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CHANGES IN PLASMA CONCENTRATION OF PROGESTERONE, ESTRADIOL AND CALCIUM ASSOCIATED WITH EGGSHELL CALCIFICATION IN LSL AND FAYOUMI HENS

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ABSTRACT

In this study, one hundred and sixty, 12 weeks old, Fayoumi and Lohman Selected Leghorn (LSL) were used. Birds were housed individually. Performance of hens was recorded from sexual maturity up to the peak of egg production. Plasma concentration of progesterone (P_4), estradiol (E_2) and ionized calcium were determined at 4 and 2 weeks before sexual maturity (SM), at SM and at the peak of egg production. During ovulatory cycle at the peak of egg production P_4, E_2 and ionized calcium were determined at zero, 6, 12, 18 and 24h post-oviposition. Eggshell quality was measured. Productive performance of LSL was better than Fayoumi hens, while eggshell quality of Fayoumi was higher than LSL. Plasma concentration of P_4, E_2 and ionized calcium were significantly increased at SM. Plasma concentration of estradiol and ionized calcium were higher in Fayoumi than LSL hens allover stages studied. Higher levels of P_4, E_2 and ionized calcium were associated with egg calcification through ovulatory cycle. The highest level of P_4 was determined at oviposition.
Keywords: Laying hen, eggshell quality, progesterone, estradiol, calcium.

INTRODUCTION

It is recognized that the laying hen possesses remarkable physiological adaptations that facilitate the absorption, deposition and storage of Ca^{2+} from feed, and utilization of CO_2 dissolved in blood to synthesize the carbonate ion in the eggshell.

Calcification of laying hen egg is associated with remarkable changes in calcium metabolism that occur with the initiation of gonadal activity (Nys *et al.*, 1986 and Ahmed Nagwa *et al.*, 1997). Shortly before sexual maturity, the formation of medullar bone (Simkiss, 1967) and a parallel increase in calcium and phosphorus retention (Nys *et al.,* 1986) are induced by the synergistic action of estrogen and testosterone. An increase in calcium absorption occurs at the onset of egg production (Hurwitz *et al.*, 1973, Radwan, 1980 and Ahmed Nagwa, 1986).

In addition, calcium retention is increased during the period of eggshell formation, between 7 and 20h after ovulation (Hurwitz *et al.*, 1973). There are also increases in both of calcium intake (Nys *et al.*, 1986 and Ahmed Nagwa, 1986) and absorption of soluble calcium in the intestine at the onset of and during egg calcification, respectively (Radwan, 1980 and Ahmed Nagwa. 1986).

Steroid hormones have been implicated in the regulation of calcium and phosphorus metabolism in the laying hen through several modes of action that may affect eggshell quality. Hens laying thick-shelled eggs have been demonstrated to have significantly greater amounts of serum calcium-binding protein, diffusible and non-diffusible calcium than hens laying thin-shelled eggs (Curl *et al.*, 1985).

Plasma progesterone concentrations have been correlated with eggshell quality at blood sampling times of 20 and 24h of the day after oviposition (Curl *et al.,* 1985). Plasma concentrations of both progesterone and estradiol are maximal about 3 to 6 h before ovulation (Etches, 1990; Etches, 1996 and Ahmed Nagwa *et al.*, 1998). It is possible that estradiol and progesterone concentrations in plasma may be related to eggshell quality during the period of maximal hormonal levels.

The objective of this study was to determine the concentrations of plasma P_4, E_2 and ionic calcium that may be involved in eggshell quality in LSL (thin-shelled eggs) and Fayoumi hens (thick-shelled eggs).

MATERIALS AND METHODS

This study was carried out in the Poultry Research Farm and Laboratory of Physiology, Animal Production Department, Faculty of Agriculture, Cairo University.

Birds

One hundred and sixty, 12 week old, Lohman Selected Leghorn (LSL) and Fayoumi birds were housed in individual cage. Hens were fed *ad libitum* laying diet containing 17% crude protein, 2800 kcal ME/ K diet, 3.5% calcium and 0.65% total phosphorus according to NRC (1984) requirements. Photoperiod was 16h.

