

## Calcium and Phosphorus Supplementation Ameliorate Growth Performance and Bone Quality of Broilers Chicken in Grower Stage

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### ABSTRACT

Worldwide calcium & phosphorus are the predominant components of broiler feed supplements in their diets and caused rickets & osteomalacia due to above minerals deficiencies.

This study aimed to investigate the effects of Calcium and Phosphorus supplementation on growth performance and bone quality of broiler chicken in grower stage. A total of 80 one-day old broilers were raised for under ambient conditions for 40 consecutive days. There were 4 groups, contain 20 chicks per group, each group consist of 4 replicates of 5 chicks. Broilers were fed with 0% of calcium & phosphorus (Control), 1% of calcium & phosphorus (low dose), 2% of calcium & phosphorus (medium dose) and 3% of calcium & phosphorus (high dose) fed for 40 consecutive days. Data were analyzed using a completely randomized design. The results revealed that low dose treated group was significantly reduced body weight gain, lameness, rickets of bone, meat production and quality of bone was affected and feed consumption was at low rate. While medium dose slightly reduced body weight gains and quality of bone was good compared to low dose. Interestingly, high dose supplemented fed group gained higher body weight, good quality of bone, higher meat productions and consumed more feed compared to low and medium doses. Our findings demonstrate that, restriction in dietary calcium & phosphorus supplementation decreased body weight gain, rickets, imbalance, reduced products and impaired bone mineralization and strength with affecting growth performance.

**Keywords-** Calcium, Phosphorus, Broilers.

### I. INTRODUCTION

Calcium (Ca) and phosphorus (P) are essential nutrients in broilers diet formulations, especially for bone formation and as enzyme cofactor (Moraes, Ribeiro et al. 2016). In addition, Ca is necessary for coagulation, eggshell formation, muscle and nerve function (Williams, Barker et al. 1999).

Calcium and phosphorus are substantial nutrients involved in many biological processes. These minerals are the most profuse elements in the body, with 99% of Ca and 80% of P stored in the skeleton as hydroxyapatite, and both play an important role in bone development and mineralization (Veum 2010). The remaining Ca is located in the extracellular fluid,

plasma, and within cells, and plays pivotal roles in blood clotting, enzyme activation, neuromuscular function, muscle contraction, intracellular signaling, cell adhesion and metabolism (Veum 2010). However, nucleotides, phospholipids, nucleic acid and phosphorylated proteins play a central role in growth, energy metabolism, acid-base balance, cellular and membrane function, and 20% of P are mainly not located in the skeleton (Wardlaw and Kessel 2002, Berndt, Thomas et al. 2007, Veum 2010).

In recent decades, the growth rate of the chicken has increased (Havenstein, Ferket et al. 1994); according to the National Chicken Council (Proszkowiec-Weglarz and Angel 2013), the average chicken weight was approximately 1.13 kg in 1925 and required 112 days to achieve mentioned weight. In 1980, the average body weight gain was 1.78 kg at 53 days, while it required 47 days in 2011 for achieving 2.63 kg body weight.

These gains in production efficiency were coupled with decreased mortality, going from 18 to 5 to 3.8%, in 1925, 1980, and 2011, respectively (Proszkowiec-Weglarz and Angel 2013). Such rapid growth requires adequate nutritional supply and, in the case of rapid bone growth, adequate Ca and P supply (Williams, Waddington et al. 2000). Insufficient supply of one or both minerals, when the deficiency or excess of one of them interferes with homeostasis of the second one (Dias, López et al. 2013), results in reduced growth rate and bone mineralization (Hurwitz, Plavnik et al. 1995, Dias, López et al. 2013).

Furthermore, Ca and P deficiencies in rapid-growing broiler lines can lead to various skeletal abnormalities, such as tibial dyschondroplasia, rickets, osteomalacia, which lead to lameness and weak bone formation as well increased morbidity and mortality in broilers farms (Edwards Jr and Veltmann Jr 1983, Edwards Jr 2000, Fleming 2008).

