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Promising Effects of Vinasse Use on Bone Strength in Laying Hens

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Abstract

This study was carried out to determine the effects of vinasse on bone strength and some material properties in laying hens with specific amounts of vinasse added to their diets. The study was the first to determine the mechanical properties of laying hens fed a diet supplemented with vinasse. The study was conducted on tibiotarsi of two hundred 94-week-old laying hens which were selected randomly from a commercial farm. Morphometric measurements were made using a digital scale and caliper, and mechanical data were collected from tibiotarsi using a three-point bending test with a compression-tension machine. The laying hens fed with vinasse had higher tibiotarsal length and weight than those in Control group. The groups fed with vinasse were significantly stiffer and had a higher breaking force than Control group ($P=0.002$, $P<0.001$). In conclusion, significant and promising bone material properties were obtained from the laying hens fed with vinasse.

Keywords: Bone strength, Laying hens, Tibiotarsus, Vinasse.

Introduction

Intensive selection for rapid growth rate or high egg production in the poultry industry production systems has a negative effect on the musculoskeletal structures of broilers and layer hens.[1,2] Although the exact etiology of leg problems is unknown, weakness of bone tissue may exacerbate leg problems by increasing the tendency for bone deformation and fragility.[3] In laying hens, especially during the laying period, bone fractures are observed due to mineral weakness in the bone structure. This is also of economic importance, especially regarding commercially produced chickens.[4,5] Osteoporosis-related bone fractures caused severe welfare problems in laying hens. Although osteoporosis depends on genetic and environmental factors, the adverse effects of osteoporosis can be reduced or eliminated by improving nutritional conditions.[6,7] Modern hybrid laying hens have been subjected to special selection for maximum egg production. Those chickens produce one egg per day for a period of approximately 50 weeks, and bone stores meet the calcium requirement of the shell glands. This calcium requirement is compensated by the medullary bone, which is formed mainly during the laying period. In this period, only medullary bone is formed, while normal structural lamellar bone formation stops. Numerous active osteoclasts are involved in resorbing and mobilizing calcium from the medullary bone, and unfortunately, cortical and trabecular bones are also resorbed at this time. Especially in older chickens (>40 weeks), the risk of osteopenia and osteoporotic fractures increases.[8] Supplementing poultry rations with microbial cultures aids in the nutrient absorption of beneficial bacteria and maintains microbial balance in the poultry digestive tract. Therefore, probiotics and by-products are used to eliminate stress-induced abnormalities in the gastrointestinal tract, so intestinal activity continues normally. Some studies have reported that probiotics support intestinal microbial balance and may help improve bone development and growth at the same time.[9–11]

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Vinasse is a by-product from the industrial production of yeasts, alcohol, citric acid, or other substances by fermentation of molasses.[12–14] Vinasse has a lower sugar concentration and higher ash and crude protein content than molasses, as most sugar is consumed during fermentation.[15,16] Modified dry vinasse from the baker's yeast industry is rich in protein and energy.[16] The chemical composition of beta vinasse consists of 35% crude protein and 31% betaine.[17] In many countries, researchers have started using vinasse as a valuable protein source in animal nutrition instead of an expensive food product, soybean.[16] Vinasse is widely used in the diet of ruminant animals since it mainly contains dissolved yeast residues, abundant soluble protein-mostly non-protein nitrogen, plenty of vitamin B, and high mineral content.[18–20] The ratio of minerals such as iron, calcium, and phosphorus in the content of fermented vinasse is also convenient for animals.[14] Because of these properties, beta vinasse can be considered a good source of nutrients and minerals. Moreover, the mineral content of vinasse may play a crucial role in developing the musculoskeletal system[21], increasing bone strength, and preventing losses due to disorders related to the musculoskeletal system in laying hens. However, the effect of vinasse on bone strength in laying hens has not yet been discovered.

The aim of this study was to determine the effects of vinasse on bone strength and some material properties in laying hens with certain amounts of vinasse added to their diets. The study was the first to determine the mechanical properties of laying hens supplemented with vinasse.

Material and Methods

Animals and feeding

This study was carried out in the Department of Anatomy with the poultry unit of Animal Health and Animal Production Application and Research Center of Bursa Uludag University Faculty of Veterinary Medicine.