Egg number and weight were recorded (from laying the first egg up to the eighth month of production) for each hen.

Eggshell quality was determined using twenty hens (10 hens/ strain) at sexual maturity (SM), one month after SM (100 eggs mean of 5 eggs per hen for 10 hens of each strain) and peak of production on 100 eggs (5 eggs / hen x 10 hens x 2 strains). The time of ovulation (±30 min.) were estimated by knowing the position of the egg in the clutch sequence, ovulation occurs 30-45 min after oviposition of the preceding egg and this relationship is used to estimate the time of ovulation. Body weight of hens was recorded at the peak of egg production.

Blood Samples

Blood samples were collected from the wing vein of 10 hens / strain using heparinized syringes at different stages of production (4 weeks before SM, 2 weeks before SM, at SM and during the peak of egg production) after oviposition. At the peak of egg production, blood samples also were collected from 16 hens (8 hens/strain) at successive stages of egg formation (0, 6, 12, 18 and 24h post-oviposition). Stage of egg formation was estimated by reference to the oviposition of the previous egg, as ovulation occurs approximately 30 min after the preceding oviposition. Blood samples were

centrifuged at 3000 rpm for 5 min to collect plasma that was stored at -20C^0 until required for assay of estradiol (E_2), progesterone (P_4) and ionic calcium determination.

Hormonal Assay

Radio immunoassay (RIA) technique was performed for plasma P_4 and E_2 (Etches *et al.,* 1981). Ready antibody coated tube kits (Diagnostic Product Corporation, Los Angeles) were used according to the procedure outlined by the manufacturer.

Estradiol antibody (at 50% binding) showed 100% cross reaction with estradiol, while it was 4.4, 10.0 and 1.8% with d-equilenin, estrone and estrone-B-D-glucuronide and ethinyl estradiol, respectively. It was less than 1% with any other steroids.

Progesterone antibody (at 50% displacement) was 100% with progesterone, while it was 2.2%, 3.4%, 9.0% and 3.2% with 11-Deoxycorticosterone, 17α-Hydroxyprogesterone, 5 α-Pregnan 3,20-dione and 5β-pregnan-3,20-dione, respectively. It also was less than 1% with corticosterone, 20β dihydroprogesterone, pregnenlone, hidroxy progesterone and testosterone and less than 0.1% with any other steroids.

Statistical Analysis

Data collected were subjected to two factor-factorial analysis of variance (MSTAT, 1986). Differences among means were tested using Duncan Multiple Range Test (Duncan, 1955).

RESULTS

Values of body weight, egg number, egg weight, shell weight and shell mass were higher significantly in LSL than Fayoumi hens through 100 days of production (Table 1). However, the percentage of shell weight to egg weight and shell thickness was significantly higher in Fayoumi than LSL .

LSL hens had significant heavier eggs and shell weights than Fayoumi ones at different productive status studied (At SM, one month after SM and at the peak of egg production). Egg and shell weight increased significantly with the advance of egg production (Table 2).

Table 1: Performance of LSL and Fayoumi laying hens through 100 days production

Item	LSL	Fayoumi	SE
Body weight (g)	1569.0 [a]	1295.4 [b]	59.2
Egg number	89.80 [a]	67.60 [b]	3.50
Egg weight (g)	55.70 [a]	38.60 [b]	0.78
Shell mass (g)	448.10 [a]	297.00 [b]	24.96
Shell weight (g)	4.98 [a]	4.48 [b]	0.27
Shell %	8.95 [b]	11.63 [a]	0.37
Shell thickness (mm)	0.353 [b]	0.423 [a]	0.012

[a,b] Means within each trait having different superscripts differ significantly (P < 0.05).

