Bones quality indices in laying hens fed diets with a high level of DDGS and supplemented with selected feed additives

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ABSTRACT: An experiment with 192 caged ISA Brown laying hens, fed a diet containing a high level of corn distillers dried grains with solubles (DDGS), was conducted to determine the influence of selected feed additives on biomechanical and geometrical indices of tibia and femur bones. At 26 weeks of age hens were randomly assigned to 8 treatments with 12 replicates (cages of two hens). To week 55, hens were fed isocaloric and isonitrogenous experimental diets either containing or not containing a high level of DDGS (200 g/kg). The diet containing 200 g/kg of DDGS was supplemented or not supplemented with feed additives, i.e. enzymes (xylanase and phytase), sodium butyrate, probiotic bacteria (*L. salivarius*), herbal extract mixtures (*Taraxaci siccum*, *Urticae siccum,* and *Salviae siccum*), inulin or chitosan. At week 55, inclusion of DDGS in the diet had no effect on biomechemical (bone breaking strength, yielding load, and stiffness) or geometrical (cortex thickness, cross-section area, weight, and length) indices of tibia and femur bones ($P > 0.05$). Some of the supplements used had a beneficial effect on bone quality in hens fed the diet with a high level of DDGS. Thus, the addition of probiotic bacteria or herb extracts increased the breaking strength of femurs and breaking strength and yielding load of tibias ($P < 0.05$). The results of this study indicate that DDGS may be included to a level of 20% in the diet of laying hens without any negative influence on bone quality, while such feed additives as probiotic bacteria and herbal extracts may improve the selected biomechanical indices of bone quality of layers fed diets with a high level of DDGS.

Keywords: layers; distillers dried gains with solubles; feed supplements; femur and tibia measurements

Osteoporosis, which is a widespread skeletal problem in modern flocks of high-productive laying hens, can be defined as a severe loss in bone mineral structure when Ca is mobilized from bone in order to contribute to eggshell formation (Whitehead and Fleming, 2000; Whitehead, 2004). Osteoporosis, in conditions of high demand for calcium for eggshell formation, often leads to increased bone fragility and high incidence of bone fractures. The consequences of this syndrome are not only related to performance and economic issues of egg production, but also constitute a significant welfare problem, causing acute and chronic pain and distress to the birds (Webster, 2004; Lay et al., 2011). Jendral et al. (2008) reported that layers kept in conventional cages, where the opportunity for exercise and movement is restricted, are particularly vulnerable to osteoporosis, exhibiting lower tibia and femur mineral density, bone mass, cortical bone area and mass, and bone breaking strength than those kept in furnished colony cages or cages modified with nest boxes and perches. Gregory and Wilkins (1989) indicated that during the end phase of laying, one or more broken bones were found in 29% of caged hens during their lifetimes. Optimization of nutrition can serve as

one of the strategies for prevention of osteoporosis in highly-producing layers. The results of some studies indicate that egg formation increases Ca mobilization from bones and decreases bone quality in layers; thus a negative correlation between bone and eggshell quality can be observed (Kim et al., 2012). It is well known that the most important nutritional factor influencing bone quality is the supply of Ca in adequate amount and form through the diet. For example, as a source of Ca for hens, particulate limestone, as compared to fine particle $CaCO_3$, can positively affect selected parameters of bone quality (Fleming et al., 1998; Koreleski and Swiatkiewicz, 2004; Saunders-Blades et al., 2009; Cufadar et al., 2011).

Distillers dried grains with solubles (DDGS) are a co-product of the ethanol industry created in the fermentation process of cereal grains, and can be defined as the feed material obtained after the removal of ethyl alcohol, through distillation, from the yeast fermentation of a grain by condensing and drying at least 75% of the resultant whole stillage by methods employed in the grain distilling industry (AAFCO, 2002). The growth of fuel ethanol production in recent years has resulted in increased availability of DDGS for feed producers. The relatively high nutritional quality of DDGS obtained from modern ethanol technology enables the use of this feed material on a large scale in poultry nutrition, as the most effective and environmentally friendly way of using DDGS (Swiatkiewicz and Koreleski, 2008). In many earlier studies, it was concluded that DDGS was a useful feed ingredient for laying hens (Swiatkiewicz and Koreleski, 2006; Krawczyk et al., 2012; Niemiec et al., 2012, 2013). Some studies indicated, however, that the utilization of such macroelements as Ca and P decreased in poultry fed diets with high levels of DDGS (Swiatkiewicz and Koreleski, 2007; Thacker and Widyaratne, 2007; Leytem et al., 2008), which could negatively affect the mineralization and quality of bones of highly-performing laying hens. Therefore, the aim of this study was to investigate the effect of a high level of dietary DDGS and selected feed additives with potential positive effects on mineral utilization, i.e. enzymes (xylanase and phytase), sodium butyrate, probiotic bacteria (*L. salivarius*), a mixture of herbal extracts (*Taraxaci siccum*, *Urticae siccum*, and *Salviae siccum*), inulin or chitosan, on biomechanical and geometrical indices of tibia and femur bones in laying hens.

