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EVOLUTION OF HORMONAL CONTROL OF CALCIUM AND PHOSPHORUS METABOLISM IN HENS ACCORDING TO AGE AND EGG PRODUCTION

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Abstract
The aim of this work was to determine the relationship between the calcium and phosphorus metabolism and the levels of the main hormones involved in their blood regulation in laying hen.
Two breeds of hens were used in this work, 22 weeks aged each one: White Cornish (CRN), as a breed of low egg production and White Leghorn (LGH), as a breed of higher egg production. The hens were raised in industrial system and they were fed according to the technologic diets. The hens were monitored from 22 to 44 weeks of age for the evolution of the parathormone, vitamin D, calcium and phosphorus levels in the blood plasma. Blood glucose concentration, total lipid, total proteins, albumins, globulins, creatinine, and uric acid were also monitored. Analysis of the hormone evolution relieves a peak of the PTH level in LGH hens, around 30 weeks of age (amounted to 392 pg/mL vs. 198 pg/mL in CRN hens). This peak of PTH is behind the laying peak and it is significantly higher in LGH hens vs. CRN hens. It was remarked that the LGH hens reached the peak of the PTH one week sooner vs. CRN hens. Regarding vitamin D, its plasma level presented a relatively constant evolution in CRN, while in LGH it presented an increase around 30 – 32 weeks-of-age up to 142 pg/mL, then it decreased slowly. Accordingly, in LGH hens, the level of calcium (in mg/dL) raised from 9.9 at the beginning of the laying cycle to 34.4 in the peak of the laying, decreasing then, to 18.0 toward the end of the monitoring period. In CRN hens, at the same age, the values of the plasmatic calcium were: 6.2, 12.9 and 18.0, respectively. The calcium/phosphorus ratio presented an ascendant evolution in both, LGH and CRN breeds, indicating an increasing of the free calcium content of the blood plasma. Plasma albumins ranged between 16.0 and 20.0 mg / mL in the LGH hens and between 19.8 and 22.8 mg / mL in the CRN hens. Uric acid plasma levels have evolved relatively parallel to the laying percentage, showing an intensified protein catabolism, according to laying percentage, in LGH hens. Total lipids followed an ascending evolution up to the peak of the laying, and then they decreased slowly in both low and high production breeds.

Keywords: *calcium, hormonal control, laying hens, metabolism, phosphorus.*

INTRODUCTION

One of the effects of the high selection pressure for the egg production is that the animals (hens in our pointy of view) become true metabolic bombs, in which, the intensity of the metabolism reach maximum acceleration. Metabolic processes are coordinated by hormonal mechanisms, so the endocrine system of the animal is maximally required. Given a maximal metabolism of an animal, the relationship between plasma levels of some elements and the activity levels of the hormonal mechanisms which regulate their plasma levels becomes more complex and more sensitive (Dojana, 2009; Larbier and Leclercq, 1992; Gardinier, 1973). The present paper analyses the interrelation between metabolic demands related to two of the organism minerals (calcium and phosphorus) and the ability of hormone regulating mechanisms to maintain their homeostasis in hens during a period of high metabolic demand (peak of the egg laying).

MATERIAL AND METHODS

Two different hen breeds have been selected to be used in these experiments: a hen breed with high egg production and a hen breed with a low egg production. Thus, research has been conducted on a group of 10 White Leghorn hens (LGH) aged 22 weeks and a group of 10 Cornish (CRN) hens, aged 24 weeks. The hens were exploited in a special industrial poultry, on a deep litter raising system, in halls having an area of 1,200 m^2, achieving a density of 7.2 capita/m^2 for LGH hens and 4.4 capita/m^2 for CRN hens. The light was common for both groups, starting from 11 hours per day and gradually increasing to 16 hours ut to the age of 27 weeks, being constantly kept up until the hens' reformation. The hens were fed with age-specific and breed-specific compound feed, in a quantity of 130 g/day, ensuring a quantity of 380 kcal EM/capita/day. The feeds contained 15.4% protein, 4.4 g% calcium and 0.66% total phosphorus, and 10,690 kJ/kG calculated metabolic energy, as shown in the manufacturing receipt.

