

The Effect of Dietary Phytase Supplementation at Different Levels on Tibial Bone Characteristics and Strength in Broilers

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Abstract: The influence of various levels of supplemental phytase on P and Ca availability was investigated using morphometric indices and the bone strength of tibiotarsi in broilers. Forty tibiotarsi were used from the Ross 308 male broilers fed corn-soybean meal based diets. Dietary treatments were: 1) Control: diet with DCP, 2) Negative control: control diet without DCP, 3) Control diet + 300 U of phytase/kg, 4) Control diet + 500 U of phytase/kg, 5) Control diet + 700 U of phytase/kg. There were no significant differences among the groups in the mean for tibiotarsal weight and length and also for the thickness of the medial wall. However, 300 U/kg diet with phytase supplementation had a greater influence on the tibiotarsal weight/length index and the robusticity index when compared to those of broilers in both control groups, and the thickness of the lateral wall and tibiotarsal index among all the groups ($P<0.05$). The percentages of tibia ash were improved by the addition of dietary phytase. Phytase supplementation slightly improved tibia breaking stress and the modulus of elasticity in broilers. Increasing phytase levels from 300 to 700 U of phytase/kg of diet provided no additional benefits for tibiotarsal bone characteristics and strength in broilers.

Key Words: Phytase, tibia, bone strength, broiler

Diyete Farklı Oranlarda Fitaz İlavesinin Broilerlerde Tibia Kemik Özellikleri ve Dayanıklılığına Etkisi

Özet: Farklı düzeydeki fitaz ilavesinin broilerlerde P ve Ca yararlanılabilirliğine etkisi morfometrik indeksler ve tibiotarsus direnci ölçümleri kullanılarak belirlendi. Araştırmada mısır-soya fasulyesi küspesi ağırlıklı rasyon ile beslenen Ross 308 erkek broylerlere ait 40 tibiotarsus kullanıldı. Denemede kullanılan rasyonlar 1) Kontrol: DCP'li rasyon, 2) Negatif kontrol: Kontrol rasyonu DCP'siz, 3) Kontrol rasyonu + 300 U fitaz / kg rasyon, 4) Kontrol rasyonu + 500 U fitaz / kg rasyon, 5) Kontrol rasyonu + 700 U fitaz / kg rasyon şeklindeydi. Tibiotarsal ağırlık ve uzunluk ile medial duvar kalınlığı ortalamaları arasında gruplar arasında bir fark bulunamadı. Bununla beraber, 300 U fitaz / kg rasyon grubuna ait tibiotarsal ağırlık / uzunluk indeksi, sağlamlık indeksi değerleri her iki kontrol grubundan, lateral duvar kalınlığı ve tibiotarsal indeks değerleri ise tüm gruplardan önemli düzeyde farklıydı ($P<0.05$). Tibia kül oranı rasyona fitaz ilavesi ile arttı. Fitaz ilavesi, broylerde tibia kırılma direnci ve esnekliğini rakamsal olarak artırdı. Fitaz düzeyinin 300 U fitaz / kg'dan 700 U fitaz / kg'a çıkarılması broylerde tibiotarsal kemik özellikleri ve dayanıklılığı üzerine ek bir yarar sağlamadı.

Anahtar Sözcükler: Fitaz, tibia, kemik direnci, broyler

Introduction

Many factors, such as growth, gender, aging, genetics, disease and nutrition, can affect bone strength through their direct or indirect effects on bone. However, the most relevant factor for poultry bone strength is nutrition. Calcium and phosphorus are primary inorganic nutrients in the bone that may be important for bone health and strength (1). Nelson *et al.* (2) reported that the addition of phytase to broiler diets resulted in a marked improvement of the utilization of phytate phosphorus as measured by bone ash. Similarly, several researchers have shown that dietary supplementation of

microbial phytase improved the availability of Ca and P (3-7).

Tests of bone strength in the literature (8,9) typically report the kilograms of force required to break various bones. However, Patterson *et al.* (10) reported that stress and modulus of elasticity are better terms to use than force in making bone strength comparisons between groups of birds that may differ in body size and bone dimension.

The cortical index (tibiotarsal index) was first proposed by Barnett and Nordin (11) to indicate bone

mineralization by morphometric measurements. Virtama and Telkka (12) and Mutus and Onar (13) showed a good correlation between the cortical index and the mineral content of bone in humans and dogs, respectively. In experimental studies of bones from rats, two indices of bone density were proposed. The robusticity index, proposed by Reisenfeld (14), and the bone weight/bone length index, used by Seedor et al (15). Both indices have shown significant changes between normal and ovariectomized (15,16) or castrated rats (17).

