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COMPARATIVE FEATURES OF THE CALCIUM AND PHOSPHORUS HOMEOSTASIS IN HENS DURING THE LAYING CYCLE

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Abstract

It was determined the evolution of the levels of calcium and phosphorus from blood plasma in White Plymouth Rock hens (a hen breed with high egg production) and White Cornish hens (a hen breed with a low egg production) during the laying cycle, beginning from 22 weeks age up to 40 weeks of age. Parallel, it has been monitored the plasma evolution of the parathyroid hormone (PTH) and vitamin D levels, and the evolution of the following blood parameters: total protein, albumin, and uric acid. The results relive significant differences, according to the breed, concerning the parallel raising of the plasmatic calcium levels and the laying egg percent. Thus, in Plymouth Rock (PLR) hens, the level of calcium (in mg/dL) raised from 10.5 at the beginning of the laying cycle to 33.3 in the peak of the laying, decreasing then, to 30.9 toward the end of the laying cycle. On the other hand, in Cornish (CRN) hens, at the same moments, the values of the plasmatic calcium were: 8.8, 22.5 and 20.4, respectively. The calcium/phosphorus ratio presented an ascendant evolution in both, PLR and CRN breeds, indicating an increasing of the free calcium content of the blood plasma. Plasma albumins ranged between 17.2 and 22.2 mg / mL in the PLR hens and between 19.8 and 22.8 mg / mL in the CRN hens, with significant differences between groups. Uric acid plasma levels have evolved relatively parallel to the laying percentage, showing an intensified protein catabolism, according to laying percentage, in PLR hens. Analysis of the hormone evolution relieves a peak of the PTH level in PRL hens, around 32 weeks of age (amounted to 353 pg/mL). This peak of PTH is behind the laying peak and it is significantly higher in PLR hens than in CRN hens (185 pg/mL at the age of 36 weeks). Regarding vitamin D, its plasma level presented a relatively constant evolution in both hen breeds, seeming to be not influenced by breed or high metabolic requirements that characterize a lay peak. It can be concluded that the high demands of calcium and phosphorus export during the laying cycle in hens with high egg production are supported by high levels of PTH, the main hormone involved in regulating the homeostasis of these two minerals.

Key words: *laying hens, calcium, phosphorus, parathormone, vitamin D.*

INTRODUCTION

Ultra specialized egg laying hen breeds have become true metabolic bombs in which specific processes have experienced maximum acceleration. Metabolic processes are coordinated by hormonal mechanisms which also stress the endocrine system of the animal. 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 (egg laying).

MATERIALS AND METHODS

To achieve the goal of this research, hens from two different breeds have been selected: 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 50 Plymouth Rock hens (PLR) aged 22 weeks and a group of 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 1200 m^2, achieving a density of 7.2 capita/m^2 for PLR 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 at 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, 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 40 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 made by means of 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 the Students′t test, according to Tacu (1978). The differences between groups were considered to be significant when the probability of the null hypothesis was less than 5% (P=0.05).

RESULTS AND DISCUSSIONS

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.

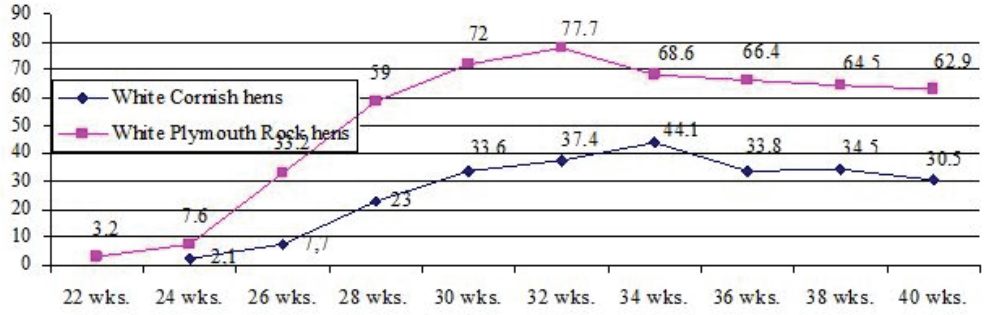

Figure 1. The evolution of the laying percentage in White Plymouth Rock and White Cornish hens during a period form 22 to 40 weeks of age

The analysis of the data presented in figure 1, shows that, in PLR 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 PLR 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 17.2 and 22.2 mg/mL of serum in the PLR 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 Plymouth Rock and Cornish hens during the laying cycle, from 22 to 40 weeks of age

