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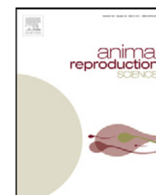

Effects of phytoestrogen supplementation in the feed on the shell gland of laying hens at the end of the laying period

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ABSTRACT

Shell quality decreases as laying hens age and the aim of present study was to investigate how a supplement of daidzein, a natural phytoestrogen in soya, affects key factors in the shell gland and eggshell quality in late-stage laying hens. Hybrids of Lohmann Selected Leghorn (LSL) and Lohmann Brown (LB), received either a daidzein diet (50 mg/kg feed) or a control diet from 60 to 72 weeks of age. Both the total number of capillaries and capillaries with carbonic anhydrase (CA) activity were higher in the LSL hybrid than in the LB. After daidzein supplementation the number of CA positive capillaries was unaffected in the LSL but increased in the LB hybrid indicating a higher sensitivity to daidzein in this hybrid. Estrogen receptor alpha and beta (ERα, ERβ) were localized and the complete picture of the two ERs can now be described in shell gland of domestic hens. Nuclear and cytoplasmic staining was generally stronger for ERβ, while membrane associated staining was present only for ERα. Interestingly, capillary endothelium contained only ERβ and since estrogen regulation of CA is well documented, the presence of an endothelial ER provides one possible route for the increase in CA positive capillaries found in LB hybrids. Eggshell quality or egg production was not affected by daidzein supplementation. The hybrids used in this study showed anatomical differences and reacted differently to daidzein supplementation, but if this can be explained by the divergences in ERβ localization noted between the hybrids remains to be clarified.

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1. Introduction

A laying period in commercial egg production last from puberty until the hens are 70–75 weeks old. Shell quality gradually decreases from the middle of the production period and reduced shell strength results in substantial economical losses [reviewed in (Etches, 1996)]. The avian eggshell consists mainly of calcium carbonate. For each egg the laying hen requires about 2–2.5 g of calcium supplied by absorption from the feed. However, bone tissue contributes with calcium during periods when intestinal absorption has ceased. The eggshell is deposited by the shell gland of the avian oviduct and blood-flow through the shell gland increases 4- to 5-fold during shell formation (Wolfenson et al., 1982).

Calcium is secreted across mucosal capillaries and epithelial cells in the shell gland by active transport and is functionally linked to bicarbonate concentration and production, which is abolished by acetazolamide, an inhibitor of the enzyme carbonic anhydrase (CA) (Eastin and Spaziani, 1978). CA catalyzes the hydration of metabolic CO_2 to HCO^{3-} which in turn is the source for the carbonate ions needed for eggshell production. Several investigations have shown that partial or complete inhibition of CA results in thin-shelled or shell-less eggs (Benesch et al., 1944; Diamants and Schluns, 1964; Lundholm, 1990).

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The complex physiological processes involved in the age related decrease of eggshell quality are still poorly understood. Intestinal calcium absorption decreases with age in hens (Al-Batshan et al., 1994), which appears to coincide with lower levels of estrogen receptor-α (ERα) in both kidney and shell gland (Hansen et al., 2003). There is no difference in circulating levels of estradiol in plasma between young and older laying hens, but old non-laying hens have lower levels (Joyner et al., 1987). An injection with estradiol increases plasma Ca^{2+} concentration (Bar et al., 1996). One main pathway for Ca^{2+} transport, at the cellular level, is the ATP-dependent plasma membrane pump (PMCA). Earlier studies have shown that estrogen treatment up-regulates PMCA in kidneys of laying hens and human uterus (Dick et al., 2003; Yang et al., 2011).

Estrogenic environmental pollutants and phytoestrogens may evoke biological responses similar to endogenous estrogen by binding to ERα and ERβ (Kuiper et al., 1998; Dusza et al., 2006). Phytoestrogens can have diverse antagonistic/agonistic effects depending on dose, type of tissue, ER subtype and the presence of endogenous hormone (Dusza et al., 2006). Embryonic exposure to either ethynylestradiol or the estrogenic pollutant o,p′-DDT disrupts the expression of CA in shell gland of laying hens and reduces shell thickness in exposed birds (Holm et al., 2006). In quail the effect on CA is still more pronounced and results in inhibition of egg production (Holm et al., 2001).

