

Mineral Profile of Broilers Birds Fed Zinc Bacitracin on Different Protein Diets

Ogboko Benson

Department of Biomedical Sciences, University of the Western Cape, private bag, X17. Bellville, 7535 South Africa

ABSTRACT

This experiment is to determine the effect of protein sources (plant and animal based) and Zinc bacitracin a growth promoter on the mineral profiles of broiler chickens. Two trials were conducted, comprising of 240 day-old chicks, and 120 day-old chicks respectively, they were housed in a battery cage in a single pen, randomly distributed into four (4) major groups and fed the following diets; A - diet composed of plant protein, B - diet composed of animal protein, C - diet composed of plant protein + Zinc bacitracin and D - diet composed of animal protein + Zinc bacitracin.

Zinc bacitracin was included at 300g per tonne. The feeding trial lasted for six (6) weeks. On terminating the feeding trial, 30 marked birds from each group were slaughtered, for bone sample collection. The left thighbone and claws were collected from each broiler for Calcium and Phosphorus analysis. The collected left thighbones and claws of each broiler were de-skinned, dried and ash in a muffle furnace and the ash used for Calcium and Phosphorus analyses.

Broilers receiving plant protein had significantly higher calcium levels in both bone and claws ($P < 0.05$). The inclusion of Zinc bacitracin lowered Ca levels significantly in both plant and animal protein diets. Phosphorus levels in both bone and claws, in all treatments showed no significant differences.

Thighbone + claws Ca:P ratio were higher for both broilers receiving plant protein ration, but no significant difference ($P > 0.05$).

KEYWORDS: Chicken, Zinc bacitracin, Feed, Calcium, Phosphorus.

1. INTRODUCTION

The growth performance of modern broiler chickens has changed considerably over the years due to genetic selection and diet provision (**NATIONAL RESEARCH COUNCIL 1994, Rama Rao et al 2009**). Calcium (Ca) and phosphorus (P) are essential for bone development and are the major minerals required for bone formation in any animal, they stand out as the two most abundant minerals in the skeletal framework of most animals including poultry. Chicken skeletons consist largely of minerals, with calcium and phosphorus comprising the highest concentration of about 28% Ca, and 12% P (**Williams B et al 2000**). Feed composition in the husbandry industry caters for high demands for these minerals in view of the rapid growth rates achieved by these methods. Minerals are either provided directly or in combination with other feed ingredients such as crushed bones, animal by-products, fishmeal, and leafy plants rich in calcium or directly as lime (**Brand TS et al 1999**).

These parameters are selected to achieve maximum growth at minimum cost, but the ratio of nutrients can produce pathological conditions such as abnormal bone development (**Abdulrahim et al 1999**). In spite of the dietary inclusion of these minerals in broiler feeds, modern broiler production is associated with several skeletal pathologies such as; bone framework deformities and calciuresis (**Abdulrahim et al 1999, Milne GNA and M Delander 2008, Susan et al 1997**).

Protein diet constitutes a major nutrients requirement for effective growth and development especially in fast growing birds (broilers). The amount of protein could be beneficial or harmful to the health of these birds as both could result in healthy bred table birds or degenerative diseases in both birds and consumers of the birds such as; skew legs, fragile bone framework, osteoporosis, cancer, calciuria and obesity (**Veith JW 1998**). The type of protein sources (plant and animal) on growth and utilization of Ca and P in poultry diet is very important, as noticed in this experiment and other experiments, protein source investigations and the role of calciuria in poultry science (**Virden WS et al 2004, Sherman AR 1992**) is constantly been highlighted.

Research has shown that animal protein increased urinary Ca in humans, and hypercalciuria was observed after supplementing human diets with peptone, gluten, or egg white (**Goulding A and Malthus RS 1969, Campbell 2007**). Using crystalline amino acid diets and diets with several protein sources with different nitrogen (N) levels, a direct relationship was found between nitrogen intake and

*Corresponding Author: Ogboko Benson, Department of Biomedical Sciences, University of the Western Cape, private bag, X17. Bellville, 7535 South Africa.

urinary Ca excretion, with no differences in calciuria due to the type of protein fed or its amino acid composition (**Bell *et al* 1975, Houndonougbo *et al* 2008 and Mondal *et al* 2009**).