The groups of hens have been monitored in terms of egg production and blood plasma concentrations of the following parameters: calcium, phosphorus, total protein, albumin and uric acid. The plasma evolution of the intact parathyroid hormone (iPTH) (biologically active parathormone) and the vitamin D level have also been monitored. Blood samples were collected every two weeks until the age of 44 weeks, on anticoagulant vacutainers, by axillary vein puncture. After collection, the samples were centrifuged at 2,500 rpm in order to fix the blood plasma which was then frozen at -12ºC until processing. The concentrations of calcium, phosphorus, protein and uric acid were determined according the methods described by Manta *et al.* (1976). The hormonal determinations were performed using an Immulite 1000 analyzer. The results have been statistically processed by determining the mean and standard error of mean. The differences between the groups have been statistically analyzed based on ANOVA single factor statistical model. The differences between groups were considered to be significant when the probability of the null hypothesis was less than 5% ($P \leq 0.05$).

RESULTS AND DISCUSSION

Figure 1 shows the evolution of the egg production from the two monitored hen groups, starting with the age of 22 weeks and up to the age of 40 weeks

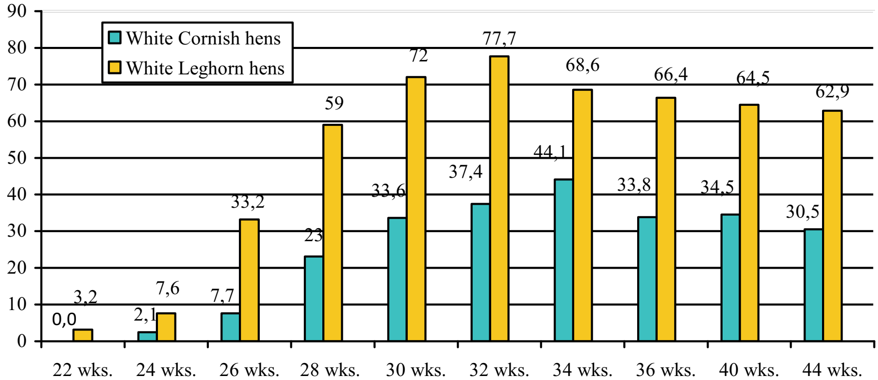

Fig. 1. The evolution of the laying percentage in White Leghorn hens and White Leghorn hens during a period from 22 to 44 weeks of age

The analysis of the data presented in figure 1, shows that, in LGH hens, the production peek was achieved at the age of 32 weeks, rate of lay amounting to a value of 77.7%. Comparatively, the CRN hens presented an egg production peek at the age of 34 weeks which amounted to 44.1% rate of lay. The statistical analysis of the differences between the two groups reveals no significant differences between the two groups during the first 4 weeks of monitoring ($P>0.05$). Starting with week 26 of age, the differences between groups became significant ($P<0.05$) and starting from week 28, the differences related to the egg production became very significant ($P<0.001$) and remain significant until the end of the monitoring period.

Table 1 shows the evolution of the main blood biochemical parameters. We found that the level of the plasma proteins was relatively constant, fluctuating around an average value of 43.17 mg/mL in the LGH hens and 46.66 mg/mL in the CRN hens. A similar evolution was also found on the level of serum albumin which fluctuated between 16.0 and 20.0 mg/mL of serum in the LGH hens and between 19.8 and 22.8 mg/mL in the CRN hens, with significant differences between the two groups ($P<0.05$). Determination of the albumin percentage provides information on the percentage fraction of bound serum calcium: an elevated albumin fraction represents a higher percentage of bound calcium.