In this study, two hundred 94-week-old Nick Chick laying hens were used, which were selected randomly from a commercial farm. Laying hens were randomly divided into one control and three experimental groups, each of which had 50 laying hens. Each group was divided into 10 subgroups consisting of 5 layers (40 replicates). The study consisted of four groups fed with a corn- and soy-based basic diet (0% Vinasse) and 1.0%, 3.0%, and 6.0% Vinasse added to this basic diet, respectively.

The raw nutrient analyses of the feeds used in the research were carried out in the Laboratory of the Faculty of Veterinary Medicine, Department of Animal Nutrition and Nutritional Diseases, according to the methods reported in the Association of Official Analytical Chemists (AOAC).[22] Laying hen diet composition was calculated according to National Research Council (NRC).[23] Animals in the study were fed a basic ration containing an average of 16.50% crude protein and 2800 kcal/kg of metabolizable energy. Unlimited (ad libitum) food and water were provided, and the study was terminated on day 90.

After slaughtering the animals by cervical dislocation as recommended by the Bursa Uludag University Animal Experimentation Local Ethics Committee (Approval No: 87799839-325.04.02-E.5641), the tibiotarsus of the left leg of each animal was separated from the body. Bones were cleaned entirely from the surrounding tissues and individually wrapped in gauze soaked with 0.9% saline solution, packed, and stored at -20°C until morphometric and mechanical measurements.[24] For the morphometric and mechanical analysis, the bones were kept at +8°C overnight to be thawed. Then morphometric and mechanical measurements were made at least one hour after the bones were kept at room temperature. Tibiotarsi weighed using a digital scale (Precisa XB4200C, Precisa Instruments Ltd., Switzerland). The tibiotarsal length of each bone was measured from the proximal end to the distal end using a digital caliper (Mitutoyo CDN-20C, Mitutoyo Corp., Kawasaki, Japan).

Mechanical measurements

Tibiotarsi specimens were submitted to three-point bending tests with the low-strength material testing machine designed and manufactured by Tufekci et al.[25] (Figure 1). Force and corresponding displacement were measured by a load-cell (100 N, Tedea Huntleigh Malvern, USA) and a Linear Variable Differential Transformer (LVDT) (10-mm stroke, Novotechnik Tr10, Germany), respectively. In addition, an oscilloscope (Nicholet-Oddysey XE, USA) recorded force and displacement data at the rate of 50 data/sec. The length between supports was adjusted to 70 mm, and the load was applied at the mid-point of the diaphysis until the bones were broken with a continual loading speed of 10mm/min.

Statistical Analysis

Statistical analysis was performed by One-way Analysis of Variance using the Statistical Package for the Social Sciences (SPSS) (SPSS, Version 23.0; Chicago, IL, USA). Differences among the groups were analyzed using the Duncan's multiple-range test.

Probabilities (P) were considered significant at $P<0.05$.

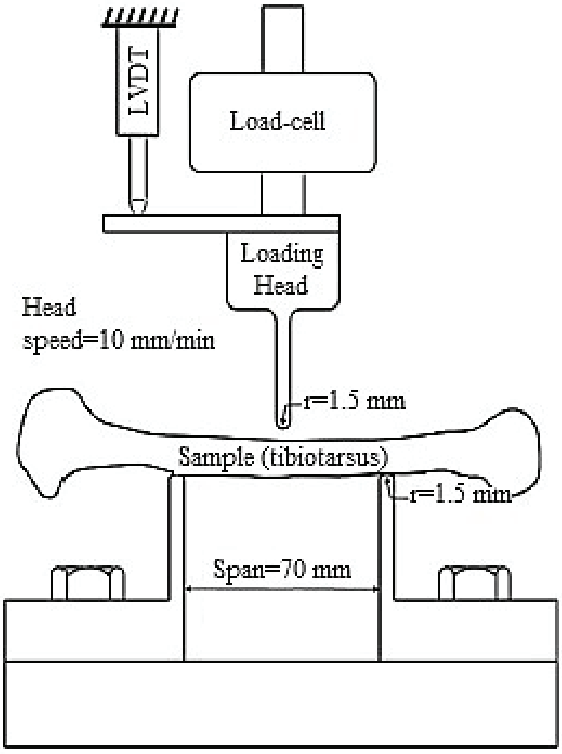

Figure 1. Schematic presentation of three-point bending test on tibiotarsus (modified from Suzer et al.26)

