium and phosphate responses for the 5 percent calcium diet have more than twice as large as for the 2.26 percent calcium diet while the stage in the egg cycle had no response.

Calcitonin

It is known that low calcium diet leads to hypertophy and hyperplasia of the parathyroid glands and that perfusion of parathyroids with calcium free blood results in the appearance of parathyroid hormone in the efferent vessels of the parathyroid gland. The opposite effect, a fall in serum calcium level following perfusion with high blood calcium has long been believed to be a result of inhibition of parathyroid

hormone release. Copp and Cheney (1962) have however observed that the fall in plasma calcium resulting from hypercalcemic perfusion of parathyroid gland occured sooner and to a greater extent than that following total surgical parathyroidectomy. These observations led Copp and Cheney (1962) to postulate a hyporcalcemic factor - calcitonin secreted in response to hypercalcemia. Subsequently, the existence of such a factor was confirmed and it was demonstrated to orginiate from the thyroid gland (Foster et al., 1964). In chickens, in which the thyroid and the ultimobrachial glands are separate, no calcitonin can be extracted from the thyroid gland.

The release of calcitonin is stimulated by high level of ionized calcium in the serum. Calcitonin is directly effective in bone, where it results in metabolic effects opposite to those of parathyroid hormone. In the kidney, calcitonin in contrast to parathyroid hormone, increases calcium excretion and inhibits synthesis of 1, 25-dihdroxy vitamin D_3, (Grodsky, 1979). Copp and Cheney (1962) and Tauber (1967) demonstrated that small doses of extracts from the ultimobrachial gland of chickens produced marked hypocalcemia in rats. This is in contrast ty thyroid extracts from chicken which failed to produce hypocalcemia

in rats (Kraintz and Paul 1967; Urist 1967). Lloyd, Petersons and Collins (1970) found that ultimobrachial extract had no detectable effect on serum calcium in laying hens. They concluded that, the function of the ultimobrachial gland in birds is still incompletely understood particularly since evidence of calcitonin in chicken is inconsistent.

CALCIUM APPETITE

Calcium appetite refers to the ability of an animal to selectively consume calcium rich substrates to meet its minimum requirements for calcium. The existence of a specific calcium appetite in rat has been well documented. When Richter and Exkert (1937) produced a state of calcium deficiency in the rat by parathyroidectomy, they found an increased preference for calcium lactate solution. A calcium-deficient state can also be induced by feeding a diet low in calcium. When two dietary treatments were fed to deficient rats, there rats showed a relative preference for food supplemented with a mixture of two percent calcium lactate and one percent calcium sulphate (Widmark, 1944).

Scott et al. (1950) demonstrated a specific hunger for calcium in rats when the animals had been deprived of calcium. In the case of the domestic fowl, Hellwald (1931) reported an experiment in which hens were deprived

of calcium, after which he divided them into two groups with one group being given macaroni with egg shell concealed in the macaroni while the other received macaroni alone. During a second meal, both groups were given egg shell, and those which had received the concealed egg shell, ate less than the more deprived group. He concluded that, fowls may be able to show a specific appetite for calcium.

Weed-Gush and Kare (1966) deprived growing chickens of calcium to ascertain whether they could exhibit a specific hunger for calcium. When their bleed calcium level were significantly lower than those of the control birds, they were allowed a choice between two mashes differing only by the absence or presence of a calcium salt. In two experiments, calcium deprived birds preferred the calcium rich mash. Weed-Gush and Kare (1966) therefore reported that the calcium-deprived birds of commercial broiler stock increased their performance to a significant degree on the 4 percent calcium carbonate enriched diet that compensated their nutritional needs. This diet, they observed, was unpalatable to the control birds and resulted in only 22 percent of the total intake, while the calcium-deprived group increased their total intake of this diet by up to 23 percent of total intake. Weed-Gush and Kare (1966) also found that

the calcium lactate in drinking water was unacceptable to both groups (control and experimental) and they reasoned therefore that the failure to select the lactate suggests that the taste factors under some circumstance might be more important than the nutritional needs. Kare and Scott (1962) also observed that palatability can take precedence over nutritional adequancy in a chicken diet. Earlier, Kare et al. (1957) had observed that the fowl is much more sensitive to taste qualities in water than food. But Calet (1965) pointed out that birds have poorly developed sense of taste and have no sense of smell. The works of Wood-Gush and Kare (1966) were later confirmed by Hudges and Wood-Gush (1971) who reported that calcium deprived chickens were albe to preferentially eat a calcium supplemented mash offered as an alternative to one unsupplemented with calcium. In the same report, deficient birds exhibited a greater interest in bizzare of edible size than the control group. This observation had been shown to lead to exploratory behaviour from which the animal might presumbly learn to correct its deficiency (Lorenz, 1965). Because several days were required before the full preference for calcium supplemented diets developed diets in deficient chickens. Hughes and Wood-Gush (1971) concluded that calcium appetite is learned

preference rather than an unlearned homeostatic control.

Research using a separate calcium source has shown that the level of dietary calcium profoundly influences calcium supplement intake by laying hens (Griminger and Lutz, 1964). However, Hughes and Wood-Gush (1972) indicated that if the opportunity existed, the fowl can regulate feed intake independent of calcium intake. Hughes and Wood-Gush (1972) suggested that calcium intake is regulated on a hour basis by the needs for egg formation and anticipated maximal requirement. Salim (1981) reported that the existence of a calcium appetite is unrelated to minimal requirements in the laying hen. He found that hens voluntarily consumed excessive amounts of calcium supplements regardless of dietary calcium level. His data showed that hens fed adequate or high dietary calcium levels did consume less supplement than those fed low or inadequate level but both consumed far more total calcium than the reported minimum requirement.

Holcombe et al. (1975) reported that hens exhibited the ability to discriminate between a high and low level of calcium in a complete feed. The hen also tended to eat more of the high calcium diet in the early morning and late afternoon, which were times of greatest calcium need or anticipated need. They concluded that,

hens regulated their calcium intake according to metabolic need. More recently, Taher et al. (1984) conducted an experiment on special calcium appetite in laying hen. Chickens on the control treatment were given 3.5 percent dietary calcium supplement. Three groups were fed 3.5 percent calcium for 13 days then, the dietary calcium level was reduced by 0.5 percent weekly until it was down to one percent. They observed that, as the dietary calcium level increased, there was a decrease free choice supplement calcium intake; however, all birds (except the control) consumed large amounts of calcium. As the level of dietary calcium was reduced, hens consumed large quantities of free choice calcium supplement and appeared to have little or no desire to reduce or stop consuming the free choice calcium supplement after their requirement had then been met. They concluded that although laying hens possessed special calcium appetite, they could not regulate calcium intake.

The ability of pullets to selectively consume calcium to meet rearing period requirements was examined by Classen and Scott (1980) using two feeding regime:

a) ad libitum consumption of 0.89 percent calcium ration.

b) ad libitum consumption of 0.35 percent calcium ration with free choice calcium source (38 percent calcium content) supplied as chick size grit.

They found that during the period from 5-18 weeks of age, the calcium grit ration fed pullets consumed significantly more calcium than the 0.89 percent calcium ration fed control (7.09 Vs 5.37g per day). Daily feed consumption recorded showed that birds fed grit supplemented diets increased calcium intake by 2 percent 19 days prior to lay, 2.88 percent on the day of ovulation and by 9.07 percent on the day of oviposition. From these findings, they concluded that pullets can selectively consume calcium to meet their changing requirements during the rearing period and prior to the onset of egg production.

<u>The influence of method of incorporation of calcium in the diet on performance</u>.

In the early 1900's, it was the practice to include in the diet of laying hens only a portion of calcium source needed for egg production, while allowing the hens free access to a calcium supplement. It was believed that hens would consume enough of this supplemented calcium to fully meet their needs. During these days, "soluble grit" was the most important form in which calcium was supplied to the birds.

Peterson <u>et al.</u> (1960) concluded that chickens could utilize the calcium more efficiently if all the calcium was included in the feed than if 2.25 percent calcium was included in the feed and free access was

given to a supplement of oyster shell. No real difference in egg production, feed utilization or hatchability was reported by Mehling (1984) when hens received all their calcium in the feed or part in their feed and part in the form of a calcium supplement. However, there was a tendency towards greater shell strength in those instances in which access to a separate calcium supplement was provided. Tyler (1955) observed that the voluntary consumption of limestone grit by caged layers was far in excess of their requirements and bore little or no relationship to egg production.

Influence of dietary calcium on feed intake

Grimmer and Lutz (1964) found considerable variation in the consumption of calcium supplement among hens housed in three different settings. Calcium consumption was found to be highest in single cage, intermediate in community cages and lowest in floor pens with litter. Hens in the floor pens preferred crushed oystershell to calcite grit, when both supplement were avilable, but consumed similar amounts of the two supplements if only one at a time was at their disposal. Birds given supplemental oystershell differed little in performance and eggshell quality from those offered a 3.0 percent calcium in the diet.

Feed consumption in chicken is governed by a number of factors. The most important may perhaps be, the energy content of the diet, but generally speaking, many important nutrients can influence feed consumption in addition to the physical properties of the diet. The influence of calcium level on feed intake is well documented. Hurwitz et al. (1969) found that on all occasions, hens, fed 3.0 percent calcium consumed greater amounts of the diet than those fed a 4.5 percent calcium diet. Scott et al. (1971) reported that a calcium intake of 2.8g per hen per day was inadequate and caused an increase in feed consumption. However, they pointed out that 5.0 percent calcium in the diet was excessive and caused a decrease in feed consumption. Naber et al. (1964) concluded that irrespective of source of particle size, these supplements when given at a higher level caused reduction in feed intake. The effect of particle size on feed intake was demonstrated by Roland and Harms (1973) who fed chicken 3 percent calcium diet and reported that hen size oystershell and pullet size limestone increased feed intake. In older birds, mean feed consumption on egg forming days was reported to be significantly higher than on non egg-forming days, whether or not calcium supplement was offered (Taylor, 1973). Feed intake was higher when no grit was provided;

the difference was significant for egg forming but not for non-egg forming days. Similarly in two experiments, Morgin and Sauver (1974) concluded that the calcium content of the diet is an important factor in regulating feed intake. Holcomb et al. (1975) found that in laying birds, feed intake increased in the morning or late afternoon which were the times of the greatest or anticipated calcium need. More recently, Jin Ho Choi (1988) monitored feed intake of layers on an hourly basis. He reported that, mean hourly intake of feed by hens showed two peaks, a smaller peak during 1000-1200 hours and a larger peak during 1700-1900hours. His study also confirmed earlier reports that hens consumed considerably more feed on egg forming than on non-egg forming days. He also reported that hourly feed intake of individual hens decreased during the hour immediately before oviposition but increased sharply during that hour of oviposition. The large amount of intake during the evening hours (1700-1900) were consistent regardless of the time oviposition.

Roland (1985, 1986) warned that birds given inadequate calcium may eat more feed in an attempt to get more calcium. This results in higher costs and the bird become obesse, develop fatty liver haemorrhagic

syndrome and lay egg with poor shell quality. Ademosun and Kalango (1973) observed that feed intake increased with increase in dietary calcium level (2.0, 2.75, 3.5 percents) up to a point and then declined. Roland (1986a) reviewed the literature concerning the influence of excess calcium in the diet on feed consumption. He reported that the findings were again inconclusive. In response to several reports that excess or high level of calcium in the diet had an adverse effect on feed consumption Roland (1986a) argued that, although excess calcium in the diet could adversely affect feed intake, these effects were due in part to the fact that:

(i) calcium carbonate source used contained high levels of other minerals such as magnesium,

(ii) some workers used calcium carbonate in flour form instead of coarse granular (this reduces feed paltability and could adversely affect feed consumption,

(iii) results of some of the studies reporting that high levels of calcium reduces feed intake were likely misinterpreted. Instead of the hens fed the higher calcium levels reducing feed intake, the lower levels had increased feed intake. He pointed out that although other factors could be involved, the above should explain most incensistences.

This is particularly true, especially if it is appreciated that excess dietary calcium simply passes unabsorbed through the digestive tract.

McIntyre et al. (1963) and Harms and Waldroup (1971) indicated that high calcium intake of between 8.93 and 9.48 gram per hen per day had no adverse effect on feed consumption.

CALCIUM SOURCE AND PARTICLE SIZE

The importance of calcium in the nutrition of birds has been recognized for a long time and different sources of calcium have been and are being tried to establish good sources and forms for poultry feeding. Brophy and Sheehy (1941) compared coral and sand containing 75-80.2 percent calcium carbonate with lime mortar containing 87.5 percent calcium carbonate and found no difference in egg production and shell thickness due to source. Gutowaka and Parkhurst (1942a) concluded that calcite was as good as oystershell for laying hens. Kennard (1944) compared oystershell with dolomitic limestone grit for egg production and concluded that the former was better. Scholes (1945) tested coquina shell containing 94.7 percent calcium carbonate and concluded that it was comparable to oystershell as far as eggshell strength was concerned.

Gutteridge and Platt (1945) compared eggshell containing 93 percent calcium carbonate with oystershell and reported that on the basis of specific gravity, eggshell as a supplement were as good as oystershell, but that the efficiency of utilization measured by the amount of supplement consumed per unit of eggshell produced was significantly less for eggshell than for oystershell. Hauser and Norris (1946) compared crushed oystershell, calcite grit and ground limestone with and without granite grit and found that, for egg production, ground limestone was inferior to oystershell or calcite grit. The poor performance of ground limestone may be due in part to particle size, since the experiment of Rau and Platt (1949) failed to show a difference in all parameters measured when limestone grit was compared to calcite grit. Tully et al. (1931) concluded that oystershell or limestone grit with steamed bonemeal proved superior to mineral salts as a source of calcium in the absence of vitamin D. Calm shell, limestone and oystershell with or without insoluble grit were compared as calcium sources by McItyre and Common (1951). They reported that ground limestone and oystershell plus insoluble grit improved appetite and rate of gain in growing male Barred Plymouth Rock chicks. On the basis of left

tibia ash content, these workers reported that all sources of the supplement allowed for good bone development. In two trials with chicks and one with laying hens, Hurwitz and Rand (1965) found that utilization of calcium was slightly poorer from gypsum than from limestone. There was no significant effect on weight, feed efficiency bone calcification, egg production or eggshell quality. Tolerance to sulphate was fairly high. The only effect of 3 percent gypsum was a slight increase in the moisture content of the droppings. Hurwitz and Bar (1968) later found that calcium sulphate supported higher concentration and activity of calcium in various intestinal segments when compared to that of dicalcium sulphate or limestone.

As can be seen from the review so far, many different compounds have been t ied over the years as sources of calcium for poultry, oyster shell and limestone were and are still, probably the most common sources of calcium employed in poultry feed industry. There are many reports from both earlier and recent experiments indicating that oystershell is more beneficial than limestone to the laying hen for eggshell quality (Scott et al., 1971; Charles et al., 1982; Bardley and Krueger, 1982). Roland (1986) pointed that part of the inconsistency in the earlier experiments dealing with various limestone sources was

due to a greater variation in their physical and chemical characteristics. For example, Hart et al. (1923), Halpin and Hayes (1925), and Kennard (1925) reported that dolomitic limestone decreased eggshell strength due to its high magnesium content. Martin and Insko (1929) found that, white limestone was more attractive to hens than dull gray limestone of similar chemical composition but both were good sources of calcium for eggshell formation. Miller and Sunde (1975) concluded that even though much data have shown that oystershell is equall or superior to limestone, the industry predominantly uses limestone in mixed rations for poultry because of the reasons that oystershell is more expensive than limestone in certain areas of the country and small feed mills are not equiped for its use calcium in poultry diets.

The influence of particle size of calcium source on growing chicks.

The content of calcium in the bodies of broilers increases by a factor of 5 in only ten days of post embryonic growth (Georgievski 1982). The poultry science association (1983) noted that the market weight of an average broiler in 1973 was 1.75kg (3.88lb) and that by 1983 it had increased to 1.86kg

(4.12lb). They project that by 1993 the weight of an average broiler bird should increase to 1.95kg (4.34lb). This demonstrate the rapid increase in growth rate of broiler birds.

To support this growth rate, the broiler chicken must have adequate bone growth. Many studies have been conducted to evaluate the best source and form of calcium that would be required for this purpose. Since Chossat (1824) recognised that pigeon required calcium supplement in the form of grain additive to ensure adequate bone strength; several studies have been conducted to evaluate the suitable particle size of calcium source that would give a good chick performance. Krishna (1971) found that chick performance as measured by tibia ash remained essentially the same when calcium was supplied by either limestone or oystershell. No significant difference in response occured when large, medium or small particle sizes were fed. However, tibia ash was greatest when the medium particle size was fed. Povic (1974) found no difference in weight gain, feed conversion and bone ash when Hybro broilers were fed to 56 days on four different limestone sources.

McNaughton et al. (1974) in three experiments evaluated the effect of particle size and source on broiler chicks from day old to 3 weeks of age. In the first two studies they reported that chicks fed oystershell were heavier on the medium (USBS 40-50) particle size diet (311g), followed by small (270-Pan) particle size (309g), while those fed limestone diet were heavier when small (270-Pan) particle size was fed. In yet another study in which oystershell was utilized, it was reported that growth rate was better with USBS 16-20 particle size. In another study, McNaughton and Deaton (1980) demonstrated significant differences in body weight and bone ash when give limestone sources, a mind marine source, argonite and oystershell were fed to broiler chicks to three weeks on "as is" basis. However, when the sources were analysed and calcium content of all sources was equalized, these differences that occured due to source disappeared. Differences in the parameters measured due to particle sizes also occured but were not observed on chickens given a similar particle sizes. They concluded that calcium utilization by the chick is dependent on particle size of calcium supplement and not source. They recommended the use of medium to fine particle size when considering both weight and tibia ash.

Anderson et al. (1984) reported that the finer particle sizes produced lower tibia ash only when fed at a higher level. Their basal diet used in the experiments contained about five times as much calcium as the test calcium added, and they suggested that the higher basal calcium level masked differences in the calcium source at the lower dietary calcium level. They reported that particle size affected gain tibia ash if the dietary calcium level was high enough. They speculated that chicks can shunt excess calcium in the form of medium particles through the gut better than can fine particles.

The influence of calcium source and particle size on laying hen performance and shell quality

Egg Production

The importance of calcium in egg production can not be over emphasised. It has been demonstrated that grossly inadequate levels of calcium in the layer diet will even lead to cesation in egg production Gilbert (1969). The question of which source is feasible for optimal production has attracted researchers attention for many years. It has been pointed out elsewhere that the chocie of calcium source for egg production in this country is between oystershell and recently, limestone. Kuhl (1976) reported that limestone and oystershell are the primary sources of calcium in the diet of laying hens because they are most economical.

Kuhl et al. (1977) reviewed the literature on calcium source and particle sizes for laying hens and remarked that, "the reviewer of published sizes in the diet of calcium sources and particles sizes in the diet of laying chickens can draw but one conclusion; findings are inconsistent".

Since Collier (1892) reported that limestone and oystershell were acceptable sources of "lime" in the diet of laying hens and Buckner (1923) later supported this findings by reporting that oystershell and limestone were equivalent source of calcium for egg production, many experiments were later undertaken with a view to ascertain the validity of these claims. Buckner et al. (1924) and McKinney (1972) used sources other than oystershell and limestone. Buckner et al. (1924) and McKinney (1972) compared calcium carbonate, calcium sulphate, calcium phosphate and calcium chloride and reported that the calcium carbonate source gave the best result in most of the criteria measured. Kennard (1925), and Halpin and Hayes (1934) fed oystershell and dolomitic limestone to laying hens and reported that oystershell gave higher egg production. Supplee (1962) and Mehring and Johnson (1965) found that the use of dolomitic limestone did not prove harmful to egg production. Stillmark and Sunde (1971) reported that a level of up 11.8 percent dolomitic

limestone could be used in the diet of laying hens without significantly affecting performance.