Table 1

The evolution of some blood biochemical parameters in White Leghorn and Cornish hens during the laying cycle, from 22 to 40 weeks of age

No	Item	Group (breed) of hens	Age (in weeks)							
			22	26	28	30	32	34	36	40
2	Total proteins (mg/mL)	White Leghorn	44.0±4.9	44.5±22.2	46.5±3.3	46.0±3.3	45.0±2.2	38.0±2.4	40.6±3..2	40.8±4.4
		Cornish	48.0±8.6	48.5±5.0	46.0±5.3	46.5±3.5	48.5±3.0	44.4±2.0	46.5±5.1	44.9±4.3
	Albumins (mg/mL)	White Leghorn	19.1±4.0	22.2±1.9	21.6±3.0	16.0±2.0	20.0±2.3	17.2±1.6	19.0±2.1	18.8±2.0
		Cornish	22.2±4.4	20.3±2.9	22.5±2.9	21.0±0.5	23.5±2.5	19.8±1.5	21.4±2.0	22.8±2.1
5	Uric acid (mg/dL)	White Leghorn	4.9±1.5	4.5±0.6	4.5±1.0	5.8±1.1	6.5±3.0	5.3±0.2	4.7±0.7	4.2±0.2
		Cornish	4.0±1.3	4.4±0.9	4.8±0.5	5.0±0.7	5.4±0.4	5.8±0.5	4.4±0.8	3.5±0.4
6	Total calcium (mg/dL)	White Leghorn	9.9±3.3	18.5±6.0	24.3±2.0	28.4±1.5	34.4±1.2	30.5±1.2	31.6±1.0	18.0±0.4
		Cornish	6.2±3.1	8.9±1.0	11.7±1.7	14.5±1.6	12.9±0.8	14.5±0.8	16.3±1.2	18.0±2.5
7	Phosphorus (mg/dL)	White Leghorn	4.2±1.1	4.4±0.9	5.4±1.0	5.9±0.8	6.3±1.2	5.0±1.5	5.6±0.5	5.0±1.4
		Cornish	2.9±0.8	3.5±0.5	3.3±0.6	4.6±0.6	4.4±0.9	4.0±1.1	3.9±0.7	3.9±1.0
8	Ca/P ratio	White Leghorn	2.5	4.2	4.5	4.8	5.4	6.1	5.6	3.6
		Cornish	3.0	2.5	3.5	3.1	2.9	4.3	4.2	4.6

So, for every 1-g/dL drop in serum albumin below 4 g/dL, measured serum calcium decreases by 0.8 mg/dL. Therefore, to correct for an albumin level of less than 4 g/dL, one should add 0.8 to the measured value of calcium for each 1-g/dL decrease in albumin. Without this correction, an abnormally high serum calcium level may appear to be normal.

For example, an animal with a serum calcium level of 10.3 mg/dL but an albumin level of 3 g/dL appears to have a normal serum calcium level. However, when corrected for the low albumin, the real serum calcium value is 11.1 mg/dL (Agraharkar, 2008).

Analysis of the evolution of the plasma level in the uric acid, as a product of protein catabolism, shows an ascending evolution (from 4.9 to 6.5 mg/mL) parallel with the increase of the egg laying percentage on the LGH hens, marking a peak around the age of 32 weeks (which coincides with the egg laying peek), followed by a descendent trend, decreasing down to 4.2 mg/mL, which was again parallel with the descendent curve of the egg laying process. The parallelism between the evolution curves of the egg laying percentage and the level of uric acid on the LGH hen shows an enhancement of the protein catabolism which is related to the enhancement of the egg production.

Analysis of the evolution of plasma concentration of total calcium shows and ascending curve on both monitored hen groups. The peak was located at a level of 34.4 mg/dL at the age of 32 weeks for LGH hens and at a level of 12.9 mg/dL at the age of 32 weeks for CRN hens. The statistical analysis of the differences between the two groups in this peak moment shows significant differences ($P<0.01$) between the two groups. Concerning the Ca/P ratio in LGH hens, it was significantly higher than in CRN hens during the entire monitoring period. The higher values of the blood calcium levels in LGH hens are in agreement with a higher production of eggs (a higher percentage of egg laying than in CRN hens). It appears that a higher production of eggs induces an increase in the plasma concentration of calcium, showing a more elevated turnover of the calcium in deposits. On the other hand, the increase of the Ca/P ratio (from 2.5 to 6.3 in LGH hens) shows an increased level of free calcium in hens with high egg production in comparison with hens with low egg production. Chen and Shen (1989) reported breed differences between ducks and Leghorn hens in terms of serum calcium levels and calcium deposits on bones. Luck and Scanes identified daily evolutions of blood calcium level in hens which were probably related to the evolution of the level of gonadotropin releasing hormones: ionized calcium showed a sigmoidal pattern over the ovulation cycle reaching a peak within 3–6 hours from oviposition and falling, as shell calcification proceeded, to a minimum 3–6 hours before the next oviposition (Luck and Scanes, 2009).

