UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

ÓRGÃOS GENITAIS E OSSO MEDULAR NA MATURIDADE SEXUAL E NO CICLO DE FORMAÇÃO DO OVO EM CODORNAS JAPONESAS

Autora: Kassiana Germani Andrade
Orientadora: Prof.ª Dr.ª Tatiana Carlesso dos Santos

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Dissertação apresentada como parte das exigências para a obtenção do título de MESTRE EM ZOOTECNIA, no Programa de Pós-graduação em Zootecnia da Universidade Estadual de Maringá – Área de concentração Produção Animal.

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Dados Internacionais de Catalogação-na-Publicação (CIP)
(Biblioteca Central - UEM, Maringá – PR, Brasil)

A553o

Andrade, Kassiana Germani
Órgãos genitais e osso medular na maturidade sexual e no ciclo de formação do ovo em codornas japonesas / Kassiana Germani Andrade. -- Maringá, PR, 2018.
xvii, 88 f.: il. color.

Orientador: Profª. Drª. Tatiana Carlesso dos Santos.
Dissertação (mestrado) - Universidade Estadual de Maringá, Centro de Ciências Agrárias, Programa de Pós-Graduação em Zootecnia, 2018.

1. Sistema reprodutor - Aves. 2. Codorna japonesa - Sistema reprodutor. 3. Ciclo de formação do ovo - Codorna japonesa. 4. Tecido ósseo - Codorna japonesa. I. Santos, Tatiana Carlesso dos, orient. II. Universidade Estadual de Maringá. Centro de Ciências Agrárias. Programa de Pós-Graduação em Zootecnia. III. Título.

CDD 23.ed. 636.6

Márcia Regina Paiva de Brito – CRB-9/1267

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TITULAÇÃO: Mestre em Zootecnia - Área de Concentração Produção Animal

APROVADA em 27 de fevereiro de 2018.

Profª Drª Simara Márcia Marcato

Prof. Dr. Luis Daniel Giusti Bruno

Profª Drª Tatiana Carlesso dos Santos
(Orientadora)

Nada é para sempre
Absolutamente nada
Nem animais
Nem seu travesseiro
Nem pessoas
Nem seus cabelos
Nem relacionamentos
Nem alegria
Nem tristeza
Apenas o conhecimento
Dura toda eternidade

Faça o bem, seja bom e amoroso, tenha respeito e gentileza por você mesmo,

pelos outros, pelos animais e pela natureza. Hoje e sempre!

À minha mãe Eunice
Aos meus avós Emílio e Elza (*in memoriam*)

DEDICO

AGRADECIMENTOS

A Deus.

À Universidade Estadual de Maringá, pela oportunidade de realizar o curso de Pós-graduação em Zootecnia.

À CAPES, pela concessão da bolsa.

À Granja Vicami, pela disponibilidade das codornas.

À minha mãe, por tudo o que faz por mim.

Aos meus avós Emilio e Elza Germani (*in memorian*).

À professora Dr.ª Tatiana Carlesso dos Santos, pela sua orientação, confiança e conhecimentos transmitidos dando-me oportunidade para crescer profissionalmente.

Aos professores que de alguma forma me auxiliaram durante o mestrado: Dr.ª Alice Eiko Murakami, Dr. Carlos Lopes de Oliveira, Dr. Gentil Vanini, Dr. Geraldo Tadeu dos Santos, Dr. Leandro Castilha, Dr.ª Lilian Iwaki, Dr.ª Nazaré Barata Mateus, Dr.ª Paula Pintro, Dr. Paulo Pozza, Dr. Ricardo Vasconcellos e Dr.ª Simara Márcia Marcato, pela enorme contribuição para minha formação.

Aos amigos do grupo de estudo, por todo apoio e pelas inúmeras horas de coletas: Christian Figueroa, Flávia Kleszcz da Cruz, Isabelle Naemi Kaneko, Lidiane Staub, Lucas Bonagurio, Lenilson Fonseca e Evandro Menezes.

A todos estagiários, pelo apoio e ajuda durante o experimento e análises: Mariana Colhado, Rodrigo Basaglia, Rafaela Machado, Luiz Felipe Bento, Guilherme Craveiro, Mateus Depieri e Wanessa Fernandes.

Aos amigos e colegas de profissão: Laura Marcela Diaz, Angélica Khatlab, Caroline Stanquevis, Ivan Camilo Ospina Rojas, Eline Finco, Francilaine De Marchi, Rodrigo Sakurada, Tainara Perini, Tatiana Diaz e Jailton Bezerra.

Aos funcionários do Laboratório de Alimentos e Nutrição Animal (LANA), Hermógenes Augusto de C. Neto e Angélica Piccioli, pelo auxílio nas análises laboratórios.

Aos funcionários da Fazenda Experimental de Iguatemi Sr. Toninho (ração), Sr. Toninho (setor avicultura) e Neno.

Aos funcionários do laboratório de Agronomia, Anderson Gonçalves de Lima, Robertos Carlos D´Avilla e Silvia Eliana Tanno Miranda, pelo auxílio nas análises laboratoriais.

Ao Milton M. Asanuma pelo auxílio nas análises histológicas.

Às funcionárias do Complexo de Centrais de Apoio à Pesquisa (COMCAP), Andressa Pelozo, Greicy Ellen de Brito Ferreira, Natalia Fier, Rosimar Maria Marques e Suzana Paiva, pela atenção e ensinamento durante a utilização dos equipamentos.

Aos meus amigos da vida privada, que graças a Deus, não consigo enumerar, pois são muitos!

BIOGRAFIA

KASSIANA GERMANI ANDRADE, filha de Eunice Angelina Germani e Rosmari Andrade, nasceu em Maringá-PR, no dia 13 de outubro de 1971.

Em março de 1996, concluiu a graduação em Zootecnia pela Universidade Estadual de Maringá, e em fevereiro de 2003, concluiu a graduação em Medicina Veterinária pela Unicesumar.

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Em 28 de fevereiro de 2018, submeteu-se à banca para defesa da dissertação.

ÍNDICE

Página

LISTA DE TABELAS

Página

LISTA DE FIGURAS

Página

RESUMO

Foram realizados dois experimentos com o objetivo de avaliar o desenvolvimento órgãos genitais e osso medular na maturidade sexual e no ciclo de formação do ovo em codornas japonesas. No experimento I, descreveu-se as mudanças durante o ciclo de formação do ovo nas codornas japonesas. Sessenta codornas (18 semanas de idade) foram distribuídas em 6 tratamentos de acordo com o ciclo de formação do ovo: 0, 2, 4, 8, 14 e 20 horas após a ovoposição. Foram analisados bioquímica sérica e aspectos morfológicos dos órgãos genitais e os ossos (fêmur e tibiotarso). O magno teve peso relativo maior nos períodos antes da ovulação (20 e 0 horas). Entretanto histologicamente, a altura e largura das pregas do magno e do útero não tiveram diferença significativa, mas as glândulas tubulares apresentaram variação na funcionalidade entre os períodos, com conteúdo eosinofílico e PAS+ abundante as 2 horas após ovulação e aspecto vazio, 4 horas após ovoposição. O epitélio da mucosa do magno apresentou células ciliadas e secretoras (1:1). O fósforo, fosfatase alcalina e cálcio iônico não variaram com os períodos, enquanto a albumina e o cálcio total tiveram maior valor 2 horas após ovoposição e menor as 8 e 14 horas, respectivamente. Para as analises ósseas, as variáveis de resistência óssea e o peso dos ossos permaneceram inalterados durante o ciclo diário do ovo. A densidade mineral óssea (mmAl) do fêmur e tibiotarso apresentou menores médias no tratamento 2 horas e maiores no tratamento 14 horas, durante a formação da casca. Não houve diferença nas variáveis da cortical dos ossos entre os períodos. Entretanto, no osso medular houve diferença para teor de cálcio, com médias menores as 14 horas após ovoposição, coincidindo com a fase ativa do útero (formação da camada paliçada), correspondendo

ao período noturno e início da manha (6h00) nas codornas. As maiores médias do teor de cálcio foi a 0h após ovoposição (16h00). Esses achados podem indiciar uma recuperação da reserva mineral do osso medular na fase inativa do útero, preparando o osso medular para o próximo ciclo do ovo. O oviduto das codornas japonesas apresentou mudanças morfológicas em função do período de formação do ovo, assim como a concentração de cálcio sanguíneo e o teor de cálcio da região medular dos ossos fêmur e tibiotarso, mantendo a exigência e a homeostase do cálcio em cada fase estudada do ciclo diário da formação do ovo. No experimento II, objetivou-se descrever a formação do osso medular e o desenvolvimento do órgão genital na maturidade sexual sob efeito de 2 programas de luz com diferentes períodos de luz/escuro, simulando as estações de inverno e verão na região de Maringá (11L:13D e 14L:10D) em 550 fêmeas de codornas japonesas de 1 a 35 dias de idade, alojadas em 11 gaiolas tipo baterias (0,80 x 0,80 m), em câmara climática, com luz e temperatura controladas. A partir de 35 dias, 30 min de luz foram adicionados a cada 3 dias até atingir 17L:7D. As coletas de sangue, ossos e órgãos genitais foram realizadas aos 17, 21, 25, 28, 31, 35, 42, 49, 56, 63 e 70 dias, em 5 aves de cada tratamento, assim como foi acompanhado o consumo de ração semanalmente. A postura das aves sob o programa de luz 11L:13D foi superior ao programa 14L:10D, com 47,89 e 32,11%, respectivamente. A maturidade sexual é caracterizada por um conjunto de eventos metabólicos. Os níveis séricos de albumina e cálcio aumentaram imediatamente antes da postura do primeiro ovo. A albumina participa do transporte de substâncias do fígado folículos em crescimento no ovário. Já o cálcio influencia a formação da casca do ovo e do osso medular. As aves do programa de luz 11L:13D apresentaram desenvolvimento do órgão genital mais precocemente que as aves submetidas ao programa de luz 14L:10D, pesos relativos de ovários aos 56 e do oviduto aos 42 dias superior e maior comprimento do oviduto aos 56 dias. Consequentemente, iniciaram a postura mais cedo. O osso medular se desenvolveu primeiro no fêmur, sendo que sua única função é fornecer cálcio para formação da casca do ovo.

Key Words: cálcio, ciclo do ovo, programa de luz, osso medular, maturidade sexual.

ABSTRACT

Two experiments were carried out to evaluate the development of genital organs and bone marrow in sexual maturity and in the daily egg-laying cycle formation Japanese quail. The experiment I, described the changes during daily egg-cycle formation in Japanese quail. Sixty quails (18 wks old) were distributed in 6 treatments according to the egg-cycle formation: 0, 2, 4, 8, 14 and 20hs post oviposition. There were analyzed the serum biochemistry, morphological aspects of genital organs and bones strength variables (femur and tibiotarsus). The magnum had relative weight higher in the periods before ovulation (20hs and 0h). However histologically, the height and width of the magnum and uterus folds had no significant difference, but tubular glands in magnum showed variation in its functionally through periods, with abundant eosinophilic and PAS+ content at 2hs and empty aspect at 4hs post oviposition. The mucosa epithelium presented ciliated and secretory cells (1:1). Phosphorus, alkaline phosphatase and ionic calcium did not vary with the periods, while albumin and total calcium had a higher value with 2hs post oviposition and were lower in 8 and 14hs, respectively. For bone analysis, the bone strength and weight variables remained unchanged during the daily egg-laying cycle. The bone mineral density (mmAl) of the femur and tibiotarsus presented lower mean at 2hs and higher at 14hs post oviposition. There were no differences in bones cortical variables in both bones. However, in the medullary bone there were differences for Ca%, with lower means at 14hs after post oviposition, coinciding with the active phase in uterus (palisade layer formation), which in the quails corresponds to the nocturnal period and early morning (06h00). The higher means of Ca % was at 0h post oviposition (16h00). This finding may indicate a recovery of minerals

reserve in medullary bone in the inactivity phase of the uterus, preparing it to next egg-cycle. The oviduct of Japanese quail had morphological changes in consequence of the daily egg-cycle as well as the concentration of serum calcium and the calcium content of the medullary bone of the femur and tibiotarsus, keeping the calcium requirement and homeostasis during daily egg-cycle. The experiment II, the objective was to describe the medullary bone formation and the genital organ development in sexual maturity under the effect of two light programs, with different light/dark periods, simulating the winter and summer seasons in Maringá (11L:13D and 14L:10D), in 550 females quails of 1 to 35 days old housed in 10 battery cages (0.80 x 0.80 m) in a climatic chamber with controlled light and temperature. From 35 days, 30 min of light were added every 3 days until reaching 17L:7D. Blood, bone and genital organs were collected at 17, 21, 25, 28, 31, 35, 42, 49, 56, 63 and 70 days in 5 birds per treatment, as well as the feed intake was observed weekly. The quail posture under the 11L:13D light program was higher than the 14L:10D light program, with 47.89 and 32.11%, respectively. Sexual maturity is characterized by a number of metabolic events. Serum levels of albumin and calcium increase immediately before sexual maturity. Albumin participates in the transport of substances from liver to the growing follicles in the ovary. Calcium, on the other hand, influences the formation of eggshell and medullary bone. The birds under the 11L:13D light program showed genital organs development earlier than the 14L:10D light program, with relative ovary weight higher at 56 days and oviduct at 42 days and oviduct length was longer at 56 days. Consequently, they started laying earlier. The medullary bone developed first in the femur, and its only function is to provide calcium for egg shell formation.

Key Words: calcium, egg-cycling, light program, medullary bone, sexual maturity.

I. INTRODUÇÃO

As codornas pertencem à ordem *Galinaceas,* família das *Fasianidae* e gênero *Coturnix*. As mais conhecidas e criadas comercialmente no Brasil, são as japonesas (*Coturnix coturnix japonica*) e europeias (*Coturnix coturnix*), e a codorna americana Bobwhite (*Colinus virginianus*) em menor escala. As codornas silvestres (*Nothura boraquina, N. menor, N. maculosa*) são encontradas desde o nordeste até a região centro-sul (Murakami & Ariki, 1998).

Segundo dados do Instituto Brasileiro de Geografia e Estatística (IBGE), o Brasil possuía 21.986.842 cabeças de codornas em 2015, sendo 8,1% maior do que o ano anterior. Representando cerca de 10% do plantel de galinhas que é de 222.121.443 cabeças, no mesmo período, com índice de -0,8% em relação a 2014. A produção de ovos de codornas (1000 dúzias) foi de 447.468 em 2015, com crescimento de 13,9%, movimentando cerca de 490 milhões de reais em 2015. A região sudeste é maior produtora de ovos de codornas no Brasil, sendo responsável por quase 80% da produção nacional, sendo a cidade de Bastos, no Estado de São Paulo, com 20,4%. No Estado do Paraná, as cidades de Apucarana e Arapongas estão entre as 20 cidades brasileiras com maior produção de ovos com 1,7 e 1,2%, respectivamente.

Considerando um período de produção de 44 semanas, as codornas japonesas produzem em torno de 300 ovos no primeiro ano de postura, sendo que o pico de postura é alcançado entre 6 a 8 semanas com 80% de produção, e se mantém superior a 80% durante 32 semanas, com média acima de 5,5 ovos/ave/semana, e em 44 semanas este índice é de 76% (Murakami & Ariki, 1998). As codornas apresentam taxas em torno de 98% de fertilidade e 86% de eclosão, para aves com 17 a 20 semanas, em uma

proporção de duas fêmeas para um macho (Santos *et al.*, 2011). Os ovos de codorna pesam em média 10g para codornas de postura e 13g para codornas de corte.

Codornas têm um metabolismo geral de formação do ovo bastante semelhante ao de galinhas poedeiras, porém com ovulação e ovoposição durante a tarde e, consequentemente, a calcificação da casca durante o dia (Holm *et al.*, 2001; Albino & Barreto, 2003; Bar, 2009). São mais difundidas para produção de ovos e sua carne, considerada exótica, é bastante apreciada. A coturnicultura está recebendo atenção dos produtores por serem animais de rápido crescimento e precocidade no início de postura (42 a 45 dias de idade), boa reprodução (fertilidade média de 90%) e pelo pequeno consumo de ração das aves cerca de 23g/dia/ave (Murakami & Ariki, 1998; Albino & Barreto, 2003).

Frangos e galinhas estão entre as espécies de aves mais estudadas. Entretanto, codornas têm sido bastante utilizadas em pesquisas por serem menores, e por isso, ocupam menos espaço, menor gasto em alimentação, além de serem resistentes e possuírem desenvolvimento rápido com intervalo curto entre gerações, e sua carne e ovos serem altamente nutritivos (Nunes *et al.*, 2016; Sreesujatha *et al.*, 2016). Codornas japonesas quadruplicam seu peso na primeira semana e aumentam quase 20 vezes o seu peso inicial aos 35 dias de idade; portanto se faz de grande importância entender suas características morfofisiológicas (Miller, 1967; Pourlis & Antonopoulos, 2011).

1.1. Órgão genital feminino em aves

Todas as aves são ovovivíparas, ou seja, produzem ovos onde a maior parte do desenvolvimento embrionário acontece fora do corpo da fêmea (Freitas *et al.*, 2011). O oviduto das aves é derivado do ducto paramesonéfrico, e é onde ocorre a fertilização do óvulo, produção de albúmen, membranas e casca para o desenvolvimento embrionário. A diferenciação morfológica das gônadas nas aves é regulada pelos níveis séricos de estrógenos, que nas codornas se inicia no quinto dia do desenvolvimento embrionário (Mattsson & Brunström, 2017), sendo que o eixo hormonal responsável por todo metabolismo reprodutivo da ave, hipotálamo-hipófise-gônadas, está completamente desenvolvido aos 13 dias de incubação (Rutz *et al.*, 2007). Além disso, somente o ovário e oviduto esquerdos se desenvolvem, provavelmente por possuir mais receptores

para estrógeno, ocorrendo regressão do oviduto direito por ação do hormônio antimulleriano (Flamini, 2014; Sreesujatha *et al*., 2016).