Investigations into the suitability of different limestone sources and oystershell as calcium source for laying hens may have arisen as a result of Kennard (1925) report; for soon after that report, the Agricultural Experiment Stations of Iowa Kentucky (Anon, 1927) both undertook similar studies into the different limestone deposits in various locations of the United States of America (USA) to determine their suitability for egg production and ereported varying results. The Iowa station report indicated that oystershell was bettern than limestone in most cases for egg production. It pointed out however, that very hard practically pure limestone, low in magnesium content was equal to or better than oystershell. Both pointed out that particle size of the caldium source was important in egg production. Messengale and Plat (1930) stated that the physical state of calcium carbonate (limestone) necessary for eggshell formation should be in the form of grit or coarse material rather than fine. Rau and Platt (1949) found that chick size, hen size and powdered limestone were equally effective as a calcium source and particle

size effect of calmshell limestone and oystershell and reported that particle size and not source affected egg production.

Hurwitz et al. (1969) found no difference in the production rate and feed efficiency of 8 month old Leghorn hens fed diets containing 4.0 percent of either limestone or eggshell. No difference in production rate of hens fed diets containing either oystershell flour or pulverized limestone was reported by Quinsenberry et al. (1969). However, mortaility and egg weight favoured oystershell. In another experiment, Quinsenberry and Walker (1970) reported no differences between oystershell and feed efficiency. Hens which had been laying for 10 months were given diets containing 3.5 percent calcium supplied by limestone or oystershell in different ratios and particle sizes. Scott et al. (1971) reported oystershell and limestone in the ratio of 2 to 1 (2:1) gave the best performance. This study indicated that mixing limestone with oystershell was a better way of improving egg production than either source given singly. Meyer et al. (1973) and Roland et al. (1974) failed to demonstrate the difference in particle size between oystershell and limestone when egg production was the parameter considered.

Miller and Sunde (1975) found no effect of both source and particle size and combination of sources on production.

Kuhl et al. (1977), Watkin et al. (1977), Christmas et al. (1977) and March and Amin (1981) fed hens different sources, particle sizes and levels of calcium to laying hens but failed to notice source or particle size effect on egg production, egg weight and feed utilization efficiency. Duke and Charles (1981) however observed that dietary oystershell resulted in a significant improvement in egg production rate but could not demonstrate the same effect in a follow-up experiment. Later, Charles et al. (1982) reported again that egg production was improved when calcium was supplied in the form of oystershell or coarse limestone to White Leghron hens. Bardley and Krueger (1982) studied the effect of calcium source and particle size on egg production characteristics of Leghorn hens and observed a numerical but non significant improvement in percent egg production when oystershell was the source of calcium. Charles et al. (1982) submitted that production rate was increased but feed efficiency decreased when calcium was supplied as oystershell or granular limestone. Recently Hamilton et al. (1985) reported a heavier

production rate in favour of oystershell as opposed to limestone.

Shell quality

While calcium is distributed throughout the entire egg, it is chiefly localised in the shell of the egg as calcium carbonate where this compound constitutes about 98 percent of the inorganic matter of the shell. The calcium content of the shell weight is about 36 percent but the actual value of the calcium in the shell and membranes varies between 1600mg and 2100mg depending on the size of the egg and laying stage (Georgievskii, 1982). The need for considering the quality of the shell becomes apparent in the light of its function which enables the egg to withstand considerable biological and mechanical assults while still maintaining its structural integrity and the wholesomeness of its contents. As domestic egg production becomes more mechanized, increasing numbers of eggs get broken as eggs move from the hen to the consumer. Conservative estimates for the incidence of broken eggs range from 6-8 percent of all eggs produced (Washburn, 1982). This estimate probably underestimates the demage because it would be difficult to detect all breakage at the point of lay or cracks in the later stages of processing and transportation to the consumer. Gleaves (1978)

estimated the breakages of eggs at about 1.5 to 2 percent when all egg handling was done by hand.

Shell breakage is related to shell quality. The term "Shell quality" is frequently used as a synonym for "shell strength" and implies, the ability of the eggshell to withstand externally applied forces without cracking or breaking. Shell quality is affected by a number of factors such as strain of hen (Hamilton et al., 1979) nutritional regimen (Wolford and Tanaka, 1970) time of day eggs are laid (Roland et al. 1973) and ambient temperature (Nwazota, 1987). The measurement of eggshell strength has been carried out for many years and a number of techniques and instruments have been developed to measure shell strength. The ability of eggshell to withstand externally applied forces is a function of the material and structural strength of the shell (Carter, 1970; Voisey and Hunt, 1974). Variables associated with shell strength are biological in nature because under optimum feeding and disease free environments, they are controlled by genetic factors which are outside the control of the egg producer. However, factors associated with insults to eggs are mechanical in nature and can be controlled to some extent by the producer.

Interest was generated in the area of shell strength or quality in the 1970's and 1980's Quisenberry et al. (1969), and Quisenberry and Walker (1970) reported an increase in shell thickness from the feeding of large particle size oystershell in the laying hen diet. Scott et al. (1971) replaced two third of supplemental calcium either from ground lime or oystershell with hen size oystershell and reported an improvement in performance and shell quality. In advising egg producers, Kim (1971) reported that "dime" size oystershell flakes in a complete diet for hens increased shell thickness and breaking strength. Scott et al. (1971) and Kim (1971) advanced a hypothesis that large particle size oystershell is retained in the gizzard which in turn "metered" out calcium into the blood even during non-feeding hours. In this way, calcium is present during all periods of shell formation for maximum shell calcification. Kim (1971) then recommended that limestone constantly used in laying hen ration be reduced to one-third and two-third be replaced by "dime" size oystershell. Meyer et al. (1973) found that the breaking strength of shells from hens fed diets containing oystershell and limestone grit were significantly stronger than eggshell from hens fed pulverized limestone as the calcium source.

Roland and Harms (1973) found that substitution of hen or pullet size limestone or oystershell for two-third of the granular limestone improved shell quality during the Summer but not during Fall. They used the "metering" out hypothesis to speculate that hens receiving diets containing only pulverized calcium carbonate must obtain all their calcium during the day time while majority of calcium derived from the large particle size calcium carbonate was "metered" from the gizzard during the early morning hours with very little metered out at night. Hamilton et al. (1985) reported an improvement in eggshell quality as measured by specific gravity from both limestone and oystershell similar particle sizes. Other experiments from different researchers such as Charles et al. (1971, 1972), Miller and Sunde (1972) all seem to lend support to the findings of Scott et al. (1971) and Kim (1971) on the "metering" hypothesis of the calcium particle size. Meyer et al. (1973) reported that oystershell gave better breaking strength of eggshell than the pulverized size. Mitoku et al. (1973) compared calcium sources (calcium carbonate, calcium lactate, oystershell and fossile shell) for layers in the summer. They observed that fossile shell gave the greatest breaking strength and shell thickness, but other parameters were not affected by calcium source. Roland et al. (1974) found that the

inclusion of pullet-size limestone in diets containing 2.75 to 3.0 percent calcium improved shell quality. But concluded also that larger particles of calcium carbonate would have no influence on shell quality if the diet contained adequate calcium.

Miller and Sunde (1975) found that egg shell from pullets 20 and 60 weeks more rigid when fed coarse limestone or oystershell. However, at the point of lay, there was no effect of particle size on shell quality. Watkins et al. (1977) also confirmed the effect of particle size an eggshell quality. Later, in 1981, Brister and associates concluded that the addition of large particle size oystershell significantly improved shell quality when substituted for a portion of pulverised calcium source. Based on this observation Bristers et al. (1981) stated that calcium from oystershell in any form is more available than that from limestone for eggshell formation. The experiment of Petersen and Høj (1981) showed that eggshell quality was a function of level of calcium in the diet. Shell quality was significantly improved by oystershell but only when diets contained up to 3.0 percent calcium. When powdered limestone was used as the only supplementary source of calcium, optimum shell quality was attained with 3.4 dietary calcium.

When the two sources were used together, optimal shell quality was attained with 2.6 - 3.0 percent calcium level. Petersen and Hoj (1981) concluded therefore that it was not only the content of calcium in the diet that determined the eggshell quality, but that the ability of laying hens to mobilize bicarbonate ions was also an important factor in shell formation.

The review on the effects of oystershell or limestone sources on shell quality by Roland (1986) was mostly lopsided in favour of oystershell. Others when oystershell and limestone were compared on equal basis of particle size and calcium content, only two out of the twelve papers reviewed extolled the extra beneficial effects of oystershell on shell quality. Roland (1986) further pointed out that while there can be several reasons for the variations in response of hens fed various sources and sizes of good quality calcium carbonate, perhaps two were worthy of mention

(1) Research workers were not always comparing calcium carbonate sources having the same particle sizes for solubilities.

(2) The hens for which oystershell was substituted for a portion of fine granular limestone in these diets were not always consuming adequate calcium.

Balnave et al. (1988) advised against administering mineral supplements in water rather than in the feed since this induces incidences of eggshell defects.

OTHER CALCIUM RELATED FACTORS THAT AFFECT EGG SHELL QUALITY.

Calcium status of the diet

Overfield (1970) worried over the downgrading of eggs because of poor shell quality reported that samples taken from different farms showed great variations in calcium content of the feed. He speculated that this may be due in part to source and formulation of diets and the different feeding methods. In another survey, poultry mash was compared at three stages, once at the mill, once just before discharging into the bulk bin on the farm and at intervals when the mash was being used. Several samples were taken at each stage over a three week period. Although the results were again varied, it was observed that the limestone had separated out during the movement and storage of the mash, particularly at the later stage. Overfield (1970) pointed out that in recent years, there has been a rapid increase in the amount of poultry feed handled in bulk at all stages from the mill to the bird and

showed from the survey an indication that when the feed is in a mash form, a great deal of physical separation can occur. Keshavara and Ackerman (1984) later pointed out that although the feed miller may incorporate an adequate or recommended level of calcium in a layer diet, separation can take place in different phases of feed handling on commercial poultry farms that may result in hens receiving different levels of calcium on different days or even for different hours of the same day.

A number of investigations have reported that the time of day that the hen receives calcium has great impact on maintaining shell quality (Lennards and Roland, 1981; Cason and Briton, 1981; Briton and Cason, 1982). It can be inferred that separation of calcium source could cause hens to eat more feed in an attempt to meet the calcium needs when the top portion of the feed is being fed whereas the accumulation of excess calcium at the bottom of bags or feeders will make feed rather unpalatable at this stage. Keshavarz (1986) found that shell quality as measured by shell thickness, breaking strength, specific gravity and percent shell in most cases was significantly reduced within 24 hours of feedin a low calcium diet (1.5 percent), apparent representing the

calcium insufficiency when separation occurs, and improved within the same period when high (5 percent) calcium diet was fed.

Carbonic anhydrase enzyme

This enzyme is closely associated with the uterine wall of the hen and was first reported by Meldrum and Roughton in 1934. Thye speculated that this enzyme might play a role in eggshell formation by influencing the rate of formation of the anion of calcium carbonate. Common (1941) found that carbonic anhydrase activity of the uterine epithelium was higher than that of other portions of the oviduct and suggested that this activity might play a part in shell secretion. Common (1941) further reported that there was a slight indication that the activity was higher in the uterine epithelium of laying birds than that of non-laying birds. However, Gutowaska and Mitchell (1945) found that there was the same amount of carbonic anhydrase in the blood of laying and non-laying hens, and that there was more in the blood than in the shell gland. Gutowaska and Mitchell (1945) also reported a correlation between shell local concentration of carbonic anhydrase in the shell gland and the ability to lay smooth and strong shelled eggs. Injection of sulphonilamide lowered the carbonic anhydrase activity of the blood and shell gland, and

thin shelled eggs were produced. Gutowaska and Mitchell (1945) then suggested that the mechanism of shell deposition was that the egg white (pH 8.2 - 9.2) provided the medium for the deposition of calcium carbonate, the deposition being brought about by calcium arising from the breakdown of blood calcium proteinate and the carbonate from bicarbonate ions. According to Gutowaska and Mitchell (1945), carbonic anhydrase favours the breakdown of bicarbonate and hence ensures a continuous production of carbonate ions. They however concluded that the shell gland does not secret a suspension of calcium carbonate since the gland has a pH of 6.2 which is near the hydrolysis point of calcium carbonate.

Taylor and Stringer (1965) also supported the view that carbonic anhydrase might be involved in shell formation by providing carbonate ions for formation of calcium carbonate. They also reported a correlation between its concentration and shell strength, this being lower in birds producing soft shell eggs. However, Heald et al. (1968) were unable to demonstrate any correlation between carbonic anhydrase concentration and shell strength and concluded that if the enzyme were essential for shell formation, its potential in the uterine epithelium is always sufficient to provide adequate carbon dioxide for shell formation.

CALCIUM REQUIREMENTS OF CHICKEN AND CALCIUM TO PHOSPHORUS RATIO.

Requirements for layers

That laying hens and indeed all animals require calcium in their diet for production may have been recogni zed before or soon after the calcium discovery in 1808 by Sir Humphrey Davy. Buckner and Martin (1920) reported that lack of a supply of calcium resulted in a decrease and finally ceasation of egg production. Buckner *et al.* (1923, 1928) and Massengale and Platt (1930) reported that egg production in laying hens depended on an adequate supply of calcium in the diet in the proper chemical form. The calcium requirements as suggested by Mitchell and McClure (1937) were based on production rate as follows:

- 100 percent egg production would require 4.6 percent calcium
- 50 percent egg production would require 2.74 percent calcium
- 0 percent production would require 0.30 percent calcium.

Stuart and Hart (1938) concluded that 1.0 percent calcium carbonate in the mash was insufficient for egg production and suggested that 5 percent was better and that 8.0 percent showed no further advantage. Gutowaska and Parkhurst (1942) showed that excess calcium in the ration decreased egg production and food utilization. They pointed out that a level of 3.96 calcium (with adequate phosphorus and vitamin D) was excessive but levels of 2.35 and 2.75 percent calcium were safe. Evans et al. (1944) recommended that 3.0 percent calcium (with 0.8 or 1.0 percent phosphorus) was best for production and shell thickness, although 2.5 percent calcium was good for production alone. After reviewing the literature at the time, the National Research Council (NRC, 1944; 1950; 1954 and 1960) in its first to the fourth editions recommended a level of 2.25 percent calcium in the diet for laying hens.

Peterson et al. (1960) reported that the 2.5 percent calcium are recommended by the N.R.C. might not be adequate for laying hens. Peterson et al. (1960) found that 3.75 percent calcium was necessary for good shell quality within the environmental temperature of 70°F and less. With higher temperature

as occurs during Summers or in the tropics, a calcium level of 4.5 to 5 percent was suggested. Mostert (1960) and Mostert and Swart (1968) found that hens in batteries required calcium from 2.5 - 3.5 percent in an all-mash diet for optimal shell quality. DeGroote and Reyntens (1967) recommended a calcium level of 3.25 percent ofr maximum performance but pointed out that for maximum shell strength 4.3 percent calcium was ideal. The National Research Council (1966, 1971) in its fifth and sixth edition, recommended a level of 2.75 percent calcium.

Scott et al. (1971) reported that when pulverized limestone was used as the only calcium supplement in a complete laying mash for hen, maximum production and shell quality were obtained when the diet contained 3.5 percent calcium. Roland et al. (1974) reported that calcium requirement for hens was not more than 3.0 percent when large particle size oystershell was fed. Ademosun and Kalango (1973) submitted that, increasing the level of calcium from 2.0 to 3.5 percent resulted in increased egg production and eggshell quality of White Rock and Rhode Island Red layers in Nigeria. They National Research Council (N.R.C., 1977) in its seventh edition recommended a calcium level of 3.25 percent of laying hens. Some

researchers are of the opinion that the level of calcium required by the laying hens should be expressed in grams per day rather than the usual expression in percentage terms. In this connection Fertuero and Cosiall (1981) and Roland (1982, 1986) after a review of the literature on the subject recommended that laying hens be fed a minimum level of 3.7 grams daily. For older hens or for hens producing eggs with poor shell quality, this value should be increased by at least 1 gram daily. Scott et al. (1983) suggested appropriate levels of calcium for feeding laying hens and based on production levels and age. They suggested that pullets (22-40 weeks of age) and matured hens (40 weeks and over) should respectively be fed 3.3g and 3.7g of calcium daily if laying at 100% rate and only 2.3g and 2.6g calcium if laying at 70 percent production rate.

Ousterhout (1980) fed diets containing 2.75 and 3.75 and 4.75 percent calcium in which ground limestone was the only source of calcium carbonate and reported that 3.75 percent gave better performance. Mathur et al. (1982, 1983) reported that on the hen day production basis, minimum requirement of calcium can be fixed at 2.75 percent but results were better with 3.5 percent with other parameters. Many other

researchers during this period recommended similar levels of calcium. Singh et al. (1981), Temnokhud et al. (1981) reported that 3.5 percent calcium was adequate for optimal performance. The National Research Council (1984) in its eighth edition suggested a calcium level of 3.4 percent. It will be noted that the National Research Council (N.R.C.) has always made an upward review of calcium level for laying hens. Roland (1986) advanced the following reasons for the difficulty in establishing calcium requirements for laying hens:

1. "Changing genetics of the hen and the continued improvement in production capacity.
2. Difference in calcium requirements among hens within strains and between strains
3. Inter-relationship of calcium with other nutrients, especially phosphorus and vitamin D.
4. Inter-relationship of particle size (solubility) of calcium carbonate to calcium availability and palatability of feed.
5. Ability of the hen to adjust feed intake partially to meet her calcium requirements.
6. Producers' or nutritionists' fears of adverse effects of excess or inadequate consumption of calcium.
7. Calcium requirement has been reported by many research workers in terms of percent in the diet without taking into account, the variation in feed intake as influenced by energy level, environmental temperature and strain or age of the bird".

Requirements for chicks

The importance of considering the calcium requirement of chicks (especially broilers) is to recommend levels that will economically ensure adequate bone growth in a healthy bird while preventing leg problems. Edwards (1982) observed that it is difficult to do calcium requirement studies using growth rate as a criterion since maximum growth rate can be obtained on rations that give poor calcification and in which severe leg problems develop.

In 1938, Branion observed that the minimum requirements for calcium is 0.70g per 100g ration but recommended 1.0g per 100g of ration to provide a margin of safety. Mitchell and McClure (1937) had suggested a year earlier that young chicks should be given a minimum of 0.7-0.75 percent calcium in the ration Couch et al. (1947) found that the requirement of chicks for vitamin D was influenced by the amount of calcium present. The higher the calcium content of the diet, the less the vitamin D needed. Research into calcium requirement was stimulated by the findings that tetracycline antibiotics were absorbed much more efficiently when low calcium ration was fed (Edward, 1982). Simco and Stephenson (1961) indicated that only 0.5 percent calcium was required in broiler diet for maximum growth rate and tee ash. Fermica et al. (1962) found that 0.6 percent calcium was adequate for optimal growth rate but 0.8 percent was required for maximum bone ash. Waldroup et al. (1963)

reported, that the requirements were greater than 0.6 but not more than 0.7 percent of the ration for maximum growth rate and bone ash. Edwards et al. (1960) had suggested calcium requirement of 0.9-1.5 percent of the ration depending on the caloric and protein content of the diet.

