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Estimation of calcitonin and calcitonin gene-related peptide-α in bone calcification in quail during laying period

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Abstract

Maintaining the balance of calcium, phosphorus, and their regulating hormones is essential in laying hens to preserve the quality of the eggs and the framework. However, the physiological regulation of these systems is quite specific. Consequently, the present study intended to estimate the level of these minerals and their regulating hormones during different egg-laying periods. Female quails were used as a model for laying hens. In this study, 60 female quails were used, divided into three age groups: the early phase (4 weeks of age), the peak phase (8 weeks of age) and the late phase (12 weeks of age), with 20 birds per group. The outcomes of the study indicate an elevation in the level of calcium and calcitonin during the peak and final period of egg production, followed by a rise in the quantity of gene-related peptide-α during the final period of egg laying, with a strong positive relationship between the level of phosphorus and calcium, as well as between gene-related peptide-α and calcitonin during the peak and final period of egg production. We conclude from this study that the level of calcium and the hormones regulating it are affected by the bird's age, in addition to a strong correlation between the level of minerals measured in this study and these hormones and the age of the bird and its productive status.

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Introduction

The quail is a basic model in many scientific investigations due to its significant economic importance in the poultry industry around the world (1) as a result of its unique characteristics represented by early sexual maturity, short productive cycle and high egg production (2), in addition to the low financial cost of its investment projects (3). The quail is used as a model animal to study the development and creation of bones, whether in pre- or post-hatching research (4). Bones are kinetic materials consisting of organic and inorganic materials in different proportions according to the age of the bird and its location within the skeleton (5). The skeleton is considered a living tissue that is permanently accumulating and reshaped by bone cells to be able to modify to mechanical loads and provide the minerals necessary for basic cellular functions (6). The function of bones in birds is not limited to providing structural support, but it also plays a crucial role in the creation of eggshells in female birds (7). Bones are considered a mineral reservoir that can control the level of minerals in the bird's body through the hormones that regulate them, and they are also considered one of the organs most affected by many endocrine signals (8). The medullary bone has multiple cavities in laying hens, representing a constant calcium source (9). The cortical bone indirectly forms the eggshell through its reabsorption to maintain the medullary bone, which provides about 40% of the total calcium needed to form the eggshell (10). The creation and reabsorption of medullary bone coincide with the daily process of oviposition, providing the necessary calcium during the night when the eggshell is mineralized, and dietary calcium is utilized in the intestine (11). The body's regulation of calcium levels is critical since calcium is responsible for many fundamental functions, including skeleton and egg development, blood clotting, muscular contraction, and glycogenolysis. It also plays a critical role in the quality of eggs (12). Many tissues regulate calcium and phosphorus balance in laying chickens, such as the small intestine, bones, and kidneys (13). Hormonal activity in the bones and small intestine leads to equivalent calcium and phosphorus rate alterations due to simultaneous increases or decreases in each intake. Conversely, the kidneys can regulate the absorption and excretion of calcium and phosphorus independently so that their rate in the blood can be variously controlled. This excretion of calcium and phosphorus in the kidneys is carried out through the influence of PTH and $1,25(OH)_2D_3$ (14). In terms of absorption and post-absorption usage, calcium and phosphorus are closely connected and are fundamental for the formation of bones (15). phosphorus is a crucial mineral that plays a role in biological processes, mainly skeleton mineralization (16) and is considered one of the essential minerals for maintaining bone growth and health (17). Calcium balance is controlled by several hormones (18), calcitonin (CT), and similar peptides, including calcitonin-gen-related peptide (CGRP), which are essential regulators of calcium levels in the bird's body (19). In mammals, the C cells of the thyroid gland secrete calcitonin, a polypeptide made up of 32 amino acids. In birds, however, the ultimobranchial gland secretes it in response to elevated calcium levels, where it functions to lower calcium levels by preventing bone resorption (20). Evidence also indicates that it affects the kidneys by reducing tubular reabsorption of calcium (21). The calcitonin family is characterized by peptides, including amylin, adrenomedullin, intermedin and CGRP (22). CGRP is a 37 amino acid peptide (23) that comes in two types: CGRP-α and CGRP-β; the calcitonin gene mainly encodes CGRP-α, which is primarily present in central and peripheral nerve fibres, while CGRP-β is primarily located in the digestive tract (24). Widely dispersed throughout bone tissue, CGRP promotes the growth of new bone, prevents bone dissolution and promotes bone repair. It also stimulates the formation of blood vessels and regulates the immune environment of the bird (25).