O ovário esquerdo das codornas e galinhas se localiza próximo ao rim, sustentado pelo ligamento mesovariano. Tem aspecto de cacho de uva e constitui de ovócitos de várias fases de maturação, formando folículos. No momento da eclosão, o ovário possui cerca de 480 mil folículos, dentre os quais, somente 250 a 500 são ovulados (Macari *et al.*, 2005; Flamini, 2014; Sreesujatha *et al.*, 2016). Estes se desenvolvem por substâncias produzidas pelo fígado por estímulo do estrógeno, produzido pelo próprio ovário, formando a gema do ovo, constituída por água (50%), lipídeos (33%), proteína (16%) e algumas vitaminas e minerais para nutrição do embrião (Freitas *et al.*, 2011; Flamini, 2014).

A hierarquia folicular é regida, principalmente, por picos hormônio luteinizante (LH) e estrógeno durante o ciclo do ovo, estimulados por fotorreceptores (Liu *et al.*, 2001), os quais influenciam a intensidade e persistência da postura (Araújo *et al.*, 2011b). Em galinhas, quando um folículo atinge 8 mm de diâmetro se torna fonte de estrógeno, entra na hierarquia e recebe estímulos hormonais para se desenvolver (hormônio folículo estimulante - FSH) e ovular (LH) (Rutz *et al.*, 2007). A luminosidade crescente estimula a secreção e liberação de FSH e LH, proporcionando o aumento do folículo F1 e sua ovulação, respectivamente (Freitas *et al.*, 2011).

Sreesujatha *et al.,* (2016) classificaram a hierarquia folicular em codornas em folículos grandes amarelos F1 (15 a 18 mm), folículos médios amarelos F2 (10 a 15 mm), folículos pequenos amarelos F3 (< 10 mm), folículos brancos grandes (< 1 mm) e folículos brancos pequenos. Quando o folículo F1 é ovulado, imediatamente o folículo F2 se torna F1 e assim sucessivamente, seguindo a hierarquia.

A hierarquia em galinhas poedeiras contém cerca de até 7 folículos acima de 8 mm, numerados de F1 a F(2, 3, 4, n) e conforme F1 ovula, a fila progride. Os folículos brancos pequenos podem ficar anos com até 1 mm, aguardando a onda folicular, e então terem um crescimento rápido de 2 meses para a iniciação da deposição do conteúdo da gema pelo fígado, por estímulo do estrógeno, e quando entra na hierarquia folicular ocorre um crescimento rápido de uns 11 dias antes da ovulação (Macari *et al.*, 2005).

A maturidade sexual nas codornas ocorre entre 5 a 6 semanas de idade (Miller, 1967; Nunes *et al.*, 2016). Antes da primeira ovulação, o metabolismo dos hormônios, principalmente o estrógeno, é responsável pelo desenvolvimento funcional do oviduto

(Holm *et al.*, 2001), acarretando na diferenciação celular do epitélio da mucosa, resultando em células glandulares tubulares, células ciliadas e células globosas, e a progesterona é responsável pela produção e secreção das proteínas ovoalbumina e conalbumina (Macari *et al.*, 2005). Por volta dos 40 dias de idade, quando se inicia a postura, ocorre a formação da gema e proteínas no fígado, formação de reserva de cálcio com osso medular, induzindo aumento da capacidade de absorção de cálcio pelos intestinos (Rutz *et al.*, 2007; Nishimura *et al.*, 2007; Bar, 2009; Freitas *et al.*, 2011).

Em galinhas poedeiras, o maior crescimento do oviduto ocorre até 8 semanas de idade, logo antes da primeira postura, atingindo o máximo de peso do meio para o final da fase de postura, e regredindo cerca de 90% do peso máximo quando cessa a postura, em 80 semanas de idade (Yu & Marquardt, 1974). Ocorrem algumas mudanças no oviduto das aves durante o ciclo reprodutivo (início, pico de postura e regressão), ativados ou desativados por estímulos hormonais. A mucosa do útero apresentou hiperplasia e/ou hipertrofia de glândulas quando estimulada por estrógeno (Yu & Marquardt, 1974; Holm *et al.*, 2001).

Finco (2015) reler sugeriu que o desenvolvimento do genital feminino em codornas japonesas começa antes e se conclui após a postura do primeiro ovo, existindo correlação negativa entre maturidade e peso, ou seja, codornas mais pesadas são mais tardias em relação à maturidade sexual, sendo o inverso verdadeiro. De uma maneira geral, o oviduto tem seu crescimento máximo aos 43 dias e o ovário aos 47 dias, supostamente porque o oviduto já deve estar preparado quando ocorrer a primeira ovulação. Concluiu também, que os segmentos do oviduto atingem o final do seu desenvolvimento em momentos distintos, sendo o útero o mais precoce aos 46 dias, seguido do ovário aos 47 dias, magno e istmo com 52 dias e infundíbulo aos 55 dias.

O oviduto está dividido em cinco partes distintas e com funções fisiológicas específicas: infundíbulo, magno (região secretora de albúmen), istmo, útero (glândula da casca) e vagina (Yu & Marquardt, 1974; Sultana *et al*., 2003; Rutz *et al.*, 2007; Moraes *et al.*, 2009). É um órgão tubular composto pela túnica mucosa, túnica muscular e túnica serosa. A túnica mucosa é mais desenvolvida e se modifica conforme o segmento, sendo que no magno as pregas são mais longas e elaboradas. Constituída por um epitélio cilíndrico simples com células ciliadas e secretoras, e lâmina própria que formam glândulas especializadas em produção e secreção das substâncias que vão constituir o ovo (Flamini, 2014).

Após a ovulação, o folículo segue pelo oviduto recebendo secreções que vão formar o albúmen (no magno), membrana da casca (no istmo) e a casca (no útero), resultando no ovo posto, levando em torno de 25 a 26 horas em galinhas poedeiras (Moraes *et al.*, 2007; Rutz *et al.*, 2007; Freitas *et al.*, 2011) (Tabela 1).

A formação do ovo ocorre em camadas, sendo conduzido até a cloaca (Freitas *et al.*, 2011). As proteínas da clara (albúmen) são produzidas e depositadas ao redor do óvulo, enquanto atravessa o magno, supõe-se que essa secreção seja estimulada pela presença mecânica do ovo, com estímulo da progesterona (Rutz *et al.*, 2007; Freitas *et al.*, 2011). As glândulas tubulares são mais numerosas no magno e são fonte de ovalbumina, que perfazem 54% do albúmen do ovo. No istmo, glândulas tubulares secretam núcleos de fibras de queratina, os quais são constituintes das membranas da casca, e são responsáveis pelo formato do ovo. Enquanto o ovo permanece no útero, a água é absorvida pelo ovo em formação, ao mesmo tempo em que recebe o revestimento de carbonato de cálcio, proteínas, pigmento e cutícula, formando a casca (Artoni *et al.*, 2001; Freitas *et al.*, 2011; Sreesujatha *et al.*, 2016).

Tabela 1 – Partes do oviduto, sua função e o tempo de permanência do ovo em galinhas poedeiras (Freitas *et al.*, 2011).

Segmento	**Função**	**Tempo**
Infundíbulo	Captação do óvulo e fertilização	15 min
Magno	Produção e secreção de albumina	3hs
Istmo	Secreção da membrana interna e externa da casca	1h30
Útero	Produção da casca	20 a 21 hs
Vagina/cloaca	Postura	1 min

1.2. Efeito da luminosidade na avicultura

A iluminação é um dos fatores ambientais mais importantes para aumentar a produção das aves reprodutoras e de postura, pois afeta vários processos fisiológicos, produtivos e comportamentais, entre eles o desenvolvimento dos órgãos genitais (Freitas *et al.*, 2005; Borille *et al,.* 2013; Nunes *et al.*, 2016; Bobadilla-Mendez *et al.*, 2016).

As aves são sazonais, ou seja, são capazes de detectar diferentes luminosidades durante as estações do ano, levando às migrações e diferentes comportamentos em relação à reprodução. Quando a luz é crescente, estimula a maturidade sexual, e os dias curtos não tem luz suficiente para estímulo adequado dos hormônios reprodutivos (Etches, 1996; Araújo *et al.*, 2011b). Rutz *et al.* (2007) esclarece que as aves entram na fase reprodutiva somente após uma idade e um peso específico. Galinhas poedeiras são refratárias à luz até 12 semanas de idade, e são mais precoces em relação à maturidade sexual se submetidas à fotoperíodos curtos durante a recria.

A luz é o principal fator ambiental responsável pelo ciclo circadiano das aves, que consiste numa série de eventos fisiológicos e metabólicos. A luz atinge a glândula pineal reduzindo a secreção de melatonina durante o dia e a noite a secreção aumenta, fazendo assim estímulos de outros hormônios que regulam o ritmo circadiano (Macari *et al.*, 2005). Maior comprimento de onda da luz (vermelha) tem maior incidência no hipotálamo e por isso oferece maior estímulo na produção hormonal, ativando mais o sistema genital como um todo, aumentando a produção de ovos cerca de 5%, sendo esse efeito semelhante à luz fluorescente (Foster *et al.*, 1985; Gongruttananun 2011; Borille *et al.*, 2013; Nunes *et al.*, 2016).

As aves detectam a luz em fotorreceptores no hipotálamo por via transcraniana, liberando GnRH, e estimula a secreção de gonadotropinas (LH e FSH), responsáveis pelo estímulo do processo de desenvolvimento do aparelho urogenital (Foster *et al.*, 1985; Gongruttananun, 2011; Bobadilla-Mendez *et al.*, 2016; Srivastava & Chaturvedi, 2016). A energia luminosa resulta em impulsos nervosos responsáveis pelo controle do ritmo circadiano das aves, em que o fotoperíodo se inicia logo na primeira luz do dia (natural ou artificial) e tem duração de 10 a 15 horas, sendo que a ave se torna refratária num período mais curto ou mais longo (Araújo *et al.*, 2011b). A fase fotossensível ocorre entre 11 e 13 horas após o primeiro estímulo luminoso, e se faz necessário que essa fase seja estimulada e ser suficiente para atingir os fotorreceptores hipotalâmicos para que haja resposta hormonal de liberação de gonadotropinas, e assim ocorrer a ovulação (Etches, 1996).

Os programas de luz e seu efeito sazonal influenciam a maturidade sexual, estimulando a produção e secreção dos hormônios sexuais responsáveis pela formação do osso medular e o desencadeamento da onda folicular, resultando na primeira ovulação e a formação do primeiro ovo, caracterizando o início da fase produtiva

(Freitas *et al.*, 2005; Nunes *et al.*, 2016). Srivastava & Chaturvedi (2016) num estudo com codornas, observaram que o mecanismo hormonal responsável pela maturidade sexual é basicamente o mesmo que regula o ciclo circadiano da reprodução nas aves em geral.

O hipotálamo possui um relógio biológico que vai ser ativado para a maturidade sexual independente do fotoperíodo, afetando somente a capacidade e a quantidade de folículos estimulados, ou seja, afetando a produção, sendo que a taxa de produção de ovos é proporcional quando a intensidade luminosa é de 0,2 a 5 lux, perdendo essa relação acima de 5 lux (Etches, 1996). A duração da luz é responsável pela idade na maturidade sexual (mesmo com variação de 1 a 500 lux) e a intensidade luminosa pela uniformidade das poedeiras, ou seja, observaram que quanto maior a intensidade da luz maior será o tamanho do ovário, mas a diferença entre 50 e 500 lux é mínima (Araújo *et al.*, 2011b).

Diferentes programas de luz têm sido amplamente estudados na avicultura de postura, pois se sabe da influência que exerce no crescimento e maturidade sexual, buscando maior produção com menor custo (Freitas *et al.*, 2005; Gongruttananun, 2011). A escolha adequada do tipo e do programa de luz faz diferença na produção e reprodução das aves (Gongruttananun, 2011; Borille *et al.*, 2013).

Freitas *et al.*, (2005) observaram aumento na postura dos ovos em galinhas poedeiras leves no programa de luz natural em contraste com luz contínua ou intermitente, sem afetar a qualidade. Embora a luz contínua tenha aumentado o consumo de ração, a melhor conversão alimentar ocorreu com luz natural.

Pesquisas recentes estão sendo feitas para avaliar o uso da lâmpada de LED (light emiting diode) na avicultura, por conta da sua durabilidade e seu baixo consumo, a fim de otimizar a produção (Freitas *et al.*, 2005; Gongruttananun 2011; Borille *et al.* 2013; Nunes *et al.*, 2016). Entretanto, Nunes *et al.*, (2016) não observaram diferença significativa na produção de ovos e consumo de ração com uso de lâmpadas LED em comparação com a lâmpada fluorescente, embora a LED azul resultou em ovos mais leves e de cascas mais finas.

1.3. Tecido ósseo em aves

O osso é um tecido conjuntivo rígido formado por aproximadamente 70% minerais, principalmente fósforo e cálcio em formato de cristais de hidroxiapatita, 20% de matriz orgânica, sendo a maior parte de osteoide de colágeno tipo I, infiltrado de glicosaminoglicanas contendo glicoproteínas específicas que ligam fortemente o cálcio, e uma pequena parte de proteínas não colagenosas, e 10% água (Van De Velde *et al*., 1984; Dacke *et al.,* 1993; Stevens & Lowe, 2001). Cobre e zinco estão presentes na formação de colágeno (Hafeez *et al.,* 2014), e a conformação física entre eles é mais importante que os cristais em si (Araújo *et al.,* 2011), pois é o colágeno que dá ao osso tenacidade, ou seja, capacidade maior de suportar carga sem afetar a rigidez (Müller *et al.,* 2012). As principais funções dos ossos são de sustentação, locomoção, proteção e reservatório metabólico de minerais (Stevens & Lowe, 2001; Vieites *et al*., 2004; Kerschnitzki *et al.,* 2014).

Osteoblastos e osteócitos produzem e nutrem o osteoide, importantes para o processo de formação do osso, depositando na matriz orgânica (colágenos e proteínas não colagenosas) que serão mineralizados posteriormente (Kerschnitzki *et al.,* 2014). Já os osteoclastos são células grandes, multinucleadas com citoplasma rico em mitocôndrias e enzimas digestivas lisossomais, tais como catepsinas e metaloproteinases. Possuem bordas onduladas que secretam os prótons e proteinases para liberar íons de cálcio e fósforo do osso e sua posterior absorção, ou seja, têm função de remodelagem óssea e digerem a matriz orgânica e inorgânica (Stevens & Lowe, 2001; Kim *et al.,* 2012; Kerschnitzki *et al.,* 2014).

Os ossos longos das aves têm crescimento endocondral, através da proliferação de condrócitos, osteoblastos e osteoclastos na cartilagem epifisária, formando o osso trabecular e ossificação intramebranosa para formação e aumento da espessura do osso cortical, com deposição de hidroxiapatita, até o início da maturidade sexual (Whitehead, 2004).

Figueroa (2015), quando avaliou o crescimento ósseo de codornas do 13º dia de incubação até 35º dia pós-eclosão, observou que a densidade aumentou com a idade, assim como a resistência óssea, avaliada a partir do 10º dia pós-eclosão, por conta da mineralização óssea. Embora a porcentagem de cálcio e fósforo não tenha apresentado

diferença significativa, constatou-se que a mineralização da diáfise do fêmur foi menor que do osso tibiotarso.

A casca do ovo é a fonte principal de cálcio para o desenvolvimento do embrião (Mello, 2015). A ossificação das codornas se inicia aos 6 dias de vida embrionária (Pourlis & Antonopoulos, 2011) e se completa a calcificação quando a cartilagem epifisária se fecha por volta de 40 dias de idade (Nishimura *et al.*, 2007). A mineralização óssea, principalmente cálcio e fósforo, confere força e resistência aos ossos (Hafeez *et al.*, 2014). O excesso ou a falta de mineralização afeta a resistência tornando o osso mais susceptível à quebra (Barnett & Nordin, 1960; Barreiro *et al.*, 2011). Quanto maior concentração de minerais na ração, mais será depositada no osso até o limite fisiológico (Hafeez *et al.*, 2014).

Três tipos de ossos são descritos conforme o arranjo de minerais e que apresentam funções distintas: osso cortical, trabecular e medular. O osso cortical é compacto e organizado, responsável pela estruturação corporal e confere resistência ao osso. O osso trabecular é poroso, tem uma arquitetura tridimensional em forma de colmeia, e está localizado nas epífises, e tem a função de fornecer força. Já o osso medular, que se localiza na cavidade da medula óssea exclusivamente das aves fêmeas, é o principal reservatório de minerais para a formação da casca do ovo. Caracteriza-se por algumas áreas de alta densidade, associadas com a presença de cálcio, chamadas de halos de cálcio, que se apresentam heterogêneos por causa de partículas minerais desordenadas (Stevens & Lowe, 2001; Kim *et al.*, 2012; Kerschnitzki *et al.*, 2014).

1.3.1. Osso Medular

O osso medular é uma particularidade de aves fêmeas e crocodilos (Whitehead, 2004), que é formado pelos osteoblastos através do estímulo do estrógeno durante a maturidade sexual, preparando o organismo da ave para início da vida reprodutiva com a formação do primeiro ovo (Whitehead, 2004; Kim *et al.*, 2012; Kerschnitzki *et al.*, 2014). Esse tipo de osso é especializado, encontrado na cavidade medular da diáfise dos ossos longos, principalmente fêmur e tibiotarso. Composto por espículas ósseas, que crescem das superfícies endósteas, também chamados de halos, com grande quantidade de cálcio que preenchem a medula óssea, além de proteoglicanos e glicosaminoglicanas (Dacke, 1979; Whitehead, 2004).