Many studies have observed the calciuretic effect of high protein diets (**Murphy EW, and BW Willis 1975, Kirk RS, Sawyer R 1991 and Kertes *et al* 2008**) and these are particularly evident in the case of protein from animal products (**Waslien CI, and RM Rehwoldt 1990**). Various reports have also shown that the nature of the protein in the diet can influence the degree of calciuria. Plant protein such as soya bean and animal protein such as beef are less calciuretic than proteins such as casein, lactalbumin and egg white (**Howe J 1986, Spencer *et al* 1987, Calvo *et al* 1982, Aletor VA, and FI Olonimoyo 1992**). The difference in the degree of calciuria has been attributed to the varying levels of nutrient intake such as Ca, Na, P (**Castenmiller *et al* 1988, Goulding A and J McIntosh 1986**), as well as concentration of sulphur containing amino acid in the protein (**Zemel MB 1988**).

An investigation into the effect of various protein diets on calcium retention showed that protein from dairy products, such as cottage cheese, caused considerable calcium loss in the urine (**Graham *et al* 1990**). When Ca loss exceeds absorption, there will be a negative Ca balance that exists and Ca will then be mobilised from bone in order to maintain plasma Ca levels in a dynamic state. This loss of Ca from the bone can eventually lead to osteoporosis. In a study carried out on sheep, it was found that diets rich in animal protein significantly affected calciures and bone formation as opposed to diets composed of plant proteins (**Brand *et al* 1999**).

Studies have been conducted on optimum dietary Ca and P contents of broilers with respect to the skeletal and bone development (**William *et al* 1998, Williams *et al* 2000**). Comparing broiler bone growth rate, mineralization (% ash), Ca and P content of a fast growing modern strains of chicken to that of slower growing old strains (guinea fowl, ducks) it was found that substantive differences exist (**William *et al* 2004**). Although both strains demonstrated similar periods of rapid cortical bone formation and mineralization, a lower ash % occurred in the fast strain and the Ca: P ratio was also higher than the expected 1.67 ratio (**Pellegrino ED and RM Biltz 1968**). It was postulated that these differences might be attributed to higher demands for P in diets of fast growing strains than what is provided by the commercial feeds.

Zinc bacitracin is an antibiotic, growth promoter widely used in the poultry industry as a feed additive to enhance growth, rapid development of broiler birds and to conserve feed can sometimes be detrimental to the health of the birds and consumers. It has been documented that the use of Zinc bacitracin will increase the body mass weight of chicken by 10.8% and reduces feed conversion with an increase in abdominal fat deposit in female at 31% (**Bernsten JO 1994, Rama Rao SV *et al* 2009**). Zinc bacitracin increase feed efficiency but has a high frequency of tibialdyschroplasia (TD) and dust bathing (**Stub C and KS Vestergaard 2001**)

This study is aimed at investigating the role of protein sources (plant and animal based protein) on growth, mineral utilization and Ca:P ratios in broilers and the role played by the growth promoter Zinc bacitracin in influencing the ratio was also monitored.

MATERIALS AND METHODS

DIETS: The basic plant and animal protein diets were formulated using the standard computerised feed formulation programme (**Stelplan Programme 1999**), used by University of Stellenbosch's Department of Poultry Science. Feed was prepared analysed and milled at the Mariendahl facility and the feed bagged and labelled for later use.

FEEDING TRIAL: In view of space and logistics, two feeding trials were conducted consecutively. In the first run 240 day-old chicks were divided into four (**William B *et al* 1998**) groups and in the second phase 120 day-old chicks were used in a repeat feeding trial. The 240 day- old chicks were obtained from a commercial hatchery, they were then housed (10 chicks per cage) in a single brooder pen, fitted with wire-framed cages, with feeders and automatic drinkers fitted to each cage.

The temperature and airflow were controlled and light was provided by fluorescent strip lights. Prior to the bird's arrival the cages and the entire pen was cleaned disinfected and fumigated to prevent infections. On the arrival of the chicks, they were immediately weighed, sexed, and randomly placed into cages with 10 birds per cage. 60 Birds per group were then placed on four different feeding regimes. Each regime was assigned six (6) groups of ten (10) birds each and the data obtained for each group of ten was pooled for feed consumption and growth determinations (**Benson 2011**).

