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TOTAL CHOLESTEROL AND CORTICOSTERONE CONCENTRATION RELATIONSHIP IN BLOOD PLASMA OF LAYING HENS

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ABSTRACT

The aim of this study was to analyze the effect of laying hens housing condition on the plasma total cholesterol and corticosterone concentrations relationship at the beginning, middle and end of the laying period. The experiments were performed in ISA BROWN egg production pullets, kept in a poultry house for rearing with deep litter technology. Blood samples (2 ml) of all hens in experimental groups were collected from brachial vein at 22, 28, 35, 41, 47, 52, 58 and 66 weeks of age. Plasma corticosterone levels were analyzed by radioimmunoassay and plasma total cholesterol by spectrometry. One-way ANOVA showed significant differences in cholesterol as well as corticosterone levels of blood plasma of laying hens during the experimental period. A very low relationship was found between individual total plasma cholesterol and corticosterone concentrations during the monitored period.

Keywords: Laying hens, furnished cage technology, blood plasma, cholesterol, corticosterone

INTRODUCTION

Cholesterol is a lipid with a unique structure consisting of four linked hydrocarbon rings forming the bulky steroid structure. Its levels in tissues reflect a balance among dietary uptake, endogenous de novo synthesis, efflux, and utilization to bile acids (**Faust and Kovacs, 2014**). With respect to cholesterol uptake, the low density lipoprotein receptors are well-known for their important roles in regulating plasma and intracellular cholesterol homeostasis (**Soto-Acosta *et al.*, 2013**), they are primarily modulated by intracellular cholesterol levels (**Liu *et al.*, 2012**). As an essential component of cell structure and the precursor of steroid hormones, the amount of cholesterol in chicken muscle will affect avian well-being, and may ultimately influence human health through dietary intake. Glucocorticoids are the counter regulatory hormones with broad effects on carbohydrate, lipid and protein metabolism (**Bamberger *et al.*, 1996**). Glucocorticoids participate in the control of whole body homeostasis and the response of the organism to stress by stimulating there lease of energy stores via promoting glucose mobilization and lipolysis (**Harvey *et al.*, 1986**). Glucocorticoid treatment is associated with increased levels of circulating free fatty acids in humans (**Macfarlane *et al.*, 2008**) and rodents (**Novelli *et al.*, 2008**). In birds it has been reported that corticosterone and insulin interact to regulate triglyceride and cholesterol levels during stress (**Remage-Healey and Romero, 2001**). Glucocorticoids, especially corticosterone, are involved in the control of appetite in poultry (**Yuan *et al.*, 2008**). **El-Letheye *et al.* (2001)** have shown that corticosterone can increase feed intake of chickens. Furthermore, there are reports of augmentation in plasma corticosterone after 24h of feed deprivation in immature chickens (**Geris *et al.*, 1999**). Hyperlipidemia and metabolic abnormalities caused by endogenous glucocorticoid excess have been well documented in mammals and birds. In mammals, increased circulating glucocorticoids together with the altered insulin sensitivity are suggested to be responsible for enhanced visceral fat deposition and hyperlipidemia (**Geraert *et al.*, 1996**). Chronic corticosterone administration did not change plasma lipid profile, except for a moderate increase of high density lipoprotein cholesterol levels in broiler chickens (**Wang *et al.*, 2013**). Chronic endogenous glucocorticoid excess in mammals is associated with metabolic dysfunction and dyslipidemia that are characterized by increased plasma triglyceride and total cholesterol levels (**Duan *et al.*, 2014**).
The objective of our study was to analyze the effect of laying hens housing condition on the plasma total cholesterol and corticosterone concentrations relationship at the beginning, middle and end of the laying period.

MATERIAL AND METHODS

Animals and experimental conditions

The experiments were performed in ISA BROWN egg production pullets, kept in a poultry house for rearing with deep litter technology. The available area, complete feeding mixture, light-dark (L:D) cycle, temperature of housing, relative humidity of air changed according to technological instructions for ISA BROWN pullets. During rearing period standard vaccinations were provided. At the age of 15 weeks, they were transferred into o furnished cage technology according to Council Directive 74/99/EC – three-tier, total area 945 cm^2/bird (8 birds kept on an area of 7560 cm^2 – 180x42x45cm), available area 643 cm^2/bird, 6 nipple drinkers, belt feeder 20 cm/bird, nest (30x35x45cm), perching area 15 cm/bird, devices for dustbathing and scratching, device for claw shortening. Experimental group consisting of 12 birds was established with the mean body weight of 1300 ± 50 g. Throughout the study, the hens were fed with balanced layer feeds that contained 875 $g.kg^{-1}$ dry matter, energy content ME_N 11.1 $MJ.kg^{-1}$, content of nitrogen substances 170.7 $g.kg^{-1}$, Ca 35.9 $g.kg^{-1}$ and P 6.3 $g.kg^{-1}$. A constant light-dark (L:D) cycle (15:9, switching on at 4.00 AM, switching off at 19.00 PM) was maintained in all three technologies as recommended in technological instructions for ISA BROWN pullets. The temperature of housing was in the range from 18 to 20 °C, relative humidity of air was ranging from 65 to 70 %. No red mite and other parasite or viral infection was presented during experimental period.