Pepper et al. (1955) reported that calcium requirements of young chickens was increased considerably by the addition of fat to the ration. But the results obtained by Biely and March (1967) showed that the addition of up 12 percent saturated fat to the chick diet did not increase calcium requirements. Lipstein and Bornstein (1968) found that neither type or level of supplemental oil had any effect on the bone calcification of chicks. Edwards et al. (1963) reported that the requirement ranged from 1.0 percent for maximum growth to 1.4 percent for maximum bone ash. Nelson et al. (1968) reported that the calcium requirement of Single Comb White Leghorn chicks given a purified diet containing no phytate was 0.5 percent. Requirements increased to 0,95 percent calcium in a diet composed of natural ingredients and containing 1.25 percent phytic acid. Hydrolysis of about 90 percent of the phytic acid with phytase reduced the amount of calcium required in the diet of natural ingredients to about 0.65 percent. Nelson and co-workers (1968) concluded therefore that the amount of calcium in the diet of growing chicks must allow for that which is inactivated by phytase.

Gendes et al. (1974) reported that male broiler chicks required more calcium than females. Based on such parameters

as weight gain, feed conversion, bleed calcium, bone ash, they showed that the best results were obtained with 0.8 percent calcium for female and 0.8-1.0 percent calcium for males. Hijikure and Merimeto (1965) and Twining et al. (1965) reported that the calcium requirement for maximum growth and bone ash was 0.83 percent for straight run broiler chicks but Twining et al. (1965) also pointed out that males required slightly more calcium than females. Lillie et al. (1964) examined the requirement of calcium as influenced by energy, sex and strain and concluded that broiler chicks required 0.7-0.9 percent. Shadra et al. (1979) reported that calcium requirement of broilers from day old to 6 weeks of age was 1.06 percent.

The ratio of calcium to phosphorus is very vital especially for young poultry. (N.R.C. 1977). Generally speaking, a ratio of about 1.2:1 calcium to phosphorus is considered to be ideal. However, ratios from 1:1 - 1.5:1 are well tolerated. The calcium and phosphorus levels as suggested for broilers by the National Research Council (N.R.C.) are shown in Table 2.

It will immediately be appreciated that there has been very little change in the level of calcium and phosphorus requirements for broilers over the years when consideration is given to the differences that have occured in broiler industry over this time period.

Table 2 : Calcium and Phosphorus requirements of broilers.

Year	Calcium	Phosphorus	Reference
-	%	%	
1944	1	0.6	N.R.C. 1944
1954	1	0.6	N.R.C. 1954
1960	1	0.6	N.R.C. 1960
1965	1	0.7	N.R.C. 1966
1971	1	0.7	N.R.C. 1971
1977	0.9	0.7	N.R.C. 1977
1984	1.2	-*	N.R.C. 1984

* Available phosphorus = 0.32%

SOLUBILITY AND BIOAVAILABILITY OF CALCIUM FROM CALCIUM SOURCES AND ITS IMPLICATION IN CHICKEN NUTRITION.

Bioavailability

The usefulness of any calcium source is dependent on how available it is to the bird as measured by such parameters as growth rate, bone ash and/or mineralization, blood calcium level, egg production and shell quality. Calcium bioavailability studies have been carried out for many decades. Waldroup et al. (1964) evaluated availabilities of several calcium salts, oystershell and limestone sources and reported that availabilities of oysteshell products varied from 93 to 117 percent and that of limestone sources varied from 86 to 100 percent as calculated by Slope Regression analysis. Reid and Wiber (1976) evaluated the availability of calcium from 5 limestone sources using body weight and bone ash of broiler chicks raised up to 4 weeks of age and reported that bone ash data showed a linear relationship between dietary calcium level and percent bone ash. Slope

Regression analysis showed percent calcium availability of ground limestone samples to range from 73.3 to 109.4 percent while that of oystershell showed an availability of 107.7 percent. In another experiment with layers, Reid and Wiber (1976) showed that availability of calcium from the limestone sources was in the range of 82.4 to 98.4 percent. Hamilton et al. (1976) determined the availability of calcium from some limestone sources processed by a new method ofor poults from day old to 21 days. Using growth rate and feed efficiency as evaluative criteria, these workers reported that finer ground limestone increased availability of calcium to poults, increase the efficiency of feed utilization of birds at low dietary calcium level and produced better weight gain. Anderson et al. (1984) found that purified and powdered calcium carbonate produced poorer performance than commercial limestone. Sibald (1982) extended the methodology of evaluating true metabolizable energy to the measurement of mineral bioavailability. Using this methodology, Kim et al. (1985) reported that availability of calcium from different calcium sources were as follows: calcium carbonate 98.12, calcium sulphate 92.63, tricalcium phosphate 83.63, dicalcium phosphate 83.58, limestone

70.7 and oystershell 66.83. From this report, it appears that limestone is more available than oystershell. However, Brister et al. (1981) had concluded that calcium from oystershell in any form was more available than limestone for eggshell formation.

Solubility

Calcium used in poultry diets must first be in solution before it can be absorbed from the gut. The solubility of a calcium source is therefore important in determining the availability of this element from the source being considered. Since the "metering" out hypothesis postulated by Scott et al. (1971) which attempted to explain why calcium from oystershell improved eggshell quality over that of limestone, other experiments have been conducted which indicate that the difference in solubility of oystershell and limestone was due to particle size and not source (Roland et al., 1972). Savage (1982) indicated that pH was involved in the solubility process and pointed out that some limestone sources react more rapidly than others with the hydrochloric acid of the gut.

Kuhl and Sullivan (1977) compared the rate of solubilities of large particle oystershell and limestone *in vivo* and *in vitro*. They force fed hens with 10 grams of either hen size oystershell or limestone and reported that retention of hen size limestone in the gizzard was significantly greater than that of hen size oystershell. However, *in vitro* solubility gave no difference between limestone and oystershell. Pullet sizes of either oystershell or limestone were more soluble than hen sizes of the two sources tested. Raben and Roland (1985) determined the solubility of limestone obtained from nine limestones or oystershell companies throughout the U.S.A. and reported that the percent solubility of limestone source having the same particle sizes vaired from 15 to 27.3 percent. Bandemer and Schaible (1942) found from *in vitro* experiments that oystershell, pure calcium carbonate bone meal and pure tricalcium phosphate remained undissolved in solution containing calcium and phosphorus at the pH of the small intestine. Tyler (1946) found that non-laying hens had a marked decrease in the solubility of calcium in the intestinal tract particularly from the anterior to the posterior part of the small inestine. This decrease corresponded to a rise in pH but this activity was not so noticable in the laying hen.

CALCIUM AND ITS INTERRELATIONSHIP WITH OTHER NUTRIENTS:

Phosphorus, calcium and phosphorus ratio and vitamin D

The role of phosphorus and vitamin D in the proper metabolism of calcium has been studied for many years. Nowotarski and Bird (1943) found that 3.47 percent calcium and 1.98 phosphorus with 20 units of vitamin D gave better three week old chick bone ash. When the experiment was carried out over a 10-week period, the higher mineral levels caused an acute decrease in bone ash. Consequently, Nowotarski and Bird (1943) concluded that high mineral levels in practical diets were less effective than vitamin D in promoting growth and preventing crooked break bone. Heuser et al. (1943) stated that non-phytin phosphorus must be incorporated in the chick ration to obtain normal growth and bone development. This observation was later confirmed and extended by McGinnis et al. (1944) who showed that when bone ash was used as criterion, 1.0 percent calcium and 0.58 percent phosphorus with varying quantities of irradiated animal sterol was best. A diet containing only phosphorus from cereal and legumes was much poorer than one containing mainly inorganic phosphorus in equivalent amount of vitamin D increased, Singsen and Mitchell (1945) showed that the ability to utilize phytin

phosphorus was influenced by vitamin D. Common (1940) estimated the phytic acid content of a number of feddstuff used for poultry ration and concluded that in ordinary poultry rations, about three quarters of the phosphorus derived from cereals is present as phytic acid.

The National Research Council (N.R.C. 1944, 1950; 1954 and 1960) did not alter the level of phosphorus requirement of 0.6 percent of the diet for starting chicks. In 1963, Waldroup et al. reported that 0.5 percent total phosphorus was adequate when calcium level was 0.6 percent with growth rate as the criterion but for better feed efficiency, 0.6 percent total phosphorus and 0.9 percent calcium were required. Lillie et al. (1964) used different strains and energy levels in a broiler experiment and concluded that from the conditions of the study 0.7 percent phosphorus with 0.9 percent calcium were adequate for optimal performance. Twinning et al. (1965) observed that for 0-6 weeks old broilers, 0.6 percent phosphorus and 0.8 percent calcium were needed when weight gain was the sole criterion. Yoshida and Hoshii (1982) reported that a combination of 1.3 percent calcium and 0.75 percent available

phosphorus should be included in the chick diet for maximum tee ash. However, for finishing such birds, a combination of 1.1 percent calcium and 0.35 percent available phosphorus was required for maximum tee ash. The National Research Council (N.R.C. 1966) reviewed the phosphorus requirement upwards to 0.7 percent total phosphorus in the case of meat-type birds while in the case of layers the level was reviewed downwards from 0.75 to 0.6 or 0.5 percent.

In poultry, the ratio of calcium to phosphorus is much more important than of either element considered singly. Branion (1938) concluded that with adequate vitamin D and responsible levels of calcium and phosphorus in the ration the Ca:P ratio may vary between 1:1 and 3:1 with 2:1 as the optimum. Carver et al. (1946) showed that in the absence of vitamin D increasing the phosphorus level from 0.5 - 0.8 percent improved growth and bone ash percentages of four week chicks at Ca:P ratio of 2:1. When however, 60 units of vitamin D was added to the diet, the Ca:P ratio of 1:1 gave better performance. Nitis and Sutrisna (1976) fed diets varying in calcium source with a constant Ca:P ratio of 2:1 to White Leghorn chicks (1-8 weeks and 8-16 weeks) and found no overall difference in performance of the two age groups. Ben et al. (1981) showed that performance

of chicken was depressed with wider Ca:P ratios and that bone Ca:P ratio was positively correlated with dietary Ca:P ratios, Hulan et al. (1985 , 1986) found that the ratio of calcium to available phosphorus rather than total calcium and phosphorus in the diet is of more important in the expression of tibia dyschondrophlasia lesions. The National Research Council (N.R.C. 1977) recommended a Ca:P ratio of 1.2:1 but stated that ratios from 1:1 to 1.5:1 are however, tolerated by the chicks. In laying hens however, wider ratios of 3.5:1 or more are advocated.

Common (1936) later found that when the hen received a high dietary calcium intake, phosphorus was no longer excreted in large amounts prior to ovulation. Morgan and Mitchell (1938) found that at the start of lay, a pullet was in a negative phosphorus balance but later returned to positive balance without a change in rate of lay. They concluded that an adequate supply of phosphorus in the diet was responsible for this change. Evans et al. (1944) showed that Single Comb White Leghorn pullets should receive 0.8 percent phosphorus at a calcium level of 2.50 percent, when the criteria were egg production and shell

thickness during the first four months of lay. Evans et al. (1944) however pointed out that with 3 percent calcium and either 0.8 or 1.0 percent phosphorus, there was no difference in shell thickness, egg weight, shell weight and porosity. These authors further suggested feeding higher levels of dietary calcium as a means of reducing calcium mobilization from the bone which in turn will reduce the phosphorus requirement. They pointed that the high phosphorus requirement of poultry was due in part to heavy excretion of phosphorus during egg laying caused by mobilization of skeletal calcium and by phytin content of poultry feedstuffs. In addition, Evans et al. (1944) speculated that high levels of calcium might lower the absorption of phosphorus.

Taylor (1965) and Hamilton and Sibbald (1977) have shwon that reducing the phosphorus level as the hen ages impreves shell quality. Several explanation have been offered to justify the above claim. Taylor (1965) attributed this improvement in shell quality to reduction in the formation of insoluble calcium phosphate complex in the digestive tract. Hunt and Chance (1970) pointed out that phosphorus acts as crystal poison in the shell gland thereby inhibiting

the rate of calcite accumulation in the egg shell. Another school of thought believes this to be due to the mobilization of calcium from the bone for shell formation. Hurwitz and Griminger (1962) and Ben et al. (1981) demonstrated that the blood level of phosphorus is directly associated with the dietary level of element. Therefore, a reduction in the phosphorus level will lead to a reduction in plasma level of this element and this will consequently result in bone resorption for the release of calcium for shell calcification.

Relationship with magnesium

The consideration of the relationship between calcium and magnesium is important since it is contained in dolomitic limestone. Hart et al. (1923), Kennard (1925) and Halpin and Hayes (1925) demonstrated the harmful effect of dolomitic limestone on poultry production. Tulley and Franks (1934) also showed that feeding of dolomitic limestone as a calcium source produced eggs with poor shell quality. They postulated that magnesium in the dolomitic limestone induced a cathartic action through the retention of water in the intestina tract, resulting in the rapid movement of the calcium ions through the intestinal tract leading to a decrease in calcium absorption. Halpin and Hayes (1925) and Kennard (1944) reported a

reduction in egg production and shell strength when dolomitic limestone was fed.

Supplee (1962) found no detrimental effect of feeding dolomitic limestone on egg production. Mehring (1965) added up to 5000ppm magnesium in the diet of broiler chicks but reported no harmful effect. Mehring and Johnson (1965) added up to 8000ppm magnesium in a layer diet without detrimental effect on production. McWard (1967) reported that performance of laying hens was not significantly influenced by the addition of 1.20 percent magnesium but eggshells were thinner when the diet contained 1.96 percent magnesium. Stillmark and Sunde (1971a) reported that a level of 11.81 perdent dolomitic limestone could be added to laying hen ration without having an adverse effect on egg production. However, they added that when magnesium was included in the diet at a level of 1.12 percent magnesium carbonate, egg production was significantly reduced. In a later experiment, Stillmark and Sunde (1971b) reported that the use of dolomitic limestone resulted in a carhartic action which probably accounted for the low performance reported earlier. Atteh and Leeson (1982) reported a harmful effect when up to 7,700ppm magnesium was incorporated in the diet of laying hens.

Relationship with manganese

Evidence is accumulating that there exists a relationship between calcium and magnesium. It has been demonstrated many times (N.R.C. 1971; 1977; Scott et al. 1982) that the deficiency of this element results in perosis in chicks, lower egg production, and reduced eggshell strength. Scott et al. (1983) and N.R.C. (1977) pointed out that perosis is markedly aggravated by high intake of calcium and phosphorus which is due in part to the absorption of manganese in the intestinal tract by precipitated calcium phosphate. Manganese deficiency in animals is characterized by reduced growth and mineralization, and deffective structure of the bones. Gaskey and Norris (1938) reported that breaking strength of eggshells increased from 3 to 4 kg when the manganese level of the diet was increased from 6.7ppm to 100ppm. Lyons (1939) observed that poor shell quality resulted when the feed contained 7ppm of manganese. Gutowaska and Parkhurst (1942a) reported that increasing the manganese level from 17 or 24 ppm to 76 or 61 ppm did not significantly affect egg production. Shell breaking strength of eggs laid by pullets fed the higher manganese feeds was significantly greater than from the birds fed the lower manganese diets.

Cox and Balloung (1969) added 33 or 66 ppm manganese to 3 diets containing either 2.5, 3 or 3.5 percent calcium and reported that there was no significant effect on shell quality. Longstaff and Hill (1971) conducted a series of trials to investigate the effect of manganese on shell quality. In one study, they submitted that feeding low level of manganese to laying hens was detrimental to eggshell quality. In the second experiment, they found that the effect of phosphorus in reducing shell strength was more marked when manganese was also incorperated in the same diet even at low level. In the third study, these workers reported that manganese had a significant effect on shell quality and that high levels of manganese increased the number of soft shelled eggs and decreased strength and thickness of the shell.

Relationship with vitamin C

Although vitamin C (ascorbic acid) is not considered essential for poultry, it has a very significant role to play in poultry nutrition. It has been demonstrated by many researchers (El-Boushy and Van Albada, 1970; Hank and Ritzel, 1977; Kechick and Sykes, 1974; Sykes 1978) that the addition of exogenous acorbic acid in varying amounts results in improvements in growth

rate, egg number, and eggshell quality, particularly under stressful conditions.

Thornton and Moregn (1958) added ascorbic acid to diet of laying hens and reported an improvement in shell thickness. Thornton (1959) reported that the presence of ascorbic acid in the feed of laying hens apparently "changed" the early calcium-45 uptake in the progeny. Thornton (1960) found that the addition of ascorbic acid to a diet containing 2 percent calcium had no influence on eggshell thickness, but when the vitamin was added to a diet containing high levels of calcium, there was an improvement in eggshell thickness. Thornton (1960) therefore concluded that vitamin C was "changing" the calcium requirement of the laying hen. Pepper et al. (1961) reported that the addition of vitamin C to diets containing varying levels of calcium up to 4.0 percent had no significant effect on specific gravity of the egg or plasma calcium. Harms and Waldroup (1961) investigated the effect of vitamin C on shell thickness and reported that up to 4.6 percent calcium did not significantly improve shell thickness. Sullivan and Kingan (1962) observed that the addition of ascorbic acid to a laying hen ration resulted in numerical but non-significant increase in shell

thickness. In another study, Sullivan and Gehle (1962) found no increase in serum calcium level with the addition of ascorbic acid.

Perek and Kendler (1962) fed 25, 75 or 400 ppm of ascorbic acid during hot weather but reported no additional benefit on shell quality. Heywant et al. (1964) also failed to get an improvement in shell quality from the addition of ascorbic acid in the diet of laying hens during hot weather. However, El-Boushy and Van Albada (1970) reported a favourable response from the addition of vitamin C on shell quality in hot weather. Nwazota (1987), and Njoku and Nwazota (1988) have demonstrated of late, the beneficial effects of the addition of ascorbic acid to laying hen rations. These workers reported a significant improvement in egg production, egg weight, feed conversion and reduction in the incidence of cracked eggs when 400mg ascorbic acid/kg feed was added to the diet alone or the combination of 5 percent palm oil with 200mg ascorbic acid/kg feed.

Thornton and Deed (1961) found that the levels of ascorbic acid synthesized in the kidney of laying hens satisfied physiological needs at optimal environmental temperatures but that, blood ascorbic acid concentrations decreased as the environmental

temperature increased from 21-31 C. The decrease in blood ascorbic acid was postulated to be due to exhaustion of endogenous stores and also to decreased kidney synthesis of the vitamin (Thorton and Deed 1961). Addition of ascorbic acid to the diet prevented an increase in body temperature as the environmental temperature increased (Lyle and Moreng, 1968). Lyle and Moregn (1968) also reported that eggshell thickness decreased when body temperature of the hen rose above normal but that the decrease in shell thickness was prevented only when ascorbic acid prevented an increase in body temperature.

LEG ABNORMALITIES IN CHICKS

Leg abnormalities cause major economic losses in the broiler industry due to increased mortality. Most of the mortalities occur as a result of chicks' failure to reach feeders and waterers. Such affected birds have to be culled because of reduced performance. At the processing plant, leg abnormalities can lead to part or whole carcass being condemned. Although the economic losses from leg abnormalities are difficult to estimate, a conservative estimate is put between 3 and 6 percent in the United States of America (Bracewell, 1982). Raising broiler chicks to roasters

stage however, greatly increases this figure (Hulan et al. 1980; Hulan and Proudfoot, 1981, 1982). Leach (1979) indicated that the two most predominant forms of leg disorders in commercial production of broiler chickens are twisted legs and tibia dyschondroplasia.