As far as we know, there is no literature concerning the effects of various levels of supplemental phytase on the bone robusticity index, bone weight/bone length index and tibiotarsal index as well as the comparison of these results with the results of the mechanical properties of tibiotarsus in broilers. Therefore, the objective of this study was to investigate the effects of various levels of phytase supplementation to broiler diets on morphometric and mechanical properties as well as the ash content of the tibia.

Materials and Methods

In this trial, 40 left tibiotarsi of Ross 308 male broilers were used to investigate the effects of phytase (Natuphos®) supplement in corn-soybean meal based diets on bone characteristics. The chicks were fed prestarter (0 to 10 days), starter (10 to 21 days), grower (21 to 42 days) and finisher (42 to 45 days) diets (Table 1). The untreated control diet was formulated for the nutritional requirements of the broiler chicken (18). Dietary treatments were: 1) Control: diet with DCP, 2) Negative control: control diet without DCP, 3) Control diet + 300 U of phytase /kg, 4) Control diet + 500 U of phytase/kg, 5) Control diet +700 U of phytase/kg. Commercial brooding management and procedures were followed during the trial. Feed and water were available *ad libitum*. Birds were exposed to continuous fluorescent light.

Left tibias were removed from individual broilers and frozen. The tibias were later thawed and stripped of soft tissues. They were then placed in boiling water for 10

Table 1. Composition of the diets (as-fed basis).