Shell percentage decreased as egg production advanced in LSL and Fayoumi (Table 2). The decrease in LSL eggshell percentage due to the increase in egg production was significant. Shell thickness of Fayoumi eggs was significantly higher than LSL at different ages (Table 2). The thickness of Fayoumi eggshell increased during one month after SM and stayed constant till the peak of egg production, while slight decrease by about 2.3% occurred in LSL eggshell thickness at the peak of egg production.

Table 2: Egg shell quality of LSL and Fayoumi laying hens at different productive stages.

Item	Sexual maturity (S.M.)	One month after S.M.	Peak of production	SE
Egg weight (g)				
LSL	41.1^{c}	54.7^{b}	59.4^{a}	1.1
Fayoumi	29.7^{d}	39.9^{c}	40.5^{c}	
Shell weight (g)				
LSL	2.42^{b}	5.28^{a}	5.46^{a}	0.16
Fayoumi	3.29^{c}	4.11^{b}	4.16^{b}	
Shell %				
LSL	10.57^{a}	9.70^{ab}	9.15^{b}	0.45
Fayoumi	11.06^{a}	10.31^{ab}	10.26^{ab}	
Shell thickness (mm)				
LSL	0.366^{b}	0.393^{ab}	0.384^{ab}	0.02
Fayoumi	0.387^{ab}	0.405^{a}	0.405^{a}	

^{abc} **Means within each trait having different superscripts differ significantly (P<0.05)**

Plasma concentration of progesterone (ng/ml), estradiol (pg/ml) and ionic calcium (mg/dl) was, generally, higher in Fayoumi than LSL hens (Table 3). The differences were more pronounced at sexual maturity.

Level of progesterone (P_4), estradiol (E_2) and ionized calcium (Ca^{2+}) increased in plasma of LSL and Fayoumi hens with the advance of age. Sexual maturity associated with significant increase in plasma concentration of P_4, E_2 and Ca^{2+} in both LSL and Fayoumi (Table 3).

Hourly changes in concentration of plasma P_4, E_2 and ionized calcium of LSL and Fayoumi hens during the ovulatory cycle are shown in Table (4). Plasma concentration of progesterone, estradiol and ionized calcium were higher in Fayoumi than LSL hens through ovulatory cycle. Each of P_4 and calcium showed two peaks at zero and 18h after oviposition, however, the initial peeks was higher in any of these components. In LSL hens, the peak of E_2 hormone was determined at 24h post-oviposition and continued till the second oviposition then the level of E_2 started to decrease from 6h to 18h post-oviposition (Table 3). While fluctuated trend was observed in the concentration of E_2 during ovulatory cycle in Fayoumi hens. The highest level of E_2 was observed at oviposition then it significantly decreased at 6h post-oviposition and significantly increased again at 12h after oviposition, and significantly decreased up to 24h post-oviposition (Table 4). Average E_2

concentration was significantly higher in Fayoumi (167 pg/ml) than LSL (117 pg/ml) during the ovulatory cycle.

DISCUSSION

Fayoumi hens had significantly less egg number, smaller eggs and thicker-shelled eggs than LSL hens (Table1). Shell percentage through 100 days production was significantly higher by about 2.7% in Fayoumi than LSL. The results of LSL and Fayoumi hens performance are in agreement with the findings of Shoukry (1987) and Ahmed Nagwa *et al.* (1997).

Table 3: Plasma progesterone, estradiol and ionic calcium of LSL and Fayoumi laying hens at different productive stages.

Item	4 weeks before S.M.	2 weeks before S.M.	At sexual maturity	Peak of production	SE
progesterone, ng/ml					
LSL	0.096^{c}	0.123^{c}	0.375^{bc}	0.613^{ab}	0.11
Fayoumi	0.150^{c}	0.118^{c}	0.767^{a}	0.782^{a}	
Estradiol,pg/ml					
LSL	37.95^{c}	58.90^{c}	126.97^{b}	124.10^{b}	17.59
Fayoumi	37.68^{c}	55.19^{c}	192.84^{a}	200.52^{a}	
Ionic calcium, mg/dl					
LSL	6.52^{bc}	7.80^{bc}	12.47^{a}	12.43^{a}	0.58
Fayoumi	6.18^{c}	8.16^{b}	12.68^{a}	13.14^{a}	

^{abc} **Means within each trait having different superscripts significantly differ (P < 0.05).**

Table 4: Plasma concentrations of progesterone, estradiol and ionic calcium in LSL and fayoumi laying hens during ovulatory cycle.