Tibia dyschondroplasia which occurs in growing broiler chickens and to a lesser extent in turkey is characterized by a plug of opaque, white, unvascularized cartilage located usually at the proximal end of tibia but occasionally in other bones. Twisted legs, sometimes referred to as angular bone deformity, is characterized by a lateral or medial deviation of the distal tibia with a corresponding deviation of the metarsal displacement, but not complete Slippage of gastrocnemius tendon. It resembles chondrodystrophy (Perosis) but without the shortening of the long bone (Hulan et al., 1986). While several nutritional factors, disease organisms and genetic traits have been identified as predisposing factors to this condition in the chick, the etiology of tibia dyschondroplasia, however,is still not fully understood (Veltmann and Jensen, 1981).

Edward and Veltmann (1983) reported that calcium and phosphorus in the diet are major nutritional factors that influence the expression of tibia dyschondroplasia. They showed that high level of dietary calcium prevented the development of the ailment whereas high level of phosphorus accentuated its development. They found no relationship between tibia dyschondroplasia incidence and percent retention of calcium and phosphorus. Later Hulan et al. (1985,1986) demonstrated a similar effect, but pointed out that the ratio of calcium to available phosphorus (Ca:P) rather than the total calcium and available phosphorus levels in the ration was important in the expression of the condition. Kling (1985) reported that a significant correlation existed between the incidence of the malady and calcium to phosphorus ratio. The lowest incidence occured on the diet with highest calcium to phosphorus ratio.

Anderson et al. (1984) showed that chicks fed the high level of calcium carbonate at low phosphorus level developed rickets. Their bones were soft and they seldom moved and when they did move, they crawled. The condition was alleviated by the use of a similar diet having medium rather than fine particle size calcium. They pointed out that the calcium and phosphorus

inbalance was more when fine particle size of calcium source was fed. Atteh and Leeson (1984) observed that the incidence of leg abnormalities was higher among chicks fed diets supplemented with oleic acid and oleic palmitic acid mixture when compared to the control group. This was attributed in part to the formation of faecal soaps as a result of calcium and fat interactions, thus rendering the calcium unavailable for absorption. Atteh and Leeson (1984) corroborated the studies of Andrews et al. (1975) which demonstrated that the birds fed oleic acid gained more weight and developed a higher incidence of leg abnormalities as compared with the control group. Andrews et al. (1975) pointed out that the weight of broilers influences the incidence of leg abnormalities.

Halley et al. (1985) examined the effect of replacing available calcium with magnesium and increasing the anion content of the diet on chick performance and leg abnormalities. They found that there was sufficient correlation between tibia dyschondroplasia and bow legs with non-phytic phosphorus in the diet. Emebe and Robertson (1985) while agreeing with the findings of Halley et al. (1985) reported also that bone ash, breaking strength, bone weight and length were greater in the normal

chickens than these with the malady. Halley et al. (1985) reported that the incidence of bowed legs, tibia dischondroplasia and body weight gain increased when the ratio of cation to anion (Cation:anion) was 1.8:1. Further increase in anion did not affect this variable. They reported that, decreasing cation to anion ratio below 1.8 to 1.6:1 did not result in the expected decrease in incidence of leg abnormality. Edward (1986) found that addition of synthetic zeolite at 1.0 percent level in a high phosphorus diet significantly reduced the incidence and severity of tibia dyschondroplasia. Also, Hulan and Proundfoot (1986) noted that dibaisc calcium phosphate fed ad libitum increases the incidence and severity of tibia dyschondroplasia and total leg abnormalities.

The fragility of bones of caged layers frequently results in excessive bone breakage and splintering during processing. This was noted by Hellwing and Waldroup (1985) who examined the short term nutritional variables so as to determine possible improvement in bone strength or reduction in bone breakage. Hellwing and Waldroup (1985) fed diets containing non-phytic phosphorus at 0.25, 0.45 or 0.65 percent with 3.5 percent calcium and reported that birds fed 0.25 percent non phytic phosphorus had more bone broken during processing, and bone ash content was reduced. Increasing

sodium from 0.15 to 0.30 percent in the same diet increased bone weight and ash content. In another study, Hellwing and Waldroup (1985) reported that increasing the oystershell in the diet for 2 weeks before slaughter at 81 weeks of age decreased the number of broken bones during processing, In yet another study, nutrients were maintained in relation to energy content and they reported that increasing calcium levels increased bone weight and shear values and decreased bone breakage during processing. From the seven experiments carried out on this subject with spent layers Hellwing and Waldroup (1985) submitted that increasing the calcium level in the diet decreased bone breakage during processing and that feeding high levels of calcium (up to 7.5 percent) from either oystershell or ground limestone for 7-14 days prior to slaughter of spent layers resulted in increased bone strength and ash content, stronger shear values and decreased number of bones broken during processing with no adverse effects on egg production or other production parameters.

TRIAL 1. THE EFFECT OF LIMESTONE SOURCES ON THE PERFORMANCE OF BROILER CHICKS (0-4 WEEKS)

OBJECTIVE

The objective of this trial was to test the nutritional adequacy of limestone from six sources in broiler chick diets from 0-4 weeks with no consideration for the variations in the calcium contents of the various limestone sources.

MATERIALS AND METHODS

Five hundred and twenty five Anak-180 straight run day old broiler chicks were utilized in a completely Randomized Design to test the six limestone sources that were procured from Ashaka in Bauchi State, Calabar in Cross River State, Jakura (marble) from Kwara State, Sokoto from Sokoto State, Ukpila in Bendel State and Yandev in Benue State. These six limestone sources accounted for the six experimental diets and a control diet which had only bone meal as the source of calcium to make up the seventh experimental diet.

Birds were vaccinated at source against Newcastle disease. Upon arrival, chicks were randomly divided into 21 groups of 25 chicks each. Three of such groups were randomly assigned to each diet. The chicks were

housed in an open sided poultry house located at the university farm. The open sides were covered to conserve heat during the brooding period. Each pen measured 3.05 x 1.22 meters. All pens used had the floors covered with wood shavings and equiped with galvanized chick feeders and plastic waterers. All chicks per pen were weighed collectively at day old before the dietary treatments were introduced.

The ingredient composition of the experimental diets and the chemical analysis are presented in tables 3 and 4 respectively. The diets were formulated to be isocaloric and isonitrogenous in corn-groundnut cake basal ingredients. Dicalcium phosphate was utilized in diets containing the tested limestone sources to supply the phosphorus requirement. Bone meal was utilized in the control diet to supply the phosphorus and calcium requirements. Bone meal was utilized in the control diet because it is the commonest source of calcium and phosphorus in poultry diets in this country presently. The main source of variation in the test diets was the different limestone sources that made up the different treatments. The feed was mixed manually at the university farm, but great care was exercised in ensuring thorough mixing.

Table 3 : The composition of the experimental diets used in Experiment 1 (Broiler starter 0 - 4 Weeks).

	Bonemeal Control diet	Limestone sources					
		Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yande
Diets	1	2	3	4	5	6	7
Maize	50.90	50	50	50	50	50	50
G/nut cake	44.62	44.27	44.27	44.27	44.27	44.27	44.27
Bone meal	3.53	-	-	-	-	-	-
Dical phosphate	-	3.40	3.40	3.40	3.40	3.40	3.40
Limestone	-	1.33	1.33	1.33	1.33	1.33	1.33
Methionine	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated Analysis:							
Protein %	23.25	23.04	23.04	23.04	23.04	23.04	23.04
M.E. Kcal/kg	2880	2840	2840	2840	2840	2840	2840
Calcium %	1.25	1.25	1.25	1.25	1.25	1.25	1.25
Total Phos. %	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Meth. + Cyst. %	0.94	0.93	0.93	0.93	0.93	0.93	0.93
Lysine %	1.34	1.32	1.32	1.32	1.32	1.32	1.32
Ca:P ratio	1.25:1	1.25:1	1.25:1	1.25:1	1.25:1	1.25:1	1.25:1
Av. P %	0.45	0.44	0.44	0.44	0.44	0.44	0.44

Premix (ZOODRY) at the rate of incorporation in the diet supplied the following additional nutrient per kilogram of feed: Vitamin A, 10,000 I.U.; Vitamin D_3 1,020 I.U.; Vitamin E, 10 I.U.; Vitamin K, 4.02mg; Vitamin B_2, 9.00mg; Vitamin B_6, 4.00mg; Nicotinic acid, 50.00mg; Calcium D-pantothenate, 16.02mg; Vitamin B_{12}, 0.03mg; Vitamin C, 40.02mg; Choline chloride, 40.20mg; Manganese, 200.02mg; Iron, 100.02mg; Zinc, 80.02mg; Copper 4.80mg; Iodine 0.38mg; Cobalt, 0.41mg; Selenium, 0.108mg.

Table 4 : Proximate Analysis of the experimental diets used in Experimental 1 (Figures on dry matter basis).

		Limestone sources					
	Control	Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yandev
	1	2	3	4	5	6	7
Protein %	24.56	24.50	24.03	23.38	24.57	24.72	24.50
Calcium %	1.24	1.19	1.19	1.22	1.15	1.18	1.12
Ether extract %	6.20	5.91	6.01	5.75	5.87	5.95	5.65
Crude fiber %	4.67	5.01	4.84	4.56	5.12	4.89	4.97
Ash %	7.13	6.94	6.89	7.27	6.78	6.45	6.65
Dry matter %	95.25	94.84	95.67	96.13	94.95	94.89	96.10

Chicks were provided with feed and water ad libitum. The pens were heated with kerosine stoves throughout the four week study period. The experiment was carried out during the months of September to October which maybe considered to be cool months. Birds were weighed collectively per pen on weekly basis.

Bone and Blood sample collection

Three birds per treatment (one per replicate) whose weights were close to the pen average were selected for blood and bone sampling. Blood samplings and bone extractions were done in the Meat Science Laboratory.

Test tubes large enough to accomodate about 10mls of blood were marked against each dietary treatment. Few drops of anticoagulant (heparin) were put in each tube. Each bird was bled by severing the bracheal vein and blood gushing out was collected in the test tube until the tube was about two-thirds full. The tubes were each sealed with celophane paper and rocked gently to mix the blood and anticoagulant in the tubes. Each blood sample so collected was immediately immersed in ice block. When all blood samples were collected, they were immediately centrifuged (within an hour of collection, Njoku 1980)

at 750g for five minutes and the blood plasma that floated on top was aspired with the aid of a plastic syring into a sterile plastic tube and stored in the freezer for later analysis.

After blood sample collection, brids were finally sacrificed and the tibia excised. Bones were soaked in warm water containing a few drops of 2 percent sodium hydroxide and allowed to simmer (Ansah 1986). This was to facilitate easy removal of residual flesh from bones. After the flesh was removed, bones were dried at 105°C overnight in the oven, it was then allowed to cool and the bone weight and length were taken. The dried bones were defatted by soaking in diethyl ether for 72 hours, air dried and stored for ash determination. The dried bones were ashed at 600°C for 24 hours in a muffle furnance, cooled in a desicator and weighed. The ash was saved for ash calcium and phosphorus determination.

Blood Plasma and Ash analysis

Blood plasma was analysed for alkaline phosphatase, and plasma calcium. Plasma alkaline phosphatase was analysed by the colorimetric method (King and King 1954), using 0.01M disodium phenol phosphate substrate in a solution of sodium carbonate and sodium bicarbonate buffer and the activity was read with the aid of

Colorimeter MODEL 252. The principle is that the phenol released by the enzyme hydrolysis from phenol sulphate at pH 10.0 reacts with alkaline oxidizing agent, 4 - amnophenazone to give a red color, the intensity of which is proportional to the activity of the enzyme.

The plasma calcium analysis was carried out as outlined by Baginski et al. (1973) with the aid of Spectrophotometer Model PYE UNICAN SP6-300. The principle is that, the calcium in a sample solution reacts with a solution containing O-Cresolphthalein complexone and 8-hydroxy quinoline in dilute hydrochloric acid. A red complex is formed between calcium and crisolphthalein complexone at a pH between 10-12 which is maintained by the addition of diethlamine buffer. The 8-hydroxyquinoline contained in the reagent binds magnesium and prevent the interference from this ion.

Calcium content of the ash was determined by atomic absorption spectrophotometer Model 290B following wet digestion method using a mixture of nitric acid, Perchloric acid and sulphuric acid as outlined by Perkin-Elmer Corp. (1968). The ecess solution left over from the wet digestion was used in determining the phosphorus content of ash using the Vanadomolybdate (Yellow method) as outlined by Hasse (1971).

Data collected were summarized and analysed statistically and where possible separation of means was carried out using Duncan's Multiple Range Test as outlined by Steel and Torrie (1980).

RESULTS

The results obtained from this experiment are summarized in tables 5, 6, 7).

The parameters on weight gain, feed intake, feed efficiency etc. are summarized in table (5). The statistical analysis of the data on body weight gain, feed intake and feed to gain ratio (feed efficiency) did not show significant differences among the dietary treatments. Figures on leg weakness (tibia dyschondroplasia) and mortality rate were calculated but were not analysed statistically to test for significant difference among treatments means.

Tibia weights, tibia lengths and tibia ash percent and the ratio of bone to body weight (table 6) did not show any statistical differences when analysed. Tibia ash weight, bone ash calcium and ash phosphorus showed significant differences among treatment means. Diet 1 gave the highest ash weight (2.17g) followed by diets 2 and 4 (1.99 and 1.95g). Diet 3 produced the least ash weight (1.58g). Percent calcium in the tibia ash was highest in diets 6 and 7 (34.95 and 34.81 percent)

Table 5 : The effect of Limestone sources on the performance of broiler starter chicks fed from 1 - 28 days of age.

Diet No	Limestone source	Total wt. gain/bird (g)	Total feed intake/bird (g)	Feed efficiency g feed/gain gain	Leg deformity (%)	Mortality (%)
1	Control	630.00	1151	1.83	1.3	9.3
2	Ashaka	632.00	1141	1.80	1.3	8.0
3	Calabar	624.32	1159	1.86	1.3	4.0
4	Jakura	611.00	1098	1.80	1.3	4.0
5	Sokoto	638.68	1196	1.87	2.7	8.0
6	Ukpila	648.32	1170	1.81	1.3	9.3
7	Yandev	622.68	1161	1.86	4.0	8.0
	*SEM	6.66	13.55	0.02	-	-

Means within a column are not statistically significant ($P > 0.05$)

*SEM = Standard error of the means.

Table 6 : Effect of Limestone sources on tibia bone parameters of broiler chicks at 28 days of age.

Diet No.	Limestone source	Bone wt. (g)	Bone length (cm)	Ash wt. (g)	% Ash	Ash calcium (%)	Ash phosphorus (%)	Ca : P ratio
1	Control	4.28	6.92	2.17^{a}	51.18	32.78^{c}	16.03^{ab}	2.05:1
2	Ashaka	4.41	6.99	1.99^{ab}	47.12	31.54^{f}	15.86^{ab}	1.99:1
3	Calabar	2.88	6.73	1.58^{e}	55.11	32.03^{d}	16.86^{ab}	1.90:1
4	Jakura	3.85	7.12	1.95^{ab}	49.01	31.80^{e}	15.33^{b}	2.07:1
5	Sokoto	4.61	7.02	1.74^{bc}	38.00	33.24^{ab}	16.85^{ab}	1.97:1
6	Ukpila	3.62	6.93	1.79^{bc}	49.91	34.81^{a}	17.02^{a}	2.05:1
7	Yandev	3.75	7.21	1.77^{bc}	47.21	34.95^{a}	16.54^{ab}	2.11:1
*	SEM	0.37	0.18	0.10	4.17	0.03	0.49	-

Means within a column with similar letter superscript or without superscripts are not significantly different ($P > 0.05$)

*SEM = Standard error of the means.

and least in diet 2 (31.54 percent). Percent phosphorus in the tibia ash in diet 6 was significantly ($P < 0.05$) superior to that of diet 4 but was not significantly ($P > 0.05$) higher than the other diets.

All dietary treatments did not result in statistical differences among each other for plasma calcium and alkaline phosphatase (table 7).

Correlations

Ash weight showed a positive and significant ($P < 0.05$) correlation with tibia weight (table 8). Ash calcium also showed a positive but not significant relationship with calcium intake. Plasma alkaline phosphtase was inversely related to bone calcium but the relationship was not significant.

Table 7 : The effect of Limestone sources on blood parameters of broiler chicks at 28 day of age.

Diet No	Limestone source	Plasma calcium mg/dl	Plasma alkaline phosphatase IU/l
1	Control	9.29	653.20
2	Ashaka	9.87	702.90
3	Calabar	10.25	667.90
4	Jakura	9.93	672.13
5	Sokoto	9.11	655.57
6	Ukpila	9.63	686.33
7	Yandev	9.75	686.33
	*SEM	0.60	12.71

Means within a column are not significantly different ($P > 0.05$)

*SEM = Standard error of the means.

Table 8 : Correlations between some parameters in Trial 1.

	Ash weight	Ash Calcium
Calcium intake	-	0.664
Plasma calcium	-	0.638
Alkaline phosphate	-	-0.128
Bone weight	0.945*	-

* Significant ($P < 0.05$)

Tabulated r value at $P < .05$ = 0.754

DISCUSSIONS

The weight gain in experiment 1 did not show significant differences among treatments. In this country, data on a similar study to enable comparison are scarce. Elsewhere however, Waldroup et al. (1964) tested the suitability of several calcium sources (reagent) calcium carbonate, oystershell, calcium sulphate and ground limestone for inclusion into broiler chick diet from day old to 28 days of age. They reported that there were no significant differences among sources when body weight was the criterion for evaluation. Anderson et al. (1984) also could not demonstrate the effect of limestone sources on weight gain. However, they speculated that the lack of significant effect might be due in part to the higher level of the basal calcium in the diet. The basal calcium level in their diets was five times that of the test sources. In this experiment, the basal calcium level furnished about 1.5 times more calcium than the test sources. According to Simco and Stephenson (1961), Formica et al. (1962) and Waldroup et al. (1964), calcium requirement for growth of broiler chicks is not more than 0.6 percent. The amount of calcium contributed by the basal diet was up to 0.75 percent and only 0.5 percent calcium came from the test sources. Dicalcium phosphate was incorporated to satisfy mainly the phosphorus requirements, the calcium from this source accounting for

most of the basal calcium. Since dicalcium phosphate is known to be highly available to chicks, it is possible that much of the calcium required for growth in this experiment was supplied from this source rather than the tested sources, hence the lack of significant effect among treatment means as reported by workers cited above. These weights compared favourably with the average weights obtained on the conventional (control) diets in this area (Dogari 1985, Nelson 1984 and Offiong 1984).

Feed intake was not significantly influenced by dietary treatment. It is possible that at the level of inclusion in the diets, the quanties of the limestone sources were not large enough to produce a significant effect on feed intake.

Feed efficiency was also not influenced by dietary treatments. Figures on leg abnormalities were calculated and were not analysed statistically since leg disorders were not distributed throughout all the replicates of each dietary treatment to enhance statistical analysis. Veltmann and Jensen (1981) pointed out that several factors apart from nutrition (disease organisms and genetic traits) have been incriminated as predisposing factors to the condition of leg weakness in chicks, but they maintain that the etiology of this malady is still

not fully understood.

The percentage mortality was calculated and not analysed statistically. Birds in this experiment were vaccinated at source. After only few days on the experimental treatments, birds in all treatments came donw with a respiratory problem which was later traced to vaccination procedure at the source of chicks. Some chicks died as a result of this problem while majority responded to treatment.