| Ingredients, % | Pre-starter | | | | | Starter | | | | | Grower | | | | | Finisher | | | | |
|------------------------|----------------|-----------------|-----------------------|-----------------------|-----------------------|----------------|-----------------|-----------------------|-----------------------|-----------------------|----------------|-----------------|-----------------------|-----------------------|-----------------------|----------------|-----------------|-----------------------|-----------------------|-----------------------|
| | C ² | NC ³ | 300 U/kg ⁴ | 500 U/kg ⁴ | 700 U/kg ⁴ | C ² | NC ³ | 300 U/kg ⁴ | 500 U/kg ⁴ | 700 U/kg ⁴ | C ² | NC ³ | 300 U/kg ⁴ | 500 U/kg ⁴ | 700 U/kg ⁴ | C ² | NC ³ | 300 U/kg ⁴ | 500 U/kg ⁴ | 700 U/kg ⁴ |
| Yellow corn | 46.60 | 48.60 | 47.20 | 47.50 | 47.60 | 50.20 | 53.50 | 50.00 | 49.90 | 50.30 | 48.70 | 53.10 | 49.80 | 50.20 | 50.70 | 48.50 | 49.00 | 49.30 | 49.80 | 50.70 |
| Soybean meal (46%) | 37.00 | 34.50 | 37.00 | 37.00 | 37.00 | 33.10 | 28.90 | 34.30 | 35.00 | 35.00 | 32.50 | 21.60 | 30.00 | 29.90 | 29.70 | 33.90 | 32.30 | 34.40 | 34.40 | 34.10 |
| Fullfat soybean meal | - | - | - | - | - | - | - | - | - | - | 4.00 | 9.00 | 6.00 | 6.00 | 6.00 | - | - | - | - | - |
| Fish meal (67 %) | 5.70 | 6.80 | 5.50 | 5.50 | 5.70 | 4.40 | 6.80 | 3.50 | 3.00 | 3.20 | 1.00 | 4.20 | 1.00 | 1.00 | 1.00 | 2.00 | 3.20 | 1.60 | 1.90 | 2.40 |
| Poultry byproduct meal | 2.50 | 3.50 | 2.50 | 2.50 | 1.90 | 3.00 | 3.00 | 3.00 | 3.00 | 2.50 | 3.00 | 5.00 | 3.50 | 3.50 | 3.50 | 4.50 | 6.00 | 4.00 | 3.30 | 2.40 |
| Vegetable oil | 4.70 | 3.80 | 4.50 | 4.40 | 4.50 | 6.00 | 5.06 | 6.00 | 6.00 | 6.00 | 6.90 | 4.30 | 6.20 | 6.10 | 5.90 | 7.50 | 6.70 | 7.50 | 7.50 | 7.50 |
| Limestone | 0.73 | 1.37 | 0.91 | 1.01 | 1.44 | 0.78 | 1.37 | 1.03 | 1.11 | 1.29 | 0.81 | 1.36 | 0.89 | 1.00 | 1.18 | 0.96 | 1.39 | 1.04 | 1.13 | 1.22 |
| Dicalcium phosphate | 1.30 | - | 0.90 | 0.60 | 0.30 | 1.10 | - | 0.80 | 0.60 | 0.30 | 1.60 | - | 1.10 | 0.80 | 0.50 | 1.20 | - | 0.70 | 0.50 | 0.20 |
| Salt | 0.22 | 0.20 | 0.23 | 0.22 | 0.28 | 0.22 | 0.17 | 0.23 | 0.24 | 0.25 | 0.28 | 0.25 | 0.28 | 0.28 | 0.28 | 0.25 | 0.25 | 0.26 | 0.26 | 0.26 |
| Premix ¹ | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| DL-Methionine | 0.25 | 0.23 | 0.25 | 0.25 | 0.25 | 0.20 | 0.20 | 0.13 | 0.13 | 0.13 | 0.21 | 0.19 | 0.22 | 0.20 | 0.21 | 0.19 | 0.16 | 0.19 | 0.19 | 0.19 |
| Phytase | - | - | 0.012 | 0.020 | 0.028 | - | - | 0.012 | 0.020 | 0.028 | - | - | 0.012 | 0.020 | 0.028 | - | - | 0.012 | 0.020 | 0.028 |
| Calculated analysis | | | | | | | | | | | | | | | | | | | | |
| ME, kcal/kg | 3050 | 3050 | 3050 | 3050 | 3050 | 3170 | 3170 | 3170 | 3170 | 3170 | 3240 | 3240 | 3240 | 3240 | 3240 | 3270 | 3270 | 3270 | 3270 | 3270 |
| Lysine, % | 1.54 | 1.56 | 1.54 | 1.54 | 1.54 | 1.46 | 1.48 | 1.45 | 1.45 | 1.45 | 1.33 | 1.35 | 1.32 | 1.32 | 1.32 | 1.38 | 1.44 | 1.37 | 1.37 | 1.38 |
| Methionine, % | 0.62 | 0.62 | 0.62 | 0.62 | 0.61 | 0.57 | 0.59 | 0.50 | 0.50 | 0.49 | 0.52 | 0.53 | 0.53 | 0.53 | 0.52 | 0.53 | 0.53 | 0.53 | 0.53 | 0.52 |
| Methionine+Cystine % | 0.92 | 0.92 | 0.92 | 0.92 | 0.92 | 0.87 | 0.89 | 0.80 | 0.80 | 0.80 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 | 0.82 |
| Av. P, % | 0.45 | 0.29 | 0.45 | 0.50 | 0.45 | 0.45 | 0.26 | 0.45 | 0.45 | 0.45 | 0.45 | 0.30 | 0.50 | 0.50 | 0.50 | 0.42 | 0.26 | 0.42 | 0.42 | 0.42 |
| Analysed values | | | | | | | | | | | | | | | | | | | | |
| CP, % | 25.43 | 25.74 | 25.11 | 25.68 | 25.36 | 24.28 | 24.32 | 24.67 | 24.90 | 24.85 | 23.07 | 23.14 | 23.27 | 23.48 | 23.66 | 23.87 | 24.73 | 23.94 | 24.15 | 23.68 |
| Ca, % | 1.08 | 1.02 | 1.18 | 1.14 | 1.07 | 1.03 | 1.19 | 1.16 | 1.05 | 1.11 | 1.04 | 1.06 | 1.12 | 1.08 | 1.17 | 1.14 | 1.18 | 1.12 | 1.18 | 1.03 |
| Total P, % | 0.72 | 0.57 | 0.78 | 0.73 | 0.74 | 0.66 | 0.55 | 0.68 | 0.62 | 0.65 | 0.61 | 0.53 | 0.63 | 0.60 | 0.66 | 0.65 | 0.53 | 0.67 | 0.62 | 0.69 |

¹ Premix provided the following per kilogram of diet: vitamin A, 9,000 IU; vitamin D3, 1,500 IU; vitamin E, 10 IU; vitamin K, .5 mg; vitamin B12, .007 mg; thiamin, .4 mg; riboflavin, 6 mg; folic acid, 1 mg; biotin, .15 mg; pantothenic acid, 12 mg; niacin, 35 mg; pyridoxine, 4mg; choline, 1,000 mg; iodized salt 2 g; manganese, 60 mg; copper, 5 mg; zinc, 50 mg; and selenium, .1 mg.

