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Bone quality, selected blood variables and mineral retention in laying hens fed with different dietary concentrations and sources of calcium

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ABSTRACT

The objective of this study was to determine the influence of limestone particle size in the diets with different contents of Ca on the biomechanical and geometrical measurements of tibia and femur bones, digestibility of nutrients, and selected biochemical blood variables. The experiment was conducted with 108 laying hens, allocated to 9 treatments of 6 replicates (cages), with 2 layers in each cage. A 3×3 factorial arrangement, with 3 dietary concentrations of Ca (3.20%, 3.70%, and 4.20%) and 3 levels of dietary substitutions (0, 25, and 50%) of fine particles of limestone (FPL; diameter, 0.2–0.6 mm) with large particles of limestone (LPL; diameter, 1.0 to 1.4 mm), was used. The hens were fed with experimental diets from 25 to 70 wk of age. At wk 45 a balance experiment was conducted, and after termination of the experiment, i.e., at wk 70, tibia and femur bones, and blood samples, were collected for analysis. Neither dietary Ca concentration nor limestone particle size had an effect on dry matter, organic matter, ether extract, N-free extracts, crude fiber, and crude ash digestibility, and P retention and excretion; however, Ca excretion increased linearly and Ca relative retention decreased linearly with increasing Ca dietary concentration ($P < 0.05$). No effect of limestone particle size on tibia and femur biomechanical and geometrical measurements, tibia and femur mineralization, serum alkaline phosphatase activity, and serum Ca and P concentration, was observed. Increased dietary Ca concentration enhanced linearly tibia and femur bone breaking strength, yielding load, stiffness, and Ca concentration ($P < 0.05$). Serum alkaline phosphatase activity decreased linearly with increasing Ca dietary concentration ($P < 0.05$). In conclusion, the results of this study demonstrated that a content of 3.20–3.70% Ca in a layer's diet is not sufficient through the entire laying cycle to maintain optimal bone quality; however, partial substitution of fine- with large-particle limestone does not improve Ca and P retention and bones quality variables.

1. Introduction

Skeletal disorders and poor bone quality, related mainly to osteoporosis, are widespread in modern laying hens. Osteoporosis is a severe decrease in mineralized structural bone in which Ca is mobilized from the bone to be involved in eggshell formation (Whitehead, 2004; Whitehead and Fleming, 2000), and which frequently results in loss of bones' strength, their enhanced brittleness and high fracture incidence. Not only performance and economical losses for the egg industry, but also important welfare problems causing acute and chronic pain and distress to the birds, are the results of osteoporotic changes (Lay et al., 2011; Webster, 2004). It was reported that almost 30% of hens experienced bones fractures during the end phase of laying (Gregory and Wilkins, 1989). Several authors indicated that hens kept in conventional cages are especially vulnerable to osteoporosis, exhibiting lower bone mineral density, bone mass, bone cross-sectional bone area, and bone breaking strength than layers kept in furnished colony cages, or cages modified with nest boxes and perches, or in floor pens (Jendral et al., 2008; Silversides et al., 2012). A study by McCoy et al. (1996) attributed 35% of mortality in high-producing caged layers to osteoporosis. Because of osteoclastic resorption and decline of structural bone content during the laying period (Fleming et al., 1998; Whitehead and Fleming, 2000), the symptoms of osteoporosis are more frequently observed in older hens.

Nutritional methods can serve as one of the tools for prevention or alleviation of osteoporotic changes and skeletal defects in highly performing layers. Because of the fact that calcification of eggshell uses extremely high amounts of Ca (approximately 2.2 g per egg), Ca nutrition, i.e., supply of the optimal amount and form of Ca, is the most crucial in order for proper mineralization of eggshell and bones in high-producing laying hens. As the skeleton

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acts as the Ca source for shell mineralization in the dark period of the day, inadequate Ca intake by hens not only leads to a considerable decrease in eggshell quality, but can increase bones brittleness and their susceptibility to fractures. For this reason several researchers (Bar et al., 2002; Castillo et al., 2004; Costa et al., 2008; Lichovnikova, 2007; Safaa et al., 2008) have suggested that the need of laying hens for dietary Ca can be higher than the value (3.25% Ca in the diet) included in NRC recommendations (NRC, 1994). On the other hand, however, it is known that too high dietary Ca concentration can negatively affect retention of some other essential minerals (Pastore et al., 2012) or efficacy of phytase supplementation to the layer's diet (Englmaierova et al., 2014).

Results of some experiments showed that replacing fine limestone with coarse limestone, as a source of Ca for hens, which is dissolved more slowly, thus providing the hen continuously with Ca (assuring maintenance of an adequate Ca blood concentration overnight, when the process of shell calcification is intensive and layers do not have access to feed), may beneficially affect selected bone quality variables (Cufadar et al., 2011; Fleming et al., 1998; Guinotte and Nys, 1991; Koreleski and Swiatkiewicz, 2004; Saunders-Blades et al., 2009). Rao et al. (1992) found that that 1.0 mm is the minimum limestone particle size required to significantly improve its selective retention in the gizzard of laying hens (Rao et al., 1992). However, the experimental data on the interactive effect of limestone particle size Ca and dietary concentration on bone quality in modern high-producing layers are limited. Therefore, this experiment was conducted to study the effect of different dietary Ca concentrations and particle size of the dietary Ca source, i.e., the level of substitution of fine-particle with large-particle limestone, on the biomechanical and geometrical measurements of tibia and femur bones, digestibility of nutrients, and selected biochemical blood variables.