Bone (tibia) weight was not influenced significantly ($P > 0.05$) by dietary treatments. Ash weight showed significant variations among treatment means. However, the percent ash content of the tibia bones were not influenced by dietary treatments. Since ash weight represent the mineral or inorganic constitutents of the bone, it could be a better measure of the mineral status of bone. It is possible that the defattening method used in these studies were not as good as conventional method of ether extraction, and bones may still contain more organic matter than was though possible (See Appendix - Fat extraction methods). Bone lengths did not show significant differences among treatment means. This is a good indication of the uniformity of birds selected for sample taking from different dietary treatments.

The percent calcium in the tibia bone ash showed significant variation among dietary treatments but the pattern of variation was not as reflected in the ash weight. However, the percent calcium in the tibia (bone) ash of all dietary treatments compares favourably with the values reported by Hulan et al. (1985) for broiler chicks of this age group. Percent phosphorus in the tibia ash also resulted in significant differences among treatment means but the values observed in all the treatment means reached average value reported by Maynard and Loosly (1962). The ratio of calcium to phosphorus in the bone (2:1) as reported by Preston et al. (1977) was essentially attained in this study.

Conclusion

The birds in this trial did not develop any ill effects that could be attributed to the sources of limestone. The performance of the birds fed diets containing limestone from the sources was comparable to that of the control diet. If these birds utilized the calcium originating from the limestone sources in actuality, it can be concluded that all the sources of the limestones tested are fit for inclusion into broiler chick rations.

TRIAL 2. THE EFFECT OF LIMESTONE SOURCES ON THE PERFORMANCE OF BROILER FINISHERS (5-8 Weeks).

INTRODUCTION

Some researchers have limited their studies on mineral nutrition of broiler chicken to the starter stage of between zero and 28 days. This is usually because of the assumption that the pattern of performance of the broiler chick is usually established at this stage and chicks are expected to respond in this manner through to market weight.

However, many studies have shown that broiler chickens do exhibit compensatory growth (Washerburn 1979, Hulan et al. 1980, Marks, 1978, and Moran 1981).

OBJECTIVE

In view of the apparent success of the first trial with broiler starter chicks 0-4 weeks, another trial was carried out with the broiler finisher chicks to ascertain the suitability of the procured limestone sources on broiler finishers (5 to 8 weeks). The following parameters were utilized for evaluation: growth rate, efficiency of feed utilization as well as bone and blood parameters.

MATERIALS AND METHODS

Four hundred and sixty two (462) Anak-180 broiler chicks of the starter stage experiment were utilized. At the end of the starter stage experiment, all the chicks were fed a common (control) diet for 7 days. At the end of this period, all chicks were mixed up and reassigned at random to 21 pens used in the previous study in such a way that each pen now had 22 chickens. Three of such pens were assigned at random to the seven dietary treatments.

The covered sides of the pens were removed to allow for cross ventilation in the poultry house and the chick feeders and drinkers were replaced with grower feeders and large waterers. The compositions of the experimental diets used in this trial are shown in tables 9 and 10. Bone and blood samples were collected at the end of the study and analysed according to the procedures outlined in trial 1. Data collected were summarized and analysed statistically and where necessary, separation of means was carried out using Duncan's Multiple Range Test as outlined by Steel and Torrie (1980).

Table 9 : The composition of the experimental diet used in Trial 2 (broiler finisher 5 - 8 Weeks of age.

	Bone meal control diet	Limestone sources					
		Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yan
Deit No	1	2	3	4	5	6	7
Corn	60.59	59.57	59.57	59.57	59.57	59.57	59.
G/nut cake	36.00	36	36	36	36	36	36
Bone meal	2.52	-	-	-	-	-	-
Dical Phos.	-	2.54	2.54	2.54	2.54	2.54	2.
Limestone	-	1.0	1.0	1.0	1.0	1.0	1.
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.
Lysine	0.15	0.15	0.15	0.15	0.15	0.15	0.
Salt	0.30	0.30	0.30	0.30	0.30	0.30	0.
Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.
Calculated Analysis:							
Protein %	20.30	20.25	20.25	20.25	20.25	20.25	20.
ME Kcal/kg	3008	2973	2973	2973	2973	2973	297
Calcium %	0.99	1.03	1.03	1.03	1.03	1.03	1.
Total P %	0.82	0.82	0.82	0.82	0.82	0.82	0.
Meth + Cyst. %	0.79	0.80	0.80	0.80	0.80	0.80	0.
Lysine %	1.02	1.01	1.01	1.01	1.01	1.01	1.
Ca:P ratio	1.21:1	1.26:1	1.26:1	1.26:1	1.26:1	1.26:1	1.26
Av. P %	0.27	0.27	0.27	0.27	0.27	0.27	0.

*The Premix (ZOODRY) at the rate of incorporation in the diet supplie the following additonal nutrient per kilogram of feed: Vitamin A, 11,012, I.U., Vitamin D_3, 4,020 I.U., Vitamin E, 20 I.U. , Vitamin K, 4.02mg, Vitamin B_2, 9.00mg, Vitamin B_6, 6.00mg, Nicotinic acid, 50mg, Calcium D-pantothenate, 1602mg, Vitamin B_{12}, 0.03mg, Vitamin C, 40.02mg, Choline chloride, 40.02mg, Manganese, 200.02mg, Iron, 100.02mg, Zinc, 80.02mg, Copper, 4.80mg, Iodine, 2.81mg, Cobalt 0.41mg, Selenium, 1.08mg.

Table 10 : Proximate analysis of the diets fed during broiler finisher study for Trial 2 (Figures on dry matter basis).

	Bone meal Control diet	Limestone sources				
		Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yandev
Diet No	1	2	3	4	5	6	7
Protein %	21.14	20.76	20.83	20.94	20.85	21.06	21.23
Calcium %	1.03	1.00	1.10	1.16	0.99	1.03	1.13
Ether extract	7.18	6.94	6.87	7.27	6.79	6.80	7.04
Crude fiber	3.94	4.06	3.41	3.76	3.81	4.14	3.89
Ash	8.51	9.01	10.11	8.68	8.43	9.35	8.96
Dry matter	95.89	96.18	94.97	94.55	96.21	95.61	94.96

RESULTS AND DISCUSSIONS

The results obtained on weight gain, feed intake, feed efficiency, leg disorders and mortality rate as presented in table 11. The analysis of variance on weight gain did not show significant differences among the various treatment means. The superior weight gain of birds fed diets 6, 2 and 1 in that order were only numerical. The desirable average final weight of 1.80kg for an eight week broiler rearing period professed by a body of literature was not attained in this experiment (the minimum weight was 1.60kg while the maximum weight recorded was 1.70kg). This may have been so because the chicks had an attack of a respiratory disease earlier (See trial 1).

Feed intake in this experiment was not influenced by dietary treatment. It is possible that the limestone sources at the levels of incorporation was not large enough to cause any significant effect on feed intake.

Feed efficiency (the amount of feed consumed per unit of gain) was not influenced by dietary treatment and this is in agreement with the studies of Pavic (1974) who found no significant effect of limestone on Hybro broilers reared to 56 days of age. The level of feed efficiencies obtained on all treatment means in this study also agrees with the World Bank Report (1983) which stated that "feed use in broilers should be kept on no more than 2.5 kilos of feed per kilo of live poultry".

Table 11 : The effect of Limestone sources on the performance of broiler finisher chickens between 5 - 8 Weeks of age.

Diet No	Limestone source	Total wt. gain/bird (g) 28 days	Total feed intake/bird (g) 28 days	Feed efficiency g feed/g gain	Leg disorder (%)	Mortality (%)
1	Control	780.33	2013.33	2.58	7.6	1.5
2	Ashaka	782.34	1898.01	2.43	1.5	1.5
3	Calabar	668.01	1746.33	2.61	3.0	3.0
4	Jakura	776.01	1933.68	2.49	4.5	3.0
5	Sokoto	764.34	1948.32	2.55	1.5	6.1
6	Ukpila	792.33	1998.99	2.52	1.5	1.5
7	Yandev	761.01	1906.32	2.50	3.0	3.0
*	SEM	16.08	28.52	0.03	-	-

Means within a column are not significantly different ($P > 0.05$)

*SEM = Standard error of the means.

Leg disorders and mortality rates were calculated but were not analysed statistically. The leg disorders that occured in this trial on diet 1 (control) were a bit high (7.6 percent). However, this diet was a control diet and not the test diets for limestone sources. Leg disorders were not distribured throughout all the replicates.

Mortality occured in all treatments in this trial but the figures were generally low. This trend was not observed for diet 5 during the second broiler finisher experiment.

Means for bone weight, tibia ash weight, percent tibia ash, tibia length, percent calcium in the tibia ash and bone to body weight ratio are presented in table 12.

Significant differences among treatment means occured in tibia bone weight. Diet 2 produced superior bone weights which were not significantly different from bones on dietss 3 and 6. Diets 1, 3, 4, 5 and 6 were not significantly different from each other, and diets 1, 4, 5 and 7 did not differ significantly. Tibia ash weight did not show significant differences among the treatment means but the numerical superiority of the treatment means for ash weights followed a similar trend like the tibia weights.

It is possible that the significant differences that occured among the treatment means in the tibia weights were due to organic matter that sill persisted in residual these bones since the significant differences disappeared on ashing the bones. Duckworht and Hill (1953) pointed out that ash weight of bones gave a more satisfactory results as for assessing the mineral content of bones than bone weight and ash percent. There were no significant differences in bone ash percent. Duckworth and Hill (1953) concluded that ash percentage and ash weight together are better criteria for assessing changes in minerals status of the adult bone than either alone.

Bone length did not show any significant ($P > 0.05$) differences among treatment means. Njoku (1980); Smith and Kabaija (1985) did not find significant differences in tibia length for different calcium and phosphorus regimens. However, Smith and Kabaija (1985) reported that the volume of bones from birds fed high calcium but low phosphorus diet was higher than those fed low calcium diets, indicating the greater thickening of bone in the former dietary treatment. This in effect means that bone length is not a good criterion for assessing calcium adequacy.

Table 12 : The effect of limestone sources on the tibia bone parameters of broiler finisher at 8 Weeks of age.

Diet No	Limestone source	Bone wt (g)	Bone length (cm)	Ash wt. (g)	Ash %	Ash calcium (%)	Ash phosphorus (%)	Bone Ca: P ratio
1	Control	14.60^{bc}	11.21	5.45	37.41	31.88^{bc}	16.29	1.96:1
2	Ashaka	17.41^{a}	11.40	6.32	36.21	31.03^{c}	17.59	1.77:1
3	Calabar	15.14^{ab}	10.87	5.98	39.41	32.38^{b}	16.54	1.96:1
4	Jakura	15.06^{bc}	11.47	5.67	37.93	32.90^{b}	17.06	1.93:1
5	Sokoto	13.84^{bc}	11.35	5.36	36.79	33.98^{a}	17.59	1.93:1
6	Ukpila	15.85^{ab}	11.31	5.96	37.64	33.97^{a}	18.13	1.87:1
7	Yandev	12.96^{c}	10.73	5.45	42.53	34.03^{a}	17.06	2.00:1
*	SEM	0.72	0.19	0.33	2.20	0.014	0.516	-

Means within a column with similar letter superscipts or without superscripts are not significantly different ($P > 0.05$).

*SEM = Standard error of the means.

The percent calcium in the tibia bone ash showed significant differences among treatment means. Since the limestone samples that constituted treatments came from different deposits, these differences in treatment means could be attributed to the sources.

The percent phosphorus in tibia ash did not show significant variations among the treatment means. Data on both the plasma calcium and plasma alkaline phosphatase are summarized in table 13. Plasma calcium showed significant differences among treatment means. The birds fed diets 1, 5 and 7 had more plasma calcium than those birds fed diet 6. The birds fed diet 6 had the least plasma calcium.

Plasma alkaline phosphatase did not show any significant differences among treatment means. Alkaline phosphatase is associated with bone calcification and functions in the transfer of phosphatase to the site of calcification. Georgivskii (1982) reported that the content of alkaline phosphatase in the blood plasma is proportional to the activity of the osteoblast. It has been reported that alkaline phosphatase enzyme deteriorates with time due to poor storage (Motzok and Waynne 1960), this may explain why the levels of alkaline phosphatase in this experiment were lower than those in the second experiment since the plasma samples were stored for a long time and were

Table 13 : The effect of Limestone sources on blood parameters of broiler finisher chicken at 8 week of age.

Diet No	Limestone source	Plasma calcium mg/dl	Plasma alkaline phosphatase iu/l
1	Bone meal control diet	12.05[a]	485.17
2	Ashaka	10.49[b]	449.67
3	Calabar	10.48[b]	440.30
4	Jakura	10.74[bc]	537.00
5	Sokoto	11.35[ab]	503.83
6	Ukpila	10.12[c]	508.17
7	Yandev	11.06[ab]	508.17
*	SEM	0.28	26.34

Means within a column with similar letter superscripts or without superscripts are not significantly different ($P > 0.05$).

*SEM = Standard error of the means.

analysed at the same time as those in all other experiments.

Conclusion

In view of the fact that the pertinent parameters of growth rate, efficiency of feed utilization, leg disorders and mortality rate which are usually monitored in assessing the performance of broiler chickens did not show significant ill-effect that could be attributed to the limestone sources it is concluded that all the limestone sources tested were good for inclusion into the broiler finisher rations.

TRIAL 3. FURTHER STUDIES ON THE EFFECTS OF LIMESTONE SOURCES ON THE PERFORMANCE OF BROILER CHICKS (0 - 4 Week).

INTRODUCTION

In the previous two trials, it was assumed that all the procured limestone sources contained the optimal or near optimal level of calcium content (38 percent). Gross studies were therefore conducted to ascertain this fact. The results of the first two trials did not show any noticable harmful effects that could be attributed to the limestone sources. It was reasoned that possibly, the dicalcium phosphate which was incorporated in the test diets to furnish the phosphorus requirement might have also supplied enough calcium for the need of birds while little use may have been made of calcium originating from the procured limestone sources incorporated into the diets.

The limestone sources were therefore analysed chemically to ascertain the actual calcium content of each source and to determine if there could be other associated minerals in quantities that maybe toxic to the broiler chickens at the levels of incorporation in the diets. The limestone sources were incorporated in the dietary treatments according to their calcium content. The level of dicalcium phosphate (phosphorus) was reduced to allow for the incorporation of more limestone in each diet so that the effect of each

limestone source (if any) may become more apparent.

OBJECTIVE

The objective of this study was to re-evaluate the effects of limestone sources on the broiler starter chicks (0-4 weeks) employing the same parameters as per trial 1.

MATERIALS AND METHODS

Three hundred and fifteen Anak-180 broiler chicks obtained from the same source as the previous experiments were utilized in a completely Randomized Design such that each treatment group was assigned 45 chicks. There were three replications per treatment and each replicate had 15 chicks. Birds were wingbanded and vaccinated against Newcastle disease (via intraoccular route) upon arrival on the farm. All birds were weighed individually before assigning them at random to the dietary treatments. The pens were heated at night with 100 watts electric bulbs.

The composition of the experimental diets is presented in Table 14. Each consisted of corn-soyabean sources was determined (by the Ballapur Glass Company Kaduna) and incorporated in the diet according to their calcium contents. The present phosphorus level in each diet was reduced from 1.0 to 0.8 while that of calcium

Table 14 : Composition of the broiler starter diets used in trial 3. (0 - 28 days of age)

		Limestone sources					
	Control	Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yand
Diet No	1	2	3	4	5	6	7
Corn	56.15	55.44	55.41	55.48	55.42	55.45	55.4
Soyabean meal	40	40	40	40	40	40	40
Bone meal	2.66	-	-	-	-	-	-
Oystershell	0.46	-	-	-	-	-	-
Dical PO4	-	2.17	2.17	2.17	2.17	2.17	2.17
Limestone	-	1.66	1.69	1.62	1.68	1.65	1.66
Methionine	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix*	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Calculated Analysis:							
Crude protein %	23	22.99	22.99	22.99	22.99	22.99	22.99
ME Kcal/kg	2950	2926	2925	2927	2925	2926	2926
Calcium %	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Total P	0.80	0.80	0.80	0.80	0.80	0.80	0.80
Ca:P ratio	1.50:1	1.50:1	1.50:1	1.50:1	1.50:1	1.50:1	1.50:1
Meth + Cyst.	0.93	0.93	0.93	0.93	0.93	0.93	0.93
Lysine	1.45	1.45	1.45	1.45	1.45	1.45	1.45

*The Premix (ZOODRY) at the rate of incorporation in the diet supplied the following additional nutrient per kilogram of feed: Vitamin A, 10,000 I.U.; Vitamin D_3, 1,020 I.U.; Vitamin E, 10 I.U.; Vitamin K, 4.02mg; Vitamin B_2, 9mg; Vitamin B_6, 4.00mg; Nicotinic acid, 50mg; Calcium D-pantothenate, 16.02mg; Vitamin B_{12} 0.03mg; Vitamin C, 40.02mg; Choline chloride 40.20mg; Manganse 200.02mg; Iron 100.02mg; Zinc, 80.02mg; Copper 4.80mg; Iodine, 2.81mg; Cobalt, 0.41mg; Selenium, 1.08mg.

Table 15: Proximate analysis of the diets - Trial 3 (Figures on dry matter basis)

Diet No	Bone meal control 1	Limestone sources					
		Ashaka 2	Calabar 3	Jakura 4	Sokoto 5	Ukpila 6	Yandev 7
Protein %	24.44	24.91	24.63	24.69	24.72	24.98	24.13
Calcium %	1.30	1.31	1.26	1.26	1.24	1.27	1.22
Ether extract %	5.88	6.91	5.18	6.01	6.21	5.95	5.50
Crude fiber %	2.96	2.68	3.12	2.73	3.25	2.82	3.64
Ash %	7.50	7.25	7.73	7.44	9.68	8.72	8.20
Dry matter %	94.58	94.57	94.40	94.56	94.71	94.07	94.54

was maintained. Oystershell was therefore employed to balance the calcium requirement of the control diet. Feed and water were supplied ad libitum. Birds were weighed individually at the end of each week and at the end of the four week trial period, the sex of each bird was determined using the comb and wattle development. Blood and bone samples, and blood and ash analysis were carried out as outlined in trial 1.

Data collected were summarized and analysed statistically and where necessary, separation of means was carried out using the Duncan's Multiple Range Test as outlined by Steel and Torrie (1980).

RESULTS

In this experiment, feed intake and feed to gain ratio (feed efficiency), table 16) did not show any statistical significance ($P > 0.05$) among the dietary treatments means. However, growth rate showed statistical significance among treatment means. Birds fed diet 1 (control) grew significantly ($P \not> 0.05$) faster than birds fed diets, 3, 5 and 7. The superior growth rate of birds fed diet 1 over those fed diets 2, 4 and 6 was only numerical and did not show any statistical significance. Morevoer, these birds fed diets 2, 4 and 6 were not statistically different from those birds fed diets 3, 5 and 7.

Table 16 : Effect of limestone sources on the performance of broiler chicks (1 - 28 day of age) 3rd broiler study.

Diet No	Limestone source	Total wt gain/ bird (g) 28 days	Total Feed intake/ bird (g) 28 days	Feed efficiency g feed/g gain	Leg disorder %	Mortality %
1	Control	596.12[a]	1118.00	1.81	0	-
2	Ashaka	536.12[ab]	999.32	1.86	0	-
3	Calabar	503.48[b]	986.00	1.96	0	2.2
4	Jakura	535.48[ab]	970.32	1.83	0	2.2
5	Sokoto	524.88[b]	1004.32	1.91	0	4.4
6	Ukpila	571.48[ab]	1034.00	1.80	0	-
7	Yandev	515.56[b]	986.32	1.92	0	-
*	SEM	5.14	8.98	0.05	-	-

Means within a column with similar letter superscripts or without superscripts are not significantly different ($P > 0.05$).