O osso medular não tem função de sustentação. Entretanto, tem uma função fisiológica única de ser uma fonte lábil de cálcio relacionado com o ciclo da formação da casca do ovo, e a ação dos osteoclastos e osteoblastos estão sincronizados com o ciclo de postura (Van De Velde *et al.*, 1984; Dacke *et al*., 1993; Kerschnitzki *et al.*, 2014), atuando na reabsorção do osso medular, quando a taxa de absorção de cálcio pelo intestino não é suficiente para a demanda do útero (Van De Velde *et al.*, 1985; Clunies *et al.*, 1993; Kim *et al.*, 2012). Cerca de 30% do cálcio da casca é proveniente do osso medular de galinhas poedeiras (Macari *et al.*, 2005).

O ciclo de postura de galinhas e codornas tem duração em média de 24 horas, e logo que o ovo é posto, ocorre nova ovulação (Van De Velde *et al.*, 1984). O estrógeno, antes da ovulação, ativa os osteoclastos e o paratormônio (PTH) no osso medular quando a concentração de cálcio total e ionizado começa a diminuir durante a formação da casca, estimulando também a absorção intestinal de cálcio (Bar, 2009). Os osteoclastos são ativados pela calcitonina quando o ovo chega no útero, para liberação de cálcio do osso medular. Logo que ocorre a ovoposição, ocorre de forma rápida a reposição do osso medular pela ação dos osteoblastos (Etches, 1987; Miller *et al.*, 1985; Kim *et al.*, 2012).

O processo de reabsorção óssea ocorre pela dissolução da matriz da hidroxiapatita, liberando íons de cálcio e fósforo inorgânico, pela ação dos osteoclastos de forma intermitente, associada ao ciclo diário do ovo e a calcificação da casca. No início da formação da casca, ocorre reabsorção das espículas da cavidade medular, mas, com a formação da casca em andamento, a reabsorção pode se estender ao osso cortical (Taylor *et al.*, 1971; Whitehead, 2004). Entretanto, a espessura da casca não é afetada pelo metabolismo ósseo e a deposição de cálcio no osso ocorre também, enquanto está se formando a casca (Buss & Guyer, 1984).

A qualidade das cascas durante o início da fase de postura foi considerada melhor, visto que a disponibilidade do cálcio nos ossos medular e cortical é maior, pelo metabolismo de reabsorção óssea ser mais rápido e mais eficiente (Whitehead, 2004). No final da fase de postura, a função hormonal vai diminuindo, causando problemas na calcificação da casca e perdas na qualidade óssea das poedeiras (Nishimura *et al.*, 2007). O efeito líquido da substituição do osso cortical e do osso medular é o enfraquecimento da força total do esqueleto e com isso, aumento do risco de osteoporose, principalmente em poedeiras mais velhas (Whitehead, 2004; Kim *et al.*,

2012). Embora o ovo de galinha seja maior conforme ela fica mais velha, a casca fica mais fina e consequentemente há diminuição da gravidade específica e da resistência do ovo (Rodriguez-Navarro *et al*., 2002; Mello, 2015).

1.4. Metabolismo do Cálcio em Aves

As aves fêmeas são os animais que têm o metabolismo de cálcio mais eficiente dentre os vertebrados, pois possuem metabolismo ósseo único diferenciado, relacionado com a produção diária do ovo, visando a formação da casca, e a atividade dos osteoclastos do osso medular está sincronizada com o ciclo de formação do ovo para suprir a demanda de cálcio, demonstrando que o remodelamento ósseo através da intensa mobilização de cálcio é bastante rápido (Van De Velde *et al.*, 1984; Whitehead, 2004; Kim *et al.*, 2012). A homeostase do cálcio provém do equilíbrio entre os processos metabólicos que regulam sua concentração sérica regida pelos principais órgãos responsáveis pela sua absorção e reabsorção: intestino, rins e ossos (Etches, 1987), e é um dos principais nutrientes/ingredientes nas rações de poedeiras, pois é componente essencial da casca dos ovos, além de fazer parte da formação de tecido ósseo e muscular (Costa *et al.*, 2010).

A ração chega ao intestino de galinhas poedeiras aproximadamente 4 horas após a ingestão, quando o cálcio começa a ser absorvido por durante cerca de 75 minutos, ou seja, parte da calcificação da casca acontece no período de escuro, quando as galinhas não estão comendo e não estão absorvendo cálcio da dieta, utilizando o osso medular como fonte de cálcio (Clunies *et al.*, 1993; Whitehead, 2004; Kerschnitzki *et al.*, 2014).

A mineralização óssea pode ser influenciada por vários fatores, principalmente o balanceamento de nutrientes da ração, pois o equilíbrio ácido-base e as mudanças de pH no sangue interferem no metabolismo geral, inclusive o crescimento e remodelamento ósseo. Ou seja, o desequilíbrio de todos os minerais, incluindo sódio, potássio, cloro, magnésio, além do cálcio e fósforo, podem acarretar problemas da qualidade óssea afetando a produtividade (Vieites *et al.*, 2004; Araújo *et al.*, 2011; Müller *et al.*, 2012; Freitas *et al.*, 2013).

O metabolismo do cálcio é controlado vários hormônios responsáveis pela absorção, transporte e deposição. Os principais são: hormônio paratireoide (PTH);

hormônio formador de vitamina D3 e a calcitonina (CTN), com o objetivo de manter sempre a homeostase do cálcio sanguíneo quando a casca do ovo estiver em formação e houver maior demanda de cálcio pelo organismo (Taylor *et al.*, 1971; Dacke, 1979; Whitehead e Fleming, 2000). PTH é secretado pela paratireoide quando nível de cálcio sérico está baixo, ou seja, ativa os osteoclastos, estimulando a mobilização do cálcio do osso medular e promove a reabsorção (Kerschnitzki *et al.*, 2014). CTN é secretada pelas células C das glândulas ultimobranquiais quando aumenta nível de cálcio sérico, ou seja, desativa os osteoclastos (Kim *et al.*, 2012). É também mediado por várias proteínas, dentre elas calbidina, osteocalcina, anidrase carbônica, que auxiliam na absorção de cálcio do alimento e o transporte de cálcio do osso para a casca do ovo (Holm *et al.*, 2001; Kerschnitzki *et al.*, 2014).

O organismo mantém a homeostase do cálcio, ou seja, evita variação na sua concentração sérica, usando o balanço eletrolítico e o osso como tamponante, através de ações hormonais, utilizando o cálcio da reserva óssea e afetando também o teor de colágeno, causando perda de qualidade óssea (Vieites *et al.*, 2004; Araújo *et al.*, 2011; Müller *et al.*, 2012; Freitas *et al.*, 2013).

Nesse ponto, as codornas têm uma diferenciação no metabolismo do cálcio, pois a maior parte da calcificação da casca ocorre durante o dia, enquanto o consumo de ração e absorção de cálcio pelo intestino está ocorrendo, não utilizando o osso medular como fonte de cálcio (Dacke, 1979; Bar, 2009). O cálcio para formação da casca do ovo é endógeno, proveniente da dieta e do osso medular (Kim *et al.*, 2012; Kerschnitzki *et al.*, 2014; Ribeiro *et al.*, 2016). Existe uma sincronia de que a absorção intestinal de cálcio aumenta conforme aumenta a exigência para a formação da casca (Clunies *et al.*, 1993). Após o inicio da calcificação do ovo (fase ativa), o cálcio usado para formação da casca é proveniente da reabsorção do osso medular (Kerschnitzki *et al.*, 2014).

Níveis altos de cálcio podem interferir na disponibilidade de outros minerais formando complexos insolúveis, diminuindo sua absorção intestinal, como o fósforo. (Costa *et al.*, 2010; Ribeiro *et al.*, 2016), por isso é importante manter a relação cálcio: fósforo na ração adequada. Alguns trabalhos encontraram valores de 1,67: 1 com balanço eletrolítico de 150 a 200 mEq/kg (Müller *et al.*, 2012). Miller (1967), estudando níveis de cálcio e fósforo para codornas em crescimento, não observou diferenças significativas em relação ao peso corporal em diferentes níveis da relação Ca: P (de 0,7

a 2,9: 1), embora o maior peso tenha sido com a relação 1,2: 1, que corresponde a 0,70% de cálcio e 0,58% de fósforo.

Minvielle *et al.,* (2000), num trabalho com 4 linhagens e seus cruzamentos, observou que a codorna produz no primeiro ano em média cerca de 222 a 290 ovos. Considerando que cada ovo pesa em torno de 11,5g, e que 0,284g são de cálcio presente na casca (Bar, 2009), ou seja, as codornas utilizam cerca de 63 a 82g de cálcio somente para formação da casca no primeiro ano.

Para codornas de postura o consumo de cálcio deve ser de 0,773 g/dia, que corresponde a cerca de 3% (Rostagno, 2017). Mello (2015) concluiu que o consumo de 640 mg de cálcio e 80 mg de fósforo, que corresponde a 2 e 0,25%, respectivamente, são níveis adequados para manutenção e produção em codornas japonesas. Masukawa *et al.* (2001) e Mello (2015) concluíram que codornas japonesas toleram bem a variação de cálcio entre 2,5-3,5% e 2,0-3,5%, respectivamente, sem afetar a produção e qualidade de ovos. Níveis adequados de cálcio (3,5%) e fósforo (0,45%) da dieta garantem qualidade da casa do ovo em codornas japonesas (Pedroso *et al.,* 1999).

1.5. Formação da casca do ovo

A função da casca do ovo é proteção física contra patógenos do ambiente e fonte de cálcio para o desenvolvimento embrionário, a qual contém cerca de 95% de carbonato de cálcio, 1 a 3% de material orgânico e o restante de magnésio e fósforo (Rodriguez-Navarro *et al*., 2002; Chien *et al*., 2009; Hincke *et al*., 2012; Rodriguez-Navarro *et al*., 2013). As células epiteliais do útero secretam um fluido rico em cálcio ionizado e bicarbonato, catalisada pela anidrase carbônica, que por sua vez é estimulada pelo estrógeno, e acarreta na precipitação de carbonato de cálcio em forma de calcita para ser depositada na casca (Holm *et al.*, 2001; Chien *et al*., 2009; Hincke *et al*., 2012).

A maior taxa de formação da casca do ovo acontece por volta de 12 a 18 horas pós-postura (Clunies *et al.*, 1993), que envolve vários mecanismos tais como, absorção e transporte de cálcio do intestino para o sangue e do sangue para osso ou para a casca, hidratação do albúmen (“plumping”) e deposição de carbonato de cálcio efetivamente (Turner & Eliel, 1978; Holm *et al.,* 2001; Bar, 2009).

A casca do ovo, considerada uma biocerâmica complexa porosa, é formada no útero por cerca de 20 horas, com a deposição em camadas de carbonato de cálcio sobre a membrana externa do ovo (Rodriguez-Navarro *et al*., 2002; Mello, 2015). Possui 3 camadas: mamilar, paliçada e a cutícula. (Hincke *et al*., 2012;). Entre as colunas paliçadas são formados poros que permitem trocas gasosas e água durante seu desenvolvimento do embrião (Hincke *et al*., 2012; Rodriguez-Navarro *et al*., 2013).

A primeira fase da mineralização já ocorre quando o ovo está no istmo e dura cerca de 5 horas, quando a matriz orgânica é depositada na membrana externa da casca, e se tornam os núcleos de cristal, que são a base dos botões mamilares (Chien *et al*., 2009). A distância e o tamanho desses botões conferem a força e resistência da casca (Hincke *et al*., 2012), quanto menor são os botões, maior é a distância entre elas, e consequentemente, maior é a resistência à fraturas (Rodriguez-Navarro et al., 2002).

A camada mamilar é organizada, formada por botões com um núcleo orgânico de mucopolissacarídeos em que é depositado radialmente microcristais de calcita, sendo fonte de cálcio para o desenvolvimento do embrião, especialmente na segunda metade da incubação. Sua forma em esferas facilita a dissolução e mobilização para liberação do cálcio, sendo q essa perda de mineral enfraquece a casca, principalmente na região equatorial, favorecendo a eclosão dos pintinhos, sendo que esta é a única camada que se modifica durante a toda fase de incubação (Chien *et al*., 2009; Hincke *et al*., 2012).

A segunda fase dura em torno de 10 horas e tem o crescimento rápido de calcita policristalina e forma a camada paliçada, que são colunas perpendiculares à casca que crescem a partir dos botões mamilares. Entre as colunas estão dispersos poros que permitem a troca gasosa e de água. Sua parte mais externa é chamada de camada de cristal vertical que é uma estrutura cristalina grande de alta densidade, com função de absorver os impactos externos para evitar rachaduras (Hincke *et al*., 2012;). Quanto maior a espessura e mais desorganizada é a camada paliçada, maior é a resistência da casca (Rodriguez-Navarro *et al*., 2002).

A terceira fase acontece nas últimas 1,5 horas antes da postura, e forma a camada mais externa da casca e a cutícula, a qual é formada de 85 a 90% de proteínas (glico e fosfoproteínas), 4% de polissacarídeos, 3% de lipídeos e pigmentos, secretados das células epiteliais do útero. Sua função principal é controlar a permeabilidade dos poros da casca contra microrganismos, contudo, sua qualidade e espessura diminuem

conforme a idade de galinhas poedeiras (Hincke *et al*., 2012; Rodriguez-Navarro *et al*., 2013).

1.6. Análises de Qualidade Óssea

Diferentes variáveis são utilizadas para avaliar a qualidade óssea: %cinzas, conteúdo mineral, densidade e dados biomecânicos a fim de determinar a capacidade da verdadeira função do osso: força, sustentação e resistência às fraturas (Kim *et al.*, 2012). Embora Buss & Guyer (1984) observaram que cinzas e teor de cálcio do osso não apresentaram relação com a qualidade óssea e da casca, concordando com Bishop *et al.*, (2000) que observaram o fator genético como mais importante para avaliar esses parâmetros. A restrição de movimento de poedeiras nas gaiolas pode acarretar atrofia muscular e consequentemente fraqueza óssea (Bishop *et al.*, 2000; Whitehead, 2004).

Antes do uso de equipamentos eram utilizadas algumas formas para avaliar resistência e força:

$$\text{Índice de robustez} = \frac{\text{comprimento do osso}}{\text{Raiz peso do osso}}$$ (Riesenfeld, 1972)

$$\text{Índice tibiotarso} = \frac{\text{diâmetro da diáfise} - \text{diâmetro do canal medular}}{\text{Diâmetro da diáfise} \times 100}$$ (Barnett & Nordim, 1960).

Densidade mineral óssea é um dos parâmetros biomecânicos mais importantes para indicar resistência (Müller *et al.*, 2012). Pode ser obtida através de radiografias (Barreiro *et al.*, 2011), fluoroscopia digital, tomografia e raio-X por absorção (DEXA) (Kim *et al.*, 2012). Nishimura *et al.* (2007) concluíram que peso corporal e o desenvolvimento do osso medular aumentam a densidade mineral do tibiotarso. Mello, (2015) observou que maiores níveis de cálcio na dieta diminuíram a densidade mineral dos ossos fêmur e tibiotarso.

O teste de resistência óssea é realizado em equipamentos que incidem cargas e magnitudes conhecidas, em que os ossos longos cilíndricos são submetidos a teste de compressão entre 2 apoios paralelos, sendo que a resistência é a força máxima que o osso resiste antes da quebra (Barreiro *et al.*, 2011; Kim *et al.*, 2012). A resistência óssea depende de sua densidade e composição. O colágeno, por exemplo, interfere na

força óssea, pois a porção orgânica do osso confere força de tensão e flexibilidade (Barreiro *et al.*, 2011; Hafeez *et al.*, 2014).

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II. OBJETIVOS GERAIS

Avaliar a influência de dois programas de luz no desenvolvimento e crescimento dos órgãos genitais e do osso medular em codornas japonesas de 1 a 70 dias, com ênfase no período da maturidade, assim como se comportam durante o ciclo diário de formação do ovo.

2.1. Objetivos Específicos

Experimento I:

- Avaliar a as mudanças morfofisiológicas do oviduto durante o ciclo de formação do ovo.
- Descrever a morfologia macro e microscópica do órgão genital de codornas japonesas.
- Avaliar o metabolismo do cálcio em relação aos níveis séricos e teor no osso medular durante o ciclo de formação do ovo.
- Identificar mudanças de conteúdo mineral, resistência e densidade dos ossos fêmur e tibiotarso durante o ciclo de formação do ovo.
- Descrever a formação da casca do ovo por meio da microscopia eletrônica de varredura.

Experimento II:

- Descrever o desenvolvimento do órgão genital em codornas japonesas submetidas a dois programas de luz de 17 a 70 dias de idade.
- Identificar a idade à maturidade sexual e descrever a morfologia macro e

microscópica do órgão genital de codornas japonesas nesta fase.

- Quantificar as mudanças de conteúdo mineral, resistência e densidade dos ossos fêmur e tibiotarso durante o crescimento de 17 a 70 dias de idade.
- Identificar o desenvolvimento do osso medular durante a fase de maturação sexual, em relação ao metabolismo do cálcio e teor no osso medular.