MATERIAL AND METHODS

The Local Ethics Committee for Experiments with Animals gave its approval to all the experimental procedures relating to the use of live animals. A total of 192 18-week-old ISA Brown hens, obtained from a commercial source, were placed in a poultry house, in cages (two birds per cage), on a wire-mesh floor under controlled climate conditions. The cage dimensions were 30 × 120 × 50 cm, equating to 3600 cm^2 of total floor space. During the pre-experimental period, up to the week 26 of hens' age, a commercial laying-hen diet (170 g/kg crude protein, 11.6 MJ/kg apparent metabolizable energy corrected for nitrogen retention (AME_N), 37.0 g/kg calcium, and 3.8 g/kg available phosphorus) was offered *ad libitum*.

At week 26, the hens were randomly assigned to one of 8 treatments, each comprising 12 replicates (cages with 2 hens in each), and fed experimental diets until week 55. During the experiment, the hens were provided feed and water *ad libitum*, and were exposed to a 14 h light : 10 h dark lighting schedule, with a light intensity of 10 lux.

Prior to formulating the experimental diets it was determined (AOAC, 2000) that the corn DDGS to be used contained 269 g/kg crude protein, 110 g/kg crude fat, 55 g/kg crude fibre, 42 g/kg crude ash, 7.40 g/kg lysine, 6.20 g/kg methionine, 10.4 g/kg threonine, 2.95 g/kg tryptophan, 0.50 g/kg Ca, 8.26 g/kg P, and 2.43 g/kg Na, respectively.

The composition of the basal diets used in the experiment is shown in Table 1. Both diets, without (control group I) or with corn DDGS (200 g/kg), were isocaloric and isonitrogenous, and were formulated to meet or exceed nutrient recommendations (National Research Council, 1994). The diet with DDGS was either not supplemented (group II) or supplemented with additives as follows (per kg of diet): feed enzymes (Ronozyme WX with endo-1,4-β-xylanase activity of 1000 FXU/g and Ronozyme NP with phytase activity of 10 000 FYT/g, both DSM Nutritional Products, Basel, Switzerland), each preparation added to the diet in the amount of 200 mg/kg – group III; sodium butyrate (GUSTOR BP-70; Norel Animal Nutrition, Madrid, Spain) in the amount of 700 mg/kg – group IV; probiotic bacteria (*L. salivarius;* Institute of Agricultural and Food Biotechnology, Warsaw, Poland), 10^8 cfu/kg – group V; mixture of herbal extracts (*Taraxaci siccum*, *Urticae siccum,* and *Salviae siccum*), 250 mg of each extract/kg – group VI; inulin (BENEO™ IPS, Orafti, Tienen,

Table 1. Composition and nutrient content of diets used in the experiment (%)

Item	Control diet (treatment I)	Experimental diets (treatments II–VIII)
Corn	36.00	29.00
Wheat	25.56	20.10
Soybean meal	24.50	16.50
DDGS	–	20.00
Rapeseed oil	2.40	2.80
Limestone	9.00	9.20
Dicalcium phosphate	1.60	1.30
NaCl	0.30	0.30
DL-Methionine	0.14	0.11
L-Lysine HCl	–	0.19
Vitamin-mineral premix[1]	0.50	0.50
Metabolizable energy (MJ/kg)[2]	11.6	11.6
Crude protein[3]	17.5	17.5
Total lysine[3]	0.86	0.86
Total methionine[3]	0.41	0.41
Digestible lysine[4]	0.74	0.65
Digestible methionine[4]	0.38	0.38
Ca[3]	3.70	3.70
Total P[3]	0.60	0.60

DDGS = distillers dried grains with solubles

[1]premix provided per 1 kg of diet: vitamin A 10 000 IU, vitamin D_3 3 000 IU, vitamin E 50 IU, vitamin K_3 2 mg, vitamin B_1 1 mg, vitamin B_2 4 mg, vitamin B_6 1.5 mg, vitamin B_{12} 0.01 mg, Ca-pantothenate 8 mg, niacine 25 mg, folic acid 0.5 mg, choline chloride 250 mg, manganese 100 mg, zinc 50 mg, iron 50 mg, copper 8 mg, iodine 0.8 mg, selenium 0.2 mg, cobalt 0.2 mg

[2]calculated according to the European Table (Janssen, 1989) as a sum of metabolizable energy content of components

[3]calculated according to the chemical composition of feed components

[4]calculated according to the digestibility coefficients determined previously in our laboratory (Szczurek, 2009)

Belgium), 5 g/kg – group VII; or chitosan (CHIMET-PASZ, Gumitex Poli-Farm, Łowicz, Poland), 3 ml/kg – group VIII.