2. Material and methods

2.1. Birds and experimental diets

A total of 108 seventeen-weeks-old hens (ISA Brown, Hendrix Genetics, Boxmeer, the Netherlands) obtained from a commercial source, were placed in a poultry house in cages (2 birds per cage) on a wire-mesh floor under controlled climate conditions. The cage dimensions were 30 cm × 120 cm × 50 cm. During the pre-experimental period, i.e., from 17 to 24 wk of age, the hens were fed a commercial diet (170 g/kg crude protein, 11.6 MJ/kg of metabolizable energy, 37.0 g/kg Ca and 3.8 g/kg available P), offered *ad libitum*. The Local Cracow Ethics Committee for Experiments with Animals approved all experimental procedures relating to the use of live animals.

At wk 25, the hens were randomly assigned to 1 of 9 treatments, each comprising 6 replicates (cages with 2 hens in each), and fed experimental diets until wk 70. During the experiment, the hens were provided feed and water *ad libitum*, and were exposed to a 14:10 h light:dark cycle, with a light intensity of 10 lx. A 3 × 3 factorial arrangement, with 3 dietary concentrations of Ca (3.20%, 3.70%, and 4.20%) and with 3 levels of substitutions (0%, 25%, and 50%) of fine particles of limestone (FPL; diameter, 0.2–0.6 mm) with large particles of limestone (LPL; diameter, 1.0 to 1.4 mm) as a Ca source, was used. The nutrient content of the diets was calculated on the basis of the chemical composition of raw feedstuffs, and metabolizable energy (ME) value was calculated based on equations in the European Tables (Janssen, 1989). The chemical composition other feed materials was determined by AOAC (2000) methods for moisture (930.15), crude protein (984.13), crude fat (920.39), fiber (978.10), and ash (942.05). Amino acids were analysed in acid hydrolysates after initial peroxidation of sulfur amino acids by color reaction with the ninhydrin reagent (Beckman-System Gold 126 AA Automatic Analyzer; Beckman Coulter, Inc., Pasadena, CA; Method 982.30; AOAC, 2000). The Ca content was determined by flame atomic absorption spectrophotometry (Method 968.08; AOAC, 2000) and total P content was determined by colorimetry using the molybdo-vanadate method (Method 965.17; AOAC, 2000). The composition of the experimental cereal–soybean diets is shown in Table 1.

Table 1
Composition of experimental diets (g/kg; as-fed).

Item	Dietary Ca		
	Reduced	Standard	Increased
Ingredient (g/kg)			
Corn	417.1	423.1	456.1
Wheat	240.0	210.0	150.0
Soybean meal	230.0	236.0	244.0
Rapeseed oil	13.0	19.0	26.0
Limestone	78.0	90.0	102.0
Monocalcium phosphate	12.5	12.5	12.5
NaCl	3.0	3.0	3.0
DL-Met	1.4	1.4	1.4
Vitamin–mineral premix[a]	5.0	5.0	5.0
Analyzed chemical composition			
Metabolizable energy (MJ/kg)[b]	11.60	11.60	11.60
Crude protein (g/kg)	170.0	170.0	170.0
Lys (g/kg)	8.35	8.35	8.35
Met (g/kg)	4.10	4.10	4.10
Ca (g/kg)	32.0	37.0	42.0
Total P (g/kg)	3.15	3.15	3.15
Available P (g/kg)	3.90	3.90	3.90

[a] Provided per kilogram of diet: vitamin A, 10,000; vitamin D_3, 3000 IU; vitamin E, 50 IU; vitamin K_3, 2 mg; vitamin B_1, 1; vitamin B_2, 4 mg; vitamin B_6, 1.5; vitamin B_{12}, 0.01 mg; Ca-pantotenate, 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; and cobalt, 0.2 mg.

[b] ME = metabolizable energy; calculated according to European Table (Janssen, 1989) as a sum of the ME content of components.

2.2. Measurements

At 45 wk of age, digestibility was determined by the total collection method. The total collection of excreta was conducted over 5 days, and the feed consumption for each cage was recorded. Excreta was stored in plastic bags at −20 °C for 2 wks and, after thawing, was dried in an oven at 50 °C to a constant weight, weighed and finely ground. The content of nutrients in the diets and excreta was estimated using the same methods as it was previously described for the feed materials. Apparent total tract digestibility coefficient of dry matter was calculated as dry matter intake − dry matter excretion/dry matter intake. Similarly, digestibility of organic matter, crude fat, N-free extracts, crude fibre, and ash was calculated. Ca (P) retention (mg) was calculated as: Ca intake − Ca excretion. Ca (P) relative retention as a % of Ca (P) intake was calculated as: Ca intake − (Ca intake − Ca excretion)/Ca intake × 100.

At the end of the experiment (70 wk of age) all of the hens were sacrificed through cervical dislocation. Blood samples were collected from the jugular vein of each hen before slaughter, centrifuged at 3000 × *g* for 15 min at 4 °C and frozen stored (−20 °C) until analysis. Serum Ca and P concentration and alkaline phosphatase (ALP) activity were measured by colorimetric assay using commercial kits (Pointe Scientific, Warsaw, Poland).

The tibia and femur from both legs were collected, cleaned of soft tissues, weighed and frozen (−20 °C) until analysis. For determination of ash, the left tibias and toes were dried for 24 h at 105 °C, weighed and dry-ashed in a muffle furnace at 600 °C. A