In United States, the level of mortality due to skeletal disorders has been reported to be approximate 3% which is 60% of the total mortality estimated in the broiler industry by the NRC (SULLIVAN 1994). Imbalances in dietary Ca and P can also result in excess amounts of P excretion that can have negative environmental effects when poultry litter is applied as a fertilizer to soil, causing eutrophication and environmental pollution (Sharpley 1999).

To minimize supplementary Ca and P droughts and prevent its consequences occurring due to deficiencies in broiler chicken productions, it is critical to formulate diets at correct Ca to P ratios. Additionally, to implement correct Ca and P ratios in diets it is essential that we understand the digestibility of Ca and P in the ingredients that we use for poultry, and its respective digestible requirements terms collectively.

Recently, a move toward the use of digestible P (dP) has been noted (Shastak, Witzig et al. 2012, Van Kernebeek, Oosting et al. 2016), but total Ca (tCa) continues to be the standard. This means the issue of low P availability from plant sources and overall availability of total P (tP) has been partially addressed, but the issue of Ca availability in the ingredients has not been addressed. Therefore, the aim of this research was to investigate the nutritional effects of calcium and phosphorus on growth performance and bone quality of broiler chicks in grower stage and its consequences occurring during the period.

## II. BIRDS AND HUSBANDRY

Broiler day-old 80 chicks, were obtained from a commercial hatchery at day of hatch and transported to the research facilities at Kunduz province. On arrival, chicks were randomized, weighed by pen, and placed in separated sheet with respect to its allocated dietary treatments. Birds were maintained on a constant lighting program and allowed ad libitum access to the treatment diets for the duration of the trial (d 0 to 40). The study was conducted to investigate the nutritional effects of calcium and phosphorus during growing stage of broilers chicken in Kunduz province. Also to identify the higher consumption of feed and significant growth performance among the treated groups respectively. In this experiment we fed 80 white broilers day-old chicks with 3 different treatments (low dose=1% of calcium & phosphorus, medium dose 2% of calcium and phosphorus and higher dose = 3% of calcium and phosphorus). The research took place in local traditional chicken farm with access to all facilities, such as constant temperature, electricity, feed and water was provided ad libitum. The start of exposure was the day of weaning until day 40.

### *Place of research*

The study was conducted in Kunduz-center district of Kunduz province. Kabul feed was supplemented to all groups with varied dose of calcium & phosphorus. The experiment begun and ended in exact place and on time.

### *Research Design*

Day-old broiler chicks, which were at first day of age, average weight was  $40 \pm 10$  grams were randomly placed in four groups, each group contain 20 chicks with having 5 replicates. There were 1) control group, 2) low dose group (1% calcium and phosphorus), 3) medium dose group (2% of calcium and phosphorus)

and 4) high dose group (3% of calcium and phosphorus). All groups were given a specific company diet. Feeding was initiated, until the end of the study (40 days). Each cage was 2 meters long, 1 meter and 2 centimeters high and also 1 meter and 2 centimeters wide. The traits which were recorded during this period were, consumed feed amount, live body weight gain, FCR (Feed Conversion Ratio), bone quality and meat productions.

A certain amount of feed was given them on daily at certain hours of the day (morning and evening). The amount of nutrients was increased subsequently because of the increase in weight gain. This method continued until the end of the study, and the remaining feed left in the grain was collected and weighed. During the study, all groups had access to clean water and were fed twice a day. The chicks were weighed each week and recorded in a research booklet before the second week started.

### *Tools needed for research*

Fortunately, all the equipment needed for a study was available in the city. The equipment used for the research includes electric lights, drinkers, feeders, thermometer, cages, digital scales, cuffs, feed and research notebooks.

### *Broiler chicks feed*

Adequate feed measurement requires knowledge and other factors such as energy balance, amino acid balance, and so on. The slightest mistake can have a direct effect on productions. Acquisition is something that an expert has access to because it is always available to them on the one hand and cheap and economical on the other. The feed can be measured to contain sufficient amounts of energy, protein (amino acids), minerals, vitamins, and essential fatty acids. Products (eggs, meat and weight) should be obtained with a minimum of nutrition.