Blood sampling

Blood samples (2 ml) of all hens in experimental groups were collected from brachial vein at 22, 28, 35, 41, 47, 52, 58 and 66 weeks of age, always between 7.00 and 8.30 am. EDTA was used as anticoagulant. Blood samples were centrifuged (20 min, 4 °C, 2500 g) and the separated plasma was stored at -20 °C until analyzed. Blood sampling was performed randomly in hens kept in standard, enriched and deep litter technology. Utmost care was given to keep the time within 2 minutes between catch and conclusion of blood sampling because this small gap is known to have little or no effect on corticosterone secretion in layers (Craig and Craig, 1985).

Plasma corticosterone and cholesterol analysis

Plasma corticosterone levels were analyzed by radioimmunoassay following dichloromethane (Merck, Darmstadt, Germany) extraction of the steroids from 100 µl aliquots of plasma and using ^{3}H-corticosterone (Amersham, UK) as described previously (**Jezova *et al.*, 1994**). Radioactivity of free corticosterone was counted using a liquid scintillation counter (Beckman LS-6500; Beckman-Coulter, USA). Corticosterone (Sigma, Steinheim, Germany, minimal purity 92%, C2505) was used as a standard. Standard curve was performed using 31.5, 62.5, 125, 250, 500, 1000, 2000, 4000, 8000 pg of corticosterone per tube. The sensitivity of corticosterone assay was 0.5 ng.ml^{-1} plasma. The intra- and interassay coefficients of variations were 6 and 8 %, respectively. All hormone measurements were performed in duplicates. Quality of the assay was controlled by repeated analysis of corticosterone in plasma pools with low and high corticosterone concentrations. Measurements of corticosterone in the same control samples were included in all assays performed. Specific antibodies were kindly provided by Prof. C. Oliver, Laboratory of Experimental Neuroendocrinology (Marseille, France). The antibody cross-reacted 100% with cortisone and cortisol, 24% with 11-deoxycorticosterone, 22% with aldosterone, 16% with 17-OH-progesterone, 4% with androstendione, progesterone and testosterone and less than 0.1% with estradiol and estrone.
Total cholesterol concentrations in blood plasma were analysed by spectrometry on the KONELAB T20xt automatic analyser (Thermo Fisher Scientific, Finland) and currently available commercial kits (Biovendor-Laboratorni medicina, Czech Republic).

Statistical analysis

Statistical analysis of the obtained data was performed using the STATISTICA 8.0 programme by single-factor analysis of variance for factor animal age. ANOVA was followed by post-hoc Fischer LSD test for pair-wise comparisons, when appropriate. Evaluation of the interdependence between the cholesterol and corticosterone concentrations was conducted using a correlation coefficient at the level of probability ($P < 0.01$).

RESULTS AND DISCUSSION

One-way ANOVA showed significant differences in blood plasma cholesterol levels of laying hens during the experimental period $F(7, 88)=3.5808$, $p=0.00195$). The Fisher post hoc testing showed a significant increase ($p < 0.01$) of the cholesterol level at the age of 28 weeks (3.81 ± 0.340 mmol.l^{-1}). Subsequently the cholesterol concentrations changed during the experimental period without any significance (figure 1). The highest concentration of cholesterol was recorded in week 47 (4.68 ± 0.918 mmol.l^{-1}). As with cholesterol, the corticosterone concentrations changed during determined period (figure 1). The age of hens significantly influenced the level of plasma corticosterone, as shown by one-way ANOVA ($F(7, 88)=7.2110$, $p=0.00035$). The Fisher post hoc testing showed a significant increase ($p < 0.01$) of the corticosterone level at the age of 52 weeks (1.38 ± 0.067 ng.ml^{-1}). A similar concentration was found at the age of 58 weeks. A significant decrease ($p < 0.01$) was recorded at the end of the experimental period (0.98 ± 0.078 ng.ml^{-1}). A very low relationship was found between the individual total plasma cholesterol and corticosterone concentrations during the monitored period, with a correlation coefficient $r = 0.178$ ($p < 0.05$).