III. Daily Egg-cycle in Japanese quail: serum biochemistry, bones and oviduct studies.

ABSTRACT: In this study, we describe the changes during daily egg-cycle formation in Japanese quail. Sixty quails (18 wks old) were distributed in 6 treatments according to the egg-cycle formation: 0, 2, 4, 8, 14 and 20hs post oviposition. There were analyzed the serum biochemistry, morphological aspects of genital organs and bones strength variables (femur and tibiotarsus). The magnum had relative weight higher in the periods before ovulation (20hs and 0h). However histologically, the height and width of the magnum and uterus folds had no significant difference, but tubular glands in magnum showed a functionally variation through periods, with abundant eosinophilic and PAS+ content at 2hs and empty aspect at 4hs post oviposition. The mucosa epithelium presented ciliated and secretory cells (1:1). Phosphorus, alkaline phosphatase and ionic calcium did not vary with the periods, while albumin and total calcium had a higher value at 2hs post oviposition and were lower at 8 and 14hs, respectively. For bone analysis, the bone strength and weight variables remained unchanged during the daily egg-laying cycle. The bone mineral density (mmAl) of the femur and tibiotarsus presented lower mean at 2hs and higher at 14hs post oviposition. There were no differences in cortical variables in both bones. However, in the medullary bone there were differences for Ca%, with lower means at 14hs post oviposition, coinciding with the active phase in uterus (palisade layer formation), which in the quails corresponds to the nocturnal period and early morning (06h00). The higher means of Ca% was at 0h post oviposition (16h00). This finding may indicate a recovery of the minerals reserve in medullary bone in the period of uterus inactivity, preparing medullary bone to next egg-cycle. The oviduct of Japanese quail had morphological changes in consequence of the daily egg-cycle as well as the concentration of serum calcium and the medullary bone calcium content of the of the femur and tibiotarsus, maintaining the calcium requirement and homeostasis during daily egg-cycle.

Key Words: calcium, femur, medullary bone, oviduct, tibiotarsus

INTRODUCTION

Female birds developed a differentiated metabolism of Ca (**Ca**) in shell formation, encompassing the bone, intestinal and hormonal systems, in order to maintain Ca homeostasis during the daily egg-cycle formation (Van De Velde et al., 1984; Etches, 1987; Whitehead, 2004; Kim et al., 2012). Blood Ca homeostasis is controlled by the parathyroid hormone (**PTH**), calcitonin, and vitamin D_3, which act on intestinal absorption and bone resorption during shell formation (Taylor et al., 1971; Dacke, 1979).

The main sources of Ca for shell formation come from diet and medullary bone, which undergo osteoblasts and osteoclasts in a synchronized manner with the egg-cycle formation, recruiting medullary bone when dietary Ca is not sufficient for shell formation (Van De Velde et al., 1984; Dacke et al., 1993; Whitehead, 2004; Kim et al., 2012; Kerschnitzki et al., 2014). The cycle of egg formation in birds last 24hs. Ovulation occurs by stimulating estrogen, which also stimulates osteoclasts, PTH and intestinal absorption to increase Ca availability for shell formation, and oviposition, osteoblasts act to recover medullary bone (Miller et al., 1985, Etches, 1987; Bar, 2009, Kim et al., 2012).

The shell formation in birds occurs in the uterus for about 20hs by the secretion of ionic Ca and carbonate, which form Ca carbonate by the action of carbonic anhydrase, that catalyzes the reaction $CO^2 + H_2O \Leftrightarrow H^+ + HCO_3^-$, then Ca carbonate is deposited in layers on the shell membrane (Holm et al., 2001; Chien et al., 2009; Hincke et al., 2012; Rodriguez-Navarro et al., 2002; Mello, 2015). The shell is composed of 95% of Ca carbonate, up to 3% of organic material and 2% of minerals (Rodriguez-Navarro et al., 2002; Chien et al., 2009; Hincke et al., 2012; Rodriguez-Navarro et al., 2013).

Similar to a grape bunch, the ovary in chickens has about 480,000 follicles at hatching, and only about 500 enter the hierarchy and are ovulated (Macari et al., 2005; Flamini, 2014; Sreesujatha et al., 2016). The follicles are developed by the action of estrogen and are formed by substances produced in the liver, resulting in the yolk with 50% water, 33% lipids, 16% proteins and vitamins and minerals (Freitas et al., 2011; Flamini, 2014). The follicular hierarchy in quails is controlled by follicle stimulating hormone, luteinizing hormone and estrogen, which develop pre-ovulatory follicles, F1,

F2 and F3, and after ovulation, F1 becomes pre-ovulatory, F2 becomes F1, and so on (Liu et al., 2001; Macari et al., 2005; Sreesujatha et al., 2016).

The oviduct of the birds derives from the paramesonephric duct and only the left oviduct is developed, because it has more receptors for estrogen, and the right is not developed by the action of the anti-mullerian hormone (Artoni et al., 2001; Rutz et al., 2007; Moraes et al., 2009; Flamini, 2014; Sreesujatha et al., 2016). The oviduct is a tubular organ composed of the serosa, muscular and mucosa tunics, and is divided anatomically into five segments with specific physiological functions: infundibulum (follicle uptake), magnum (albumin), isthmus (shell membrane), uterus (shell) and vagina (oviposition) (Turner and Eliel, 1978; Yu and Marquardt, 1974; Sultana et al., 2003; Parizzi 2006; Moraes et al., 2009).

Quail has a general metabolism of egg formation very similar to that of laying hens, but with ovulation and oviposition during the afternoon and, consequently, the shell calcification during the day (Holm et al., 2001; Albino and Barreto, 2003; Bar, 2009). Thus, the largest period of shell formation occurs at times when the bird has access to the food. The objective of this study was to evaluate the morphophysiological changes of the oviduct and Ca metabolism in blood and bone of Japanese quail throughout the egg-cycle formation from the time of ovulation and during the phases of shell formation.

MATERIAL AND METHODS

Birds and Ethical Approval

This experiment was carried out in Poultry house at Experimental farm of Iguatemi (FEI) of Maringá University (UEM) and approved by ethical committee (CEUA protocol No. 7006280815).

Sixty female Japanese quail (*Coturnix coturnix japonica*) UEM/2015, 18 wks old, standardized by weight (170g ± 5%) and egg production (95%), which were housed individually in galvanized laying cages (17.5 x 14.5 x 9.0 cm). The light program was 17 hs (natural + artificial), during summer time, with a temperature and humidity of 22.96°C and 89.63%, respectively. The diet was according to the requirements for quail in laying phase (Rostagno et al., 2011), based on corn and soybean meal (CP 18.80%, ME 2,800, Ca 2.92% and P 0.30%), with food and water *ad libitum*.

The experimental design was completely randomized with 6 treatments according to physiology periods of egg-cycle formation: 0, 2, 4, 8, 14 and 20 hs post oviposition (**POV**), with 10 replicates and each bird was considered as experimental unit. Each quail was observed daily to determine the exact oviposition time and according with the time of egg laying, quails were distributed in treatments (Table 1).

Table 1. Description of egg position and estimated day-time of each treatment

Treatment	Description	Estimated Day time
0h	Post-oviposition, just before the next ovulation	16h00
2hs	Egg in magnum and albumen secretion	18h00
4hs	Egg in Uterus (inactive phase)	20h00
8hs	Egg in Uterus (beginning of active phase)	24h00
14hs	Egg in Uterus (active phase)	06h00
20hs	Egg in Uterus (final egg formation)	12h00

Tissue and Blood Collection

The quails of each treatment (n=10) were weighed and blood collected by venipuncture of the jugular vein in tube without anticoagulant, centrifuged 3,000 rpm for 15 min to obtain 2 mL of blood serum. Then this was frozen at -20°C and analyzed by Gold Analisa® commercial kits for the determination of total calcium (**Cat**), phosphorus (**P**), total protein (**Pt**), albumin (**Alb**) and alkaline phosphatase (**AP**) levels in a UV-VIS Evolution 300 spectrophotometer. The ionic calcium (**CaI**) was determined by the formula $CaI = 6\ x\ Ca - (0{,}19\ x\ Pt) + A/3)/(0{,}19\ x\ Pt) + A + 6$, where CaI in mg/dL, Ca (Ca md/dL), Pt (total protein g/dL) and A (albumin (g/dL). Quails were anesthetized with intraperitoneal barbiturate injection (sodium thiopental 10mg/kg BW) and sacrificed by cervical dislocation. Bones and viscera were collected.

Morphology of Genital Organs. The left ovary and oviduct were weighed together. The magnum, isthmus, uterus (shell gland) and liver were isolated and weighed to obtain absolute and relative weight (relative weight % = (absolute weight / weight of the bird) x 100). The magnum and isthmus were isolated and measured with a digital pachymeter. The number of developing macroscopic follicles and follicular diameter in ovaries were evaluated. The eggs in development inside magnum, isthmus or uterus were weighed and the shell + shell membrane were prepared for analysis in scanning electron microscopy.

Fragments of the magnum and uterus were opened longitudinally, attached to styrofoam surface with pins exposing the mucosa and immersed in 10% formaldehyde fixative solution, 0.1M phosphate buffer pH 7.4. The fragments were histologically processed, paraffin embedded, cut (3 μm) and stained with hematoxylin and eosin (HE), for histological analysis. Then by histochemical reactions of periodic acid Schiff (PAS), they were used to identify secretion of neutral glycoproteins, and by Alcian blue pH 2.5 (AB), to identify polysaccharides.

To determine the height, width and area of magnum and uterus folds, digital images were obtained by light microscopy (Motic BA400) coupled to the Moticam 2500 5.0MP USB 2.0 image capture camera and the images were analyzed in Motic software Images plus 2.0 (Motic Chine Group Co. Ltd. software). The variables were measured in at least 5 folds/bird/segment.

Scanning electron microscopy. The shells of the developing eggs, present in post-posture treatments 4, 8, 14 and 20hs were washed, cut into strips or fragments in the equatorial region of the egg and dried in an oven at 37ºC for 72 hours. After that, they were stuck in stub aluminum and metallized with gold for 60 seconds to be analyzed by a Shimadzu scanning electron microscope model Superscan SS-550, Hadono, Kanagawa, Japan.

Bones analyzes

In the same birds, the right femur and tibiotarsus bones were removed, dissected, weighed individually and stored at -20°C wrapped in gauze humidified in saline solution for further analyzes of mineral composition and biomechanical aspects.

Bones Mineral Density. For bone mineral density (BMD) analysis, the bones (femur and tibiotarsus) of the same treatment birds were x-rayed along with the aluminum penetrometer (with 8 levels from 1 to 8 mm) at the CDVET - Veterinary Diagnostic Center, in radiographic equipment Tecno-Design 300/150 with the technique Kv 40, MA 50 and MAS 40 in digital radiographic film CR - Carestream, Direct View Vita CR veterinary. The radiographs were taken to determine bone density using the tool "Histogram" of the software Adobe Photoshop CC, in the diaphysis of each bone, since it was the same area used to the strength test.

Seedor index. The Seedor index is an indication of the bone density and for its determination (Seedor *et al*., 1991), the same bones are used for the bone strength and then measured at their greatest length, by pachymeter and weighed in a precision scale.

The index was obtained by dividing the result of the weight of the bone by its length. Seedor Index = Weight (mg) / Length (mm).

***Bones Strength*.** For the maximum strength measure of bone breakage, which is called bone strength, the tibiotarsus and femur bones of the birds were analyzed at the Food Engineering Laboratory in Brookfield Engineering Laboratories Inc. MA, USA, with TexturePro CT V1.4 build 17 software. The parameters used were compression test with 5% deformation, Trigger 500g, test speed 0.05 mm/s, TA7 and T7TPB device. The device a three-point bend rig, where the bones were positioned with support only in the bone epiphyses region, being the probe applied in the diaphysis region, always at the measuring the force applied at the time of bone rupture (kg). Hardness means resistance to permanent deformations. Fractureness ou fractureability means fragility, that is, susceptibility to fracture and deformability before breaking.

Mineral content. A longitudinal cut was made in each bone and the medullar bone (with the marrow bone) was cured and weighed and analyzed separately from the rest of the bone. The ashes were obtained by calcining in a muffle at 600°C for 6 hs and after the cooling they were weighed. To decomplex the hydroxyapatite crystals and release the minerals, 10 mL of hydrochloric acid (6M) were added to the ash and placed on a heating plate and the solution evaporated in the exhaust hood until completely dry. The precipitate was dissolved by adding distilled and deionized water and in the filtered solution to the volume was completed for 100 mL (Müller *et al*., 2012).

The solution was used to determined mineral content. Calcium concentrations was analyzing using a Varian Atomic Absorption AA240FS spectrophotometer with SpectrAA software from Victoria, Australia, and phosphorus concentrations by colorimetry on a UV-VIS Evolution 300 spectrophotometer.

Statistical analysis

On the basis of the results, normally distributed variables were subjected to ANOVA using Tukey test while the not normally distributed parameters were analyzed by Kruskal-Wallis test, using R Studio program with 5% significance level.

RESULTS

There was a significant effect ($P<0.05$) for body weight, ovary + oviduct, magnum, isthmus and egg weight. However, there was no significant effect on uterus and liver weight of Japanese quails throughout the egg formation cycle. The body weight was higher in the period of 20hs in average 11.5g, probably corresponding to the weight of the egg present in the uterus of the same period. However, soon after the laying (0hs) the body weight was lower. The weight of the ovary + oviduct (without egg) was higher to the post-posture treatment 20hs, due to the higher weight of the magnum and isthmus segments, besides the larger size presented by the ovarian follicles, in these same periods. The magnum presented higher weight in the periods of 0 and 20hs, next to the oviposition. The isthmus weight was lower after 14h of oviposition (inactive phase). The weight of the egg was considered from the beginning of the shell membrane formation, presenting a gradual increase, reaching almost double up to 20hs (Table 2).

The vitellogenic ovarian follicles (yellow) are denominated as a function of their size from largest to smallest as F1, F2, F3, F4 and F5 (Figure 1). The follicular diameter data in the 04 largest ovarian follicles are shown in Table 2. The diameter of the F1 was larger at oviposition (0h) and again at 20hs post oviposition. The follicles in general presented variable sizes during the daily egg-laying cycle as a function of the follicular hierarchy (Fig. 1 - 0hs), with larger diameter in the periods closer to the laying and smaller in the period 2hs post oviposition (Table 2).

Table 2. Means of the body weight (BW) and eggs in formation (g) and relative weights (%) of the ovary + oviduct (Ova+Ovi), magnum, isthmus, uterus and liver, and follicular diameters (mm) F1 to F4 in Japanese quail during the daily egg-laying cycle.

	Time post-oviposition									
	0h	2hs	4hs	8hs	14hs	20hs	Means	CV%	SEM	*P*
BW (g)	161.40^{b}	168.30^{ab}	171.10^{ab}	165.40^{ab}	163.10^{b}	177.70^{a}	167.96	6.12	1.35	0.011
Egg (g)	-	-	6.63^{d}	9.50^{c}	10.39^{b}	11.50^{a}	9.66	7.06	0.11	<0.001
Ova+Ovi	8.16^{d}	9.35^{c}	9.81^{c}	11.89^{b}	13.54^{a}	14.44^{a}	11.25	7.58	0.11	<0.001
Magnum	2.33^{a}	1.76^{b}	1.60^{b}	1.61^{b}	1.84^{b}	2.21^{a}	1.90	13.48	0.03	<0.001
Isthmus	0.53^{a}	0.50^{abc}	0.47^{abc}	0.46^{bc}	0.43^{c}	0.55^{a}	0.49	11.85	0.01	<0.001
Uterus	1.27	1.32	1.27	1.30	1.39	1.39	1.32	14.57	0.03	0.530
Liver	3.71	3.36	3.55	3.54	3.46	3.52	3.53	11.78	0.05	0.943
	Follicular diameters									
F1	18.31^{a}	15.99^{c}	16.39^{bc}	16.92^{abc}	17.67^{ab}	18.36^{a}	17.25	6.65	0.15	0.012
F2	14.50^{ab}	11.55^{c}	12.80^{bc}	12.39^{c}	14.26^{ab}	15.38^{a}	13.40	9.85	0.18	<0.001
F3	10.86^{a}	7.55^{b}	8.17^{b}	8.09^{b}	10.10^{a}	10.96^{a}	9.26	13.75	0.17	<0.001
F4	7.26^{a}	4.69^{c}	5.02^{c}	5.54^{bc}	5.89^{abc}	6.77^{ab}	6.03	17.98	0.16	<0.001

^{abc}Means with different letters within the same line differ significantly in Tukey test ($p < 0.05$).
SEM: Standart error of the mean

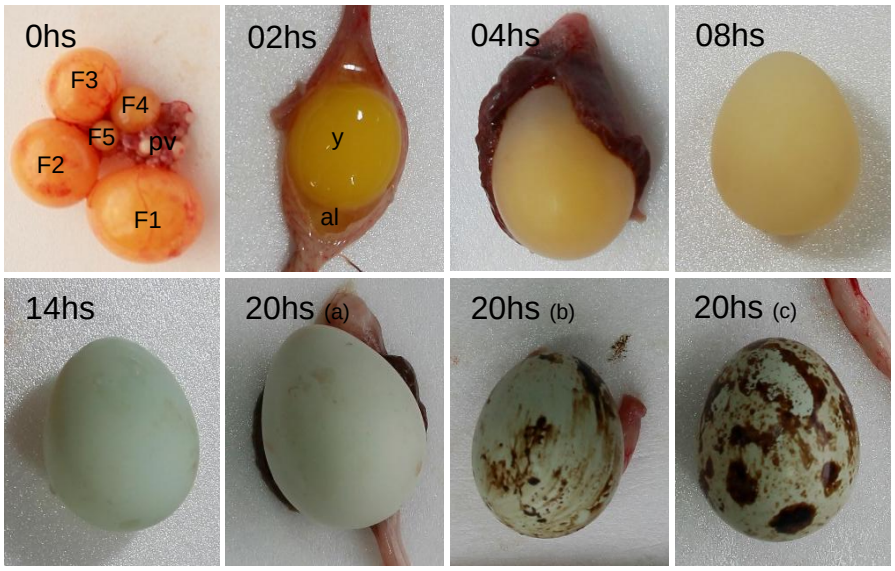

Figure 1 - The daily egg-laying cycle formation. At the moment of oviposition (0hs) is visible the follicular hierarchy (F1-F5), pre ovulatory follicle (F1) and pre vitellogenic follicles (pv). At 2hs post-oviposition, the magnum is secreting albumen (al) around yolk (y). At 4 to 20hs post-oviposition it occurs the formation of membrane fibers and eggshell. At 20hs there were observed eggs in final period of eggshell formation, that in some quails, with carbonate calcium deposition (a), pigment deposition (b) and ready egg with cuticle (c).