Table: 1. Feeding Regimes

A	Plant based diet	(60 Chicks) without Zinc bacitracin
B	Animal based diet	(60 Chicks) without Zinc bacitracin
C	Plant based diet	(60 Chicks) with Zinc bacitracin
D	Animal based diet	(60 Chicks) with Zinc bacitracin

The above feed regimes were administered to (60Chicks) of four different diets (A, B, C and D). Diets were in either plant or animal based, with addition of Zinc bacitracin (growth promoter) at the ratio of 300gm per tonne.

Table 2. : Composition of plant and animal diets of broiler chickens

PLANT PROTEIN DIETS			
RAW MATERIALS	KG	NUTRIENTS	%
Lime	1.15	Calcium	0.90%
Cocsidiostart	0.1	Arginine	1.65%
Maize (High grade)	49.159	Isoleucine	1.02%
MonoCalcium	1.225	Leucine	1.96%
Phosphate oil (21 Day)	8.674	Linoleic acid	1.07%
Lycine	0.011	Lycine	1.20%
Methionine	0.2661	Metabolic energy	13.2MJ
Soya cake	39.003	Methionine	0.55%
Salt	0.217	Methionine+Cystine	0.96%
Vitamines/microminerals	0.2	Sodium	0.10%
		Avail. Phosphorus	0.40%
		Avail. Protein	20.92%
		Avail. Threonine	0.80%
		Avial. Tryptophan	0.25%
ANIMAL PROTEIN DIETS			
RAW MATERIALS	KG	NUTRIENTS	%
Poultry by-products	5	Arginine	1.25%
Lime	2.452	Calcium	1.90%
Cocsidiostart	0.1	Isoleucine	0.95%
Maize (High grade)	66.617	Leucine	2.34%
Lycine	0.031	Lycine	1.20%
Methionine	0.131	Linoleic acid	1.46%
Soya cake	5	Metabolic energy	13.3MJ
Fish meal	2047.00%	Methionine+Cystine	0.96%
Vitamin/microminerals	0.2	Sodium	0.20%
		Avail. Phosphorus	0.705
		Avail. Protein	25.05%
		Avail. Threonine	0.95%
		Avail. Tryptophan	0.25%

* Formulated feed result, (Stelplan programme, 1998).

The birds were fed daily at 9.00am and both feed and water was provided ad-libitum (**Mirosh LW et al 1981**).

The feeding procedure continued for the six weeks of the feeding experiment and on the final day of the feeding trial, the mass gain, total feed consumption and mortality rates during the trial were recorded. The condition of the birds and anatomical abnormalities were also noted. The birds were then transferred to the abattoir for slaughtering, sample collection and determination of carcass parameters. In the second run the same procedures were followed, except that 120 day-old chicks were used and 30 birds were assigned to each treatment consisting of three replicas of 10 birds each. On completion of the experiment, 20 marked birds from each dietary regime were selected for analyses. Slaughtering and organ collection was also the same as for the first trial (**Benson 2011**).

COLLECTION OF SAMPLE AND CHEMICAL ANALYSIS: On terminating the feed intake experiment, 30 birds from each dietary regime were used for mineral profile analysis. Each birds thighbone and claw was collected for both calcium and phosphorus analysis.

CALCIUM AND PHOSPHORUS DETERMINATION IN BROILER BONE

EXPERIMENT

METHODOLOGY: The percentage of calcium and phosphorus was determined in the bone and claws of the experimental broiler chickens, the following procedures were followed ; The thighbones and claws of each broiler were collected after slaughtering, and stored at -15 °C for later analysis. All tissues were removed from the bone and the bone and claw then dried in a drying oven at 80 - 120°C for 24hrs. The bone was weighed to the nearest 0.1mg, ashed in a Muffel furnace at 500°C for 12hrs, and ash weight determined to the nearest 0.1mg.

0.5gm of ash (bone) was used for Calcium determination using the Nitrous Oxide/acetylene flame of an Atomic Absorption-spectrophotometer, after standered acid bone digestion using the technique described by (Moor PD and SD Chapman 1986)

PHOSPHORUS ANALYSIS.

Phosphate was determined using the Murphy and Riley phosphorus determination method (Murphy and JP Riley 1962). The same solution prepared for Ca analysis was used for phosphate determination using the spectrophotometer reading at 884nm wave length.