The plasma level of the intact parathyroid hormone (iPTH) o LGH hens had an initial value of 126.46 ± 44.45 pg/mL (Figure 2).

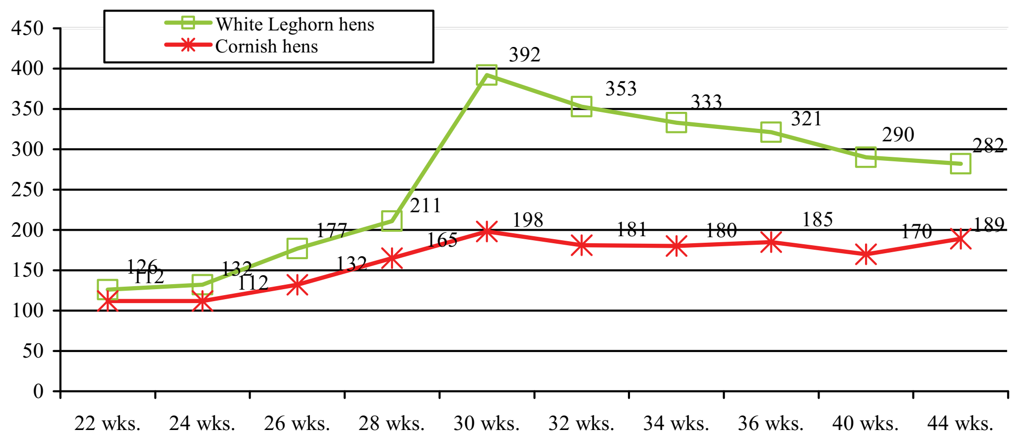

Fig. 2. The evolution of the plasma levels of parathyroid hormone (in pg/mL) in White Leghorn hens and White Cornish hens during the laying eggs cycle

The values remained relatively constant during the following determinations, subsequently increasing, reaching a peak at the age of 30 weeks, when the egg production peak was also marked. This peak amounted to a value of 392.16 ± 86.85 pg/mL. Subsequently, the plasma level of the intact parathyroid hormone (PTH) on LGH hens slowly decreased towards the values registered at the beginning of the monitoring period (reaching a value of 282 pg/mL at the end of the monitoring period). On CRN hens, the plasma level of the iPTH had not an ascending trend, but an evolution which was rather unspecific to the respective physiological period. However, this correlates with a much more reduced egg laying percent. The plasma level of the iPTH on this hens group oscillated between a minimum value of 112 pg/mL at the age of 22 weeks and 198 pg/mL at the age of 30 weeks. Clinically speaking, when the calcium level is high the PTH level needs to be low.

An elevated level under this conditions shows and intense activity in the thyroid gland (the producing PTH C-cells). In the case of our experiment, the increase of the PTH concentration in parallel with the plasma calcium level might be explained by an eventual positive feedback mechanism. Rahman *et al.* (2005) found that increased iPTH level occurs even early in the course of CRF and progressive hypocalcemia and hyperphosphatemia are the initiating factors for the development of hyperparathyroidism. This is explained by the occurrence of a push pull mechanism, well known in the specialized literature (Dojană, 2009).

Vitamin D dosage was made taking into account its involvement in the calcium metabolism along with the PTH (Figure 3). Its normal plasma level is amounted to 35 – 40 ng/mL (Larbier and Leclercq, 1992; Mundy and Guise, 1999). Because of its long half-time and a higher concentration, vitamin D is commonly measured to assess and monitor vitamin D status in hens. The plasma level of vitamin D had an unspecific evolution which was not connected to the evolution of the egg laying process for both hen groups (with high egg production and with low egg production) and seemed to not have been influenced by breed or by the intense metabolic stress which characterize an egg laying process peak. Therefore, in LGH hens, the levels of this vitamin (hormone) fluctuated between 54 and 143 pg/mL and in CRN hens, these levels fluctuated between 54 and 154 pg/mL. This aspect differentiates hens from other species of animals on which it was found significant correlations between the level of vitamin D and the level of blood calcium (Tsao *et al.*, 1985).

