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Effects of dietary energy and calcium levels on performance, egg shell quality and bone metabolism in hens

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ABSTRACT

This study investigated the effects of dietary energy and calcium levels on laying performance, eggshell quality and bone metabolism of layers. One hundred and sixty-two 19-week-old Hy-Line brown laying hens in 54 battery cages were allocated to one of nine dietary treatments with control, middle and high levels of energy (11.50, 12.68 and 13.37 MJ/kg, respectively) and low, control and high levels of calcium (2.62%, 3.7% and 4.4%, respectively) for 60 days, using a 3 × 3 factorial arrangement.

Compared with the control energy diet, high- and middle-energy diets increased fat deposition and egg weight, decreased feed intake and bone quality and had no effects on eggshell quality. The high-energy diet reduced the serum phosphate concentration and elevated osteocalcin mRNA expression in the keel bone without increasing osteocalcin protein. Dietary calcium intake did not affect fat deposition, feed intake or egg weight. Low dietary calcium resulted in weaker eggshells and poorer bone quality than that from hens fed the control diet. High dietary calcium increased serum calcium concentration, osteoprotegerin mRNA and osteocalcin protein and inhibited serum alkaline phosphatase activity and decreased its mRNA compared with low or control dietary calcium. The high-energy and high-calcium diet significantly reduced egg production.

Compared with the control energy diet, high- and middle-energy diets increased fat deposition but had negative effects on bone metabolic homeostasis. Dietary calcium did not influence fat deposition but a high-calcium diet benefited bone homeostasis, while a low-calcium diet was associated with poorer eggshell quality and bone homeostasis.

Introduction

Bone remodelling is a physiological process in which old or damaged bone is removed by osteoclasts and new bone is formed by osteoblasts (Feng and McDonald, 2011). An imbalance between the two processes may result in osteoporosis, a bone disease defined as a weakening or progressive loss of mineralized structural bone (Whitehead and Fleming, 2000). Osteoporosis is common in caged layers, approximately 30% of which experience a bone fracture at least once during their lifetime (Fleming, 2008). Such fractures cause economic losses and negatively affect welfare (Kim et al., 2012; Swiatkiewicz and Arczewska-Wlosek, 2012).

In laying hens cortical and cancellous bone provide structural integrity, but there is also have a third type of non-structural bone, medullary bone, which is formed in the long bones of hens when they reach sexual maturity (Dacke et al., 1993; Whitehead and Fleming, 2000; Fleming, 2008). During the laying period, structural bone formation ceases and only medullary bone is formed; however, both structural and medullary bones continue to be resorbed. The net effect is osteoporosis (Whitehead and Fleming, 2000; Fleming, 2008).

Nutrition is an important factor in avian osteoporosis (Fleming, 2008) and recent research has shown that dietary energy is important for bone. Zernicke et al. (1995) and Patsch et al. (2011) demonstrated that obesity induced by a high-fat diet in rats decreased bone mineral content, mechanical properties and microarchitecture and increased bone resorption. Cao et al. (2010) also found that obesity induced by a high-fat diet in mice increased bone resorption compared with normal weight mice. Atteh et al. (1983, 1984) stated that a high-fat diet in growing broiler chicks deleteriously affected bone mineralization, and Wohl et al. (1998) found similar results in adult roosters. However, these findings conflict with previous findings that bodyweight (BW) and fat mass favour bone density and prevent osteoporosis (Reid, 2002, 2008). In the current study, our aim was to research the effects of dietary energy level on performance, shell quality and bone metabolism in laying hens.

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Calcium is the critical nutritional factor for eggshell formation and bone health. The recommended amount of calcium for laying hens varies. The U.S. National Research Council (1994) suggested a calcium level of 3.25% for laying hens consuming feed at 100 g/day, whereas the standard is 3.5% in China (Ministry of Agriculture of the People's Republic of China, 2004) and 4% in the Hy-Line commercial management guide (2006). Castillo et al. (2004) stated that the biological optimum level for maximum egg production and egg specific gravity were 4.34% and 4.62% calcium, respectively, and the economic optimum level of calcium was 4.38% for 23-week-old Hy-Line W-98 hens. Roland et al. (1996) indicated that increasing the dietary calcium level (5%) increased bone quality without any adverse effect on egg production.

As dietary calcium and energy may affect bone metabolism, this research also studied the influence of three levels of dietary calcium and three levels of dietary fat on laying performance, shell quality, femoral and tibial quality, as well as bone specific genes and protein in the keel bone.

Materials and methods

Experimental design

The study was conducted under the guidelines approved by the Animal Care and Use Committee of the Nanjing Agricultural University.

One hundred and sixty-two Hy-Line brown layers, 19 weeks of age, from Qinglong Mount Hen Company were fed in 54 conventional cages ($40 \times 35 \times 35$ cm, $L \times W \times H$; three birds/cage) for 60 days, with each hen having 467 cm^2 of floor space. All hens were randomly allocated into one of nine diet treatments, with six cages per treatment. During the experimental period, hens received light for 16 h/day. All birds were each given 110 g feed per day. Water was provided ad libitum by a nipple drinker.