The magnum data showed that there was no difference in length between the periods studied, with a mean of 14.49 cm. Histologically, the magnum presented 11 broad longitudinal folds with rounded edges of 1.19 mm in height and 0.68 mm in width (Table 3).

The epithelium of the magnum presented a significant difference in height, being higher 2hs post-oviposition, and lower after 14hs. It is characterized by the same proportion of secretory and ciliated cells (1:1) arranged alternately (Table 3/Figure 2B, 3H), being that in the ciliated cells the nucleus is in the central region and the secretory ones are in the basal region. They were evaluated in regions where the cut was well perpendicular, thus not exposing cells overlapping, evidencing a single layer of cells.

Magnum presented a lot of tubular glands filled with many eosinophilic and PAS+ and AB- granules in the cytoplasm (Figure 2, 3). The glandular lumen showed secretion in all treatments PAS and AB+. In the period of 2hs it was fuller, since its content is being secreted in high quantity for the glands lumen and from these to the magnum lumen (PAS and AB+) through ducts, which were formed by active epithelium with higher concentration of secretory cells showing intense PAS and AB+ reaction, when egg was present, being evident its partial emptying in the following period (Figure 3C). Consequently, the tubular glands have fewer granules in the cytoplasm. Therefore, in the treatment 4hs, tubular and lumen glands are observed with low secretion and clearer areas were visible in PAS stain. (Figure 3 - white arrows).

The uterus was characterized macroscopically by an expanded region of the oviduct, located after the isthmus, whose weight did not vary among the periods studied (Table 2), presenting dark brown mucosa in which the folds are not distinguishable. Through histological slides (Figure 4) they have been found to be numerous, thin and branched. These folds had about 1.13 mm in height and 0.20 mm in width (Table 4). The uterine epithelium did not present alteration with height of 20.24 μm and is composed of secretory cells with PAS content + (Figure 4 black arrows), weak AB + and hair cells negative for both stains. The submucosa has eosinophilic glands and PAS- and weakly AB + (Figure 4).

Eggshell obtained during formation and analyzed by scanning electron microscopy showed that the beginning of mammillary bodies formation occurred 4hs post-oviposition, however still evidencing membrane fibers (Figure 5). Eggshell analyzed 8hs post-oviposition showed the final formation of mammillary layer and the

beginning of palisade layer. In this stage, the mammillary bodies grew forming columns and results palisade layer. Gas exchange pores were formed among columns randomly. The calcium carbonate crystals grew with a ridge display, observed as consecutive layers (Figure 6). At 14hs post-oviposition the palisade layer is evident and in the outer view shows the surface irregular spongy appearance and it is possible to observe projections of the columns that make up this layer. The junction of these columns gives the layer uniformity, interrupted only in the gas pores exchange (Figure 7).

At 20hs post-oviposition, gas exchange pores and all layers can be identified, mammillary, palisade and cuticle. The last layer, cuticle, gives a smoother surface appearance (Figure 8). Only two quails presented pigmented eggshell, one of them with cuticle and another one without fixation of the pigment (Figure 1).

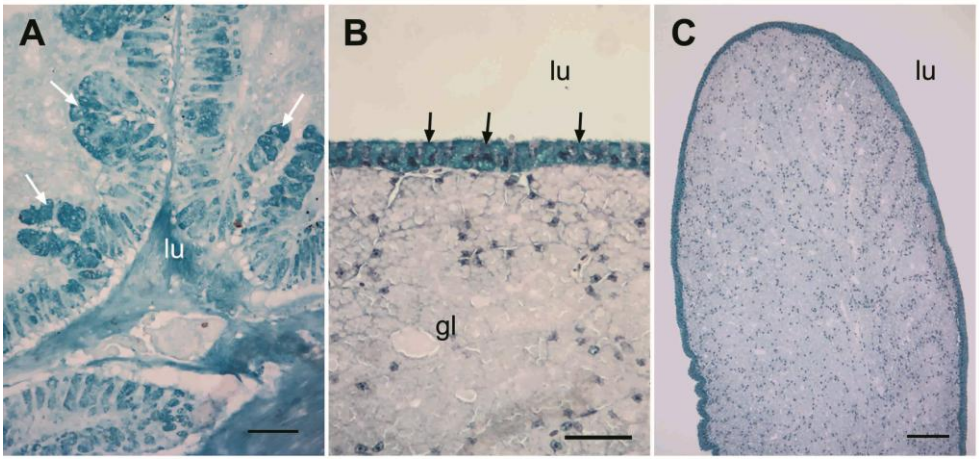

Figure 2 – Photomicrography of microscopical slides of magnum in Japanese quail in AB pH 2.5 stain. The image A represented the treatment 0h and B, C 20hs. Note the glands (gl) in mucosas AB- and positive reaction in secretory cells (black arrows) and cytoplasm of the ducts cells (white arrows). The secretion in lumen (lu) is AB+. Scale bar: A-B) 30µm, C) 100 µm.

Table 3. Measures of the magnum and uterus in Japanese quail during the daily egg-laying cycle.

	Time post-oviposition								
Variables	0h	2hs	4hs	8hs	14hs	20hs	Means	SEM	*P value*
				Magnum					
Lenght (cm)	13.96	15.33	13.49	14.58	14.79	14.61	14.49	0.19	0.136
Folds									
Number	10.40	9.80	10.20	11.60	10.40	14.50	11.03	0.48	0.119
Height (µm)	1125.07	1006.37	1178.31	1306.24	1251.76	1289.83	1192.93	41.82	0.319
Widht (µm)	733.01	611.39	706.39	555.65	696.76	767.43	678.27	25.74	0.202
Epithelium									
Height (µm)	12.36^{ab}	19.45^{a}	18.45^{ab}	11.93^{ab}	9.68^{b}	19.05^{ab}	15.15	0.96	0.019
% ciliated	50.00	49.67	50.40	50.00	50.00	49.80	50.00	0.57	0.999
% secretory	50.00	50.33	49.60	50.00	50.00	50.20	50.00	0.57	0.999
				Uterus					
Folds									
Height (µm)	1075.84	1324.43	1341.40	977.20	1029.37	1031.58	1126.79	41.00	0.057
Widht (µm)	179.60	200.51	224.51	194.69	186.88	230.08	203.60	10.15	0.631
Epithelium									
Height (µm)	18.79	20.71	21.83	19.19	20.80	20.11	20.24	0.40	0.293

^{ab}Means with different letters within the same line differ significantly in Tukey test ($p < 0.05$).

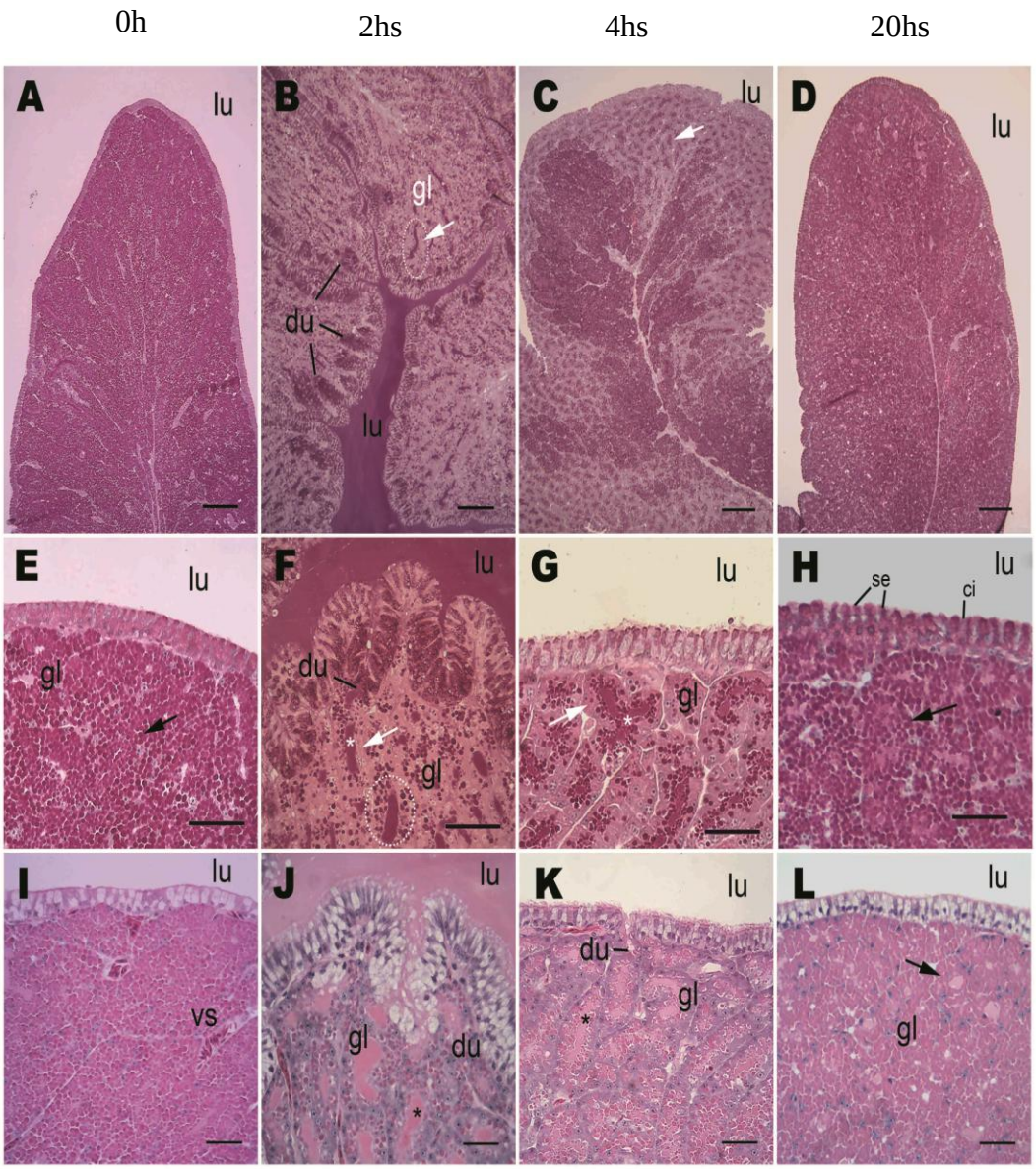

Figure 3 – Photomicrography of microscopical slides of magnum in Japanese quail in PAS (A-H) and HE (I-L) stain. Note that in treatments 0 and 20hs the cytoplasmic granules in tubular glands (gl) of the submucosa and the secretory cells (se) of the epithelium are abundantly filled with strongly PAS + content and eosinophilic granules (arrows). In the 2hs treatment, it could be observed that the epithelium of ducts (du) and lumen of the glands (*) were in intense secretory activity to the lumen (lu). In the 4hs treatment, the partial emptying of the tubular glands can be easily visualized, and then the filling again at 20hs, to start a new cycle. Ciliated cells (ci) and blood vessels (vs) could be identified. Scale bar: A-D) 100µm, E-L) 30µm.

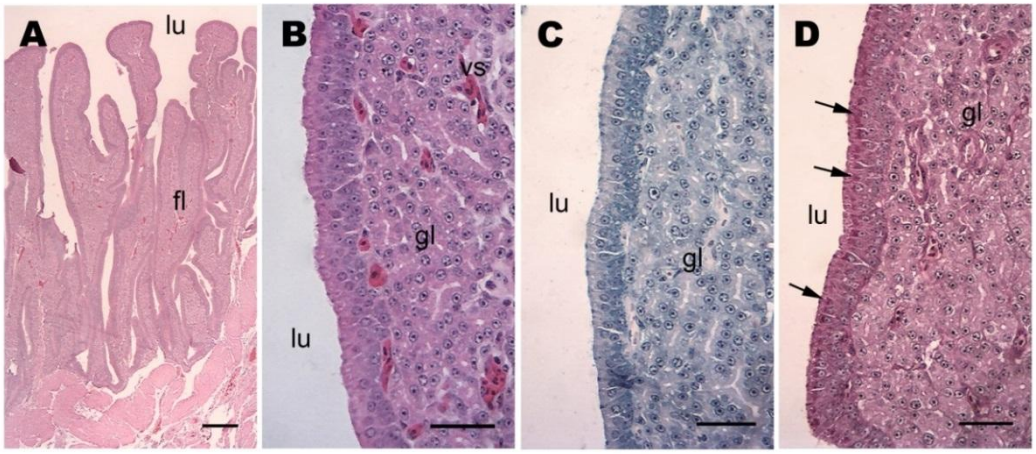

Figure 4 – Uterus photomicrography in Japanese quail in HE (A-B), AB pH 2.5 stain (C) and PAS (D) stain in 20hs treatment. The branched character of the uterine folds (fl) was evident. The mucosa epithelium shows secretory and ciliated cells (arrows) PAS and AB+. The submucosa glands (gl) had eosinophilic, AB- and weak PAS+ cytoplasmatic granules. Scale bar: A) 200µm, B-D) 30µm.

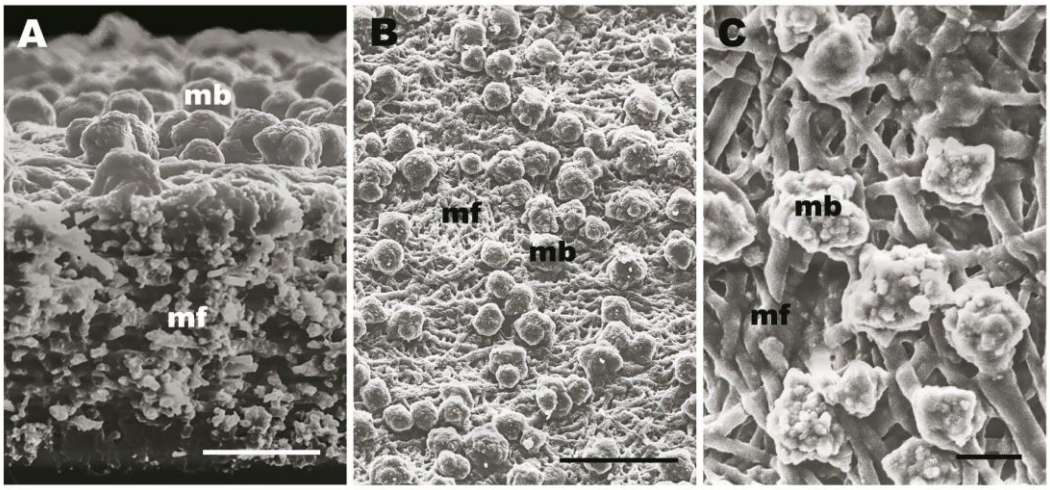

Figura 5 – Scanning electron microscopy in eggshell at 4hs post-oviposition in cross-section (A) and outer (B-C) views. Notice the beginning of egg shell formation. The mammillary bodies (mb) are rounded formations arranged randomly on the outer shell membrane fibers (mf), which is still visible at this stage of shell formation. Scale bar: A) 20µm; B) 50µm; C) 10µm.

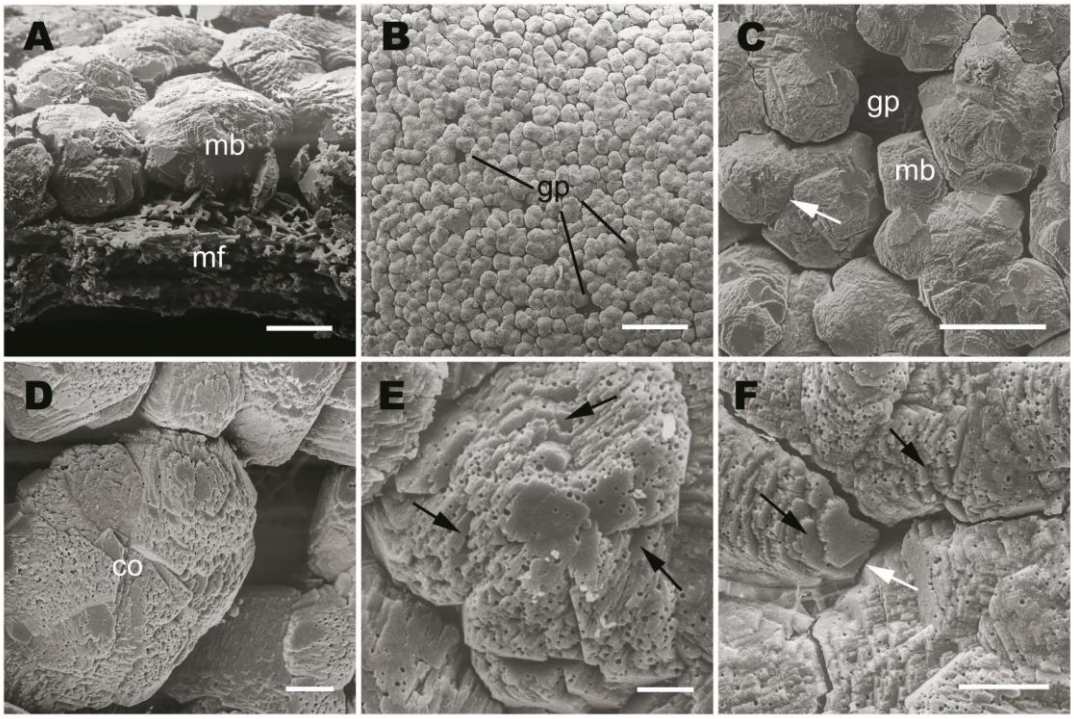

Figure 6 – Scanning electron microscopy in eggshell at 8hs post-oviposition in cross-section (A) and outer (B-F) views. At this stage was possible to observe several stages of egg shell formation. The mammillary bodies (mb) were well developed and fully cover the outer shell membrane (mf) by external vision. It is visible (D) the growth of columns (co) through the successive deposition (E) of crystal layers (black arrows), and later the junction boundary (C, F) of these (white arrows) forming random spaces characterizing the gas pores (gp). Scale bar: A) 20µm, B) 200µm, C) 50µm, D-F) 10µm.