² C: Control; ³NC: Negative Control; ⁴U/kg: U of phytase/kg of diet

min. Bones were defleshed by hand and the bone cap was removed. They were then air dried for 24 h at room temperature. The tibiotarsal length was measured with a dial caliper and bones were weighed on a precision balance. Following morphometric measurements, breaking stress and modulus of elasticity were determined by the formula used in the material analysis of wood, plastic and metal structural members in engineering handbooks (19,20) (Figure). Stress accounts for the area over which the force is applied and the geometric shape of this area. Prior to breaking, each bone was marked at the midpoint and outside diameters were measured both perpendicular and parallel to the direction of the applied force using a dial caliper. After breaking, diameter measurements were made inside and outside the midshaft of the bone both perpendicular and parallel to the direction of the applied force to calculate the area moment of inertia. This term was used together with elastic deformation force and bone deflection to elastic deformation to determine the stress (kg/cm²) and modulus of bone elasticity (kg/cm²). The thickness of the medial and lateral walls was measured as close as possible to the midpoint mark using a dial caliper. Medullary canal diameter was calculated by subtracting the thicknesses of the medial and lateral walls from the diameter of diaphysis. The bone weight/bone length index was obtained by dividing the tibia weight by its length (15). The tibiotarsal index and the robusticity index are determined using the following formulas: tibiotarsal index = diaphysis diameter - medullary canal diameter / diaphysis diameter X 100 (11), robusticity index = bone length / cube root of bone weight (14, 21).

The bone fragments were oven-dried at 105°C for 24 h and ashed in a muffle furnace at 600°C for 6 h in porcelain crucible (22). The percentage of ash was determined relative to the dry weight of the tibia.

Data were evaluated by ANOVA (23). Significant effects of treatment means were separated using Student's t-test. Differences were considered to be significant at 0.05.

Results

Increasing dietary supplementation of phytase from 0 to 700 U of phytase/kg of numerically increased body weight in comparison to control groups (Table 2). There were no significant differences among the groups in