### *Importance of Phosphorus*

National Research Council (Council 1930) recommended dietary phosphorus level for laying hens at 0.32% available phosphorus per kilogram of diet. Low dietary phosphorus during the laying period can lead to increased incidence of cage layer fatigue, reduced bone ash, increased severity of osteoporosis and diminished bone strength. Phosphorus plays key role in carbohydrate metabolism, fat metabolism and the regulation of acid-base balance in body.

Deficiency of phosphorus, calcium or vitamin D, or all of these leads to lameness, stiff legs, ruffled feathers, reduction in growth, leg bones appear rubbery, joints become enlarged, poor egg shell quality, reduced egg production, thin shelled eggs, and shell less eggs. The phosphorus nutritional role relates to calcium. Hence, the absorption of these elements depends upon the ratio of dietary calcium to phosphorus. An excess of either can interfere this process and causes production losses. Excess calcium in the diet leads to the formation of calcium phytate complexes, which are highly insoluble and poorly digested.

**Source of Phosphorus**

Dicalcium phosphate (DCP), Monocalcium phosphate (MCP), Defluorinated phosphate (Rock), Meat and Bone meal, and Fish meal are some of the sources of phosphorus.

**Plant materials:** Corn grain, wheat grain, wheat bran, broken rice grain, rice polish, groundnut meal, soya extract, sunflower solvent extract, bajra grain, and barley grains, etc., are the plant sources for phosphorus. The phosphorus content of typically used plant materials ranges from 0.09% to 1.09%. As this is largely in phytate form, its digestibility varies from 27% to 66% in poultry.

**Animal sources:** The total phosphorus content is as much as 30%. The amount of available phosphorus provided by animal sources like meat, fish, and bone meal varies from 59% to 74% depending on the material's origin and production process.

These sources run a greater risk of possible contamination.

**III. DATA ANALYSIS**

The data obtained are analyzed statistically using 5 Digo, Ca., USA (Graphpad. Prism.) All available data are recorded in tables and graphs based on the

average deviation standard. Based on statistical experience and meaning findings are (Significant) data, each group data using One-Way (One-Way Analysis Variance) ANOVA and Takey test as  $P < 0.05$  has a meaningful meaning and is accepted in each experimental group. The data also show the weekly weight gain and meat production of the experimental groups using the two-way ANOVA. FCR Feed Conversion Ratio is calculated in each group.

**IV. RESULTS**

**Organ Weight Measurements**

The results on the heart, liver and lungs weight gained showed that 1% of calcium and phosphorus (Low Dose) group significantly ( $p < 0.05$ ) gained weight compared to control group (Table 1). Conversely, the organ weights gained for the heart, liver and lungs in 2% of calcium and phosphorus (medium dose) significantly ( $p < 0.05$ ) increased organs weight gaining compared to control and low dose during the experiment. However, the organs weight gained in 3% of supplemented calcium and phosphorus (High dose) comparatively higher than all treated doses of calcium and phosphorus (Table 1).

**Table 1. Organs weight measurement in starter and grower stages.**

Stages / Days	Treatments	Heart (g)	Liver (g)	Lungs (g)	P-Value
Starter (day 10)	Control	1.04±0.04 <sup>b</sup>	3.04±0.04 <sup>b</sup>	0.94±0.04 <sup>b</sup>	<0.001
	Low Dose (1%)	1.86±0.02 <sup>b</sup>	4.86±0.02 <sup>b</sup>	1.16±0.02 <sup>b</sup>	<0.001
	Medium Dose (2%)	2.41±0.04 <sup>c</sup>	5.41±0.04 <sup>c</sup>	1.98±0.04 <sup>c</sup>	<0.001
	High Dose (3%)	3.43±0.02 <sup>a</sup>	6.43±0.02 <sup>a</sup>	2.43±0.02 <sup>a</sup>	<0.001
Grower (Day 40)	Control	4.04±0.04 <sup>b</sup>	8.04±0.04 <sup>b</sup>	4.04±0.04 <sup>b</sup>	0.005
	Low Dose (1%)	12.86±0.02 <sup>c</sup>	14.86±0.02 <sup>c</sup>	11.86±0.02 <sup>c</sup>	0.005
	Medium Dose (2%)	13.41±0.04 <sup>b</sup>	18.41±0.04 <sup>b</sup>	12.41±0.04 <sup>b</sup>	0.005
	High Dose (3%)	18.43±0.02 <sup>a</sup>	25.43±0.02 <sup>a</sup>	26.43±0.02 <sup>a</sup>	0.005