The experimental diets were fed to birds from 26 to 55 weeks of age. The nutrient content of the diets was calculated on the basis of the chemical composition of raw feedstuffs (AOAC, 2000), and metabolizable energy value in line with equations from the European Table (Janssen, 1989).

At the end of the experiment all hens were sacrificed through cervical dislocation. The tibia and femur from the right leg were collected, cleaned of soft tissues, weighed, and frozen (–20°C) pending the analysis. Measurement of the bones' biomechanical properties was done by means of the three-point bending test using an Instron 5542 testing machine (Instron Ltd., High Wycombe, UK) (constant speed of crosshead 10 mm/min; distance between supports 50 mm). Bone breaking strength and yielding load were measured as a graphical record from post-deformation curves. Stiffness in elastic conditions was calculated as a yielding load/elastic deformation ratio.

The tibia length, cortex thickness, external and internal diameters (for cross-section area calculations) were measured at the breaking place using an electronic slide caliper. The cross-section area was calculated as

$$3.14 (HB – hb)/4$$

where:
H = external vertical diameter
B = external horizontal diameter
h = internal vertical diameter
b = internal horizontal diameter

The data were subjected to statistical analysis using a completely randomized design in accordance with the GLM procedure of STATISTICA software (Version 5.0, 1995). All data were analyzed using One-Way ANOVA. When significant differences in treatment means were detected by ANOVA, Duncan's Multiple Range Test was applied to separate means. Statistical significance was considered at $P \leq 0.05$.

RESULTS AND DISCUSSION

As presented also in our previous paper (Swiatkiewicz et al., 2013), the incorporation of 200 g/kg DDGS to the diet did not affect laying performance parameters. The bone breaking strengths of tibias and femurs (Tables 2 and 4) of ISA Brown hens obtained in our study (175 and 159 N, averaged across all dietary treatments) were similar to the values observed in our earlier experiment with Bovans Brown layers (Swiatkiewicz et al., 2010a), but higher than in the experiment with Hy-Line Brown hens of a similar age (Koreleski and Swiat-

Table 2. Effect of corn distillers dried grains with solubles (DDGS) and experimental additives on biomechanical indices of tibia bones

Treatments	Bone breaking strength (N)	Bone breaking strength/cross section area ratio (N/mm^2)	Yielding load (N)	Stiffness (N/mm)
I	172.8^{a}	10.07^{a}	107.8^{ab}	136.6^{a}
II	172.2^{a}	9.84^{a}	104.3^{a}	135.0^{a}
III	181.6^{ab}	10.68^{ab}	111.0^{ab}	139.5^{a}
IV	166.2^{a}	9.77^{a}	114.0^{ab}	139.3^{a}
V	196.2^{b}	11.62^{b}	129.0^{c}	163.6^{b}
VI	176.9^{ab}	10.47^{ab}	119.8^{bc}	148.5^{ab}
VII	164.3^{a}	9.57^{a}	112.3^{ab}	136.9^{a}
VIII	169.7^{a}	9.55^{a}	116.8^{ab}	144.2^{a}
SEM	2.76	0.160	1.65	2.19
P-value	*	**	**	*

treatments: I = control diet, II–VIII = experimental diets containing 200 g/kg DDGS; supplementation: II = not supplemented, III = feed enzymes (xylanase + phytase), IV = sodium butyrate, V = probiotic bacteria, VI = herb extracts mixture, VII = inulin, VIII = chitosan

$^{a–c}$within a column, values not sharing a common superscript significantly differ ($P \leq 0.05$)

$^{*}P \leq 0.05$, $^{**}P \leq 0.01$

kiewicz, 2004). These differences may indicate the significant influence of genetic selection for egg production on bone quality.