Materials and methods

Ethical approve

The approval of the College of Veterinary Medicine, University of Mosul's Scientific Ethical Committee on Animal Experimentation, UM.VET.2024.023 was required to proceed with this work.

Laying quail and husbandry

This investigation was conducted in the laboratory of the University of Mosul's College of Veterinary Medicine's animal house during the period (from April to June). Female quails were raised in an open-type hall, and suitable conditions were provided with a temperature of 25±2 and lighting represented by 12 h of light and 12 h of darkness. According to the N.R.C (26), food and drink were given out without restriction. (27). In this study, sixty female quails were employed, with 20 birds in each of the three age groups: early (4 weeks of age), peak (8 weeks of age), and late (12 weeks of age).

Assessment of serum biochemistry

At every age of laying, jugular vein blood samples were taken., using gel tubes placed in a centrifuge for 15 minutes at 3500 rpm to get serum samples and stored at -20°C till serological tests were performed (28). Serum calcitonin and CGRP-α levels were measured using Sandwich ELISA (29,30). Calcium and phosphorus were measured by adding 50ul of Ca reagent (Giesse diagnostics), then adding 30ul of serum at 37 room temperature, mixing well with a specific cuvette, and reading the result at 600 nm for Ca and 340 nm for P using Smart -120 Automatic Chemistry Analyzer (31).

Statistical analysis

One-way analyses were executed via SPSS statistics 26. The mean with standard error (S.E) was used to evaluate quantitative data. A personal two-tailed correlation test was used to test whether variations between parameters studied were statistically significant when compared at different laying stages (32,33).

Results

Firstly, in the ratios of egg-laying for the three periods, the rate of egg was highest in the peak and late oviposion phases and slightly lower in the early oviposion period (Table 1). We assessed the serum Ca, P, CT and CGRP-α in laying quail during the different egg-laying stages. The analyzed data exhibited significant elevation in Ca and CT levels at $P \leq 0.05$. during the late and peak stages of laying compared with the earlier period, with overcome in the level of Ca and CT in the late stage (Figures 1 and 2). It was observed that the level of P at three periods of laying did not appear to have any significant elevation in its level at $P \leq 0.05$., but mathematically, it shows a rise during the peak and late phase (Figure 3). The analysis of CGRP-α shows a significant elevation in its level during the late stage at $P \leq 0.05$ in combination with the early and peak periods (Figure 4). The study of correlation between the studied data in the laying quail shows a strong positive relationship between P and Ca with a correlation value ($r= 0.965$, $P =0.000$). At the same time, P with CT and CGRP-α with Ca exhibited negative non-significant correlation ($r=- 0.058$, $P =0.837$) ($r= - 0.115$, $P = 0.684$), respectively, during the earlier period of laying (Table 2).

Table 3 displays the positive significant relationship between the P and Ca during the peak laying stage with a correlation ($r=0.566$, $P = 0.02$). Also, the result shows a strong positive relationship between CGRP-α and CT with ($r=0.839$, $P =0.000$). Meanwhile, P with CT and CGRP-α with Ca show negative non-significant correlation with (r=0.240, *P*= 0.388) (r= 0.269, *P* = 0.333), respectively (Table 3). It is noteworthy that during the late stage of laying, P show a positive relationship with Ca at (r= o.885, *P* =0.000), with a powerful positive relationship between CGRP and CT with (r= 0.782. *P* = 0.001), while P with Ca and CGRP-α with CT did not exhibit any relationship during the late stage of laying (Table 4).