*SEM = Standard error of means.

Table 17 : Effect of limestone sources on tibia bone parameters of broiler at 28 days of age .

Diet No	Limestone source	Bone wt (g)	Bone length (cm)	Ash wt. (g)	% Ash	Ash Calcium	Ash phosphorus %	Ca : P
1	Control	5.17	6.73	1.90	37.02	30.28^{d}	16.60^{b}	1.84 : 1
2	Ashaka	5.16	6.78	1.78	34.59	32.28^{a}	17.06^{b}	1.89 : 1
3	Calabar	5.41	6.70	1.96	36.22	31.70^{ab}	17.05^{b}	1.86 : 1
4	Jakura	4.90	6.51	1.79	36.53	29.39^{d}	14.69^{c}	2.00 : 1
5	Sokoto	5.98	6.86	2.25	37.52	31.00^{c}	15.53^{c}	2.00 : 1
6	Ukpila	5.68	6.97	1.91	33.38	30.53^{bc}	16.54^{b}	1.85 : 1
7	Yandev	4.72	6.44	1.74	37.02	30.51^{bc}	18.40^{a}	1.66 : 1
*	SEM	0.30	0.22	0.18	2.44	0.07	0.29	-

Means within a column with similar letter superscripts or without superscripts are not significantly different ($P > 0.05$).

*SEM = Standard error of means.

Table 18 : The effect of limestone sources on blood parameters of broiler chicks (at 28 days of age) 3rd broiler study.

Diet No	Limestone source	Plasma calcium mg/dl	Plasma alkaline phosphatase IU/l
1	Control	9.37	1888.6
2	Ashaka	9.75	1796.30
3	Calabar	10.36	1952.50
4	Jakura	9.74	2059.00
5	Sokoto	9.27	1917.00
6	Ukpila	9.13	1980.90
7	Yandev	9.13	1909.90
*	SEM	0.33	51.07

Means within a column are not significantly different ($P > 0.05$).

*SEM = Standard error of means.

The results on the analysis of bone parameters are shown in Table 17. Tibia bone weight, ash weight, ash percent and bone length, did not show significant differences among the treatment means. However, percent calcium and percent phosphorus in bone ash showed significant differences among treatment means. The percent calcium in the bone ash was highest in diet 2 but did not differ from that of diet 3. Diets 3, 6 and 7 were not statistically different from each other. Diets 5, 6 and 7 differed only from diets 1 and 4. Statistical analysis of plasma calcium and alkaline phosphatase were not significantly different between treatment means. (Table 18).

CORRELATIONS

Ash weight showed in significantly ($P<0.05$) positive correlation with tibia weight. Plasma alkaline phosphatase was inversly related to bone calcium and this inverse relationship was significant ($P<0.05$).

DISCUSSIONS

Significant differences in weight gain occured among the dietary treatments. These differences are believed to represent real treatment effect on weight gain, for the different limestone sources were analysed

Table 19 : Correlations between some parameters in experiment 3. The effects of some limestone sources on the performance of broiler chicks (28 days of age).

	Ash weight	Ash calcium
Bone weight	0.889*	-
Plasma calcium	-	0.348
Alkaline phosphatase	-	-0.801*

*Significant (P/0.05)

Tabluted r valur at P/0.05 = 0.754.

and incorporated into the diets according to their calcium contents. Moreover, the calcium to phosphorus ratio was widened to incorporate more limestone sources in the different diets. The calcium being contributed by the test sources furnished about 1.4 times more calcium than that contributed by the basal diet (by calculations). The growth rate of birds in this study was slightly less than those in trial 1. However, the average weight gain of birds obtained in this study compares favourably with that described as "good growth" by Shard et al. (1979) that is, 500g versus 550g. Feed intake was not influenced significantly ($P > 0.05$) by diets.

Feed efficiency was not influenced significantly by dietary treatments. Birds in this trial did not develop any visible signs of leg abnormalities through out the 28 day study period. The percentage mortality was calculated and was not subjected to analysis of variance because mortality figures did not reflect dietary influence since deaths that occured were not distributed throughout the replicates of the various diets. Most of the deaths occured when birds were still under two weeks of age. Farrell et al. (1973) suggested that mortality measurements should start at two weeks of age

to remove hatching and other effects that are independent of dietary treatments.

Bone weight, ash weight, tibia ash expressed as percent of the whole bone, and tibia length, all did not respond significantly to dietary treatments. The percent calcium in the tibia ash showed significant variations among diet means but were in general agreement with the values reported by Hulan et al. (1985). The percent phosphorus in the tibia ash also showed significant variations among treatment means but generally agreed with the known values for chickens.

Conclusion

The apparent lack of ill effects on the starter chicks (0 - 4 weeks) that can be attributed to the limestone sources is in agreement with the findings of trial 1 that the limestone sources tested are in effect suitable for use in the diets of this class of poultry.

TRIAL 4 . Further studies on the effects of limestone sources on the performance of broiler finishers (5 - 8 weeks of age).

INTRODUCTION

The apparent success of the previous broiler finisher study utilizing the limestone sources as test material necessitated a repeat of such a study to further ascertain whether these sources could be justifiably certified good and safe for inclusion into the broiler finisher diets. The limestone sources used in this study were chemically analysed as in trila 3 before they were incorporated in the diets.

OBJECTIVE

The trial was carried out to determine whether the incorporation of the analysed limestone sources could produce any noticable adverse effect on the broiler finisher (5-8 weeks) using similar parameters as per the other broiler studies.

MATERIALS AND METHODS

Two hundred and ninety four Anak - 180 broiler chicks that had been used in trial 3 were utilized in a Completely Randomized Design to evaluate these

limestone sources on the broiler finisher stage. Birds were separated into sexes before the start of the trial using the comb and wattle development. Each treatment group had equal number of males and females to eliminate variations in feed intake and growth rate due to sexes. As in the previous trials, there were seven dietary treatment groups and each treatment group was made up of 42 birds. There were three replicates per treatment and each replicate group had 14 birds. The covered sides of the poultry house were removed to allow for cross ventilation of the poultry house and the chick feeders and waterers were replaced with grower feeders and large waterers. The composition of the experimental diets are shown in Tables 20 and 21.

Bone and blood samples were collected at the end of the study and analysed as outlined in trial 1. Data collected were summarized and analysed statistically and separation of means was carried out using Ducan's Multiple Range Test as described by Steel and Torrie (1980).

Table 20 : Composition of the experimental diet used in trial 4 (broiler finisher 5 - 8 weeks).

		Limestone sources					
	Control	Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yandev
		Diet No					
Ingredients	1	2	3	4	5	6	7
Corn	62.86	62	61.98	62.03	62.0	62.01	62.0
Soyabean meal	33.0	33.0	33.0	33.0	33.0	33.0	33.0
Bone meal	2.50	-	-	-	-	-	-
Oystershell	0.61	-	-	-	-	-	-
Dical phosphate	-	2.52	2.52	2.52	2.52	2.52	2.52
Limestone	-	1.45	1.47	1.42	1.45	1.44	1.45
Methionine	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix*	0.63	0.63	0.63	0.63	0.63	0.63	0.63
Calculated analysis:							
Protein %	20.12	20.04	20.04	20.04	20.04	20.04	20.04
ME Kcal/kg	3041	3011	3010	3012	3011	3011	3011
Calcium %	1.13	1.13	1.13	1.13	1.13	1.13	1.13
Total P %	0.82	0.84	0.84	0.84	0.84	0.84	0.84
Ca : P ratio	1.40:1	1.35:1	1.35:1	1.35:1	1.35:1	1.35:1	1.35:1
Meth + Cys. %	0.83	0.83	0.83	0.83	0.83	0.83	0.83
Lysine	1.24	1.24	1.24	1.24	1.24	1.24	1.24

*The Broiler Premix (Peter Hand) at the rate of inclusion in the diet supplied the following per kilogram of feed : Vitamin A, 11,630 IU; Vitamin D_3, 3,876 IU; Vitamin E, 14 IU; Vitamin K_3 1.94mg; Vitamin B_1, 0.97mg; Vitamin B_2 7.75mg; Pantothenate acid 9.69mg; Nicotinic acid, 29.08mg; Vitamin B_6, 1.94mg; Vitamin B_{12}, 2mg; Folic acid 0.97mg; Biotin, 72.69mg; Copper, 4.85mg; Iron, 14.5mg; Manganese, 67.85mg; Selenium, 0.15mg; Zinc, 58.15mg.

Table 21 : Proximate analysis of the diet for Trial 4
(dry matter basis)

Diet No.	Bone meal Control	Limestone sources				
		Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yandev
Diet No.	1	2	3	4	5	6	7
Protein %	21.5	21.0	21.0	21.75	21.2	21.30	20.88
Calcium %	1.13	1.15	1.03	1.04	0.93	1.16	1.18
Ether extract %	3.99	3.40	3.59	3.7	3.19	3.56	3.74
Crude fiber %	2.81	2.86	2.95	3.16	3.14	2.71	3.17
Ash %	8.71	9.01	10.11	8.11	7.97	8.19	8.27
Dry matter %	96.12	95.75	94.86	95.14	95.61	95.92	94.90

RESULTS AND DISCUSSION

There were no significant treatment effect on weight gain (Table 22) but birds reached the 1.80kg wiehg (market weight) at the end of the eight week experimental period. The overall lack of significant treatment effect in the two broiler finisher experiments is in agreement with the studies of Pavic (1974), and Nitis and Sutrisna (1976) who failed to demonstrated significant effect of limestone (and seasshell) sources on weight gain for broiler finishers and leghorn growers. Smith and Kabaija (1985) failed to demonstrate the effect of calcium level on weight gain even when the calcium to phosphorus ratio (Ca:P) was very wide (4.8:1). Edward (1982) pointed out the difficulty associated with using growth rate as a criterion for evaluating the adequacy of calcium adequacy in experiments since maximum growth can be achieved on rations that give poor calcification of bones. Since these birds grew well on the tested limestone sources as compared to the control group, the sources can be dcertified as good for use.

Table 22 : The effect of limestone sources on the performance of broiler finisher chickens (5 - 8 weeks of age) Trial 4

Diet No	Limestone source	Total wt gain/ bird (g) 28 days	Total feed intake/bird (g) 28 days	Feed efficiency g feed/g gain	Leg disorder %	Mortality %
1	Control	923.34	2307.33	2.50	0	0
2	Ashaka	966.66	2292.33	2.37	0	0
3	Calabar	948.00	2327.31	2.45	0	14.3
4	Jakura	976.68	2379.66	2.44	0	0
5	Sokoto	926.67	2341.32	2.53	0	0
6	Ukpila	1054.68	2381.01	2.36	7.4	0
7	Yandev	939.99	2328.00	2.48	0	7.14
*	SEM	12.45	23.54	0.06	-	-

Means within a column are not significant ($P > 0.05$)

*SEM = Standard error of means.

Feed intake was not significantly influenced by dietary treatments. Intake of feed was generally good and agreed with the intake values reported by Offiong (1984). Feed efficiency (that is feed per unit gain) was not significantly influenced by dietary treatments.

The incidence of leg abnormalities was recorded for diet 6 (7.4 percent). Though, the figure expressed as a percentage look a bit high, there were however, only three birds which showed the signs and these came from only two of the three replicate groups. It is therefore unlikely that this condition could be attributed to the limestone source.

Mortality figures were recorded for diets 3 and 7. These diets occured towards the end of the trial and gross postmortem findings were not evident. However, since there were no mortality figures on other diets one can infer that the two dietary treatments played a role in these deaths.

Means for tibia bone weight, tibia ash weight, ash expressed as percent of bone weight, bone length, percent calcium in the ash, percent phosphorus in the ash and the ratio of bone to body weight are presented in Table 23. Tibia weights did not show significant differences between treatment means. Tibia ash

weight however, showed significant ($P<0.05$) differences between treatment means. Treatments 1, 2, 3, 4 and 5 were not significantly different from each other. Likewise, treatments 1, 4, 5 and 7 and there were no significant differences between mean ash weights for treatments 1, 4, 6 and 7. Diets 2 and 3 were significantly different from diets 6 and 7. Since ash weights gives a better measure of the mineral status of bone (Duckworth and Hill 1953) calcium or limestone effect has been demonstrated in this trial when ash weight is the parameter for comparison. However, it can be seen that great overlaping of non significant differences occured among the treatment means, and together with the absence of significant differences among the treatment means for percentage ash in thisntrial, it can be concluded that all the limestone sources treated allowed for good bone development. This is also in view of the fact that the control diet was not significantly different from the control diet was not significantly different from the testlimestone sources.

Tibia bone ash expressed as percentage of tibia bone weight (ash percent), did not show significant variations between treatment means. Georgvskii (1982)

Table 23 : The effect of limestone sources on the tibia bone parameters of broiler finisher chickens at 8 weeks of age.

Diet No	Limestone source	Bone wt (g)	Bone length (cm)	Ash wt (g)	Ash %	Ash calcium %	Ca : P ratio	Phospherus %
1	Control	15.52	10.96	5.31^{abc}	34.19	30.61^{f}	1.90 : 1	16.28
2	Ashaka	15.69	10.68	5.81^{a}	37.53	31.90^{b}	1.93 : 1	16.54
3	Calabar	15.49	10.68	5.92^{a}	38.38	32.28^{a}	2.07 : 1	15.52
4	Jakura	14.11	10.70	5.18^{abc}	36.81	29.23^{g}	1.80 : 1	16.23
5	Sokoto	15.27	10.26	5.65^{ab}	37.28	31.52^{c}	1.91 : 1	16.54
6	Ukpila	13.96	10.48	4.83^{c}	34.68	31.00^{a}	1.90 : 1	16.28
7	Yandev	13.73	10.41	5.02^{bc}	36.49	30.78^{e}	1.92 : 1	16.03
*	SEM	1.04	0.22	0.23	1.24	0.02	-	0.425

Means within a column with similar letter superscripts or without superscript are not significantly different ($P > 0.05$).

*SEM = Standard error of the means,

pointed out that when minerals are lost from the bone, the percent ash of the bone still remain unchanged. This in effect means that the ash percentage is not a sensitive tool in assessing the calcium adequacy of a calcium source. However, Duckworth and Hill (1953) suggested that ash weight should be employed in conjunction with ash percentage when assessing calcium adequacy of a diet. From the parameters on tibia bone weight and percent ash which together have not shown conclusive evidence of superiority of one limestone source over another, it can be said that all the sources of the tested limestone are fit for incorperation into broiler finisher diets.

Bone length did not result in significant differences between treatment means. The percent calcium in the tibia ash showed significant differences among treatment means. Treatments 3 and 6 were not significantly different from each other but all other treatment means were significantly different. The values for calcium in the tibia ash in this trial agree with ash calcium values reported by Hulan et al. (1985). However, these values are generally lower than the values reported in the gross experiments of trial 1 and 2 which utilized the limestone sources that were not analysed prior to incorperation

in the test diets and the levels of dicalcium phosphatase incorperated was higher. This may mean that calcium from the dicalcium phosphate source was more available to broiler chickens than the limestone sources used in trial 1 and 2. Percent phospherus in the tibia ash did not result in significant differences between treatment means but the means generally agreed with the known values for chickens. The ratio of bone to body weight did not result in significiant differences among treatments means.

Data on plasma calcium and plasma alkaline phosphatase are presented in Table 24. Plasma calcium did not showed significant differences among treatment means. However, alkaline phosphatase showed significant differences among treatment means. Diets 2 and 3 and 4 were not significantly different from each other but diet 4 only, differed from all other treatments means. High levels of alkaline phosphatase in the serum indicates calcium inadequcy in the diet (Paul and Snetsinger 1969, and Garlich et al. 1984). However Ogura gave excessive amounts of calcium in the form of calcium carbonate to broiler chickens and reported among other findings, a remarkable increase in alkaline phosphatase

Table 24 : The effect of limestone sources on blood parameters of broiler finisher chickens at 8 weeks of age. Trial 4.

Diet No	Limestone source	Plasma calcium mg/dl	Plasma alkaline phosphatase IU/l
1	Control	9.95	1725.3[a]
2	Ashaka	9.83	1682.7[ab]
3	Calabar	10.12	1672.6[ab]
4	Jakura	10.33	1547.8[b]
5	Sokoto	10.47	1789.2[a]
6	Ukpila	10.00	1796.3[a]
7	Yandev	9.64	1732.4[a]
*	SEM	0.40	46.56

Means within a column with different superscripts differ ($P<0.05$) and those without do not differ ($P>0.05$).

*SEM = Standard error of the means.

activity in the serum. He explained that the excessive calcium interfered with phosphorus metabolism leading to poor bone mineralization. Alkaline phosphatase enzyme is therefore a function of phosphorus nutrition and bone calcification and its association with calcium metabolism is only in an indirect manner.

Conclusion

Ash weight which is a good indication of mineral status of bone differed significantly between diets. Diet 6 (Ukpila source) that produced significantly the least ash weight showed greater leg disorders. It is therefore concluded that the calcium from the Ukpila limestone source is less available to broiler chickens for optimal bone mineralization but adequate for optimal growth. It is suggested that this limestone be ground to pulverized (powdered) form for inclusion in the diet since the form in which it was incorporated in the diets, was still more granular than others.

EXPERIMENT 5. THE EFFECTS OF LIMESTONE SOURCES ON THE PERFORMANCE OF LAYERS.

INTRODUCTION

While the broiler chicken in the previous trials was expected to respond to the inclusion of the different limestone sources in the diets, the levels at which the tested materials were incorporated were apparently not large enough to give a true picture of the influence of the limestone sources on the parameters measured in view of the narrow calcium to phosphorus ratio in this breed of chickens, which limit the incorperation of large percentage of limestone sources in the diets.

The calcium to phosphorus ratio in the diet of laying hens is much wider than that in the broiler chicken because of the laying hens' ability to metabolize large amounts of calcium for shell calcification. Therefore more limestone from each deposit can be incorporated in the diet without fear of disterting the calcium to phosphorus ratio, thereby producing the true picture of the limestone effects on the parameters to be measured. An experiment was therefore conducted with layers in which up to 8 percent limestone from each deposit was incorperated in the diets.

OBJECTIVE

The objective of this study was to determine the effects of limestones sources as calcium sources for laying hen perfirmance and eggshell quality taking the following as response parameters: egg production rate, egg weight, feed efficiency, feed intake etc.

MATERIALS AND METHODS

One hundred and sixty eight (168) pullets of Shaver Star Cross 579 x Hubbard Golden Comet were used in this study. Pullets were 30 weeks old and were laying at 50 percent hen day production when they were put on the study. Birds of equal groups were randomly assigned to seven dietary treatments with each treatments have three replicate pens. Each replicate pen contained 8 birds. Birds were leg banded and weighed individually. The birds were housed in an open sided poultry house located in the University farm. The pens were equiped with laying nests, galvanized hanging feeders and plastic waterers. Feeders were routinely adjusted so that the base pan levels with the back of the hens to minimize feed wastage. The pens were provied with woodshavings as litter material . Birds were debeaked

and each left wing feathers clipped to prevent birds from flying into adjacent pens. The only source of light for the birds was the day light.