STASTICAL ANALYSIS.

Data was subjected to Repeated measures ANOVA, using the Levene’s Test of Homogeneity of Variances, of the Statistical Analysis system (SAS 1982).

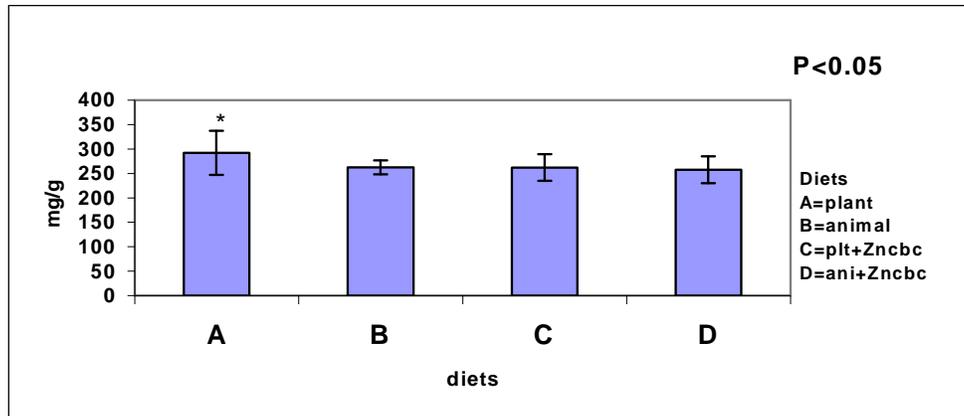
RESULTS

Table 3: Calcium and phosphorus levels in broiler’s thighbones

Treatments	Thighbones Ca(mg/g)	Thighbones P(mg/g)
A	292.05	2.38
B	262.63	2.25
C	262.16	2.21
D	257.46	2.21

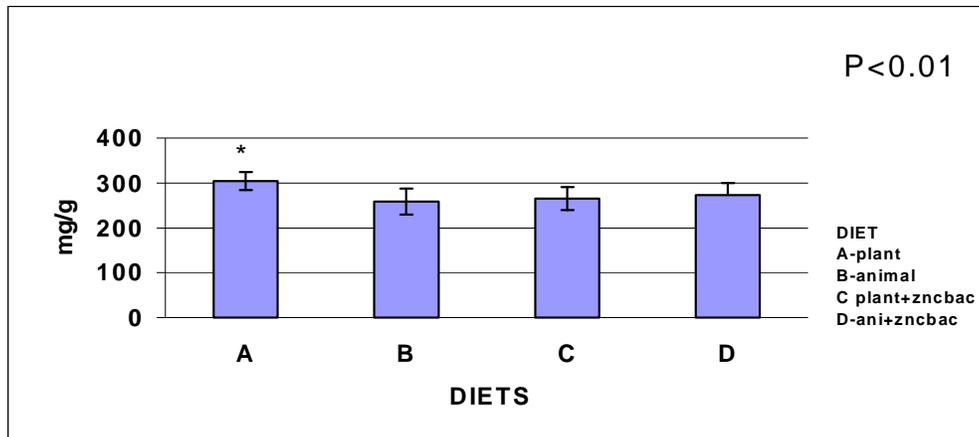
Mean mg/g of calcium and phosphorus levels in thighbones in treatment (A,B,C,D).

Fig. 1. Mg/g Ca in thighbone of broilers receiving different dietary regimes.