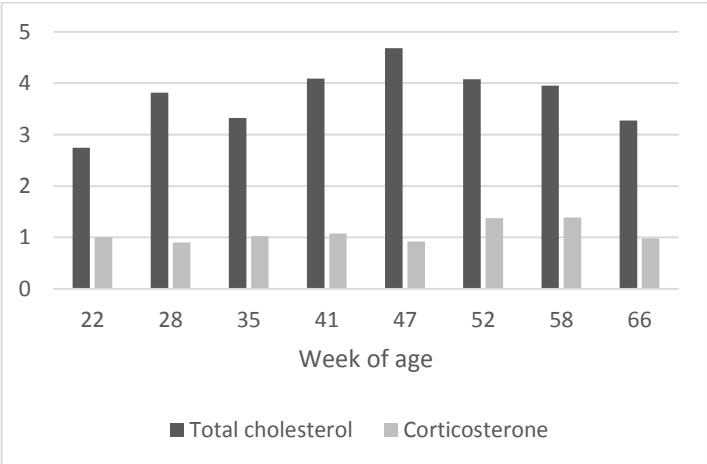

Figure 1 Total cholesterol and corticosterone concentrations in blood plasma of laying hens during experimental period. Total cholesterol is presented in mmol.l^{-1}, corticosterone in ng.ml^{-1}. Data in columns represent mean.

In our experiment, plasma corticosterone levels increased with age, showing some variation in the middle of the trial and a slight decrease at the week 58 up to week 66 of age. Changes in corticosterone levels in the laying period have been attributed to the seasonal effects during breeding (**Koelkebeck *et al.*, 1984**). Also, animals are exposed to many external factors. Factors that may induce stress responses include stocking density, temperature, transport, feed restriction, feed contamination, fear and diseases (**Nicol *et al.*, 2006**), which can activate the hypothalamic-pituitary-adrenal axis to enhance the release of glucocorticoids from the adrenal gland (**Carsia and Harvey, 2000**). Elevated release of corticosterone from the adrenal cortex in response to stressful stimuli has been proposed to promote gluconeogenesis and lipolysis to break down fat tissues to provide the bird with more energy (**Scanes, 2009**). In our experiment, the concentration of total cholesterol in the blood plasma of laying hens increased in all groups from week 22 to 47 of age, with a subsequent decrease to the end of the monitored period. Intracellular cholesterol content depends on three major factors: cholesterol uptake into cells, de novo cholesterol synthesis within cells, and efflux of cholesterol out of cells (**Feeney *et al.*, 2013**). With respect to de novo cholesterol synthesis, multiple mechanisms for the feedback control of cholesterol biosynthesis converge at the rate-limiting enzyme 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) (**Garevik et al., 2012**). Glucocorticoids elevate the HMGCR activity in cells (**Cavenee and Melnykovych, 1979**). The chicken HMGCR gene is present in many tissues, including the brain, liver, intestine and skeletal muscle (**Burtea *et al.*, 1991**). The liver is a key organ involved in the control of plasma cholesterol, which is largely determined by the rate of removal of low density lipoproteins from the circulation via hepatic low density lipoprotein receptor (**Myant *et al.*, 1991**). The number of hepatic low density lipoprotein receptors directly governs plasma low density lipoprotein cholesterol (**Rudling *et al.*, 2002**). Glucocorticoids stimulates a concentration- and time-dependent increase of low density lipoprotein receptor biosynthesis in cultured fibroblasts (**Filipovic and Buddecke, 1985**). Receptor-mediated cholesterol uptake is suggested to play a role in maintaining the intracellular free cholesterol pool. **Duan *et al.* (2014)** found that muscular low density lipoprotein receptors levels in corticosterone treated chickens were significantly increased compared with controls. High glucocorticoids levels enhance lipid accumulation into macrophages cultured in vitro by increased cholesterol ester synthesis and decreased cholesterol ester breakdown without altering cholesterol influx or efflux (**Cheng *et al.*, 1995**). **Duan *et al.* (2014)** recorded, that chronic corticosterone administration induces cholesterol and triglyceride accumulation in chicken muscle by upregulating their intracellular synthesis and uptake. **Yeon-Hwa Kim *et al.* (2015)** determined significantly higher plasma corticosterone concentrations in corticosterone-treated group, compared with control. Increased concentrations were found also for cholesterol.

CONCLUSION

In this study we analyzed the effect of laying hens housing condition on the plasma total cholesterol and corticosterone concentrations at the beginning, middle and end of the laying period. In conclusion, our experiment revealed no relationship between the blood plasma corticosterone and cholesterol concentrations.

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