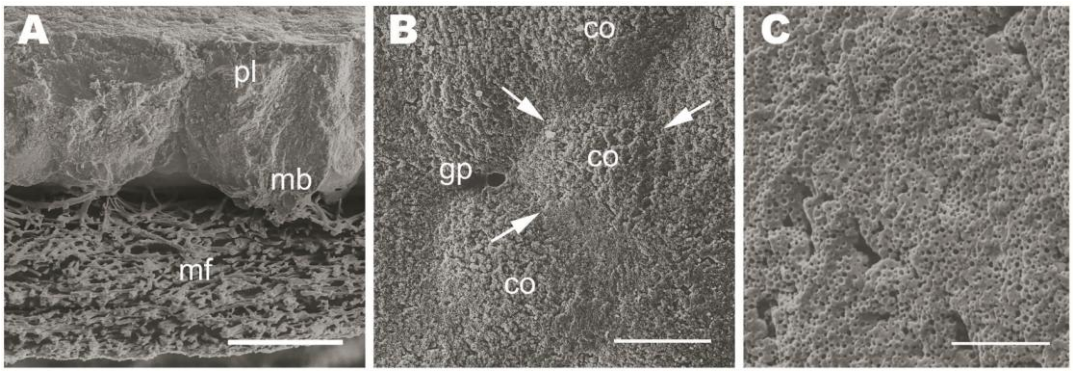

Figure 7 – Scanning electron microscopy in eggshell at 14hs post-oviposition in cross-section view (A) and outer (B-C) views. At this stage of egg shell formation was observed the total junction boundary (white arrows) among columns (co) that were still growing (B), forming the intermediate (A) of the palisade layer (pl) above the mammillary bodies (mb). The outer view of the shell has an irregular sponge appearance (C) and gas pores (gp). Scale bar: A) 50 µm, B) 20 µm, C) 10 µm.

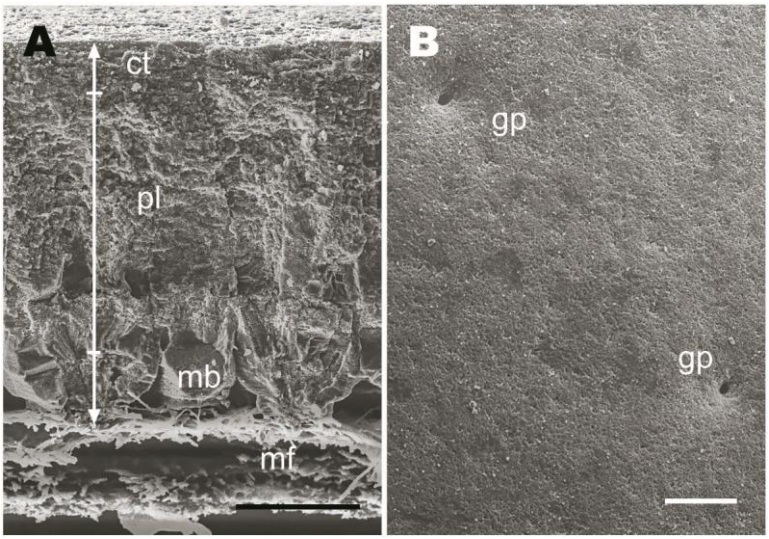

Figure 8 – Scanning electron microscopy in eggshell at 20hs post-oviposition in cross-section view (A) and outer (B) views. In this final phase, it was possible to observe all the layers of egg shell formation (A), with distinction of the fibers membrane (mf), mammillary bodies (mb), palisade layer (pl) and the cuticle, which this final layer gave a smooth appearance to the shell (B), where the presence of sparse gas pores (gp) could be easily distinguished. Scale bar: A) 50 µm, B) 100 µm.

The results of serum biochemistry are described in table 4. Plasma protein averages were statistically different between the treatments, being higher in 2, 4 and 8hs, which coincide with the production and secretion of albumen during the passage of the follicle by magnum. The treatments 0, 14 and 20hs, close to ovulation, had lower averages. Albumin also showed a significant difference between treatments, being higher in treatment 2hs and lower in 0 and 8hs. Serum calcium levels presented a difference between the periods, with the highest mean in the 2hs treatment, decreasing gradually until reaching the lowest mean in the treatment 20hs, maintaining an average of 26.15 mg/dL. There were no significant differences in the serum variables of phosphorus, alkaline phosphatase and ionic calcium.

Table 4 Serum biochemistry in Japanese quail during the daily egg-laying cycle.

	Time post-oviposition								
	0h	2hs	4hs	8hs	14hs	20hs	Means	SEM	*P*
Pt (g/dL)	4.77^{b}	5.61^{ab}	5.43^{ab}	4.90^{ab}	4.65^{b}	4.72^{b}	5.01	0.078	0.002
Alb (g/dL)	1.22^{b}	1.65^{a}	1.42^{ab}	1.33^{b}	1.46^{ab}	1.42^{ab}	1.42	0.027	0.001
AP (U/L)	1202.89	1311.98	1488.06	1384.58	1280.45	1237.86	1316.87	55.59	0.702
P (mg/L)	9.26	8.26	8.80	8.69	8.82	8.59	8.74	0.303	0.963
Ca (mg/dL)	25.56^{abc}	27.98^{a}	26.73^{abc}	26.20^{abc}	25.37^{b}	24.96^{bc}	26.15	0.316	0.037
CaI (mg/dL)[1]	18.76	19.17	18.88	18.91	18.16	17.94	18.65	0.215	0.537

^{abc}Means with different letters within the same line differ significantly in Tukey test ($p < 0.05$).

[1] calculated by formula

The variables related to the bone strength of the femur and tibiotarsus (Table 5), such as hardness and fractureness, which represent the maximum weight that the bone supports before the first break, did not present differences between the treatments of both bones, as well as the time of the first break and the Seedor index. It was found that the femur resists up to an average of 2.65 kg of weight for almost 7 seconds before fracturing, which represents almost 5 times its weight. The tibiotarsus bone resists on average 2.42 kg for 9.7 seconds, before the break, that is, 4.40 times its weight.

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Table 5. Means of da hardness, fractureness–, time of first break, density by radiography and Seedor index to evaluate the strength bones in Japanese quail during the daily egg-laying cycle.

	Time post-oviposition								
Variables	0h	2hs	4hs	8hs	14hs	20hs	Means	SEM	*P value*
				Femur					
Hardness (kg)	2.61	2.97	2.69	2.15	3.14	2.46	2.66	0.11	0.144
Fractureness (kg)	2.49	2.85	2.39	2.13	3.12	2.44	2.56	0.13	0.269
Time (s)	7.20	8.08	7.47	6.18	7.25	5.84	6.97	0.26	0.136
Density (mmAl)	0.99^{abc}	0.35^{c}	0.57^{abc}	0.71^{abc}	1.06^{a}	0.99^{ab}	0.79	0.07	0.012
Seedor (mg/mm)	14.43	14.56	15.23	15.16	14.29	14.82	14.77	0.28	0.863
				Tibiotarsus					
Hardness (kg)	2.51	2.57	2.37	2.47	2.34	2.55	2.47	0,08	0.948
Fractureness (kg)	2.37	2.57	2.24	2.47	2.34	2.51	2.42	0.09	0.088
Time (s)	7.56	10.42	8.54	10.66	9.18	10.50	9.65	0.31	0.064
Density (mmAl)	0.92^{ab}	0.43^{b}	0.51^{ab}	0.61^{ab}	1.02^{a}	0.84^{ab}	0.73	0.059	0.039
Seedor (mg/mm)	11.99	12.12	12.74	11.52	11.73	11.34	11.90	0.20	0.39

[ab]Means with different letters within the same line differ significantly in Tukey test ($p < 0.05$).
SEM: Standart error of the mean

The density measured by the radiographs with penetrometer showed a similar difference for the femur and tibiotarsus, with a higher average in the 14hs period, in the active phase of eggshell formation, and a lower average in 2hs, when the albumin formation in the magnum is occurring (table 5).

The mineral evaluation data of the femur and tibiotarsus bones are shown in table 6. The total weight of the femur bone was not significant among treatments, being its mean 0.540 g, as well as its parts: cortical and medullary, with averages of 0.42 and 0.12 g, respectively. The weight of the femur medullary bone increases until 4hs, in order to recover this reserve and prepare for a new cycle. There is an increase in the weight of the bone medullary in the treatment 20hs, probably because there is deposition of calcium from the shell at the same time as it is absorbed in the diet in order to maintain the homeostasis. The weight percentage of the femur cortical bone of the femur is around 78.42% and the medullary bone is 21.58%. Although the proportion did not present a significant difference, it can be observed that the lower mean of the medullary bone is in the treatment 0h, right after post oviposition, that is, at the end of the eggshell formation, suggesting the use of the bone reserve as a source of calcium. The ash content of the cortical and medullary bone was 64.06 and 46.80%, respectively.

The calcium and phosphorus bone concentration of the femur cortical portion showed no significant difference between the treatments. The femur medullary bone presented a significant difference for calcium and phosphorus concentration. The treatment 0h had the highest means of calcium and phosphorus (16.43 and 8.15%, respectively), right after the laying, when the uterus is inactive, and recovering the concentration, being this period of higher absorption of these minerals. The lowest averages of the same variables occurred in the 14hs treatment, when the egg is in the uterus, peaking forming shell, demonstrating the intense recruitment of calcium and phosphorus from the medullary bone. In the period 20hs also presented higher averages of phosphorus when the shell formation is at the end and the shell deposition velocity has decreased and the medullary bone is recovering its reserves.

Table 6. Means of weight, ashes, calcium (Ca), phosphorus (P) and proportions in cortical and medullary bones in femur and tibiotarsus in Japanese quail during the daily egg-laying cycle.

	Time post-oviposition								
Variables	0h	2hs	4hs	8hs	14hs	20hs	Means	SEM	*P*
				Femur					
Weight (g)	0.524	0.565	0.564	0.554	0.522	0.516	0.540	0.01	0.480
Cortical (%)	80.60	79.99	76.31	77.69	79.25	76.99	78.42	0.60	0.269
Medullar (%)	19.40	20.01	23.69	22.31	20.75	23.01	21.58	0.60	0.269
Ash cortical (%)	67.01	63.65	63.53	63.63	61.54	64.90	64.06	0.80	0.516
Ash medullar (%)	56.09	52.17	42.09	46.38	40.47	44.68	46.80	1.91	0.184
Ca cortical (%)	12.00	12.21	13.34	11.81	10.71	9.38	11.55	0.85	0.812
Ca medullar (%)	16.43^{a}	13.48^{ab}	11.83^{ab}	11.48^{ab}	9.42^{b}	13.20^{ab}	12.61	0.57	0.034
P cortical (%)	8.09	7.23	7.91	7.73	7.78	7.42	7.71	0.52	0.997
P medullar (%)	8.15^{a}	6.92^{ab}	6.27^{ab}	5.60^{ab}	4.35^{b}	7.45^{a}	6.44	0.29	0.010
				Tibiotarsus					
Weight (g)	0.548	0.561	0.594	0.533	0.548	0.523	0.551	0.01	0.359
Cortical (%)	82.81	79.49	81.23	77.82	81.28	80.59	80.54	0.50	0.117
Medullar (%)	17.19	20.51	18.77	22.18	18.72	19.41	19.46	0.50	0.117
Ash cortical (%)	72.22	70.42	69.54	73.21	69.89	70.29	70.93	0.55	0.346
Ash medular (%)	50.79	47.53	45.50	46.44	42.07	44.13	46.08	1.18	0.406
Ca cortical (%)	16.93	16.14	15.86	15.41	17.29	17.97	16.63	0.71	0.903
Ca medullar (%)	13.30^{a}	9.92^{ab}	11.83^{ab}	9.22^{ab}	6.45^{b}	10.41^{ab}	10.20	0.57	0.036
P cortical (%)	9.77^{ab}	8.06^{b}	9.78^{ab}	9.87^{ab}	11.86^{a}	11.48^{a}	10.08	0.32	0.032
P medullar (%)	7.56	6.87	5.90	7.27	5.19	6.20	6.50	0.26	0.124

^{ab}Means with different letters within the same line differ significantly in Tukey test ($p < 0.05$).
SEM: Standart error of the mean

The mean weight of the tibiotarsus bone was 0.551g being the same among treatments ($p<0.05$). The weights of the cortical and medullary portions were 0.44 and 0.11g, corresponding to 80.54 and 19.46%, respectively. The ash content of the cortical and medullary bone was 70.93 and 46.08%, respectively.

There was no significant difference between treatments of calcium concentration of the cortical portion and phosphorus of the medullary bone of the tibiotarsus. The calcium content of the medullary bone was lower in the 14hs treatment, when the eggshell formation in the uterus is occurring, due to the higher recruitment of calcium, and gradually increases in the treatment 20hs, until reaching the highest average in the treatment 0h, for recovery of its reserve for the next egg cycle. The phosphorus concentration of the cortical region showed a significant difference between the treatments, being the means higher in the treatments 14 and 20hs and smaller in 2hs.

Although the tibiotarsus bone is on average almost 10 mm larger than the femur bone, the weight of the cortical and medullary regions of each is very similar, except for the ash content of the cortical region that is higher in the tibiotarsus bone, because it contains more calcium and phosphorus. In contrast, the femur medullary bone is a better source of calcium.

DISCUSSION

The ovary may represent about 3% of the body weight of broiler breeder hens in the reproductive phase (Macari *et al*., 2005). The isolated weight of the ovary was not obtained but in the analysis of the relative weight of ovary + oviduct it is possible to observe the increase in weight during the cycle of eggs formation as a function of the increase in the weight of pre-ovulatory follicles that accumulate vitellogenic content and increase in size, consequently. The mean values found in this study of follicular measurements (F1 to F4, 17.25, 13.40, 9.26 and 6.03 mm, respectively) are very similar to those found by Sreesujatha *et al*. (2016) in follicles F1, F2, F3 of 16.20, 12.48 and 7.95 mm respectively. In addition to being classified according to the classification of follicular hierarchy in F1 quail (15 to 18 mm), F2 (10 to 15 mm), F3 (<10 mm), differentiating only in the fact that it does not consider the F4 follicle, in consideration of the pre-ovulatory follicle >19 mm. When the largest follicle is ovulated, the follicle F2 in the hierarchy to be F1 and so on, so that the largest follicle will always be the next to be ovulated and the others increase in volume in sequence. In a study in laying hens it was observed that the follicles can remain in rest for months, and when they reach 6 to 8 mm they enter the hierarchy where only one follicle develops and ovulates of 5 to 10 days (Johnson, 1993).

The oviduct of quail is similar to chickens and turkeys, has a mucosal layer lined by simple cylindrical ciliated epithelium, lamina propria of connective tissue, smooth outer and inner circular smooth muscle layer of connective tissue and mesothelium (Hodges, 1974; Moraes *et al*., 2009).

The weight of different segments of the oviduct were similar to those described by Turner & Eliel (1978), who obtained mean weights of the magnum, isthmus and uterus, with 3.26, 0.84 and 2.02g, respectively. The magnum presented higher weight in

the periods of 0 and 20hs after the oviposition, demonstrating that in these periods the magnum is preparing for the development of the next egg, fact that provides an increase of its weight as the oocyte moves along the oviduct towards the magnum. Studies have stated that there is a difference in weight of the magnum during the egg cycle formation (Artoni *et al*., 2001), perhaps this increase in weight is also related to the increase in the circulatory supply of the muscular layer that increase the contraction to promote the passage of the egg and the spermatozoa (Artoni *et al*., 2001, Moraes *et al*., 2007). The egg remains in the magnum for about 3 hours (Artoni *et al*., 2001; Moraes *et al*., 2007), resulting in changes related to the passage of the egg, providing a series of physiological events that promote the release of albumen, which is deposited around the egg (Parizzi, 2006; Artoni *et al*., 2001) since the magnum and isthmus are responsible for the proteins production during the eggs cycle formation (Yu & Marquardt, 1974).

As shown in this work, the magnum has a large number of tubular glands with eosinophilic cytoplasm (Carneiro *et al*., 2014), which secrete a large variety of proteins for the formation of albumen (Artoni *et al*., 2001), that are synthesized by endoplasmic reticulum and stored in granules in the Golgi complex and secreted via exocytosis (Kato *et al*., 1987), PAS + because they are glycoproteins present in the albumen composition (Hodges, 1974). The results confirmed that the entire oviduct epithelium consists of equal numbers of ciliated and glandular cells (Davidson, 1973). After the goblet cells secrete their contents, they retract and are suppressed by the hair cells, causing the impression of the surface is only ciliated (Hodges, 1974).

There was observed that the quantity of granules in the cytoplasm of the tubular glands were full in the 0 and 20hs treatment, and that although the presence of albumen secretion was constant, the production and secretion was more intense during the 2hs treatment, after ovulation and during the passage of the egg through the magnum, being according to the literature. The peak of secretion occurred at the time of ovulation, when there was a maximum amount of secretion in the tubular glands. These events are not only caused by the mechanical stimulus, but also by the nervous and mainly by the hormonal stimulus, that modulate the secretory granules. Studies have shown through biochemical and structural analysis that the secretion of albumen occurs only through the glands that are directly in contact with the egg and that the concentration of proteins is extremely variable during the egg cycle. Although some studies have shown that

albumen proteins are secreted and produced continuously (Hodges, 1974; Laugier & Brard, 1980; Kato *et al*., 1987).

The size and shape of the magnum folds are according to Carneiro *et al*. (2014) who found slightly higher average values of the height of the magnum folds and similar values of width of 1.684 and 0.711 mm, respectively. Its mucosa presented large folds, broad and visible macroscopically, compared with another region of the oviduct (Hodges, 1974; Moraes *et al*., 2007) and there was no difference in the number of magnum folds using different protein levels in the diet in quails (Artoni *et al*., 2001).