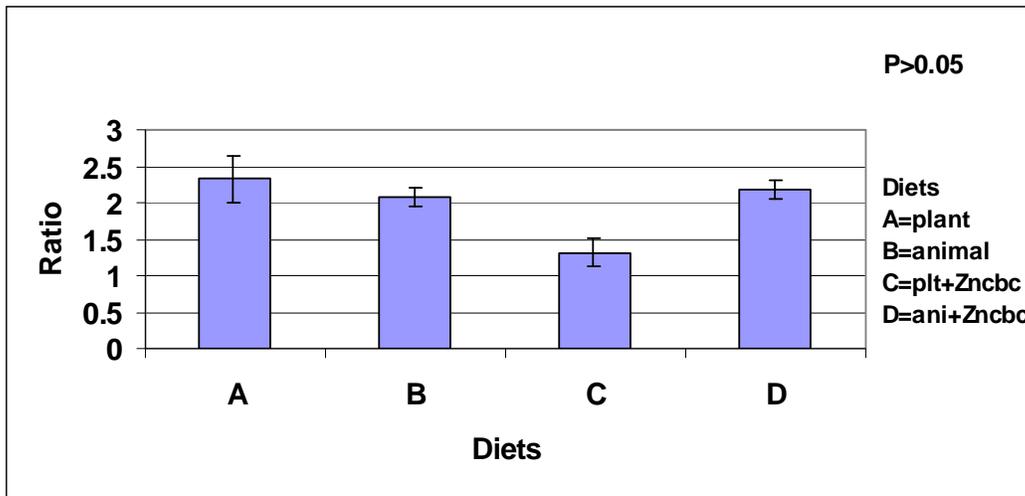
* = (P<0.05) significantly different.

Fig. 2. Mg/g Ca in thigh claws of broilers receiving different dietary regimes.



* = (P<0.05) significantly different.

Fig. 3. Ca/P ratio in thigh claws of broilers receiving varied dietary regimes.



The dietary regimes significantly affected calcium levels in both thighbone and claws. Ca levels in broilers on the plant protein regime (treatment A) had significantly higher ($p < 0.05$), Ca/g dry bone than all other treatments. The average value of calcium in thighbones was 292.05mg Ca/g dry bone. The addition of Zinc bacitracin as in treatment C (plant based diet + Zinc bacitracin) caused the mean value to decrease significantly ($p < 0.05$) to 262.16mg Ca/g dry bone, and observed in animal protein regime.

The thighbones of broilers on treatment B (animal based diet) showed an average calcium level of 262.63mg Ca/g dry bone and the addition of Zinc bacitracin (treatment D animal based diet + Zinc bacitracin), further decreased the levels to (257.46mg Ca/g dry bone), as shown in fig.1. The P-value did not differ between treatments ($P < 0.05$). While there is no significant difference ($p > 0.05$) in phosphorus levels in all treatments

The results of claws follow similar trend to those of the bone, but mineral levels were even higher than those of bone. Ca levels were significantly affected by dietary regime ($p < 0.01$) with treatment A (plant based diet), 304.34mg Ca/g dry claws producing significantly higher Ca levels than the rest. The addition of Zinc bacitracin to the diet as in treatment C (plant based diet + Zinc bacitracin), also decreased Ca levels to 265.34mg Ca/g dry claws. While the animal based diet (treatment B) produced the lowest levels with 258.55mg Ca/g in claws. The addition of Zinc bacitracin, as in treatment D (animal based diet + Zinc bacitracin) increased calcium levels (272.03mg Ca/g dry claws), (fig 2).

This results show that plant based diets tend to increase Ca levels in bone and claws (shanks), while animal based diets, decrease Ca levels in both bone and claws. The addition of Zinc bacitracin also decreased Ca deposition in bone but the increase in claws was not significantly different. Phosphorus levels in broiler claws showed no significant difference between treatments.

The ratio of Ca: P in broiler's thighbones of all the treatments showed no significant difference ($p > 0.05$) between treatments. Treatment A with a value of 2.38 (plant-based diet) was the highest, and it was closely followed by treatment D with a value of 2.25 (animal based diet). The addition of Zinc bacitracin, reduced the ratio plant protein diets ratio to 2.21, followed by the animal protein diet without Zinc bacitracin at 2.21 with no significantly different.

Similar trend occurred in (fig 3). Were the Ca:P ratio of Claws is similar to that of the bone, as treatment A (plant based diets) shows the highest ratio value of 2.33, followed by treatment D (animal based + Zinc bacitracin) with 2.18. Followed by treatment B (animal based diet) with 2.08 and finally treatment C (plant based diet + Zinc bacitracin) with the least value of 1.32, with no significant difference.

DISCUSSION

The results obtained in this feeding trial showed a decline in bone calcium deposit when Zinc bacitracin was added to plant or animal diets. However, in spite of the fact that the plant based diet had lower Ca levels (0.90%) than the animal protein based diet (1.90%), broilers on the plant based diet were able to convert the lower Ca in their diet to higher bone Ca as against the broilers on the animal based diet. The addition of Zinc bacitracin to the diet had a further negative effect on the mineral

utilization of broilers and could result in bone abnormalities as corroborated in a similar experiment conducted by Brand et al on sheep (**Brand TS et al 1999**).