Results

The tibiotarsal length, weight and mechanical measurements of the laying hens were shown in Table 1. It was observed that vinasse had positive effects on bone weight and length in animals. The mean tibiotarsal length was 110.049±2.715 mm, and the tibiotarsal weight was 8.081±0.475 gr for Control group. Hens fed with vinasse had longer bone lengths than those not fed vinasse. The mean tibiotarsal length was 112.811±2.726 mm, and the tibiotarsal weight was 8.226±0.375 g for 1.0% Vinasse group. The mean tibiotarsal length was 113.946±2.513 mm, and the tibiotarsal weight was 8.724±0.701 g for 3.0% Vinasse group, and the length was 114.683±2.158 mm, and tibiotarsal weight was 8.507±0.444 g for 6.0% Vinasse group. It was observed that bone length and weight increased in direct proportion to the vinasse added to the diet. No significant differences were observed in terms of bone lengths between the groups fed with vinasse.

The three-point bending tests were performed on the bone samples, and stiffness was identified. The stiffness for the bones in Control group was 87.394±13.763 N/mm. Stiffness for the other groups was found 92.640±11.578 N/mm fed with 1.0% vinasse diet, 115.400±14.268 N/mm fed with 3.0% vinasse diet, 113.560±18.328 N/mm fed with 6.0% vinasse diet. It was seen that diets with vinasse added had a positive effect on bone stiffness (Figure 2). The average fracture force for those fed with a standard diet (0% Vinasse) was 122.153±16.216 N. Forces were 143.082±19.213 N, 155.721±24.641 N, 165.590±34.099 N for groups fed with a 1.0%, 3.0% and 6.0% vinasse diet respectively and overall P values less than 0.001 were considered statistically significant. It was observed that Control group was statistically significantly more displaced, more ductile, and fractured at a lower force than the groups fed with vinasse (Figure 3). According to this result, it could be said that Control group had less compressive strength. It was observed that the groups fed with vinasse were significantly stiffer, and fractured at a higher strength than Control group. This showed that it had a higher compressive strength and tends to break without much deformation (bending).

Table 1. The tibiotarsal length, weight and mechanical measurements in control and laying hens were fed different doses of vinasse.

	Breaking Deflection (mm)	Stiffness (N/mm)	Breaking Force (N)	Bone Length (mm)	Bone Weight (g)
Control	2.627±0.276[a]	87.394±13.763[a]	122.153±16.216[a]	110.049±2.715 [a]	8.081±0.475 [a]
1% Vinasse	2.355±0.183[ab]	92.640±11.578[a]	143.082±19.213[ab]	112.811±2.726 [ab]	8.226±0.375 [ab]
3% Vinasse	2.227±0.219[b]	115.400±14.268[b]	155.721±24.641[b]	113.946±2.513[b]	8.724±0.701[b]
6% Vinasse	2.255±0.264[b]	113.560±18.328[b]	165.590±34.099[b]	114.683±2.158[b]	8.507±0.444 [ab]
P-value	0.005	0.002	>0.001	0.001	0.038

- Values in the table were presented as Mean ± Standard deviation of the mean.
- The mean significant difference level was 0.05.
- Different superscripts stated the significant differences between groups.

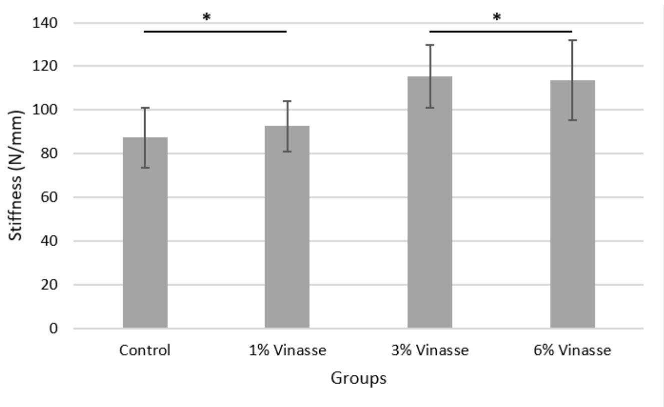

Figure 2. Comparison of stiffness in control and laying hens were fed different doses of vinasse.

* indicates the significant differences between groups.

In addition, no significant difference was found between the groups fed with different doses of vinasse in terms of breaking deflection, stiffness, and breaking force. However, it was observed that the amount of breaking force that the bone could carry without breaking tends to increase in direct proportion to the increase in the dose of vinasse.