Item	Hours post-oviposition					SE
	0	6	12	18	24	
progesterone, ng/ml						
LSL	0.681^{b}	0.223^{d}	0.278^{cd}	0.583^{b}	0.357^{cd}	0.054
Fayoumi	0.973^{a}	0.310^{cd}	0.345^{cd}	0.410^{c}	0.282^{cd}	
Estradiol, pg/ml						
LSL	130^{bcd}	117^{bcd}	111^{cd}	107^{d}	124^{bcd}	21.0
Fayoumi	190^{a}	164^{ab}	177^{a}	156^{abc}	149^{abcd}	
Ionic calcium, mg/dl						
LSL	12.43^{ab}	11.27^{abc}	10.52^{bc}	12.09^{abc}	10.02^{c}	0.73
Fayoumi	13.14^{a}	11.30^{abc}	10.70^{bc}	11.84^{abc}	10.97^{abc}	

^{abcd} **Means within each trait having different superscripts significantly differ (P< 0.05).**

Eggshell Quality

Eggshell weight increased in both strains as egg production advanced (Table2), while the opposite trend was observed with shell percentage. The decrease in shell percentage from laying the first egg to the peak of production was 1.42% and 0.80% in LSL and Fayoumi, respectively. The

initial shell thickness, at SM was thicker in Fayoumi and LSL. The thickness increased in both strains till one month of production, followed by decrease in case of LSL only at the peak of production (Table 2). However, it is worthy noting that the period from laying the first egg to the peak of egg production was longer in Fayoumi than in LSL hens. These results are inconsistent with other studies that demonstrated that shell quality declined as egg production advanced (Muller *et al.*, 1960; Curl *et al.*, 1985; Ahmed Nagwa, 1986 and Shoukry, 1987).

Hormones and Calcium Level at Successive Production

Progesterone hormone concentration sharply increased at sexual maturity in plasma of both LSL and Fayoumi hens (Table 3). The concentration of P_4 hormone in Fayoumi at sexual maturity was almost double that of LSL hens (0.767 vs. 0.375 ng/ml). After sexual maturity, P_4 hormone kept its concentration until reaching the peak of egg production in plasma of Fayoumi hens, while in LSL hens, the hormone increased significantly to reach its peak (0.613 ng/ml) at the same productive stage (Table 3). This result is in harmony with the results of Ahmed Nagwa *et al.* (1997), who found that P_4 concentration increased significantly at the age of sexual maturity in LSL and Fayoumi hens. Etches (1996) reported that the concentration of P_4 remained at the basal levels until one week prior to lay, when production was rapidly accelerated to the level that characteristics of a laying hen. The significant increase in the level of P_4 hormone at sexual maturity was attributed to the increase of gonadotrophic releasing hormone (GNRH) from pituitary gland (Etches, 1984 and 1996). Also, sexual maturation in the pullet is accompanied by an increase in the ability of P_4 to stimulate LH release. This increase in the ability of P_4 to stimulate LH secretion is presumed to be mediated by the hypothalamus via the production of GNRH.

Estradiol hormone had similar trend as that observed in P_4 concentration in hens plasma. The first egg laid was associated with significant increase in E_2 concentration (Table 3), and then the level of E_2 remained nearly constant till the peak of production. However, Tixier-Biochard *et al.* (1990) found that E_2 level peaked two weeks before the first egg, remaining relatively constant thereafter. The stability of E_2 hormone concentration was attributed to the formation of yolk protein and deposition of calcium in eggshell (Gruber, 1972 and Tixier-Biochard *et al.*, 1990). The concentration of E_2 in plasma from sexual maturity up to the peak of egg production ranged from 192.84 to 200.52 pg/ml in Fayoumi and from 126.97 to 124.10 pg/ml in LSL. Shoukry (1987) and Ahmed Nagwa *et al.* (1997) concluded that the significantly higher level of E_2 hormone in Fayoumi than LSL hens might be related to the high shell quality of Fayoumi eggs. Similarly, Soares (1984) reported that plasma estradiol concentrations were somewhat higher in hens that lay eggs with high eggshell quality than in those with low shell quality.