Studies on humans with regard to Ca metabolism, relates largely to osteoporosis (**Peacock M 2010**), and has highlighted the role of diet in mineral utilisation and the consequence of Zinc bacitracin. Osteoporosis is a serious degenerative problem, especially in postmenopausal woman. Low dietary calcium intakes in childhood could result in lower bone density at puberty and it is assumed that they are more susceptible to osteoporosis in later years. It is now clear that adequate calcium intake by adults can at least retard the development of this disease (**NATIONAL RESEARCH COUNCIL 1994**). High incidence of osteoporosis among the aged has resulted in an increased focus on dietary source of Ca and the controversial use of Ca supplements for controlling age related bone loss (**Heaney RP et al 1982, Albrink MJ 1991, Khandare AL et al 2005, Yoneyama K, and J Ikeda 2010, Dawson-Hughes B, and SS Harris 2002**).

Low calcium intake raises circulating levels of calcitriol and improves the efficiency of calcium absorption, resulting in the efficient use of dietary calcium. This could explain why calcium deficiency among humans is so rare throughout the world, in areas where calcium intake is low by American standards. The average American woman apparently consumes in excess of 800mg of Calcium/day and in Africa consumption can be as low as 450- 500mg/day (**Harville EW et al 2004, Gordon GS and C Vaughan 1986**). American women are told to supplement their Ca intake by increasing their milk consumption to about a quarter litres per day or to take calcium supplements, however Gordon and Vaughan (**Gordon GS and C Vaughan 1986**) emphasised that there is no data available to demonstrate that high calcium intakes do, in fact, help prevent osteoporosis. True calcium deficiency would of course be expected to produce osteoporosis and this has been produced in animals fed a very low calcium diet (**Jowsey J and J Gerson-cohen 1964**). The question constantly ask is not whether calcium is an essential nutrient; the issue is how much calcium is required to maintain health and whether or not calcium intake is related to development of osteoporosis (**Lewandowski S et al 2001, Matsumoto ED et al 2006**).

In this study, high protein diets tend to lower Ca:P ratio in the bone and increased the incidence of skew-leg-syndrome as noticed in experiment on sheep receiving animal protein (fish meal and blood meal as is standard practice in the animal husbandry industry) (**Brand TS et al 1999**). It has shown that the addition of animal protein to the diet severely compromised bone development, although there were weight gain but food consumption did not differ significantly between the two groups.

Although no significant difference exist ($p > 0.05$) between treatments, the average bone Ca:P ratio for plant based diet without Zinc bacitracin (treatment A) was higher than any of the other treatments. This indicates that broilers on plant based diet were more successful in incorporating Ca into bone in spite of the lower calcium availability in this group. Similarly results were also found in sheep on plant diets versus animal protein diets (**Brand TS et al 1999**), and high animal protein in the diet can be calciuretic (**Sherman AR 1992**). Zinc bacitracin places greater demands on the broilers in view of its growth promoting qualities. There is therefore the possibility of weak and fragile skeletons resulting in deformities, as could be noticed in some broilers fed diets containing Zinc bacitracin in this experiment,

Ca:P ratio is an indication of strength and high ratios can be associated with stronger bones (**Brand TS et al 1999**). Pathological assessment supports (**Rennie JS 1993**) the incidence of increase fibria dyschondroplasia and hypocalcaemic rickets when both low Ca and high P was added in a diet, also blood ionic Ca and bone Ca:P ratio were reduced by a higher dietary P, an incidence that can have effect on the mineral utilization in broilers.

High phosphorus levels in bone increased BMD but lowered bone strength. High plasma phosphorus decreases calcium absorption from the gut and calcium mobilization from the bone. Phosphorus is an integral part of the acid-base balance in the body. The proper ratio of calcium to phosphorus (Ca: P ratio) for growing birds is 1.5- 2.0, as growing birds cannot synthesise high level of calcium in the diet as these interfere with the absorption of phosphorus, zinc and manganese. Moreover it may lead to kidney damage and possible hampering of the proper development of the parathyroid gland by increasing gut PH which in turn decreases absorption of calcium. Feed formulation and consumption are very important in determining calcium requirement when expressed as a percentage of diet (**Abdulrahim SM et al 1999**).