Treatments were allocated using a 3×3 factorial arrangement with three levels of energy (control-, middle- and high-energy:11.50, 12.68 and 13.37 MJ/kg, respectively) and three levels of calcium (low-, control- and high-calcium: 2.62%, 3.7% and 4.4%, respectively) (Table 1). The diets had the same protein and available phosphorus. Diet 4 (control energy and control calcium: 11.50 MJ/kg and 3.7%) was formulated according to the commercial management guide (Hy-Line UK, 2006).

Sample collection

Hen week-egg production was recorded from day 30 to 60. Feed intake was recorded and calculated from day 26 to 29 and 56 to 59. BW was measured at the beginning of the experiment and on day 60. During days 57–59, eggs from each cage were collected and stored at 4 °C for subsequent measurements.

Five millilitres of blood from the wing vein of one randomly sampled bird per cage were obtained after oviposition on day 60. Serum was separated by centrifugation of the blood at 1500 *g* for 15 min at 4 °C and stored at −70 °C until use. Following cervical dislocation, the liver and abdominal fat pads were measured, and relative liver or fat weight was calculated using the following formula:

$$\text{Relative liver or fat weight} = \text{liver or fat weight (g)}/\text{BW (kg)}.$$

Femurs and tibiae were dissected out and frozen with flesh intact at −20 °C until further analysis. In addition, a sample of the keel bone (1.5×2 cm, at the front edge of the bone) was collected from each bird and stored at −70 °C for real-time PCR and Western blot analysis.

Eggshell traits

Eggshell thickness was measured to the nearest 0.001 mm at the blunt and sharp ends and the equatorial region using a micrometer (211-101EK; Guilin Guanglu Measuring Instrument). Eggshell breaking strength was determined on a vertical axis using Material Testing Machines (LR10K Plus; Lloyd Instruments), with a standard 100 N load cell.

Bone traits

Before analysis, femurs and tibiae were cleaned of all tissue and weighed. Relative bone weight was calculated using the following formula:

$$\text{Relative bone weight} = \text{bone weight (g)}/\text{BW (kg)}.$$

All bones were then stored in 70% alcohol until measurements of radiographic density were made using computerized densitometry (Luo et al., 2012), using a HF50-R32 X-ray apparatus (44 kV and 4 mA s, Wandong Medical Equipment). Briefly, the bones were placed on a 35×43-cm X-ray plate along with a 16-step standard scale with a 0.25-mm increment aluminium step-wedge for calibration purposes. Images were collected by using a Regius model 110 Computed Radiography (Konica Minolta Medical and Graphic) and analysed with ImageJ software (National Institute of Health). The ImageJ program calibrated mean radiographic density of each excised bone in terms of mm of aluminium equivalence.

The breaking strength tests were carried out by 3-point bending (Norrdin et al., 1995; Kocamis et al., 2000) with Material Testing Machines (LR10K Plus, Lloyd Instruments) using NEXYGEN Plus software (Lloyd Instruments) and a standard 10 kN load cell. The distance between the two fixed points supporting the bone was 60 mm (tibia) or 40 mm (femur). The weight load was applied to the midpoint of the shaft under a crosshead speed of 5 mm/min until failure. Mechanical data were collected, and force deflection curves were analysed with NEXYGEN Plus software. The values for stiffness (N/m), Young's modulus (MPa) and mixed force (N) were recorded.

Table 1
Ingredients and chemical composition of experimental diets.

Item	Treatment								
	1	2	3	4	5	6	7	8	9
Ca	Low			Control			High		
Energy	Control	Middle	High	Control	Middle	High	Control	Middle	High
Corn	61.08	54.70	50.94	61.08	54.70	50.94	57.12	50.73	46.99
Soybean	26.02	27.31	28.07	26.02	27.31	28.07	26.82	28.11	28.87
Soybean oil	1.90	7.00	10.00	1.90	7.00	10.00	3.12	8.22	11.21
Limestone[a]	6.05	6.02	6.01	9.05	9.02	9.01	10.98	10.96	10.94
Monocalcium phosphate	1.29	1.31	1.32	1.29	1.31	1.32	1.30	1.32	1.33
Salt	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Zeolite	0	0	0	3	3	3	0	0	0
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Premix[b]	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Nutritional composition									
Energy (MJ/kg)	11.50	12.68	13.37	11.50	12.68	13.37	11.50	12.68	13.37
Crude protein (%)	16.50	16.50	16.50	16.50	16.50	16.50	16.50	16.50	16.50
Ca (%)	2.62	2.62	2.62	3.70	3.70	3.70	4.40	4.40	4.40
Available P (%)	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Lysine (%)	0.78	0.80	0.81	0.78	0.80	0.81	0.79	0.81	0.82
Methionine (%)	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37	0.37

[a] Calcium content of limestone was 360 g/kg of particle size 850–1000 μm.

[b] Premix supplied the following per kilogram of feed: vitamin A, 3,750,000 IU; vitamin D_3, 1,250,000 IU; vitamin E, 2500 IU; vitamin K_3, 480 mg; vitamin B_1, 400 mg; vitamin B_2, 1920 mg; vitamin B_6, 1500 mg; vitamin B_{12}, 3 mg; niacin, 5000 mg; pantothenate, 2500 mg; folacin, 200 mg; biotin, 50 mg; Fe, 25,000 mg; Cu, 3500 mg; Zn, 33,250 mg; Man, 31,800 mg; I, 150 mg; Se, 50 mg; Co, 40 mg.