The macro aspect and microscope of the uterus are in agreement with the literature (Artoni *et al*., 2001; Moraes *et al*., 2007; Carneiro *et al*., 2014). Carneiro *et al*. (2014) observed mean values of height and width of 1256.95 and 261.01 μm, respectively for uterine folds. Although it was not significant, it presented a higher weight with 20hs, when the egg is present and less weight in the treatment 0h, soon after the laying, justifying the morphological changes that occurred in the uterus between the periods of 4 to 20hs, in which the egg made present, by the intense activity of this organ while it produces the eggshell.

The epithelium of the pseudostratified uterus was composed of ciliated and secretory cells and the lamina propria is filled by tubular glands of cubic to cylindrical epithelium (Hodges, 1974; Moraes *et al*., 2009), where the cells have apical granules in their cytoplasm (Hodges, 1974; Moraes *et al*., 2007). The uterus of quail is similar to that of laying hens, except for the coloration (Artoni *et al*., 2001).

In the uterus there is the hydration of the albumen and the shell formation by the deposition of calcium carbonate, proteins, pigments and cuticle on the shell membrane (Yu & Marquardt, 1974, Artoni *et al*., 2001) where the egg stays the longest, about 20 hours, the last 5 hours for deposition of the pigment, characteristic of quail eggs (Moraes *et al*., 2007).

Although Nunes *et al*. (2016) observed that the eggshell represents about 15% of egg weight, the weight gain found is probably due to a process called “plumping”, which consists of hydration of the albumen, it begins in the isthmus and continues to occur when the egg arrives in the uterus through the pores that are formed, while the deposition of calcium carbonate for the shell formation occurs (Hicke *et al*., 2012).

The shell composition is basically calcium carbonate, formed by a reaction catalyzed by carbonic anhydrase, which in turn is stimulated by estrogen, considered a

natural and porous ceramic formed in layers on the shell membrane (Holm *et al*., 2001; Hincke *et al*., 2012; Mello, 2015). The highest eggshell formation rate occurs around 12 to 18 hours post-laying (Clunies *et al*., 1993), which involves various mechanisms such as absorption and transport of calcium from the intestine to the blood and from blood to the bone or to the shell, hydration of albumen ("plumping"), oviduct muscle contraction for egg movement and laying, and secretion of the shell protein matrix, mediated by hormones and proteins (Turner & Eliel 1978; Holm *et al*., 2001; Bar, 2009; Hincke *et al*., 2012).

Plasma proteins play an important role in the albumen formation and therefore, probably the peak of albumen production is in the periods when the magnum is in the active phase of secretion, that is, in treatments 2 and 4hs, increasing serum protein levels. Albumin, together with globulin, compose the fraction of total plasma proteins, so it presented similar behavior, suggesting that secretion and albumen production are constant, being larger or smaller according to the position in which the follicle is in the oviduct.

The serum calcium values are in agreement with Schreiweis *et al*. (2004) who reported that during the shell calcification of laying hens, plasma levels of total and ionized calcium decrease, indicating that there is a drainage in the calcium reserves, which is presumed is not fully offset by bone mobilization. Clunies *et al*. (1993) analyzed activity with labeled calcium and obtained average values of blood calcium similar to this work and stable during the egg cycle formation.

Kerschnitzki *et al*. (2014) observed that during periods with light, the laying hen, through ingestion of calcium from diet, keeps her body in homeostasis. Until 9 hours after oviposition, the concentration of serum ionic calcium increases and phosphorus decreases. Between 9am and 9pm after oviposition, the plasma concentration of ionic calcium decreases, and bone medullary calcium is reabsorbed and transported to the uterus to form calcium carbonate for shell mineralization. As phosphorus is not required for the calcium carbonate, its plasma concentration increases reaching peak around 12 hours post oviposition, which did not happen in this work, where it was observed that the highest concentration of calcium in the blood reached peak 2hs after the oviposition and gradually decreased until 20hs. It is noteworthy that this variation is small enough to active other mechanisms of calcium metabolism maintaining homeostasis.

The calcium metabolism is controlled by various hormones responsible for absorption, transport and deposition. The main ones are: parathyroid hormone (PTH); vitamin D3-forming hormone and calcitonin (CTN), in order to maintain the blood calcium homeostasis when the eggshell is in formation and the body has a higher demand for calcium (Taylor *et al.*, 1971; Dacke, 1979).

In laying hens the eggshell formation occurs mainly during the night, during which the birds stay in the dark and are not eating. In the evening, where there is no calcium offering if the laying hen is forming the shell of the egg, there is a bone mobilization, enough to avoid bad shell calcification. Calcium levels in laying hens show a circadian rhythm as a function of ovulation stage and eggshell formation (Etches, 1987). Van de Velde *et al*. (1984) identified two metabolic periods: peeling period (active period) and when the shell is not in formation (inactive period). The bone medullary adds calcium during the day and reduces this reserve during the period when calcification is active. When calcium intake is present, the bird ingests large amounts of calcium at the end of the light period. The circulatory system serves as a reserve and transport, with an average of 25 mg of calcium, which is constantly recruited. The synthesis of the vitellogenic yolk occurs continuously and is not altered by the stage of the ovulatory cycle. Similarly, urinary calcium excretion is continuous from the vascular system, although less calcium is excreted during the shell formation periods (Taylor & Kirkley, 1967).

In pigeons, during the cycle of eggshell formation, there is an intense process of decreasing the medullary bone through the activity of osteoclasts, and a total reduction can also occur (Bloom *et al.*, 1958). In quail metabolism the bone dynamics are similar to laying hens (Taylor & Dacke, 1984). Quails have a differentiation in calcium metabolism, since most of the shell calcification occurs during the day, whereas dietary intake and calcium absorption by the intestine is occurring, not using medullary bone as a source of calcium (Dacke, 1979; Bar, 2009).

Calcium for eggshell formation is endogenous, about 10%, half of it comes from the diet, complementing the available source of calcium from the medullary bone (Kim *et al*., 2012; Kerschnitzki *et al*., 2004; Ribeiro et al., 2016). There is a synchrony that intestinal calcium absorption increases as the requirement for shell formation increases (Clunies *et al*., 1993). After initiation of egg calcification (active phase), the calcium

used to form the shell comes from reabsorption of the medullary bone (Kerschnitzki *et al*., 2014).

The strength of bone did not change during egg formation, even in the shell formation phase by the deposition of calcium carbonate in the periods 4 to 20hs. Mello (2015) observed that higher calcium levels in diet decreased the mineral density of the femur and tibiotarsus bones. The general anatomy of the bone observed in this work does not change with the absorption and reabsorption of daily calcium, necessary for the calcium mobilization used for eggshell formation, the density and mineral concentration of calcium are more reliable parameters for evaluation of the calcium mobilization daily.

The medullary bone is a fast-turnover structure, with a large number of osteoclasts on its surface, and the osteoclastic activity in this bone can offer about 40% of the calcium in the shell (Van De Velde *et al.*, 1984) and 60% would come from diet (Van De Velde *et al.*, 1985), and can be metabolized at a rate of 10 to 15 times faster than the cortical bone. Its destruction and reconstruction are extremely fast and in cases of calcium deficiency at the expense of the cortical bone. Its total volume does not change during the poultry posture cycle, only its degree of calcification (Dacke *et al.*, 1993), explaining the findings in this study where the medullary calcium presented a lower mean in the treatment 14hs when eggshell formation is occurring, that is, when there is higher recruitment of calcium.

After oviposition, the osteoblasts replace the osteoclasts and regenerate the medullary bone. Although medullary bone is important for shell formation, there is no direct relation between shell and bone quality (Whitehead, 2004). This calcium mobilization occurs initially in the medullary bone followed by the cortical bone (Abdul-Aziz, 1998). Justifying the premise that the cortical bone has structural function and the medullary bone has a function of labile reserve of minerals, mainly calcium and phosphorus, to form eggshell daily.

At the beginning of the cycle, laying hens ingest a large amount of calcium from diet, which causes an increase in ionic calcium in the blood with a peak around 6 hours after oviposition, as well as an increase in medullary bone mineralization, but there is no change in trabecular bone thickness (Kerschnitzki *et al.*, 2014).

After the onset of calcification of the next egg (after 6 hours of oviposition) there is a decrease in the serum ionic calcium, while a serum increase of phosphorus is

observed. In this period, there is also a decrease in the mineral content of the medullary bone, with the presence of a large number of active osteoclasts (Van De Velde *et al*., 1985; Kerschnitzki *et al.*, 2014; Rodriguez-Navarro *et al*., 2018).

When evaluating the mineral content of the medullary bone, we can observe the dynamics of the bone during the daily cycle of egg formation (Kerschnitzki *et al.*, 2014). The reduction of the calcium percentage in the ashes of the medullary bone analyzed 14 hours post oviposition suggests that during the hours before that period this was probably the main mineral source for the shell formation. In quails by the evening time of the posture this comprises the dark hours of the light program. In this period about ¼ of the shell thickness is deposited as evidenced by scanning electron microscopy.

CONCLUSION

The oviduct of Japanese quail presented morphological variations in function of the period of egg formation, as well as the blood concentration of calcium and the calcium content of the medullary region of femur and tibiotarsus bones, maintaining the calcium requirement and homeostasis in each evaluated phases of the daily egg-laying cycle.

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IV. Light effect on bones and oviduct development in Japanese quail.

ABSTRACT: The objective of this study was to describe the medullary bone formation and the genital organ development in sexual maturity under the effect of two light programs with different light/dark periods, simulating the winter and summer seasons in Maringá, (11L:13D and 14L:10D), in 550 females quail of 1 to 35 days old housed in 10 battery cages (0.80 x 0.80 m) in a climatic chamber with controlled light and temperature. From 35 days, 30 min of light were added every 3 days until reaching 17L:7D. Blood, bone and genital organs were collected at 17, 21, 25, 28, 31, 35, 42, 49, 56, 63 and 70 days in 5 birds of each treatment, as well as the feed intake was observed weekly. The quail posture under the 11L:13D light program was higher than the 14L:10D light program, with 47.89 and 32.11%, respectively. Sexual maturity is characterized by a number of metabolic events. Albumin and calcium serum levels increased immediately before sexual maturity. Albumin participates in the transport of substances from the liver to the growing follicles in the ovary. Calcium, on the other hand, influences eggshell formation and medullary bone. The birds under the 11L:13D light program showed genital organs development earlier than the 14L:10D light program, with relative weight of the ovary at 56 days, oviduct at 42 days and oviduct length was longer at 56 days. Consequently, they started laying earlier. The medullary bone developed first in the femur, and its only function is to provide calcium for eggshell formation. At 63 days all birds analyzed for both lighting program presented ovary and oviduct developed.

Key words: bones, light, medullary bone, oviduct, sexual maturity.

INTRODUCTION

For the best productive and reproductive performance of the birds, it is necessary to know the factors that influence its genetics and response of the bird: management and nutrition (Rutz et al., 2005). The initial stages should have special attention to this aspect, since it depends on the maximum potential expressing in the egg production phase (Freitas et al., 2010; Makiyama, 2013).

The light in poultry is critical to performance because it directly affects the hormonal stimulation, production (Rutz et al., 2005; Srivastava et al., 2007; Freitas et al., 2010; Molino, 2013), sexual activity, feathering, growth and vigor (Murakami and Ariki, 1998). It is recommended 24 hours of light in the initial phase, 12 hours in the growing phase and 17 hours in the production phase, although 15 hours light in the laying phase is enough to maintain egg production and quality in Japanese quails (Gewehr et al., 2005; Molino, 2013) and laying hens (Freitas et al., 2010).

The onset of sexual maturity or laying is around 5 to 6 weeks of age in quails (Miller, 1967; Makiyama, 2013; Nunes et al., 2016). The age of sexual maturity directly influences the performance, since early laying quails have a lower yield (Makiyama, 2013). The pituitary gland controls the hormones responsible for sexual maturity. For female birds, before the first ovulation, the ovary development is related to the androgen and estrogen hormones, which are responsible for the functional development and metabolism of the reproductive system (Holm et al., 2001; Rutz et al., 2005; Molino, 2013), as well as calcium reserve formation of medullary bone, which induces increase of calcium absorption capacity on the intestines (Bar, 2009; Rutz et al, 2007; Freitas et al., 2011).

The photoperiod also affects the feed intake of the birds and consequently the weight and fat deposition, which may affect the onset of lay and egg size (Makiyama, 2013). Temperature affects hormone physiology, especially at high temperatures, decreasing hormone luteinizing (LH), estrogen and progesterone release, perhaps because of high cortisol levels caused by heat stress (Rutz et al., 2005).

In the region of Maringa, longitude 51:56:19, latitude -23:25:31, altitude 596 m, time zone -3 hours, the light on the shortest day (June 22) goes from 7h08 to 17h51 (10 hours and 43 minutes), and the longest day (December 22) goes from 5h38 to 19h15 (13 hours and 53 minutes) (National Observatory, 2016). Light programs are critical for laying quails production, but there are not enough studies specifically for this type of breeding, using adapted data from other species (Makiyama, 2013; Molino, 2013).

This study aims to determine ovary and oviduct development of quails during and after sexual maturation, medullary bone formation and laying performance until 70 days of age in Japanese quail submitted to two different light programs.

MATERIAL AND METHODS

Birds and Ethical Approval

The experiment was carried out in Bioclimatology Sector of Experimental Farm of Iguatemi (FEI) of University of Maringa (UEM) and approved by ethical committee (CEUA protocol No. 8295280616). The Japanese quails with 1-day old came from Granja Vicami, Assis, SP.

A total of 550 birds were used, housed in galvanized cages, 0.80 x 0.80 m, with heating and temperature control system, where they received food and water *ad libitum*. The temperature and humidity of the environmental chamber were maintained at $24.63^{o}C \pm 1.8$ and 72.4 % ± 5.1, respectively.

The experimental period was 1 to 70 days in 2 phases. Two diets were formulated according to the requirements of each phase (Rostagno et al., 2011) based on corn and soybean meal. The diet for 1 to 42 days consisted of crude protein 25%, metabolizable energy 2,900 Mcal/kg, Calcium 0.85% and Phosphorus 0.32%, and the diet for 42 to 70 days was composed of crude protein 22%, metabolizable energy 2,800 Mcal/kg, Calcium 3.5% and Phosphorus 0.32%.

The experimental design was a completely randomized with 2 treatments and 5 replications of 55 birds. The treatments consisted of two lighting programs simulating day length in Maringá located latitude -23°25'31", longitude 51°56'10" and altitude 596 m, during summer with 14 hours light:10 hours dark (14L:10D) and winter with 11 hours light:13 hours dark (11L:13D). The 1-day quails were housed in galvanized cages according to the light program. The light was provided in an artificial way, with fluorescent lamps, and the light period was controlled with analog timer G20 TMP10111.

From 1 to 35 days in treatment 11L:13D the light period started at 7h00 and ended at 18h00. In the treatment 14L:10D the light period started at 5h30 and ended at 19h30. After 35 days it was increased every 3 days, 30 minutes of light, fractionated between the beginning and the end of the day (15 min each) until they reached 17 hours of light (17L:7D) for both treatments (table 1).

Table 1. Light Program

	Light Program			
	11L:13D		14L:10D	
Day	Day Time	Light Duration	Day Time	Light Duration
1-35d	07h00 to 18h00	11h00	05h30 to 19h30	14h00
36d	06h45 to 18h15	11h30	05h15 to 19h45	14h30
39d	06h30 to 18h30	12h00	05h00 to 20h00	15h00
42d	06h15 to 18h45	12h30	04h45 to 20h15	15h30
45d	06h00 to 19h00	13h00	04h30 to 20h30	16h00
48d	05h45 to 19h15	13h30	04h15 to 20h45	16h30
51d	05h30 to 19h30	14h00	04h00 to 21h00	17h00
54d	05h15 to 19h45	14h30	04h00 to 21h00	17h00
57d	05h00 to 20h00	15h00	04h00 to 21h00	17h00
60d	04h45 to 20h15	15h30	04h00 to 21h00	17h00
63d	04h30 to 20h30	16h00	04h00 to 21h00	17h00
66d	04h15 to 20h45	16h30	04h00 to 21h00	17h00
69d	04h00 to 21h00	17h00	04h00 to 21h00	17h00
70d	04h00 to 21h00	17h00	04h00 to 21h00	17h00

Live performance

During the breeding and rearing phase, the birds and the feed leftover were weighed weekly to determine body weight, feed intake, body weight gain and feed conversion. After the beginning of the laying, the eggs production were evaluated.

Tissue and Blood Collection

Blood, bones and viscera were collected 17, 21, 25, 28, 31, 35, 42, 49, 56, 63 and 70 days of the experimental period, from one female per repetition (n=5). The collection period was around 14h00 coinciding with the final stage of eggshell formation in the uterus (shell gland).

Blood tests. Blood was collected by venipuncture of the jugular vein in tube without anticoagulant, centrifuged 3000 rpm for 15 min to obtain 2 mL of blood serum. Then it was frozen at -20°C and analyzed by Gold Analisa® commercial kits for the determination of total calcium (**Cat**), phosphorus (**P**), total protein (**Pt**), albumin (**Alb**) and alkaline phosphatase (**AP**) levels in a UV-VIS Evolution 300 spectrophotometer. The ionic calcium (**CaI**) was determined by formula $CaI = 6\ x\ Ca - (0{,}19\ x\ Pt) + A/3)/(0{,}19\ x\ Pt) + A + 6$, where CaI in mg/dL, Ca (Ca md/dL), Pt (total protein g/dL) and A (albumin (g/dL). The quails were sacrificed by cervical dislocation after losing consciousness from loss of blood volume.