Ionized calcium concentration in blood plasma of LSL and Fayoumi hens increased four weeks before sexual maturity, with a sharp increase at onset of the first egg formation. Etches (1996) concluded that, although ionized

calcium represents only about 20% of total calcium in blood, it was the most important form in terms of shell formation since only the ionized molecule is sequestered by the shell gland and deposited in the shell gland fluid.

The present results showed a relationship between E_2, P_4 and calcium at the age of sexual maturity. Etches (1996) reported that as sexual maturity approached, the production of $E_2$and P_4 stimulates deposition of calcium in the medullary bone. In addition, the storage depots of calcium in bone developed under the influence of estrogen stimulation as the concentration of this hormone rises at SM. On the other hand, to meet the needs of eggshell calcification, the concentration of the calcium binding protein in the shell gland increases at SM in response to increased concentration of estrogen (Etches, 1984 and 1996). The increase in the plasma concentration of estrogen stimulates the production of 1, 25, dihydroxycholecalciferol by increasing the activity of enzyme to convert its precursor in the kidney.

Plasma concentration of E_2, P_4 (Ruschkowski, 1993; Etches, 1996 and Ahmed Nagwa, 1998) and ionic calcium (Singh *et al.*, 1986; Frost *et al.*, 1991 and Ruschkowski, 1993) varied according to the stage of egg formation. Maximum peaks of E_2, P_4 and ionic calcium were determined immediately post-oviposition. Ruschkowski (1993) reported that a transitory peak in plasma estradiol 17β concentration immediately following oviposition.

Hormones and Calcium Level During Ovulatory Cycle

The trend of P_4 and estradiol hormone concentration in plasma of LSL and Fayoumi hens at different periods during ovulatory cycle was illustrated in our previous study (Ahmed Nagwa *et al.*, 1998).

In the present study, the highest plasma P_4 concentration (ng/ml) was determined immediately at oviposition (0.827), then sharply decreased at 6h (0.262) post-oviposition and started to increase again at 12h (0.312) to be 0.496 at 18h and decreased significantly again at 24h post-oviposition (0.319). The difference in the overall average of plasma P_4 concentration between LSL and Fayoumi hens is not significant during the ovulatory cycle, the concentration was 0.425 and 0.464 ng/ml in LSL and Fayoumi, respectively. However, the concentration of P_4 hormone was significantly higher in LSL (higher producing layer) than Fayoumi at 18h post-oviposition. This result reflects a relationship between P_4 concentration in plasma and egg production sequence. Etches *et al.* (1981) reported that the increase in plasma P_4 concentration at 4-6h before ovulation was almost directly related to progesterone secretion by the largest pre-ovulatory follicle.

The ovary of Fayoumi hens has a large proportion of small follicles that mature at a slower rate (low producer) than that of LSL (high producer). Senior and Furr (1975) stated that E_2 is not an important secretion of large follicle and the largest quantities of E_2 were present in the numerous small follicles and ovarian stroma. Also, the higher plasma E_2 in Fayoumi may be involved in eggshell quality through a relationship with calcium deposition. Taylor (1965) suggested that E_2 might help in building up the reserves of calcium in preparation for eggshell formation. Moreover, Palmer and Bahr

(1992) reported that E_2 regulated yolk formation and calcium deposition in hens.