No	Item	References values	Group of hens	Age (in weeks)							
				22	26	28	30	32	34	36	40
2	Total proteins (mg/mL)	35-40*	Plymouth Rock	44.0± 4.9	44.5±	46.5±	46.0±	45.0±	38.0±	40.6±	40.8±
			Cornish	48.0± 8.6	48.5±	46.0±	46.5±	48.5±	44.4±	46.5±	44.9±
	Albumins (mg/mL)		Plymouth Rock	19.1± 4.0	22.2±	21.6±	18.9±	20.5±	17.2±	19.0±	18.8±
			Cornish	22.2±4.4	20.3±	22.5±	21.0±	23.5±	19.9±	21.4±	22.8±
5	Uric acid (mg/dL)	1-7**	Plymouth Rock	4.9± 1.5	4.5±	4.5±	5.8±	6.5±	5.3±	4.7±	4.2±
			Cornish	4.0± 1.3	4.4±	4.8±	5.0±	5.4±	5.8±	4.4±	3.5±
6	Total calcium (mg/dL)	4.5-6[a] 20-98[b]**	Plymouth Rock	10.5± 3.3	18.5±	24.3±	28.4±	33.3±	30.5±	31.6±	30.9±
			Cornish	8.8± 3.1	8.9±	11.7±	14.5±	22.5±	22.5±	20.3±	20.4±
7	Phosphorus (mg/dL)	3-6	Plymouth Rock	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	3.5/1	Plymouth Rock	2.5	4.2	4.5	4.8	5.2	6.1	5.6	6.2
			Cornish	3.0	2.5	3.5	3.1	5.1	5.6	5.2	5.2

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 PLR 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 PLR 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 33.3 mg/dL at the age of 32 weeks for PLR hens and at a level of 22.5 mg/dL at the age of 32 and 34 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 PLR hens, it was significantly higher than in CRN hens during the entire monitoring period. The higher values of the blood calcium levels in PLR 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 6.1 in PLR hens) shows an increased level of free calcium in hens with high egg production in comparison with hens with low egg production. Chen and Chen (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 of 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 PLR hens had an initial value of 126.46 ± 44.45 pg/mL (Figure 2).

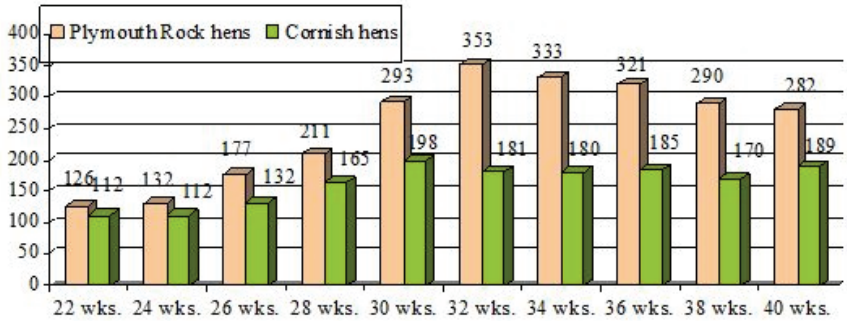

Figure 2. The evolution of the plasma levels of parathyroid hormone (in pg/mL) in White Plymouth Rock and White Cornish hens during the laying eggs cycle

The values remained relatively constant during the following determinations subsequently increasing, reaching a peak at the age of 32 weeks, when the egg production peak was also marked. This peak amounted to a value of 353.16 ± 86.85 pg/mL. Subsequently, the plasma level of the intact parathyroid hormone (PTH) on PLR 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 notve an ascending trend, butan 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 (Dojana, 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 PLR hens, the levels of this vitamin 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).

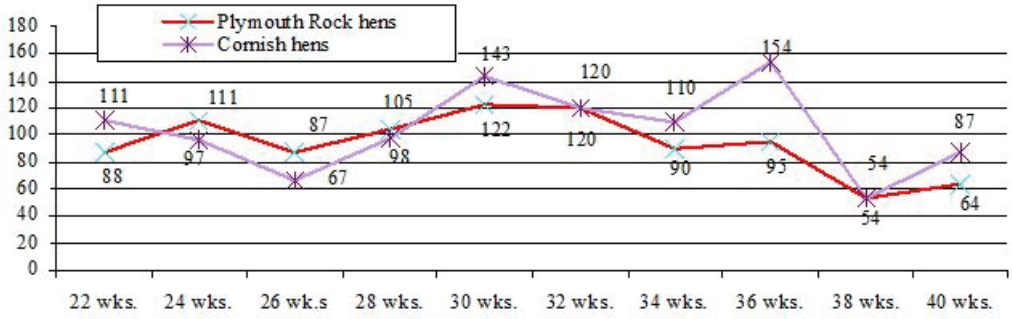

Figure 3. The evolution of the plasma levels of vitamin D (in pg/mL) in White Plymouth Rock and White Cornish hens during the laying egg cycle

CONCLUSIONS

Maximum metabolic requirements of the calcium and phosphorus metabolism during the laying egg peak are supported by an increased parathormone secretion and by the corresponding growth of some blood plasma parameters (calcium and albumins).

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