The composition of the experimental diets is provided in table 25. Diets were formulated to contain essentially all the desired nutrients. The only source of variation in the experimental diets was the different limestone sources which were incorporated according to the analysed calcium contents. The basal diet consisted of the corn-cottenseed cake as sources of energy and protein respectively. Oystersshell and bone meal were used in the control diet (diet 1) to provide the required level of calcium and phosphorus. These two sources of calcium and phosphorus are commonly employed in laying hen rations in Nigeria. Dicalcium phosphate was used in the experimental diets to balance the phosphorus levels. Feed and water were provided ad libitum to the birds.

Eggs were collected twice daily at about 800 and 1400 hours. Eggs collected from each pen were weighed collectively at the end of each days collection using a Mettlers H balance (Model P. 163).

Table 25 : **Composition of the experimental layer diets used in Experiment 5 (Layers).**

Ingredients	Control	Limestone sources: Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yande
		Diet NO					
	1	2	3	4	5	6	7
Corn	56.59	56.29	56.34	56.48	56.19	56.34	56.26
Delinted & decorticated cotton seed cake	33.0	33.0	33.0	33.0	33.0	33.0	33.0
Oystershell	7.8	-	-	-	-	-	-
Bone meal	1.35	-	-	-	-	-	-
Dical Phosphate	-	1.30	1.30	1.30	1.30	1.30	1.30
Limestone	-	8.15	8.10	7.76	8.25	8.10	8.18
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Lysine	0.18	0.18	0.18	0.18	0.18	0.18	0.18
Premix*	0.65	0.65	0.65	0.65	0.65	0.65	0.65
Calculated analysis:							
Protein %	17.0	17.0	17.0	17.0	17.0	17.0	17.0
ME Kcal/kg	2723	2712	2714	2719	2709	2714	2711
Calcium %	3.51	3.50	3.50	3.50	3.50	3.50	3.50
Total P %	0.73	0.73	0.73	0.73	0.73	0.73	0.73
Meth + Cyst %	0.86	0.86	0.86	0.86	0.86	0.86	0.86
Lysine %	0.87	0.87	0.87	0.87	0.87	0.87	0.87

*The layer **Premix** (Peter Hand) at the rate of incorporation in the diet supplied the following per kilogram of feed: Vitamin A, 12,000 IU; Vitamin D_3, 4,000 IU; Vitamin E, 15 I.U; Vitamin K_3, 2mg; Vitamin B_6, 1mg; Vitamin B_2, 8mg; Pantothenic acid, 10mg; Nicotinic acid, 30mg; Vitamin B_6, 2mg; Vitamin B_{12}, 2mg; Folic acid, 1mg; Biotin, 75mg; Copper, 5mg; Iron, 15mg; Manganese, 70mg; Iodine, 0.5mg; Selenium, 0.15mg; Zinc, 60.00mg.

Table 26 : Proximate analysis of the layer diets (dry matter basis)

	Control	Limestone sources: Ashaka	Calabar	Jakura	Sokoto	Ukpila	Yandev
	Diet No: 1	2	3	4	5	6	7
Protein %	18.14	17.88	17.59	18.06	17.19	18.11	18.09
Calcium %	3.74	3.67	3.61	3.79	3.71	3.71	3.84
Ether extract %	5.57	4.95	5.54	5.62	5.08	4.54	5.84
Crude fiber %	6.51	7.06	6.84	6.14	6.58	6.66	6.94
Ash %	10.93	10.71	10.03	10.34	10.94	10.35	10.41
Dry matter %	93.68	94.13	93.90	94.20	93.33	94.13	93.90

Soft shelled egg and broken or cracked eggs were also recorded. At the end of each 28 day period, an egg was selected at random from each pen's collection and the 3 eggs treatment were selected each day for three successive days for eggshell quality determination (Keshawarz 1984). Each egg was broken out at the equiterial region (Keshavarz 1986). Each eggshell was cleaned out with a toilet tissue paper and dried at 100°C for about 20 minutes, cooled and the shell weight and shell thickness taken. Each shell was measured at three different places along the equatorial region with the aid of a micrometer (Ames, Walthan, Massachusetts). Feed intake was determined at the end of each 28 day period and efficiency of feed utilization was determined. At the end of the study which lasted for 4 months, birds were individually weighed and the change in weight was determined. One bird was selected at random from each pen such that three birds per treatment were selected for bone and bleed sample analysis as outlined in trial 1.

The experimental parameters were summarized and subjected to the analysis of variance. Significant differences between treatment means were identified using the Duncan's Multiple Range Test as described by Steel and Terrie (1980).

RESULTS AND DISCUSSIONS

The results from statistical analysis of data on hen-day production, egg weight, feed per dozen eggs, feed intake per bird per day, eggshell thickness, percent eggshell, initial and final bodyweight treatments means are presented in table 27.

Hen-day Production

There were no significant differences ($P>0.05$) among treatment means for hen-day egg production. However, diets 2, 4, 7 and 6 in that order produced numerically higher figures comparable to the control. Diet 5 produced the least figure. Contrary to published reports that oystershell is superior to limestone in enhancing egg production (Duke and Charles 1981, Charles 1982, and Hamilton *et al.* 1985), there were no significant differences between the limestone containing diets and that of the control which contained oystershell. This experiment is in agreement with those reported by Kuhl *et al.* (1977), Watkin *et al.* (1977), Christmas *et al.* (1977) and March and Amin (1981), who failed to demonstrate the effect of calcium source and particle size on egg production.

Table 27 : The effect of limestone sources on the performance of layers

Diet No	Limestone source	Average Hen day production %	Average Egg wt (g)	Average Feed/ dozen egg (g)	Average Feed intake/ bird/day (g)	Average Shell thickness (mm)	Average Shell %	Average initial body wt kg	Average Final body wt (kg)
1	Control	67.49	56.11^{c}	1917	102^{d}	0.372	9.07	1.54	1.73^{c}
2	Ashaka	67.37	56.92^{bc}	1875	105^{b}	0.368	9.12	1.56	1.77^{bc}
3	Calabar	62.94	58.13^{ab}	2120	110^{a}	0.382	9.26	1.64	1.89^{ab}
4	Jakura	67.41	55.93^{c}	1976	111^{a}	0.378	9.37	1.57	1.87^{ab}
5	Sokoto	61.03	58.35^{a}	2004	97^{e}	0.379	9.16	1.58	1.80^{abc}
6	Ukpila	65.31	57.04^{bc}	1997	104^{bc}	0.362	9.04	1.58	1.93^{a}
7	Yandev	66.13	58.73^{a}	2023	103^{cd}	0.372	8.92	1.53	1.88^{ab}
*	SEM	2.63	0.42	35.20	0.5	0.01	0.32	0.04	0.04

Means within a column with similar letter superscripts or without superscripts are not significantly different ($P > 0.05$).

*SEM = Standard error of the means.

Egg weight

Significant (P/0.05) differences occured among the treatment means for egg weight. Birds fed diet 3, 5 and 7 produced eggs with significantly superior egg weights. Birds fed diets 2, 3 and 6 were not significantly different from each other, and birds fed diet 2, 1, 4 and 6 did not differ in this parameters. Further examination of the results of egg production and egg weight indicates generally that birds with lower production figures produced heavier eggs. Kuhl (1977) also observed a similar effect in his study. He speculated that the result of heavier egg weight was not a dietary function but a function of egg number. Eggs in a clutch tend to get smaller as the eggs are laid. Thus birds with lesser number of eggs in the clutch will lay heavier eggs. The effect of dietary treatments on egg weight may therefore be an indirect one by way of influencing the mechanism that control the clutch in the hen.

Contrary to the documented evidence that oystershell improved egg weight over limestone (Quisenberry et al, 1979) the diet containing oystershell (diet 1) actually produced the least egg weight.

Feed intake : was also influenced by dietary treatments. Birds fed diets 3 and 4 consumed the most feed which was significantly (P/0.05) greater than the amount of feed consumed by those birds fed other diets. So also were those of birds fed diets 2, 6 and 7 and those fed diets 1 and 7 respectively. Diet 5 resulted in the least feed intake value. The limestone source in diet 5 was highly pulverized and was very bulky. Birds tended to select the larger feed particles resulting in the physical separation of the limestone which usually settled at the bottom of the feeders. The same effect on other dietary treatments was not very noticeable. Generally, the feed consumption values were lower than those reported on floor experiments. This maybe due to the more efficient feed management system employed.

Feed efficiency

Feed per dozen eggs was not influenced by dietary treatment. However, diet 3 produced numerically the poorest feed conversion ratio (2.12:1) while diet 2 produced the best conversion ratio (1.88:1). Oluyemi and Roberts (1988) gave the standard for tropical countries as 1.8:1. The trend in feed conversion ratio followed that of egg weight values. Birds that

had higher rate of egg production tended to have lower egg weights and birds that had higher egg weight gave poorer feed utilization efficiency per unit number of eggs. The apparent lack of treatment mean differences in this parameter is in agreement with the experiments of Quisenberry and Walker (1970), Hurwitz et al. (1969), Olomu (1979) and Nwazota (1987) who also reported feed conversion ratios similar to those reported in this study.

Egg shell thickness

All eggshell thickness values represent the eggshell plus the shell membrane. There were no significant differences among treatment means. Owing et al. (1977) reported the value of 0.325mm; Jensen et al. (1963) gave a value of 0.325-0.372mm for the turkey breed and Balloun and Miller (1964) reported a value of 0.367-0.400mm for turkey breed also. Brister et al. (1981) reported a value of 0.380mm for thickness. The eggshell thickness values obtained in this study are in good agreement with these authors.

Shell percentage

The eggshell weight expressed as percentage of total egg weight was not influended by dietary treatments. This result agrees with that of Brister et al. (1981) who tested different calcium source and particle sizes and found no significant differences among the means. Reynnells (1979) worked on calcium requirements of pheasants and reported similar mean values for this parameter. Shell quality is basically governed by the amount of shell deposited per unit surface area of the egg. Tayler (1961) concluded that, it is difficult to obtain an accurate value for mean shell thickness because of variations in shell thickness over different parts of the same shell. Shell percentage is therefore a better measure of shell quality than shell thickness measurement since it took into account the weight of the whole shell relative to the weight of the egg. Oystershell has been shown to produce eggs with superior shell quality over limestone (Stuart and Hart 1938; Meyer et al. 1973). This superior advantage of oystershell was not evident in this study. Hamilton et al. (1985) reported an improvement in eggshell quality from both limestone and oystershell of similar particle sizes. Peterson

et al. (1960) could not demonstrate the effect of physical form of calcium supplement on eggshell quality.

The eggshell contains calcium in the form of calcium carbonate where this compound constitutes 98 percent of the inorganic matter of the shell (Simkiss 1967). Common (1934) speculated that only half of total calcium needed for shell calcification comes from dietary sources, the remaining amount of calcium needed for this purpose is met through the skeletal tissue. Comar and Diggers (1949) and Mueller et al. (1964) showed that even on a diet which were adequate in calcium content 30 - 40 percent of the eggshell calcium was supplied by the skeleton. Farmer et al. (1986) put the percentage calcium derived from the bones for shell calcification at least at 15 percent. However, Hurwitz and Bar (1969) stated that the function of bone in supplying calcium in eggshell has been considerably overestimated and that sufficient amounts of calcium for shell formation could be supplied by the intestine if the dietary source is not limiting. The feed was formulated to conform with National Research Council (NRC 1984) standards for calcium and other nutrients. Moreover, when shell calcification is not taking place, calcium absorbed from dietary source is stored in the bone for future use. It can therefore be said that all shell calcium is of the dietary origin.

Since the parameters on eggshell quality agree with the values suggested as ideal, and in view of the fact that there were no significant differences among treatment means in both parameters, the test caldium source are fit for inclusion intoppoultry diets.

Body weight

The initial body weight of birds at the start of the study did not show significant differences among groups indicating the level of uniformity of the birds at the start of the study. However, after four months on the different dietary treatments birds responded to dietary treatments. There were significant differences among treatment means.

Bone parameters

The mean values for bone length, bone weight, ash weight, ash expressed as percentage of bone weight (% ash), bone to body weight ratio, percent calcium in the bone ash, and percent phosphorus in the bone ash are presented in table 28.

Bone length

Tibia length did not show significant variation among the treatment means. Njoku (1980) observed

Table 28 : The effect of limestone sources on tibia bone parameters of layers

Diet No	Limestone source	Bone length (cm)	Bone wt (g)	Ash wt (g)	Ash %	Ash calcium %	Ash phesphorus %
1	Control	10.64	14.04	6.40	44.83	36.38[b]	18.87
2	Ashaka	10.64	13.99	5.84	41.33	36.33[b]	18.52
3	Calabar	10.39	14.30	7.14	50.32	38.53[a]	18.87
4	Jakura	10.94	16.02	7.54	46.47	36.42[b]	18.98
5	Sokoto	10.47	14.57	6.97	47.69	34.79[c]	17.55
6	Ukpila	10.32	14.71	7.77	51.46	38.42[a]	17.55
7	Yandev	11.06	15.04	7.05	45.89	38.33[a]	18.33
*	SEM	0.33	0.28	0.32	0.42	0.14	0.56

Means within a column with similar letter superscripts or without superscripts are not significantly different ($P > 0.05$)

*SEM = Standard error of the means.

significant differences in tibia bone length of growing turkey fed different calcium and phosphorus levels. Birds with longer bones resulted in heavier weight gains. Birds in this study were apparently matured and their bones had stopped growing.

Bone weight

Fat free tibia weight did not show significant differences between treatments. Generally, the bones of layers contained less fat (ether extracts) than the broiler breed. This may be as a result of the usually high energy contents of broiler diets. The bone weight of pullets increases considerably as the pullet approaches maturity as a result of the formation of the medullary bone which accounts for 30-40 percent of calcium stored at this stage (Hurwitz 1973). The apparent lack of treatment effect on tibia bone weight in this study in which the limestone sources tested accounted for about 8 percent of the ration and supplied over 90 percent of the total calcium in the diet is a testimony to the suitability of the sources for inclusion into poultry rations.

Ash weight

This parameter was also not influenced by dietary treatments. However, diets 6, 4, 3 and 7 in that order produced numerically higher ash weight values,

while diet 2 produced the least ash weight. Ash weight represents a better measure of the mineral status of bone than the fat free bone weight. In these adult birds used in this study, the bones were already formed before the experiment started and so the final values of ash and mineral contents would be indicators of the degree of depletion or repletion of the bones due to egg production. The absence of significant differences in tibia bone ash between the limestone sources and the control (oystershell) maybe ab indication that all the calcium sources tested supplied similar quantities of calcium for laying hen metabolism.

Ash percent

Ash weight expressed as percentage of fat free bone weight did not show significant ($P > 0.05$) differences between treatment means. Percent ash values followed a similar trend like that of ash weight. Duckworth and Hill (1953) stated that ash percent and ash weight together are better criteria for assessing changes in mineral status of adult bone than either alone. Hinsken *et al.* (1970) found no differences between means for ash percent due to dietary calcium levels (0.9, 1.8, 2.5 and 3.7

percents). King (1978) working with pheasants, also did not find differences in ash percent of the femur and tibia adult female pheasants fed diets with calcium at 1.4-3.8 percents. The levels of calcium content of this diet was 3.5 percent. Assuming that the sources tested did not supply all the 3.5 percent calcium intended, there could still be little difference in ash percent between the treatment means as shown above. Bloom et al. (1941) pointed out that when minerals are lost from skeleton, bone weight and ash weight should be reduced but not ash percentage. Since the parameters of bone wieght, ash weight and ash percent respectively, did not respond differently to the tested calcium sources, it can be said that all the sources involved in this study are acceptabl for poultry rations.

Ash calcium

The percent calcium in bone ash responded to dietary treatments. Diets, 3, 6 and 7 produced significantly ($P < 0.05$) higher percent calcium values. Diets 1, 2 and 4 were not significantly ($P > 0.05$) different from each other but were significantly different from other treatments means. Diet 5 produced

the least percent ash calcium value. The mean values although, significantly different, generally agree with the values reported by Tayler et al. (1969) and Field et al. (1974). The treatment response exhibited by birds in this study also agrees with the findings of Reynnells (1979) who fed pheasants different calcium levels but obtained similar percent calcium values (36.3-38.5).

Percent phospherus in the ash

There were no apparent effect of dietary treatment on percent ash phosphorus. The level of phosphorus in the bone ash agree with those reported by Rennells (1979) and Maynard and Loosli (1962).

Blood parameters

The results of the effect of different sources of calcium on plasma calcium and plasm alkaline phosphatase enzyme are given in table 29.

Plasma calcium

Result indicate that there were no treatment effect on plasma calcium values. A careful examination of the data on production rate and plasma calcium values shows that some degree of relationship existed between production

Table 29 : The effect of limestone sources on blood parameter of layers.

Diet No	Limestone source	Plasma calcium mg/dl	Alkaline phosphatase iu/l
1	Control	29.53	421.27
2	Ashaka	28.14	257.97
3	Calabar	26.63	312.40
4	Jakura	27.94	325.33
5	Sokoto	25.73	402.33
6	Ukpila	26.67	418.90
7	Yandev	27.07	357.37
*	SEM	0.53	58.10

Means within a column are not significantly different ($P > 0.05$).

*SEM = Standard error of the means.

rate and this parameter. These diets that had the greatest hen-day production figures also had higher plasma calcium values. Thus diets 1, 2, 4, 7 and 6 which produced numerically the highest plasma calcium values also had greatest hen-day production figures. Scott et al. (1971) reported that plasma calcium of hens in lay which were fed adequate calcium diet after 8 months was 28.1 and 27.1 mg per 100ml at 4.00am and 4.00pm respectively on limestone diet, and 32.9mg and 29.5mg per 100ml during the same period on oystershell diets. In this experiment bleed sample collection was carried out in the afternoon but before 4.00pm. However both the plasma calcium values for limestone and oystershell (diet 1) diets agree with the report of Scott et al. (1971).

Plasma alkaline phosphatase level

Alkaline phosphatase values did not show any significant variations among treatments. Gutowska and associates (1943) speculated that plasma alkaline phosphatase activity of laying hens seemed to be related to their productivity but not clearly to their eggshell strength. The mean values for plasma alkaline phosphatase reported herein do not follow the above

suggestion since diets 2, 4 and 7 that resulted in numerically greater productions showed lower values for the enzyme. It has been pointed out that at least part of the calcium required for eggshell formation is of skeletal origin (Common 1934; Comar and Driggers 1949; Mueller et al. 1964 and Farmer et al. 1986), hence the rise in plasma alkaline phosphatase level during the period of lay. The apparent lack of treatment effect on this parameter is an indication that all the calcium sources responded in a similar manner and therefore are good sources.

Correlations

The correlation table for the results of the layer study is given in table 30. Egg production and egg weight resulted in an inverse relationship. This was expected since it has been shown in this study and that of Kuhl (1977) that dietary treatments with greater hen-day egg production had smaller egg weights.

Egg production was inversely related to shell thickness ($r = -0.417$) although this was not significant. This was unexpected since it has been demonstrated that the eggshell thickness is a function of eggsize (North 1983; Oluyemi and Roberts 1988); but the production of

Table 30 : Correlations between some parameters in the layer study

	Bone weight	Ash weight	Shell thickness	Calcium intake	Ash calcium	Egg production
Bone weight	-	0.847*	-	-	-	-
Egg weight	-	-	-	-	-	-0.704
Shell %	-	-	0.622	0.536	-	-
Shell thickness	-	-	-	-	-	-0.417
Plasma Ca	-	-	-	-	-	0.626
Alkaline phosphate	-	-	-	0.579	0.804*	-
Ash Ca	-	-	-	0.739	-	-
Egg production	-	-	-0.704	0.609	-	-

* Significant (P/0.05)

Tabulate value at P/0.05 = 0.754

calcium by the shell gland for eggshell formation remains more or less constant (Roland, 1980).