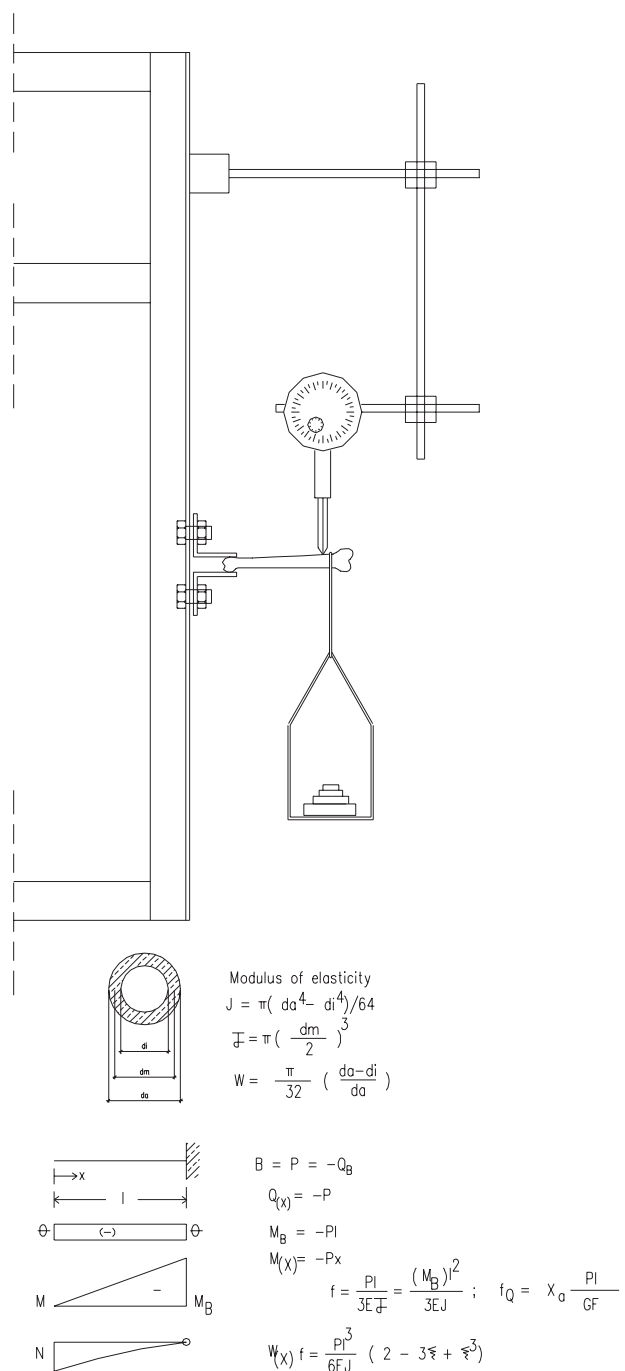


Figure. Mechanical design and formulas for calculating the mechanical properties of bone (19,20).

tibiotarsi weight and length. The tibiotarsi weight/length index was found to be significantly higher (P<0.05) in the three experimental groups than in the control groups, with the highest readings coming from the 300 U of

Table 2. The effect of various phytase levels on morphometric parameters of tibia.

| | Control | | Negative control | | 300 U of phytase/kg | | 500 U of phytase/kg | | 700 U of phytase/kg | |
|---|--------------------|-------------|-------------------|-------------|---------------------|-------------|---------------------|-------------|---------------------|-------------|
| | \bar{X} | S \bar{x} | \bar{X} | S \bar{x} | \bar{X} | S \bar{x} | \bar{X} | S \bar{x} | \bar{X} | S \bar{x} |
| Body weight, g | 1894 ^{bc} | 14.6 | 1864 ^c | 14.4 | 1987 ^a | 10.5 | 1926 ^b | 17.8 | 1972 ^a | 8.1 |
| Weight, mg | 5801 | 340 | 5376 | 131 | 6599 | 237 | 6214 | 194 | 6559 | 406 |
| Length, mm | 92.2 | 1.0 | 89.4 | 0.9 | 90.5 | 1.0 | 91.5 | 0.7 | 92.0 | 1.4 |
| Tibiotarsi Weight / Length index, mg/mm | 63.0 ^{bc} | 3.8 | 60.2 ^c | 1.7 | 72.9 ^a | 2.4 | 67.9 ^{ab} | 1.8 | 71.1 ^{ab} | 3.7 |
| Robusticity index | 5.1 ^a | 0.1 | 5.2 ^a | 0.1 | 4.8 ^b | 0.1 | 5.0 ^a | 0.0 | 4.9 ^{ab} | 0.1 |
| Diaphysis diameter, mm | 7.8 | 0.2 | 7.3 | 0.3 | 7.6 | 0.2 | 7.9 | 0.2 | 8.1 | 0.2 |
| Thickness of the medial wall, mm | 1.2 | 0.1 | 1.0 | 0.1 | 1.3 | 0.2 | 1.2 | 0.1 | 1.2 | 0.1 |
| Thickness of the lateral wall, mm | 2.1 ^b | 0.1 | 1.8 ^c | 0.1 | 2.3 ^a | 0.1 | 2.0 ^{bc} | 0.1 | 2.0 ^{bc} | 0.1 |
| Medullary canal diameter, mm | 4.5 ^a | 0.1 | 4.5 ^{ab} | 0.2 | 4.0 ^b | 0.2 | 4.7 ^a | 0.2 | 4.9 ^a | 0.3 |
| Tibiotarsal index | 42.3 ^b | 1.1 | 38.0 ^c | 1.5 | 47.0 ^a | 2.2 | 40.1 ^{bc} | 1.8 | 39.0 ^{bc} | 2.4 |

^{a-c} Values with different superscripts in a row differ significantly (P<0.05).

Table 3. The effect of various phytase levels on ash content and mechanical measurements of tibia.

| | Control | | Negative control | | 300 U of phytase/kg | | 500 U of phytase/kg | | 700 U of phytase/kg | |
|---|--------------------|-------------|-------------------|-------------|---------------------|-------------|---------------------|-------------|---------------------|-------------|
| | \bar{X} | S \bar{x} | \bar{X} | S \bar{x} | \bar{X} | S \bar{x} | \bar{X} | S \bar{x} | \bar{X} | S \bar{x} |
| Ash, % | 42.0 ^{bc} | 0.8 | 41.5 ^c | 0.8 | 44.1 ^a | 0.5 | 43.8 ^a | 0.6 | 43.6 ^{ab} | 0.8 |
| Modulus of elasticity, kg/cm ² | 4039 | 892 | 4155 | 775 | 6009 | 1192 | 5728 | 2207 | 7279 | 805 |
| Breaking stress, kg/cm ² | 85 | 8 | 83 | 12 | 119 | 16 | 114 | 14 | 90 | 13 |