In the present experiment there were no statistically significant differences in the biomechanical and geometrical parameters of tibia and femur bones between hens fed diets with or without a high level of DDGS (Tables 2–5). To date, the experimental data on the effect of dietary DDGS on bone quality in laying hens have been limited. Deniz et al. (2013), correspondingly to our findings, found no differences in tibia crude ash between hens fed diets without or with 100 g DDGS/kg. Some authors have indicated that mineral availability may be decreased in poultry when birds are fed a diet containing DDGS (Swiatkiewicz and Koreleski, 2007; Thacker and Widyaratne, 2007; Leytem et al., 2008), which can potentially decrease the mineralization of bones and their resistance to fractures. However, other authors found no negative effect of DDGS on P retention (Masa'deh et al., 2011, 2012) and the lack of observed differences in our study between bone quality in hens fed diets with or without DDGS may indirectly support the hypothesis that dietary DDGS does not affect mineral availability. The results of the broiler study of Lumpkins and Batal (2005) indicated that P bioavailability in corn DDGS is much higher even than in corn grain.

In our study there were no statistically significant differences between dietary treatments in geometrical indices of tibia and femurs (Tables 3 and 5); however, some of the additives used had a positive effect on the biomechanical characteristics of bones in birds fed diets with DDGS (Tables 2 and 4). Thus, layers fed the high DDGS diet supplemented with probiotic bacteria had a significantly higher ($P < 0.05$) breaking strength, yielding load, and stiffness of tibia (Table 2) and breaking strength of femur (Table 4) than those in the unsupplemented group. In the case of the herb extracts, significant positive effects were found for yielding load of tibia (Table 2) and breaking strength of femur (Table 4). Other additives used, i.e. feed enzymes, sodium butyrate, inulin, and chitosan did not affect any of the analyzed bone parameters.

To date the number of published results from poultry experiments on the effect of probiotic bacteria or herb extracts on bone quality have been limited. The mechanism of the effect of probiotic bacteria observed in our study can probably be related to their positive influence on mineral utilization, which can be attributed in turn to increased solubility of minerals due to the bacteria's increased production of short-chain fatty acids, alteration of intestinal mucosa, and increase of the absorption surface through the beneficial

Table 3. Effect of corn distillers dried grains with solubles (DDGS) and experimental additives on geometrical indices of tibia bones

Treatments	Cortex thickness (mm)	Cross section area (mm^2)	Tibia weight (g)	Relative tibia weight (g/100 g of body weight)	Tibia length (cm)
I	0.844	17.2	11.2	0.628	117.5
II	0.834	17.7	11.4	0.668	121.0
III	0.834	17.1	11.1	0.671	119.9
IV	0.855	17.1	10.7	0.684	118.4
V	0.887	17.1	10.7	0.634	119.3
VI	0.883	17.1	10.9	0.639	120.7
VII	0.845	17.3	10.2	0.612	118.2
VIII	0.889	17.9	11.0	0.634	119.9
SEM	0.015	0.298	0.139	0.008	0.478
P-value	ns	ns	ns	ns	ns

treatments: I = control diet, II–VIII = experimental diets containing 200 g/kg DDGS; supplementation: II = not supplemented, III = feed enzymes (xylanase + phytase), IV = sodium butyrate, V = probiotic bacteria, VI = herb extracts mixture, VII = inulin, VIII = chitosan; ns = nonsignificant

effect of bacterial fermentation products on the proliferation of enterocytes, increased expression of Ca-binding proteins, release of bone modulating factors, degradation of phytates by probiotic bacteria enzymes, and overall improvement of gut health (Scholz-Ahrens et al., 2007). Recently, positive effects of probiotic bacteria (*Bacillus subtilitis*) on bone quality (i.e. tibia weight, density, and ash content) in laying hens have also been found by Abdelqader et al. (2013). Other authors reported that probiotic addition positively affected such eggshell indices like breaking strength, thickness, and weight (Xu et al., 2006; Yousefi and Karkoodi, 2007; Panda et al., 2008); this was probably related to better utilization of minerals. Nahashon et al. (1993) reported that probiotic bacteria added to a

Table 4. Effect of corn distillers dried grains with solubles (DDGS) and experimental additives on biomechanical indices of femur bones

Treatments	Bone breaking strength (N)	Bone breaking strength/cross section area ratio (N/mm^2)	Yielding load (N)	Stiffness (N/mm)
I	151.5^{ab}	8.35^{ab}	106.5	128.1
II	139.2^{a}	7.87^{a}	95.0	117.5
III	$158.9^{a–c}$	8.43^{ab}	112.2	137.7
IV	149.9^{ab}	8.30^{ab}	101.8	124.4
V	183.3^{c}	9.93^{c}	116.5	143.0
VI	172.2^{bc}	9.31^{bc}	116.8	141.6
VII	149.0^{ab}	7.95^{ab}	104.5	128.3
VIII	$166.7^{a–c}$	9.36^{bc}	112.0	137.5
SEM	3.72	0.178	2.29	2.99
P-value	*	*	ns	ns

treatments: I = control diet, II–VIII = experimental diets containing 200 g/kg DDGS; supplementation: II = not supplemented, III = feed enzymes (xylanase + phytase), IV = sodium butyrate, V = probiotic bacteria, VI = herb extracts mixture, VII = inulin, VIII = chitosan; ns = nonsignificant