Heart, liver and lungs weight unit (g, n = 10); the table shows different treatments, 1% of calcium and phosphorus (Low Dose), 2% of calcium and phosphorus (Medium Dose) and 3% of calcium and phosphorus (High Dose). Values shown are mean ± SE. (Standard Errors), <sup>a, b, c</sup> means in a row with different superscript differ significantly ( $p < 0.05$ ).

**Live body weight gain**

The live body weight gained was increased subsequently according to the value of treatment

supplemented through feed. The higher the treatment is supplemented the more the weight has gained in all groups. Therefore, in starter stage, the live body weight in higher dose was comparatively higher than low and medium doses. While, in grower stage the live body weight gained was higher in higher supplemented dose of calcium and phosphorus (3%) compared to all other treated groups. This live body weight gaining indicated that there is a direct relationship between dose and weight gaining respectively (Table 2).

**Table 2. Live body weight measurement in starter and grower stages.**

Groups/ Treatments	Live body weight gained in Starter stage (day 10)	Live body weight gained in Grower Stage (day 40)	P-Value
Control	360.94±0.79 <sup>b</sup>	1200.94±0.79 <sup>b</sup>	<0.001
Low Dose (1%)	389.34±0.41 <sup>a</sup>	1456.34±0.41 <sup>a</sup>	<0.001

Medium Dose (2%)	415.64±0.88 <sup>c</sup>	1563.64±0.88 <sup>c</sup>	<0.001
High Dose (3%)	460.02±0.98 <sup>d</sup>	1863.64±0.88 <sup>c</sup>	<0.001

Live body weight gaining measured in both starter and grower stages, unit (g, n = 10); the table shows different treatments, 1% of calcium and phosphorus (Low Dose), 2% of calcium and phosphorus (Medium Dose) and 3% of calcium and phosphorus (High Dose). Values shown are mean ± SE. (Standard Errors), <sup>a, b, c</sup> means in a row with different superscript differ significantly ( $p < 0.05$ ).

#### Feed intake

Feed intake among the treatments were significantly positively affected ( $P > 0.05$ ). Feed intake was numerically higher in higher supplemented doses of calcium and phosphorus. The feed intake and body weight gaining has direct relationship. Therefore, during the starter and grower stages, the higher feed intake and body weight gaining was observed in higher treated dose of calcium and phosphorus. This indicate that, the feed intake also has direct relationship with treatment and there was dose-depending relationship.

#### Feed conversion ratio

Lowered or poor FCR in control and low dose groups may possibly be due to less feed intake in the control group and lower calcium and phosphorus based diets. It is likely that lowered calcium and phosphorus supplementation may affect the feed consumption and cause lower feed conversion ration in all groups. It has been observed that the higher feed intake and body weight gaining is linked with higher feed conversion and its depending on treated doses of calcium and phosphorus. Better FCR in millet fed birds was also reported earlier (Ibitoye, Oloredo et al. 2012).