Table 1: Laying rate at three stages of the laying period

Laying stage	Early	Peak	Late
Laying rate%	70	100	95

Laying rate total number of eggs/total number of female quail X 100.

Table 2: Correlation between Ca, P, CT and CGRP-α during the early stage of laying

	Ca		CT	
	R values	P values	R value	P values
P	**0.965	0.000	- 0.058	0.837
CGRP-α	-0.115	0.684	0.453	0.090

**Correlation is significant at the 0.01 level.

Table 3: Correlation between Ca, P, CT and CGRP-α during the peak stage of laying

	Ca		CT	
	R values	P values	R value	P values
P	*0.566	0.02	-0.240	0.388
CGRP-α	-0.269	0.333	**0.839	0.000

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

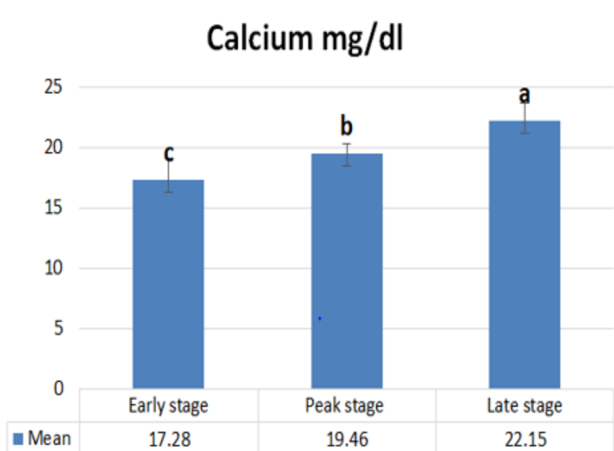

Figure 1: Changes in levels of calcium with age throughout the laying cycles.

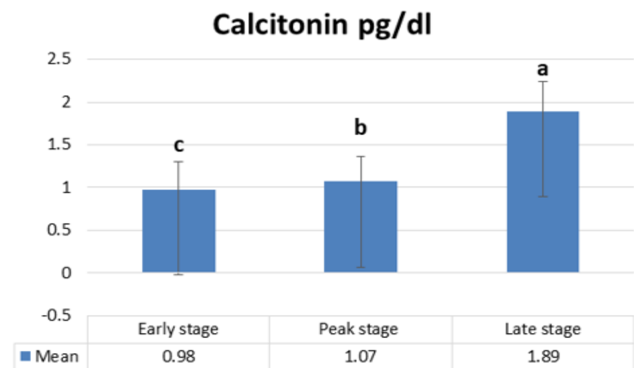

Figure 2: Changes in levels of calcitonin with age throughout the laying cycles.

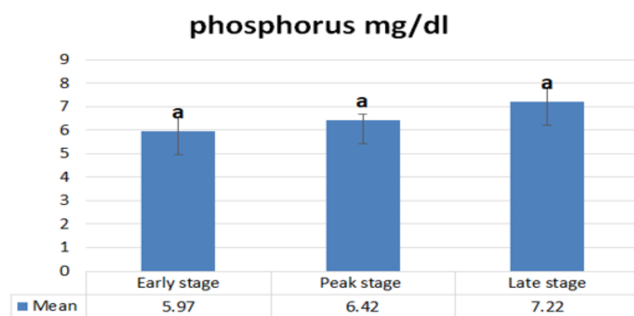

Figure 3: Changes in levels of phosphorus with age throughout the laying cycles.