Although soya bean or soya bean meal is commonly used as a protein source for layers, the response to phytoestrogens in poultry is poorly investigated. However, supplementation of the diet with daidzein, a natural phytoestrogen found in soya beans, has been found to improve the laying performance of Shaoxing duck during post peak laying stage (Zhao et al., 2005). In a study made on ISA hybrid layers the amount of cracked eggs decreased and egg production and eggshell thickness was improved by a supplementation of 10 mg daidzein/kg feed (Ni et al., 2007).

These findings led us to further investigate the role of phytoestrogens in laying hens. More specifically, to study if daidzein, when added to the feed late in the laying period, influences shell quality and oviducal anatomy, with special emphasis on the structure and function of the shell gland. To achieve this, localization of CA activities, ERα, ERβ and the calcium transporter PMCA was performed on excised shell gland, in addition to measurement of eggshell quality and egg production.

2. Materials and methods

2.1. Animals

Laying hens of two different hybrids, 32 Lohmann Selected Leghorn (LSL) and 32 Lohmann Brown (LB) (Gimranäs AB, Sweden) arrived at the stable at 15 weeks of age. The layer hens were held for an entire production period in the university poultry research facility under conditions similar to commercial egg production. The hens were held in furnished cages in groups of 8 hens in each cage, except one control group LSL with nine hens and one control group LB in which one hen died just before the start of the experiment. At arrival the hens received 9 h light/24 h. The light period was gradually increased to 14 h light/24 h at 23 weeks of age.

Up to 60 weeks of age the hens were fed according to a phase feeding program for commercial hens, distributed by a Swedish feed manufacturer (Lantmännen). From 60 to 72 weeks of age hens were fed either of two experimental diets with the same nutritional composition, differing only by adding 50 mg/kg daidzein to one of them (LC Laboratories, Woburn, MA 01801, USA). Each diet was distributed to two 8-hen groups, i.e., 16 hens, of the two different hybrids. The experimental diets did not include any soya products, and were ordered in two batches. A diet with a content of 2.640 kcal/kg (11.0 MJ) metabolic energy, 165.3 ± 5.2 g crude protein, 32.0 ± 0.5 g Ca and 5.8 ± 0.4 g P. The cages fulfilled the Swedish Animal Welfare Directives and the study was approved by the Uppsala Local Ethics Committee.

2.2. Egg and eggshell measurements

Total egg production and indoor temperature was monitored in the stable throughout the experiment. Eggs were collected each morning and number of eggs and egg weight was recorded. Laying % was calculated as number of eggs laid/day and hen × 100. Eggs for shell quality measurements were collected each morning for five consecutive days in three periods when the hens were 61, 66 and 71 weeks old. Each egg were marked on three points approximately 120° apart along the equator of the egg with a pencil. Shell deformation was calculated from the average value of measurements on each marking point, after a load of 1000 g was applied on the egg (The Canadian Egg Shell Tester, Otal Precision Company Ltd, Ottawa Ontario, Canada K1G3N3). The shell breaking strength was recorded for each egg using the same instrument. The shell pieces were rinsed clean of albumen and yolk with distilled water and dried over night at 120 °C. Shell weight including shell membranes was recorded. Shell thickness was measured with a digital micrometer (Mitutoyo Absolute, No. 7360; Mitutoyo Corp., Stockholm, Sweden) on the marking points along the equator. Shell membranes were removed by boiling the shells in 2.5% (w/v) NaOH for 8 min, rinsing in distilled water and drying overnight at 120 °C. Shell thickness was measured once more on the same three places.

2.3. Dissection and tissue preparation

The hens were killed at 72 weeks of age by an intravenous injection of pentobarbital sodium (100 mg/ml, Apoteket AB, Umeå, Sweden). Body weight was recorded for each bird and the left oviduct was rapidly removed, the ventral part of mesoviductus was cut and the oviduct straightened. The length was measured from the vaginal orifice to the fimbriated infundibulum and the location of an egg was recorded. The shell gland was cut open lengthwise and pieces from the center were removed for fixation. All tissues were divided in two and nailed on a small piece of cork to minimize tissue distortion. One piece was fixed in 2.5% glutaraldehyde in 0.067 M phosphate buffer (pH 7.2) and the other piece in 4% paraformaldehyde in 0.067 M phosphate buffer (pH 7.2) for 24 h at 4 °C. After rinsing in

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