#### Bone quality

Broiler chickens are particularly more susceptible to bone abnormalities and minerals deficiencies compared to egg-laying birds. As broiler chickens known “rapid-grower chicken” therefore, the mineralization is essential for their bone formation to load the body weight accordingly. The performance of the modern broiler has changed considerably in recent years but their diets have changed little (Williams, Waddington et al. 2000). The most common leg disorders in commercial chicken production are “twisted legs”, rickets and tibial dyschondroplasia (TD) (Hulan, Proudfoot et al. 1984). All three of these problems are associated with Ca and P deficiencies or imbalances. The other bones like cortical of fast growing broilers are also highly absorbent which may lead to bone deformity (Thorp and Waddington 1997, Hamdard, Lv et al. 2019, Hamdard, Shi et al. 2019). The bone quality of all supplemented groups were good and there was no deformity of bone structure. It’s important that, not only supplementation of calcium and phosphorus are essential for bone formation but also vitamin D and light are directly affecting the construction of bone during growing stages of chickens. Based on proper bone

formation, (Driver, Pesti et al. 2005), suggested that diets should contain 1.1 to 1.3% Ca and 0.3 to 0.6% available P.

## V. DISCUSSION

In this study, the dietary supplementation effects of calcium and phosphorus on growth performance, bone quality and feed intake were examined. It was found that the growth performance, live body weights, organs weight gaining and bone quality was improved depending on supplemented doses respectively. The higher the supplemented dose of calcium and phosphorus were used, the better the body weight and growth was enhanced.

Calcium is the most abundant mineral in the body of animals (McDonald et al., 1995). Well over 90% of Ca is found in the bones where it combines with P, the second most abundant mineral in bone, to form calcium phosphate crystals or hydroxyapatite with the molecular formula  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  (Scott et al., 1982). Other elements including Na, Mg, Fe and F1 may also be incorporated into the hydroxyapatite crystal (Matuszewski, Łukasiewicz et al. 2020). The small amount of Ca present in the soft tissues has several important functions. Calcium is essential for the activity of a number of enzyme systems including those that hydrolyze polysaccharides, phospholipids, and proteins (Driver 2004). Calcium is also required for a number of the blood-clotting proteins where it supports the interactions between macromolecules, such as protein-protein binding or protein-phospholipid binding via various Ca-binding proteins (Driver 2004). Other Ca-binding proteins include components of the cytoskeleton. Calcium ions also participate in cell signaling as signals induce an increase in cellular Ca concentration, either from extracellular fluid, in the case of nerve cells, or from cellular Ca storage organelles, in the case of muscle cells (Driver 2004). Calcium is therefore essential for both transmission of nerve impulses and muscle contraction (Hamdard, Zahir et al. 1925, Liem 2009, Matuszewski, Łukasiewicz et al. 2020).

Phosphorus, the other major constituent of bone, is an essential component of almost all metabolic processes (Matuszewski, Łukasiewicz et al. 2020). The P content of the body is somewhat less than that of the Ca content and 80 to 85% of the total body phosphorus is found in the bones. Phosphates are used for energy storage and metabolism in the formation of sugar phosphates and adenosine di- and tri-phosphates (Gautier 2016). In addition, P forms part of phosphoproteins, nucleic acids and phospholipids (Gautier 2016).

Therefore, dietary supplementation of calcium and phosphorus on broilers chicks from day one until day 40 (grower stage) has direct relationship with better production and growth performance and the live body weight, organs weight gaining, feed intake and bone quality were dose-dependent and improved consequently. These findings suggest that there is a strong relationship between growth and mineralization which need to be further investigate for new innovations.

## VI. CONCLUSION

Calcium & Phosphorus are an essentials nutrient that not only comprises about 30% of the poultry skeletal ash content but also is involved in a great number and variety of metabolic pathways.

Plant ingredients are the main basis for compound poultry feed. Phosphorus is a component of organic compounds involved in energy, carbohydrate, amino acid and fat metabolism, nervous tissue metabolism, blood chemistry and lipid transport. The requirement of phosphorus in birds can be affected by environmental conditions, genetic strains and other nutrients in the diet. Chicks and grower birds require more phosphorus than layers, because of the phosphorus needs for skeletal growth. Therefore, mineralization and feed should be balanced considering the standards of formulation for potential growth performance and broiler productions.

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