^{a-c} Values with different superscripts in a row differ significantly (P<0.05).

phytase/kg diet group. The robusticity index was lowest in the 300 U of phytase/kg diet group (P<0.05). The diaphysis diameter data revealed no significant difference between the groups. There were no differences between the groups as to the thickness of the tibia’s medial wall. In contrast, the highest thickness of the lateral wall was found in the 300 U of phytase/kg diet-supplemented group (P<0.05). The mean of the medullary canal diameter of the 300 U of phytase/kg diet was statistically lower than in other groups, except for the negative control group (P<0.05). The highest mean for the tibiotarsal index was also found in the group treated with the 300 U of phytase/kg diet (P<0.05).

Dietary phytase supplementation improved tibia ash content when compared with the control groups (P<0.05). However, this effect was numerically the

highest in the broiler fed diet supplemented with 300 U of phytase/kg. Dietary phytase supplementation numerically improved tibia breaking stress and the modulus of elasticity in birds (Table 3).

Discussion

The tibia weights and lengths of broilers fed graded phytase supplements were no different from those of broilers fed the control diets (Table 2). However, Qian *et al.* (6) reported that the tibias of broilers fed with supplemental phytase and inorganic P were longer and wider than those of broilers fed P-deficient diets.

The tibiotarsi weight/length index was found to be significantly higher (P<0.05) in the three experimental groups than in the control groups. There was no

difference between the groups with regards the thickness of the medial wall of the tibia. In contrast, the thickness of the lateral wall was different and the highest value for that was found in the 300 U phytase/kg-supplemented group ($P < 0.05$) (Table 2). The lowest robusticity index was also found in the 300 U phytase/kg diet group. The lower the robusticity index and the higher bone weight/bone length index as well as the tibiotarsal index are, the denser is the bone (11,14,15,21). The highest tibiotarsal index was found in the tibia of broilers fed a diet supplemented with 300 U of phytase/kg ($P < 0.05$). These results suggest that supplemental phytase increases the availability of P and Ca and promotes the growth and development of the bone.

Because bone mineralization provides compressional strength to bones, the bone ash content have been used as the indices of bone strength (1). The percentages of tibia ash were significantly improved by dietary phytase supplementation in this experiment (Table 3). These data are in an agreement with Nelson *et al.*, (2); Perney *et al.*, (5); and Qian *et al.* (6). Increased bone ash suggests an improvement in bone mineralization due to increased P and Ca utilization, which was caused by the liberation of inorganic P and Ca from the phytate molecule by the phytase enzyme in the gastrointestinal tract.

The stress at yield reflects the rigidity of bone as a whole, whereas the slope of the linear region of the stress versus strain-curve is called Young's modulus or elastic modulus and reflects the intrinsic stiffness or rigidity and material properties of bone. High modulus may indicate bone to be more rigid, whereas low modulus could mean the bone is more ductile (1). Although some small improvement in tibia breaking stress and the modulus of elasticity were observed due to supplemental phytase, these differences were not significantly different (Table 3). This result was consistent with the previous reports that the inclusion of phytase in the diet improves bone

strength (5-7). Bones from the 300 U phytase/kg diet treatment had the highest mean tibia breaking stress value. However, the modulus of elasticity maximized with dietary phytase at 700 U of phytase/kg (Table 3). These observations were supported by ash data. This is because the percentage of bone ash is usually positively correlated with bone breaking strength (24). In a similar study, bone strength and modulus of elasticity were reduced in the tibiotarsi of broilers by feeding a low Ca and low P diet (10).

The greatest tibiotarsal weight/length index, the thickness of the lateral wall, tibiotarsal index, bone breaking strength, bone ash and also the lowest robusticity index appears to occur when broilers are fed a 300 U phytase/kg supplemented diet. Increasing phytase levels from 300 to 700 U phytase/kg in a diet provided no additional benefit. The discrepancy in these results may have resulted from large variations in growth rate and bone mineralization among individual broilers.

In conclusion, dietary phytase supplementation numerically improved bone strength by promoting bone mineralization in broilers. These findings were supported with the results of three morphometric indices as parameters of bone density. However, graded phytase supplementation did not show an increased effect on most of the parameters in the present study.

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