The broiler claws ratio showed similar trends to those of the bone with a higher mineral value compared to those of bone. The upper levels of the chicken skeleton tend to have a higher bone density than the lower extremities (claws) with more cartilage in the upper bone (**Gordon GS and C Vaughan 1986**). Protein values were not significantly affected by the diets and Zinc bacitracin also did not significantly alter protein incorporation in bone or claws. Diets rich in animal protein increase acidity (**Cao JJ, and FH Nielsen 2010**) which is buffered by Ca^{++} released from bone. Ca^{++} is thus the

mineral that will be affected by the high protein diets rather than phosphorus and the type of protein will also have an effect. Since animal proteins are rich in sulphur containing amino acids (**Swain JL, and IL Rouse 1999**), they tend to produce higher acid loads, as noticed in this experiment.

In poultry there is still a controversy over optimum dietary mineral contents, particularly since bone formation impairs growth performance (**Gordon GS and C Vaughan 1986, Cao JJ, and FH Nielsen 2010**) and this can result in a conflict of interest between bone health and market mass. Similar study has showed that there is a slight tendency for higher weights to be achieved at lower dietary calcium intake and higher % ash to occur with a higher dietary Ca, there is also an evidence of a conflict between bone mineralization and final body weight, (**Malden CN et al 1997, Williams B et al 2000, Williams B et al 1998**), also in a study carried out on sheep showed that bone density was not a good indicator of bone strength, as sheep with the highest BMD were worst affected with the limb deformity (**Brand TS et al 1990**).

In formulating diets for growing birds and laying hens it must be considered that the calcium requirement of different breeds vary and currently acceptable ratios of Ca: P are set at 1: 5 for broilers and 3: 4 for laying hens (**Abdulrahim SM et al 1999**). The matter is further complicated in that other minerals also impact on bone development and utilization (**Rama Rao SV et al 2009, Fisher H et al 1953, Bar A et al 2003**). Diets with high concentration of phosphorus and low concentration of magnesium are known to promote nephrocalcinosis in rat (**Du Bruyn DB 1972, Hoek AC et al 1988, Mars YWHM et al 1988**). Decreasing the intake P or increasing that of Mg not only prevents nephrocalcinosis but also causes magnesiora (**Draper HH et al 1972, Schneider W and E Menden 1988, Hitchman AJ et al 1979**), this is supported by in-vitro observations that Mg inhibits the precipitation of calcium phosphate. Hence more studies is needed to validate this findings, especially in poultry as it constitute the most consumed animal protein product world wide.

CONCLUSION AND RECOMMENDATIONS

Poultry feed are primarily designed to maximize profit, with the factors like; physiological profiles, source of ingredients etc which could be detrimental to consumers are not necessarily a priority of the farmer.

Feeding broilers growth promoters, as well animal protein and Ca sources such as poultry by-products comprising the following; claws, feathers, offal' and dead birds, fish meal, carcass meal may be economically viable, but this acid forming diets will take their toll in terms of mineral utilization and incorporation into bone tissue.

The poultry industry also feeds broilers non-traditional grains, which require engineered enzymes for digestion. These may also result in adequate growth but often carry with them a legacy of negative side effects (such as intestinal bleeding for animals). Since meat quality is not directly affected, the effect of these physiological adjustments in broilers are not known, the possible side effects for consumers are even less apparent and should receive attention particularly if growth promoters (Zinc bacitracin) are used.

In view of the short maturing period of broilers (+/- six weeks) the time required for adequate drug withdrawal periods to enable the chicken get rid of all trace of drugs administered before marketing is inadequate and should be increased.

Chickens form one of the most consumed animal protein sources and it would be of value if farmer could be encouraged to use plant based protein sources in the ration of broilers since these produces the best profiles in this study.

Moreover, withdrawal period for drugs that might be administered should be legislated. The use of poultry by-products, animal waste and carcass meal should be prohibited, particularly in view of the increased risk of disease transmittance via this rout. Legislation to this effect is already in place in some countries. Moreover, the use of these products can lead to biological magnification of substances detrimental to human health.

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