Eggshell percentage had a positive relationship with eggshell thickness.

Egg production had a positive and non-significant ($P > 0.05$) relationship with calcium intake. This is to be expected since calcium has a great influence on egg production.

The relationship between calcium intake and egg shell percentage were positive and non significant.

Calcium intake was also correlated positively with percent calcium in the bone ash. This agreed with the result obtained by Greeley (1962) who stated that direct relationship existed between ash content of the bone and the amount of calcium in the diet.

Plasma calcium was positively correlated with egg production. The correlation was not significant.

Plasma alkaline phosphatase was negatively and significantly correlated with percent calcium in the bone ash, negatively and non significantly correlated with calcium intake.

Body weight was positively and significantly correlated with ash weight but not significantly in the case of bone weight. The significant correlation between body weight and ash weight is in agreement with the findings of Roland *et al.* (1974).

EXPERIMENT 6. SOLUBILITY AND BIOAVAILABILITY STUDIES OF LIMESTONE SOURCES.

INTRODUCTION

The availability of calcium from any calcium soucre is dependent on the absorption and retention of this nutrient from such a source. Solubility plays an important role in absorption since highly soluble sources will pass rapidly through the gastro intestinal tract, thereby allowing mineral time for contact with the hydrochloric acid (Hcl) of the G.I.T. which liberate calcium ions for absorption (Savage 1982).

Sources which are less soluble will be retained in the gizzard and proventriculus for a longer period of time, "metering" out only small quantities of the less soluble source at a time to react with the Hcl of the G.I.T. resulting in the subsequent release of calcium ions for absorption.

With these assumptions in mind, solubility and bioavailability studies were carried out employing the calcium sources that were uaed in formulating the poultry diets in the previous experiments to further evaluate the limestone sources.

OBJECTIVES

Solubility test: The boejctive of this study was to find out which sources of calcium used were most soluble, to give an insight into which source may be most available to the chicken.

Bioavailabiluty study: The objective of this study was to find out whether availabilities of the calcium sources were indeed influenced by their solubility rates.

MATERIALS AND METHODS

Solubility test

Six limestone sources (procured from Ashaka Calabar, Jakura, Sokoto, Ukpila and Yandev), oystershell, bone meal and dicalcium phosphate were tested to find out which source was most soluble using 12N hydrochloric acid as outline by Roland (1986). One gram of each calcium source wasnweighed out and placed in a clean 250ml beaker. 100ml of 12N hydrochloric acid solution was added to each sample. The calcium source and the Hcl solution were allowed to react for exactly 30 minutes (Roland 1986, suggested 15 minutes time interval). After the time limit for reaction, the mixture was immediately filtered through

a filter paper held by a plastic funnel in a 500ml flask. The soluble (fractions were) filtered completely by the continuous flushing with deionized water, while the insoluble fractions remaining on the filter paper were dried at 100°C for 30 minutes, cooled in a dessicater and weighed. The amount of soluble calcium source was determined and recorded. The procedure was repeated using 0.5g calcium sources to react with 50ml of 12N HCl solution but all the other steps were as described above. The last part was repetation of all the procedure using 0.25g of each calcium source to react with 25ml 12N HCl solution. The percent solubility of each calcium sources was calculated from the equation:-

$$\% \text{ solubility} = \frac{\text{amount of Ca source solubilized}}{\text{original wt Ca source}} \times \frac{100}{1}$$

BIOAVAILABILITY EXPERIMENT:

Sixty hens in lay were housed in pairs in battery cages. The same nine calcium sources used in experiment one above were utilized and together with a control which made the tenth treatment. Each treatment was randomly assigned to three replicate cages. All the birds were fed a calcium free diet (Kim et al. 1985, Myung et al. 1983) for 48 hours. At the end of

this period birds were force-fed a calculated amount (3g) from each calcium source and the source was placed in the crop using a copper pipe (25cm long and 1.25cm diameter). A plastic funnel the calcium source was introduced into the crop. The pipe was kept from blocking by means of a piece if wire long enough to reach the crop end of the pipe from the funnel. This wire when fully pushed in was bent at the distal end of the copper pipe to prevent injury to the crop. The calcium source particles that may adhere to the inner sides of the pipe were washed down with water and bird returned to the cage to continue on the calcium free diet. The tenth group was not force fed but continued throughout on the calcium free diet. After force feeding, droppings were collected on a clean sheet of polythene. Feed and water were provided ad libitum during the study period. Twenty four hours after force feeding, all faeces on the polythene sheets were collected separately according to their replicate groups in polythene bags and were marked clearly on the bag for further identifications. After all faeces were collected, they were taken immediately to the laboratory and dried at 105°C overnight; cooled

in a dessicater and ground to pass threugh 1mm sieve and saved for calcium determination as described previously in trial 1.

The data collected was summarized and subjected to the analysis of variance and where appropriate, significant treatment means were separated using the Ducan's Multiple Range Test as outlined by Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

Solubility (*in vitro*) experiment.

Results indicate that highly significant differences occured between means (Table 31). Dicalcium phosphate resulted in the highest solubility rate which was highly significant ($P<0.01$). Bone meal was significantly ($P<0.01$) lower than dicalcium phosphate only but significantly higher rate and this than all the other sources tested. The Ashaka source was significantly higher in solubility than the Calabar and Ukpila sources but was not different from the other limestone sources. The Calabar limestone source was not significantly different ($P>0.05$) from Jakura, Sokoto, Yandev limestone sources and the oystershell.

Table 31 : The degree of solubility of different calcium sources in 12N HCl.

Source	% Solubility	Dicalcium Phospate = 100%
Ashaka	39.87^{c}	50.53
Calabar	37.53^{d}	47.57
Jakura	38.13^{cd}	48.33
Sokoto	38.77^{cd}	49.14
Upkila	35.50^{e}	44.99
Yandev	38.40^{cd}	48.67
Dicalcium PO_4	78.90^{a}	100.00
Bone meal	43.98^{b}	55.74
Oystershell	38.17^{cd}	55.74
SEM*	0.49	-

Means with different superscripts differ ($P < 0.01$)

*SEM = Standard error of the means.

Oystershell and bone meal sources had larger particles removed by sieving but it must be emphasized that this represented the size that was usually incerperated in the dietary treatments. Savage (1982) advised that the sources to be tested should be measured on "as incerperated" basis since regrinding te same particle size may present a false picture ef the actual solubility as eccurs in mixed rations. The percentages selubility values fer limestene seurces in this study de net agree with these reperted by Raben and Reland (1985). This maybe due te time facter employed in allewing the sources to react with the hydrechleric acid. In this study, 30 minutes reaction time was allewed while Raben and Reland (1985) allewed enly 15 minutes. It was however, ebserved that the reaction was still progressing after 15 minutes. Moreever, Savage (1982) suggested that the peried ef time feed (calcium source) stays in the preventiculus and gizzard is extremely variable and can range frem few minutes te about half an hour depending en many facters. Savage (1982) further stated that the difference in solubility rate was a functien ef surface area which is due apparently to both perosity and particle size in the case of limestone sources.

It is striking to observe that Ukpila limestone source had the least solubility value. The physical appearance of this source also showed that is was a highly crystaline source of limestone and "as incorperated", it was apparently more granular than most sources tested. Oystershell has been shown to be retained in the gizzard for a longer period of time due to particle size (Scott et al. 1971, Kim 1985). This implies that, it is usually soluble than limestone. Kuhl and Sullivan (1977) also could not demonstrate any difference in solubility rates between limestone and oystershell of similar particle sizes. The superior solubility rate of dicalcium phosphate was expected since this is a manufactured product with specification standards. This is actually why it is usually selected as a yard stick for measuring the suitability of other products as source of calcium and phosphorus.

When dicalcium phosphate was utilized as a standard and its value was taken as hundred percent soluble, the solubility values expressed relative to this standard improved but since they were calculated, these values followed the same significant trend as those expressed earlier.

Bioavailability (in vivo) study

The different calcium sources (Table 32) showed highly significant variations between treatment means. The Jakura limestone source had the highest availability rate (92.8 percent) which was significantly ($P<0.01$) different from those of Ashaka and Ukpila limestone sources. It was also significantly ($P<0.01$) different from that of dicalcium phosphate which resulted in the least solubility value. The Jakura source was however, not significantly different ($P>0.01$) from the Yandev, Calabar and Sokoto limestone sources. Values for the control group were not shown in the tables since these birds were not force-fed any calcium source but were utilized to estimate the amount of calcium voided in the faeces of the calcium deprived birds to permit the estimations for calcium availability from the calcium sources fed in the other treatments.

The results obtained in this study with respect to the availability of calcium from oystershell and limestone sources agree with the report of Waldroup et al. (1964) although these authors employed different criteria for evaluation. The values for the limestones are also in generall agreement with those reported by Reid and Wiber (1976) who used a similar breed of poultry and employed

a similar methodology for evaluation of the availability of the limestone sources. The value obtained from this study for dicalcium phosphate is in good agreement with that of Kim et al. (1985) who used Japanese quails as experimental animals. However, the values for limestone and oystershell reported by Kim et al. (1985) were low compared to the values obtained in this experiment. This difference may be due to particle size of both limestone and oystershell (which was not indicated in their study) used and/or time of calcium starvation employed by them. Kim et al. (1985) starved the birds of calcium for only twenty four hours while birds in this study were starved of calcium for forty eight hours to presumbly get them into negative calcium balance so that the calcium from the limestone sources can actually be extracted by the deficient birds. Scott et al. (1971) and Kim (1971) suggested that the usually larger particles of oystershell due to crushing resulted in its being retained in the gizzard for a longer period of time and "metering" out only a gradual amount of calcium at a time for absorption as opposed to the usually pulverized limestone which passes faster through the gut. The oystershell and bone meal were sieved to removed large particles and this may have contribured to the differences between the results of this experiment and that reported by Kim et al. (1985).

Dicalcium phosphate was highly soluble in the solubility experiment (*in vitro*) and was expected to behave in like manner in the *in vivo* experiment. Despite the fact that the value obtained in the *in vivo* experiment is in agreement with that of Kim *et al.* (1985), it had the lowest numiercal value and was significantly ($P<0.01$) different from other limestone sources while in the experiment of Kim *et al.* (1985) dicalcium phosphate was significantly superior to the limestone and oystershell sources. This differences may be explained in terms of the high solubility rate of dicalcium phosphate which may have passed rapidly through the gut as compared to the less soluble limestone and oystershell sources which allowed more contact with the HCl in the proventriculus and gizzard thus releasing more calcium ions for absorption. Savage (1982) stated that once a calcium source enters the duodenal loop, the pH is theoretically too high for calcium release and the calcium from that source becomes unavailable. Since dicalcium phosphate presumbly has production standards, it is not suprising that the dicalcium phosphate in this experiment and that of Kim *et al.* (1985) responded in a similar manner even though the time of calcium starvation in the two experiment was different. It was observed that the faeces of hens fed

Table 32 : The availability of calcium from different calcium sources.

Source	% Availability
Ashaka	87.0^{cd}
Calabar	88.6^{abcd}
Jakura	92.8^{a}
Sokoto	90.0^{abc}
Ukpila	87.3^{bcd}
Yandev	90.0^{abc}
Oystershell	92.6^{ab}
Bone meal	89.6^{abc}
Dicalcium PO_4	84.5^{d}
SEM*	1.05

Means with a column with different superscripts differ ($P<0.01$).

*SEM = Standard error of the means.

this calcium source sppeared actually whitish and more loose to the touch than those from other calcium sources.

The main trust of this study is the evaluation of the limestone sources as calcium source for layers. The results obtained from both the solbulity and availability experiments showed significant variations between treatment means. However, all the values fall within values recognised as adequate by other authors.

CONCLUSIONS

The result of the solubility study indicated that, as incorperated in the diets, some limestone sources were more soluble than others. Savage (1982) pointed out that the solubility of limestone sources is a function of particle size and porosity, and that the less soluble the source, the more it is retained in the gizzard and proventiculus, thereby allowing more time for hydrochloric acid reactions with the source for the release fof calcium ions for absorption. Thus it follows that the more soluble the source(s) the less available they appear to be. This fact is reflected in the availability study where

the dicalcium phosphate used as a standard in the solubility study, for its high solubility rate became less available in the availability study.

While the result of the statistical analysis on availability indicated that the Jakura source only was significantly ($P \leq 0.01$) more available than the Ashaka and Ukpila limestone sources, the results of the production parameter of the layer study did not show such significant variations. It is therefore concluded that all the limestone sources tested are good and can be recommended for use in their various localities in layer rations.

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Appendix 1 : Analysis of variance of 4-week body weight gain of broiler chickens (Trial 1).

Source of variation	Degree of freedom	Sum of squares	Mean squares	F value	Probability
Treatment	6	6186.810	1031.135	1.935	0.09
Interaction	18	7086.524	393.696	0.733	0.76
Error	56	29834.00	532.750	-	-
Total	83	3815736.81	-		

Period omitted out = 4 ∴ Period D.F. = 3

Appendix 2 : Analysis of variance of 8 week body weight of broiler chickens (Trial 2).

Source of variation	Degree of freedom	Sum of squares	Mean squares	F value	Probability
Treatment	6	23628.540	3938.09	1.692	0.15
Interaction	12	19008.508	1584.04	0.631	0.76
Error	42	97756.667	2327.54	0	-
Total	62	2950927.429	-		

Period = 3 omitted ∴ Period D.F. = 2

Appendix 3 : Analysis of variance of 4 week body weight gain of broiler chicken (Trial 3)[a]

Source of variation	Degree of freedom	Sum of squares	Mean squares	F value	Probability
Treatment	6	4749.373	791.562	2.494	0.033*
Interaction	18	7045.393	391.411	1.23	0.27
Error	56	17776.400	317.436	-	-
Total	83	245847,104	-		

a = Periods = 3 not included

* = Significant P/0.05

Appendix 4 : Analysis of variance of 8 week body weight gain of broiler chicken (Trial 4)

Source of variation	Degree of freedom	Sum of squares	Mean squares	F value	Probability
Treatment	6	274.319	45.72	0.229	0.96
Interaction	12	2001.102	166.759	0.836	0.61
Error	42	8374.907	199.403	-	-
Total	63	19355.403	-		

*Period = 3 but not included in the ANOVA

Appendix 5 : Analysis of variance of ash weight of broiler chickens 4 week old (Trial 1)

Source of variation	Degree of freedom	Sum of squares	Mean squares	F values	Probability
Treatment	6	0.663	0.114	3.773	0.019**
Error	14	0.422	0.030	-	-
Total	20	1.104	-		

**Significant P/0.01

Appendix 6 : Analysis of variance of ash weight of broiler chickens 8 week old (Trial 2).

Source of variation	Degree of freedom	Sum of squares	Mean squares	F values	Probability
Treatment	6	2.263	0.377	1.174	0.37
Error	14	4.500	0.321	-	-
Total	20	6.763	-		

Appendix 7 : Analysis of variance of ash weight of broiler chickens 4 week old (Trial 3)

Source of variation	Degree of freedom	Sum of squares	Mean squares	F values	Probability
Treatment	6	0.522	0.087	0.316	0.51
Error	14	1.331	0.095	-	-
Total	20	1.854	-		

Appendix 8 : Analysis of variance of ash weight of broiler chicken 8 week old (Trial 4).

Source of variation	Degree of freedom	Sum of squares	Mean squares	F values	Probability
Treatment	6	3.097	0.516	3.355	0.029*
Error	14	2.167	0.155	-	-
Total	20	5.264	-		

*Significant P/0.05.

Appendix 9 : Analysis of variance of solubility study (Trial 6)

Source of variation	Degree of freedom	Sum of squares	Mean squares	F value	Probability
Treatment	8	4413.67	551.708	773.37	0.01*
Replications	2	1.18359	0.591797	0.829	
Error	16	11.414	0.713		
Total	26	4426.27			

*Significant (P/0.01)

Appendix 10 : Analysis of variance of the Hen day Production (Trial 5)

Source of variation*	Degree of freedom	Sum of squares	Mean squares	F value	Probability
Treatment	6	454.895	75.816	0.912	0.49
Periods	3	329.928	109.976	0.322	0.28
Interaction	18	427.786	23.786	0.285	0.99
Error	56	4657.536	83.170	-	-
Total	83	5869.611			

Appendix 13 : Mineral composition of the Limestone samples

Limestone source	Ca %	Mg %	Fe Mg/kg	Mn Mg/kg	Zn Mg/kg	Cu Mg/kg
Ashaka	38.54	0.30	1508	557.1	Trace	Trace
Calabar	37.80	0.71	761.7	703.5	Trace	Trace
Jakura	39.50	0.16	2583	354.3	28.1	Trace
Sokoto	38.15	0.50	254.9	Trace	Trace	Trace
Ukpila	38.80	0.25	2324	400.9	18.2	Trace
Yandev	38.46	0.44	338.5	Trace	74.3	Trace

APPENDIX 14

Fat Extraction using Conventional and non conventional methods.

Introduction

In Nigeria today, as a result of difficulties encountered in the procurement of (necessary and conventional) equipments for some laboratory analysis, and even where available, many of these equipments are not flexible enough to permit the use of modified methods that may be required in certain situations. The case in point is that of fat extraction of bones for mineral studies. The fat extraction of compounded feed or feedstuffs is well known and poses no great problem. However, fat extraction of bones which can not be carried out using the conventional methods poses a great problem in mineral studies. While at extraction of bones has been carried out successfully in developed countries using such modified equipment as side chambers (Njoku, 1980), the availability of such an equipment in this country is very limited. It is thus necessary to attempt to develop a cheap and practical method that will permit the conduct of mineral studies in animals tc keep pace with other areas of animal research programmes.

Materials and Methods

Nine spent layers were utilized in a paired experiment to test if any difference existed between two methods of fat extraction. Birds were sacrificed by severing the head and tibia bones excised for the study. The pair of tibia bones from each chicken were tied together during the course of the study to avoid identification difficulties. Bones were immersed in simmering water in which two percent sodium hydroxide solution was added (Ansa 1986) to enable easy removal of flesh from the bones. Flesh as removed by screpping with a scapel blade and the clean bones were dried in the oven at 110°C overnight, cooled in a dessicator the following morning and individual bone weight taken. Individual bones from each chicken were crushed in a mortar with pestle. One bone from each pair was assigned to one of the two methods of fat extraction in question. These methods were either Soxhlet method or immersion of the crushed bone in ether contained in a 250ml flask that was sealed to prevent escape of ether and the process allowed to stand for 72 hours with occasional shaking of the bottle. At end of this period (72 hours), the content of the flask was filtered through a previously weighed filter paper and allowed to dry in open air and weighed. The extraction processes was repeated for each pair of bones. The results obtained were summarized and analysed statistically as outlined by Steel and Torrie (1980).

Results and Discussions

The average difference between the two methods of fat extraction used was 0.08 ± 0.13 grams. The observed mean difference was not significant at five percent level of probability. The average value of fat fraction for the immersion and Soxhlet methods were 0.88 ± 0.09 and 0.96 ± 0.11 grams respectively. It is therefore evident that the fat extraction by Soxhlet method was numerically superior to that of the immersion method. Where ashing of the bone is required it may be advisable to use the immersion method where an entire bone can be used.

Conclusion

This experiment demonstrates that either methods of fat extraction can be employed successfully in mineral studies with bones. The use of the immersion method is recommended over the Soxhlet method when dealing with large bones.