Ionic calcium level was higher in plasma of Fayoumi than LSL from 18 to 24h during the ovulatory cycle, but there were no remarkable changes in the concentration of ionic calcium between LSL and Fayoumi from 6 to 18h post-oviposition. Ionized calcium had similar trend in LSL (lower shell quality) and Fayoumi (higher shell quality) through ovulatory cycle (Table 4). Maximum plasma ionic calcium level was determined immediately after oviposition (before the next ovulation), and then it significantly declined up to 12h post-oviposition and significant increase was occurred at 18h after oviposition and decreased again at 24h post-oviposition.

The fluctuations in the concentration of calcium in plasma during ovulatory cycle are attributable to several factors. Urist *et al.* (1960) correlated increased protein-bound calcium levels in the blood with yolk formation. Taylor (1966) and Solomon (1971) showed a cyclic deposition and destruction of medullary bone which is synchronized with the egg-laying cycle, bone formation being initiated shortly after oviposition and continuing at a high rate during the first few hours of calcification. Etches (1996) found that the ability of the shell gland to accumulate ionized calcium from blood and transfer it to the shell gland fluid is truly remarkable, and throughout the calcification period, a supersaturated concentration of Ca^{2+} is maintained in this fluid to support the crystals precipitation of calcium carbonate in the shell. The demand for Ca^{2+} during shell formation reduces both the total and ionic calcium concentration in plasma. Furthermore, the maximum concentration of ionic calcium in the shell gland fluid occurs when the level of it in plasma is minimal.

Etches (1996) declared that the movement of Ca^{2+} from gastrointestinal tract to the shell is influenced by the production of vitamin D in kidney and PTH from parathyroid glands. Although the molecular mechanisms that support the enormous flux of Ca^{2+} among the various physiological compartments are not understood, Etches (1996) reported that the epithelial cells of shell gland may play a part in the transfer of ionized calcium from blood into the shell gland fluid, although details of their role not yet clear. It has been suggested that ionic calcium is transported into the shell gland fluid by the ciliated and/or non-ciliated cells in the shell gland epithelium, and that cells of the tubular glands produce carbonic anhydrase. The higher level of calcium at different physiological status that correlated with good shell quality of Fayoumi egg than LSL may be related to the action of carbonic anhydrase enzyme that is believed to be the key enzyme in the production of carbonate for shell formation.

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التغيرات فى تركيز هرمونات البروجستيرون و الاستراديول و الكالسيوم الأيونى و المصاحبة لتكلس قشرة البيضة فى دجاج LSL و الفيومى

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أستخدم فى هذه الدراسة عدد 160 دجاجة LSL و الفيومى عمر 12 أسبوع. وضعت الدجاجات بصورة فردية. سجلت المظاهر الإنتاجية للدجاج من عمر النضج الجنسى و فى قمة الإنتاج. تم تقدير تركيز هرمونى البروجستيرون و الاستراديول و الكالسيوم الأيونى على عمر 4, 2 أسبوع قبل النضج الجنسى و عند النضج الجنسى و فى قمة الإنتاج. أثناء دورة التبويض فى قمة الإنتاج تم تقدير كل من البروجستيرون و الاستراديول و الكالسيوم الأيونى على فترات صفر, 6, 12, 18, 24 ساعة بعد وضع البيضة و تم قياس جودة قشرة البيضة.

كان المظهر الإنتاجى لدجاج LSL أعلى من الفيومى بينما كانت جودة قشرة البيضة الفيومى عالية الجودة عن قشرة بيضة دجاج LSL. حدثت زيادة معنوية فى تركيز هرمونى البروجستيرون و الاستراديول و الكالسيوم الأيونى عند النضج الجنسى. كما كان تركيز هرمون الاستراديول و الكالسيوم الأيونى فى بلازما الدجاج الفيومى أعلى من LSL فى كل المراحل التى تم دراستها. تم تقدير أعلى مستويات لهرمون الاستراديول و الكالسيوم الأيونى مصاحباً لتكلس قشرة البيضة أثناء دورة وضع البيضة. و كانت أعلى مستويات لهرمون البروجستيرون فى بلازما الدجاج عند وضع البيضة.