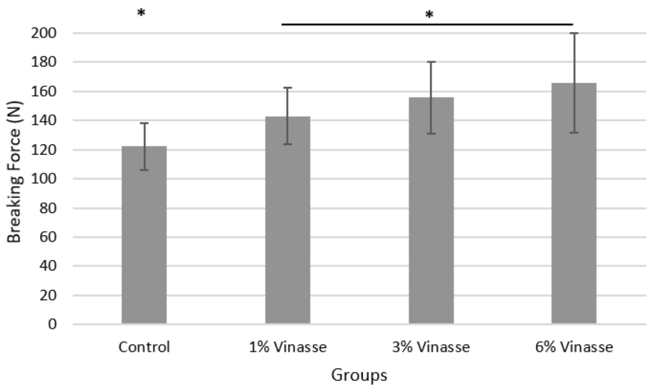

Figure 3. Comparison of breaking force in control and laying hens were fed different doses of vinasse.

* indicates the significant differences between groups.

Discussion and Conclusion

Vinasse obtained from molasses, a by-product of the sugar industry, is an important alternative for other protein feeds in the feed industry with its high betaine, mineral and crude protein content.[27] In the present study, when Control and groups fed with vinasse were compared, it was seen that the laying hens in the Control group had a shorter tibiotarsal length. Additionally, although there was significant difference between Control group and all experimental groups, no significant difference was observed in terms of bone lengths between the groups fed with vinasse. However, the bone length increased in direct proportion to the vinasse amount added to the diet, although it was not statistically significant (P>0.05). Similarly, Bilal et al.[27] reported that there were no significant changes in tibiotarsal length and diameter in broilers supplemented with 2.5% and 5% vinasse. Similar to the bone length results of our study, we found that tibial weight was higher in animals fed with vinasse compared to Control group. It was also observed that tibiotarsal bone weight increased in direct proportion to the vinasse-supplemented diet. However, there was no significant difference in terms of bone weight between the groups fed with vinasse.

In the present study, significant differences were found in breaking force between Control and vinasse groups. The present study showed that hens fed with vinasse appeared to have higher strength than those not fed vinasse. According to that result, it could be said that Control group had less bending strength. It was observed that the groups given vinasse were significantly stiffer and fractured at a higher force than Control group. This showed that vinasse groups had a higher breaking force and tended to break without much deformation. In addition, no significant difference was found between the groups given different doses of vinasse in terms of breaking deflection, stiffness and breaking force. However, it was observed that the amount of breaking force that the bone could carry without breaking tended to increase in direct proportion with the dose of vinasse.

In our literature search to date, we could not find a reference to compare our study data, as no bone strength publications were studied with vinasse. However, we thought that the positive effects of vinasse on bone strength were due to the high mineral and betaine content of vinasse. Consistent with this argument, Cetin et al.[17] reported that the chemical composition of beta vinasse consists of 31% betaine. Those researchers also mentioned that betaine caused an increase in mineral substance absorption in the intestines. Similarly, it was also reported that dietary betaine supplementation in laying quails might increase the digestibility of dry matter, crude protein, crude fiber, and crude ash, which positively affects the intestinal mineral absorption and digestive capacity of the intestinal epithelium.[28,29] Gerimipour et al.[21] also reported that vinasse contained betaine and the calcium concentration of vinasse was also high. The positive effects of vinasse on mineralization was observed by Cetin et al.[17] that egg shell thickness and shell breaking resistance increased in quails fed with the addition of vinasse compared to quails without vinasse, and it was reported that this positive effect may be due to the effect of betaine on the increase of mineral substance absorption in the intestines. Consistent with those researchers, Maidin et al.[30] reported that betaine supplemented diet reduced plasma homocysteine concentrations in blood and improved bone strength.

In conclusion, the present study was the first report measuring the effects of vinasse on some biomechanical traits of tibiotarsus in laying hens. Since tibiotarsal breaking strength is a good indicator and has a high correlation with other bone properties[31], we observed that there was a significant and promising results that the tibiotarsus had stronger and better properties in the laying hens fed with vinasse. Besides, the addition of dietary vinasse increased bone strength in laying hens, which could be used as an alternative feed additive to reduce welfare concerns related to leg problems in laying hens.

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