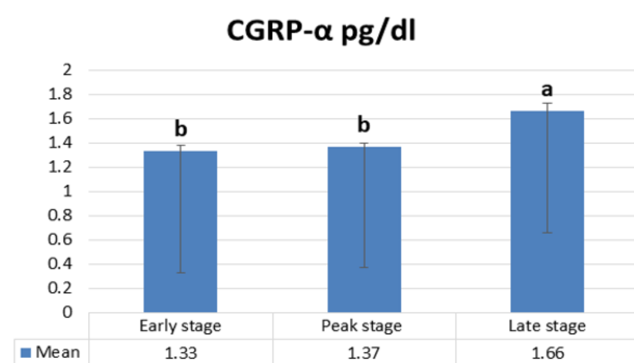

Figure 4: Changes in levels of CGRP-α with age throughout the laying cycles.

Table 4: Correlation between Ca, P, CT and CGRP-α during the late stage of laying

	Ca		CT	
	R values	P values	R value	P values
P	*0.885	0.000	0.656	0.842
CGRP-α	0.452	0.091	**0.782	0.001

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level.

Discussion

The research indicated that calcium, calcitonin, and CGRP-α quantities surged at egg production's peak and end phases. In contrast, the amount of phosphorus did not change throughout the three egg-laying phases. These outcomes are reliable based on the observations indicated by San *et al*. (34), which showed significantly higher calcium levels in adult laying hens during peak production than younger hens, with no differences in phosphorus levels between the two age groups. This increase in calcium levels can be attributed to several factors affecting its absorption, including the age and productive status of the bird (35). The small intestine absorbs calcium through both paracellular and transcellular channels. The tight junction proteins occludin and claudins facilitate the direct calcium exchange between the intestine and blood in the transcellular pathway (36). Transcellular calcium transport comprises three main stages: entry of calcium across the brush border into the enterocyte, translocation from the apical to the basement membrane, and extrusion across the basement membrane into the blood (37). Previous studies indicate that the ability of the intestine to absorb calcium changes with age due to changes in the tight junction protein gene regulation (38).

In laying hens, the active stage of calcium transfers from the circulation to the shell gland persists for calcification (39). This process requires abundant calcium in the blood and imposes an increasing demand for its presence, thus activating the various transport mechanisms to transfer large amounts of calcium (40).

The jejunum and ileum represent the two main parts of calcium absorption in poultry (41), where the absorbed calcium is stored exclusively in the bones. More than 99% of the body's calcium reserves are found in the bones, while the remaining 1% is found in free ions in soft tissues and body fluids (42). When calcium is absorbed through the intestinal epithelium, it mixes quickly with body fluids, and if the blood calcium level is within the natural range, about half of the absorbed calcium will be deposited in the bones to replace the calcium that was previously released, leading to a continuous exchange between the blood and bone tissue (43). Studies on the impact of age on the ability of minerals to digest in chickens are scarce, in contrast to other important nutrients (43). Most studies have focused on the impact of age on blood mineral levels in chickens and have shown contradictory results, with some researchers reporting a declining trend in calcium digestibility with age (44). In contrast, Morgan (45) indicated that ileal calcium digestibility was higher with age in poultry.

Although the physiological regulation of calcium and phosphorus is limited and complex in poultry, maintaining the balance of these minerals is essential for maintaining skeletal integrity and eggshell quality (46). Although phosphorus is not found in large amounts in eggshells, it is crucial to producing eggshells because it helps to balance blood acidosis and bicarbonate levels by encouraging the excretion of excess hydrogen ions (47).

The study showed a relationship between calcium, calcitonin and CGRP-α rate during the peak and final ovipostion period. Calcitonin is an important hormone in bone balance and calcium levels (48). This hormone reduces the calcium level in the circulation in response to its high rate by inhibiting bone resorption or suppressing calcium release from the bones. The primary modulator of the process of bone resorption is calcitonin (49). Osteoclasts are responsible for resorbing bone, and mature osteocytes are created when hematopoietic cells are incorporated. Osteoclast receptors bind to calcitonin, which causes the ridged boundaries to disappear in a matter of minutes, resulting in the removal of cells, limitations on their ability to move, and the disintegration of bone (50).