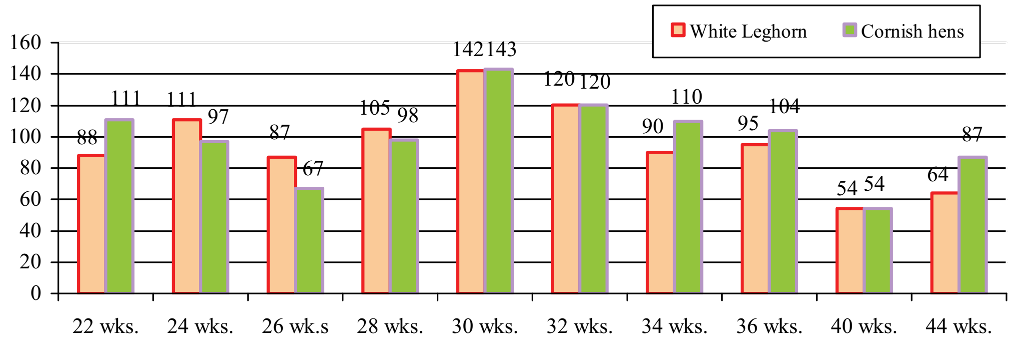

Fig. 3. The evolution of the plasma levels of vitamin D (in pg/mL) in White Leghorn hens and White Cornish hens during the laying egg cycle

CONCLUSION

Higher demands of calcium and phosphorus during the laying cycle in hens with high egg production are supported by high levels of PTH and vitamin D, the main hormones involved in regulating the homeostasis of these two minerals. In the same time, the levels of vitamin D seem to be not essentially modified in lower egg percentage hens.

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REFERENCES

1. Agraharkar, M., 2008. *Hypercalcemia*, Medscape. Available form http//emedicine. medscape.com/ article.
2. Chen W-L., Shen T-F., 1989. Comparative studies on the utilization of calcium between laying Tsaiya duck and Leghorn hen. *A.J.A.S.*, 2, 67-75
3. Dojană, N., 2009. *Tratat de fiziologia animalelor de fermă*. Editura Academiei Române, București.
4. Gardinier, E.E., 1973. Inorganic phosphorus, organic phosphorus, and inorganic calcium in blood plasma from breed chickens fed various levels of dietary calcium and phosphorus. *Canadian Journal of Animal Science*, 53: p. 551-556.
5. Larbier, M. și B. Leclercq., 1992. *Nutrition et alimentation des volailles*, INRA Editions, Paris.
6. Luck, M.R., C.G. Scanes, 2009. Plasma levels of ionized calcium in the laying hen (*Gallus domesticus*). http://www.science direct.com.// dx.doi.org/10.1016/0300-9629(79)90645-5.
7. Manta, I., M. Cucuianu, G. Benga, A. Hodârnău, 1976. *Metode biochimice în laboratorul clinic*. *Ed. Dacia*, Bucureşti.
8. Mundy R.G., T.A. Guise, 1999. Hormonal Control of Calcium Homeostasis. *Clinical Chemistry*, vol. 45 no. 8 1347-1352.
9. Rahman.,M.H., M.M. Hossain, S. Sultana, C.Y. Jamal, M.A. Karim, 2005. Correlation of serum parathormone level with biochemical parameters in chronic renal failure. Indian Pediatr. 42(3):250-4.
10. Preda Claudia, N. Dojană, 2013. Comparative features of calcium and phosphorus homeostasis in hens during the laying cycle. *Scientific works, Series C,* vol. LIX (1), 2013.
11. Tsao, C.S., M. Young, S.M. Rose, P.Y. Leung, M. Davies, V. Andrews, 1985. Effect of ascorbic acid on plasma calcium in guinea pigs. Int. J. Vitam. Nutr. Res. 55(3):309-1.