$^{a–c}$within a column, values not sharing a common superscript significantly differ ($P \leq 0.05$)

*$P \leq 0.05$

Table 5. Effect of corn distillers dried grains with solubles (DDGS) and experimental additives on geometrical indices of femur bones

Treatments	Cortex thickness (mm)	Cross section area (mm^2)	Femur weight (g)	Relative femur weight (g/100 g of body weight)	Femur length (cm)
I	0.828	18.28	8.82	0.493	84.6
II	0.776	17.74	8.57	0.509	85.5
III	0.829	18.83	8.97	0.531	85.1
IV	0.846	18.15	8.35	0.534	83.3
V	0.846	18.51	8.76	0.529	84.4
VI	0.837	18.45	8.63	0.505	84.9
VII	0.839	18.90	8.38	0.485	84.5
VIII	0.802	17.85	9.11	0.527	85.5
SEM	0.013	0.291	0.107	0.008	0.290
P-value	ns	ns	ns	ns	ns

treatments: I = control diet, II–VIII = experimental diets containing 200 g/kg DDGS; supplementation: II = not supplemented, III = feed enzymes (xylanase + phytase), IV = sodium butyrate, V = probiotic bacteria, VI = herb extracts mixture, VII = inulin, VIII = chitosan; ns = nonsignificant

layers' diet increased retention of Ca and P. Chen and Chen (2004) noted that diet supplementation with prebiotics (oligofructose or inulin), which are substrates for intestinal probiotic bacteria, increased the total ash, Ca, and P in the tibia of layers. Correspondingly, the positive effect of inulin on some eggshell quality parameters reported in the previous work (Swiatkiewicz et al., 2010b) may indicate that prebiotics, by modulating intestinal microbiota, improve mineral utilization in laying hens.

Findings corresponding to our results were noted in broiler chickens by Mutus et al. (2006) who reported that diet supplementation with probiotic bacteria (*Bacillus licheniformis* and *Bacillus subtilis*) significantly increased the thickness of the medial and lateral walls of the tibia, the tibiotarsal index, percentage ash, and P content, without any effect on tibia weight, length, weight/length index, robusticity index, diaphysis diameter, modulus of elasticity, yield stress parameters, or percentage Ca content. Angel et al. (2005) found that the incorporation of probiotics with low calcium and phosphorus levels into broilers' diets significantly increased the retention of Ca and P along with tibia breaking strength and ash content. In a more recent experiment, feeding broilers with a diet containing a low level of Ca negatively affected performance and tibia characteristics, whereas the addition of probiotic bacteria had a positive effect on these parameters and helped to overcome the problems related to a low-Ca diet (Houshmand et al., 2011). The beneficial influence of probiotic bacteria on tibia breaking strength and mineralization in broilers, attributed by the authors to higher assimilation of calcium in the bones, was also demonstrated when a diet with standard levels of Ca and P was used (Panda et al., 2008).

Similarly to the positive effect of herb extracts on certain bone quality indices observed in our experiment, Zhou et al. (2009) found that the dietary addition of a mixture of four traditional Chinese herbs (*Herbal Epimedii*, *Rhizoma Drynariae*, *Rhizoma Atractylodis,* and *Radix Astragali*) increased breaking strength of the tibia as well as bone weight, radiographic densities and bone indices of humerus, tibia, and femur in older ISA caged layers. They indicated that the mechanism of positive effect of herbs on bone quality was possibly associated with minimizing structural bone loss and stimulating bone mineral absorption in osteoporotic laying hens (Zhou et al., 2009). Correspondingly, it was demonstrated in a model experiment with rats, that essential oils and monoterpenes of such herbs as sage, rosemary, and thyme are efficient inhibitors of bone resorption (Mühlbauer et al., 2003).

In conclusion, the results of the present study show that a high dietary level of DDGS (200 g/kg) had no negative effect on bone quality; however,

such feed additives as probiotic bacteria and herb extracts can positively affect selected mechanical properties of the tibia and femur bones in aged, high-producing laying hens.

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Received: 2013–06–13
Accepted after corrections: 2013–11–14

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