Pre-osteocytes mature into grown-up osteocytes that create the bone matrices and then mineralize them. Osteocytes can either undergo apoptosis or become trapped in the bone matrix, which leads to their differentiation into osteocytes. Mononuclear bone-forming cells called osteoblasts differentiate from mesenchymal stem cells of the bone marrow (51). Several research studies have reported the effect of calcitonin on osteocyte separation and bone advancement (52).

One of the most important peptides that have obtained increasing attentiveness in last years because of its potential role in regulating bone regeneration is CGRP-α (53), a neuropeptide stated in sensory nerve fibres that innervate bone tissue and has a role in upregulating genes associated with ossification and bone remodelling with improving bone quality (54). In a physiological setting, CGRP-α largely acts locally, especially in the peripheral nerve and circulatory systems, where it principally causes vascular dilation. It is also extensively dispersed in skeleton tissue, where it encourages bone building and hinders bone resorption (55). Osteoclasts are principally bone-forming cells, and CGRP-α promotes their gene expression by binding to the CRL receptor on their cell surface. By raising the level of OPG, it can also suppress the RANKL/RANK/NF pathway (56), preventing the proliferation of osteoclasts and decreasing bone resorption (57).

Conclusion

Our data indicate an increase in serum calcium, calcitonin and CGRP-α levels during the peak and end periods of egg laying, with a stable phosphorus level, with a correlation between the study parameters. These dynamic changes in the level of minerals and hormones can be attributed to the effect of the bird's age and the increasing need for them during the different periods of egg laying, in additionally to the mechanisms participating in the secretion and absorption of these minerals and hormones, which require further research studies.

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Conflicts of interest

The writers did not disclose any potential conflicts of interest.

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تقدير الكالسيتونين والببتيد- ألفا المرتبط بجين الكالسيتونين في تكلس العظام في طائر السمان أثناء فترة وضع البيض

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الخلاصة

يعد الحفاظ على توازن الكالسيوم والفسفور والهرمونات المنظمة لها أمرا ضروريا في الدجاج البياض للمحافظة على سلامة الهيكل العظمي وجودة البيض، على الرغم من أن التنظيم الفسيولوجي لهذه الأنظمة محدد تماما، لذا هدفت الدراسة الحالية الى تقدير مستوى هذه المعادن والهرمونات المنظمة لها خلال الفترات المختلفة لوضع البيض وتم استخدام إناث السمان كنموذج للدجاج البياض. استخدم في هذه الدراسة ٦٠ طائر من إناث السمان قسمت الى ثلاث مراحل عمرية، الأولى المرحلة المبكرة (بعمر ٤ أسابيع)، الثانية مرحلة الذروة (بعمر٨ أسابيع) والمرحلة الثالثة المرحلة المتأخرة (بعمر١٢ اسبوع) وبواقع ٢٠ طائر لكل مجموعة. أظهرت نتائج الدراسة ارتفاع في مستوى الكالسيوم والكالسيتونين خلال فترة الذروة والنهاية من إنتاج البيض يرافقه زيادة في مستوى الببتيد- ألفا المرتبط بجين الكالسيتونين خلال الفترة النهائية لوضع البيض مع وجود ارتباط موجب قوي بين مستوى الفسفور والكالسيوم وكذلك بين الببتيد- ألفا المرتبط بجين الكالسيتونين والكالسيتونين خلال فترة الذروة والنهاية من إنتاج البيض. نستنتج من هذه الدراسة بان مستوى الكالسيوم والهرمونات المنظمة له تتأثر بعمر الطائر إضافة الى وجود علاقة ارتباط قوية بين مستوى المعادن المقاسة في هذه الدراسة وهذه الهرمونات وعمر الطائر والحالة الإنتاجية له.