Morphology of Genital Organs. The genital organs were isolated, the left ovary and oviduct, measured and weighed, for obtaining the absolute and relative weight (relative% = (absolute weight / weight of the bird) x 100). Fragments (0.5 cm) of the magnum and uterus were obtained on each female and fixed in 10% formaldehyde, 0.1M pH 7.4 phosphate buffer, processed in histological routine, embedded in paraffin, cut with 0.5 µm and stained with hematoxylin and eosin (HE). The sections were analyzed qualitatively to determine the physiological state of the oviduct segments.

Bones analyzes

In the same birds, after collection of the genitals, the femur and tibiotarsus bones were removed, dissected, weighed individually and stored at -20°C wrapped in gauze humidified in saline solution for lather analyzes of mineral composition and biomechanical aspects.

Bones Mineral Density. The bones were submitted to computed tomography on the i-Cat Next Generation (Imaging Sciences International, Hatfield, Pennsylvania, USA). All images selected for the research followed the protocol of a field of view

(FOV) of 8x8cm, *voxel* of 0.125mm and acquisition time of 26.9 seconds and were submitted to the same conditions of brightness and contrast, for standardization. The images generated were analyzed by the *XoranCatVersion* 3.1.62 image program (*Xoran Technologies, Ann Arbor, Michigan, USA*), where measurements were obtained (mm) and values of bone density, according to the Hounsfield Unit (HU), -1000 to +1000, where -1000 is the density corresponding to air, 0 to water and +1000 corresponds to bone density, to obtain the mineral density of the medullary and cortical bone, being the means of 3 points. The variables were obtained in cross-sections of the digital images in the middle region of the diaphysis.

***Seedor index*.** The Seedor index is an indication of the bone density and for its determination (Seedor et al., 1991), the same bones will be used for the bone resistance and then measured at their greatest length, by a pachymeter and weighed in a precision scale. The index was obtained by dividing the result of the bone weigh by its length. Seedor Index = Weight (mg) / Length (mm).

***Bones Strength*.** For the maximum strength measure of bone breakage, which is called bone strength, the tibiotarsus and femur bones of the birds were analyzed at the Food Engineering Laboratory in Brookfield Engineering Laboratories Inc. MA, USA, with TexturePro CT V1.4 build 17 software. The parameters used were compression test with 5% deformation, Trigger 500g, test speed 0.05 mm/s, TA7 and T7TPB device. The device, a three-point bend rig, where the bones were positioned with support only in the bone epiphyses region, being the probe force applied in the diaphysis region, always at the same point, measuring the force applied at the time of bone rupture (kg). Hardness means resistance to permanent deformations. Fractureness ou fractureability means fragility, that is, susceptibility to fracture and deformability before breaking.

Mineral content. A longitudinal cut was made in each bone and the medullar bone (with the marrow bone) was cured, weighed and analyzed separately from the rest of the bone. The ashes were obtained by calcining in a muffle at 600°C for six hours and after the cooling they were weighted. To decomplex the hydroxyapatite crystals and release the minerals, 10 mL of hydrochloric acid (6M) were added to the ash and placed on a heating plate and the solution evaporated in the exhaust hood until completely dry. The precipitate was dissolved by adding distilled and deionized water and in the filtered solution the volume was completed for 100 mL (Müller et al., 2012).

The solution was used to determined mineral content. Calcium concentrations were determined using a Varian Atomic Absorption AA240FS spectrophotometer with SpectrAA software from Victoria, Australia, and phosphorus concentrations by colorimetry on a UV-VIS Evolution 300 spectrophotometer.

Statistical analysis

On the basis of the results, normally distributed variables were subjected to ANOVA factorial 2 x 11 (light program X age) comparing means by Tukey test and not normally distributed parameters were analyzed by methods of the generals linear models, using R Studio program with 5% significance level.

RESULTS

Live Performance

The performance results are shown in table 2. For body weight and weight gain weekly, differences were observed between light programs at 35 and 42 days and in the range of 1 to 35 and 1 to 42 days respectively, where the birds in program 11L:13D gained more weight, and consequently, higher body weight than 14L:10D, although feed conversion was higher in the 14L:10D program in the same periods. The means of body weight showed peak at 56 days and remained stable for up to 70 days. Feed intake was influenced by the light program in the period 1-28 days, and the quails created with 14L:10D had higher consumption.

Table 2. Live performance in Japanese quail during 70 days under two light programs (n=55).

Age	Light program		Means	SEM	CV%	*P value*
	11L:13D	14L:10D				
			Body weight (g)			
28d	74.06	74.33	74.19	1.10	4.93	0.906
35d	104.57[a]	96.39[b]	100.85	1.69	5.57	0.039
42d	116.27[a]	107.36[b]	112.22	1.00	2.97	0.002
70d	139.93	140.49	140.18	1.02	2.42	0.792
			Weight gain (g)			
1-28d	68.67	68.94	68.80	1.10	5.31	0.906
1-35d	99.18[a]	91.00[b]	95.46	1.69	5.88	0.039
1-42d	110.88[a]	101.97[b]	106.83	1.00	3.12	0.002
1-70d	134.54	135.10	134.79	1.02	2.51	0.792
			Feed intake (g)			
1-28d	233.10[b]	244.24[a]	238.16	2.23	3.10	0.034
1-35d	360.78	369.42	364.71	2.67	2.43	0.142
1-42d	487.83	487.62	487.74	3.68	2.50	0.977
1-70d	1224.96	1212.33	1219.22	12.49	3.40	0.627
			Feed convertion (kg/kg)			
1-28d	3.40	3.55	3.47	0.05	4.88	0.182
1-35d	3.65[b]	4.06[a]	3.84	0.07	6.13	0.018
1-42d	4.40[b]	4.79[a]	4.58	0.06	4.11	0.008
1-70d	9.11	8.98	9.05	0.09	3.21	0.488

[ab] Means with different letters within the same line differ significantly in Tukey test ($p < 0.05$).

Regarding egg production evaluated weekly, it was observed that the quails under light program 11L:13D that the laying started at 40 days and at 70 days presented 47.9% of laying, while those raised in the 14L:10D the laying started with 42 days reaching 32.1% at 70 days. Figure 1 shows an increase in laying up to 63 days in both treatments, presenting a slight decrease in the following week.

There was no mortality, cannibalism or uterine prolapse problems in the repetitions when daylight hours were increased, between 35 and 70 days.

Genital organs

There was a significant interaction ($P < 0.05$) between the light programs and age of birds for body weight and oviduct relative weight (Table 3). Body weight showed a gradual increase up to 49 days, remaining stable and reached an average weight of 131.87g and 146.40g at 70 days, in the light programs 11L:13D and 14L:10D, respectively. At 35 and 49 days the quails submitted to the 11L:13D light program were heavier, while at 70 days this occurred in the 14L:10D light program.

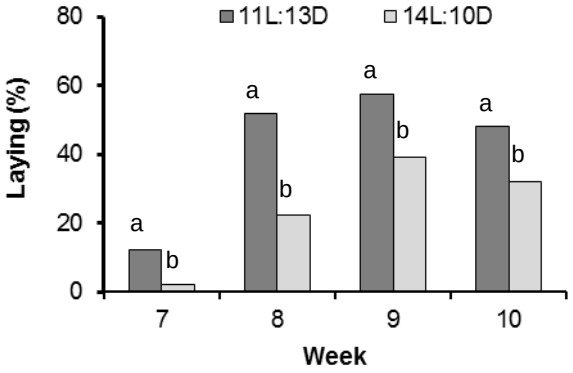

FIGURE 1 Weekly Laying (%) in Japanese quail 42 to 70 days under 2 light programs. The two programs presented similar behavior of 7 to 10 weeks, however the 11L: 13D light program always had higher posture.

Table 3. Relative weights of genital organs in Japanese quail until 70 days under two light programs (n=5).

	Body weight (g)		Ovary (%)		Oviduct (%)		Liver (%)	
Age (days)	A	B	A	B	A	B	A	B
17	55.12^{f}	54.27^{e}	-	-	-	-	2.76^{ab}	2.86^{ab}
21	63.12^{ef}	68.10^{de}	-	-	-	-	2.43^{b}	2.91^{ab}
25	76.5^{de}	77.33^{cd}	-	-	-	-	2.48^{b}	2.55^{ab}
28	78.31^{de}	73.98^{cd}	-	-	-	-	2.55^{b}	2.77^{ab}
31	86.31^{d}	85.76^{cd}	-	-	-	-	2.63^{b}	2.35^{b}
35	106.67^{cA}	91.89^{cB}	0.11^{b}	0.11^{c}	0.83^{b}	0.70^{b}	2.50^{b}	2.32^{b}
42	116.05^{bc}	113.42^{b}	1.68^{ab}	0.18^{c}	3.34^{aA}	0.87^{bB}	2.49^{b}	2.21^{b}
49	141.41^{aA}	127.07^{abB}	1.67^{ab}	0.87^{bc}	3.24^{aA}	1.94^{abB}	2.83^{ab}	2.35^{b}
56	129.73^{ab}	131.13^{ab}	3.84^{aA}	0.98^{abcB}	4.57^{aA}	2.10^{abB}	3.19^{ab}	2.39^{ab}
63	136.29^{a}	137.98^{a}	3.33^{a}	3.47^{a}	3.61^{a}	3.73^{a}	3.26^{ab}	2.97^{ab}
70	131.87^{abB}	146.40^{aA}	2.72^{a}	2.82^{ab}	3.52^{a}	3.11^{a}	3.54^{a}	3.34^{a}
Means	110.67	101.94	2.22^{A}	1.40^{B}	3.19^{A}	2.07^{B}	2.79	2.64
SEM	0.85		0.16		0.13		0.04	
CV (%)	9.22		68.47		38.83		16.43	
Light	0.615		0.019		<0.001		0.090	
Age	<0.001		<0.001		<0.001		<0.001	
Light *vs* Age	0.041		0.072		0.013		0.130	

Means with different small letters within the same column and capital letters within same line differ significantly in Tukey test ($p < 0.05$).
A: 11L:13D; B: 14L:10D

Table 4. Length (mm) of left oviduct in Japanese quail until 70 days under two light programs.

	Infundibulum		Magnum		Istmus		Uterus		Total	
Age (days)	A	B	A	B	A	B	A	B	A	B
42	33.28^{b}	23.10^{c}	87.04^{bA}	52.12^{bB}	48.55^{a}	38.28^{b}	26.6	22.13	195.47^{b}	135.61^{b}
49	34.01^{b}	35.68^{abc}	93.95^{ab}	83.46^{b}	50.73^{a}	41.09^{ab}	26.47	23.3	220.00^{ab}	183.53^{b}
56	49.48^{abA}	33.16^{bcB}	135.43^{aA}	80.18^{bB}	65.78^{aA}	43.61^{abB}	27.24^{A}	18.49^{B}	277.93^{aA}	175.43^{bB}
63	60.14^{aA}	46.04^{abB}	131.70^{a}	137.37^{a}	57.43^{a}	59.31^{a}	28.53	22.08	277.80^{a}	270.21^{a}
70	66.14^{a}	55.67^{a}	124.47^{ab}	129.62^{a}	60.87^{a}	56.36^{ab}	27.64	29.07	279.12^{a}	270.72^{a}
Means	48.61^{A}	38.73^{B}	114.52^{A}	96.55^{B}	56.67^{A}	47.73^{B}	27.30^{A}	23.01^{B}	250.06^{A}	207.10^{B}
SEM	1.56		3.41		1.47		0.84		6.39	
CV (%)	25.11		22.46		20.00		23.37		19.72	
Light	0.004		0.019		0.006		0.022		0.004	
Age	<0.001		<0.001		0.004		0.447		<0.001	
Light *vs* Age	0.446		0.029		0.154		0.389		0.133	

Means with different small letters within the same column and capital letters within same line differ significantly in Tukey test ($p < 0.05$).
A: 11L:13D; B: 14L:10D

For the oviduct relative weight, the means values were higher in the 11L:13D light program for 42, 49 and 56 days, where the difference could be observed between 35 and 42 days in the birds under the 11L:13D light program, and in birds under the 14L:10D light program this occurred between 56 and 63 days of age. There was an isolated effect of age and light program on ovary relative weight. From 35 days onwards there was an increase in ovary weight in both light programs, so that in the 11L:13D light program the ovary weight was higher at 56 days, and the birds in the 14D:10L light program showed an increase of ovary weight relative to 63 days of age (table 3). Suggesting that not all birds light program 14D:10L had reached sexual maturity, that is, they were later quails.

The relative liver weight showed significant differences in age, showing a small decrease between 21 and 42 days to 31 to 49 days, the 11L:13D and 14D:10L light programs, respectively, presenting an increase in the proportion, with a mean of 3.44% at 70 days (table 3).

To the morphometric analysis of the oviduct segments (table 4), between 42 and 70 days, only the magnum length showed interaction between the light program and age, being the 11L:13D light program the longest at 42 and 56 days, reaching almost 125 mm at 70 days. The infundibulum, isthmus and total oviduct length increased with the age of the birds and were longer in the 11L:13D light program. The uterus presented difference only in relation to the light program, where it was higher in birds under the 11L:13D light program.

Macroscopically, it was observed that the quails raised at 11L:13D light program had an onset of genital maturation prior to those raised in the 14L:10D treatment. The first signs of ovary enlargement occurred at 28 days. At 35 days the oviduct had an increase in its thickness, with visible pre-vitellogenic follicles (whites with 1 mm), eventual vitellogenic follicles (yellow up to 3 mm) and pre-ovulatory follicles (yellow plus 3 mm) on the ovary surface. The quails at 14L:10D light program presented this feature only at 49 days, and in some birds the ovary and oviduct were still at the found at the onset of maturation. At 63 days all birds analyzed for both lighting program presented ovary and oviduct developed (Figure 2).

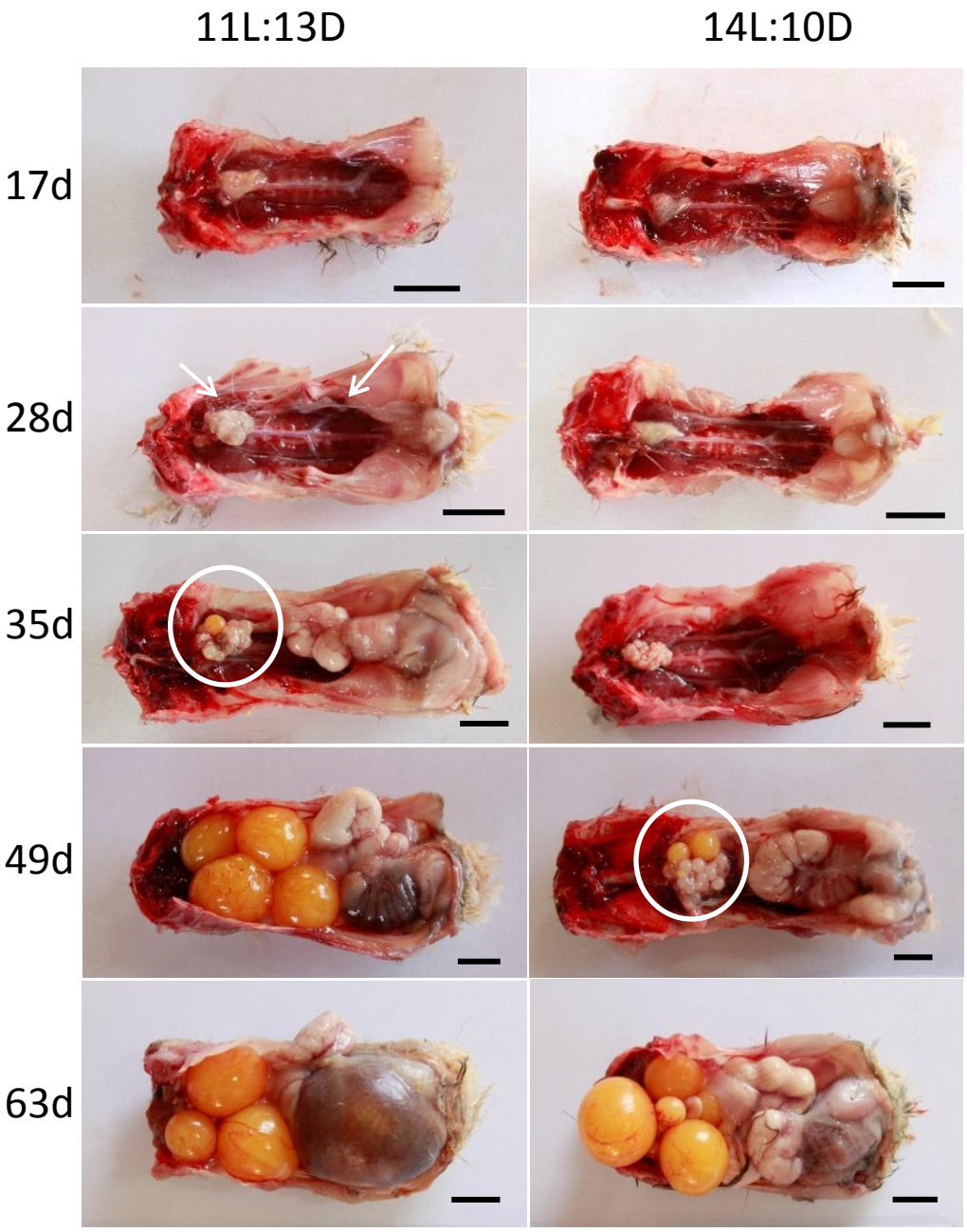

FIGURE 2 Macroscopy aspect of genital organs in Japanese quails submitted to 2 light programs. At 28 and 35 days the ovary and oviduct had started to development in the 11L:13D and 14L:10D light program, respectively (white arrows). Even as, the first yellow follicle and oviduct development showed at 35 and 49 days in the at 11L:13D and 14L:10D light program, respectively. At 63 days, all quails had functional genital organs.

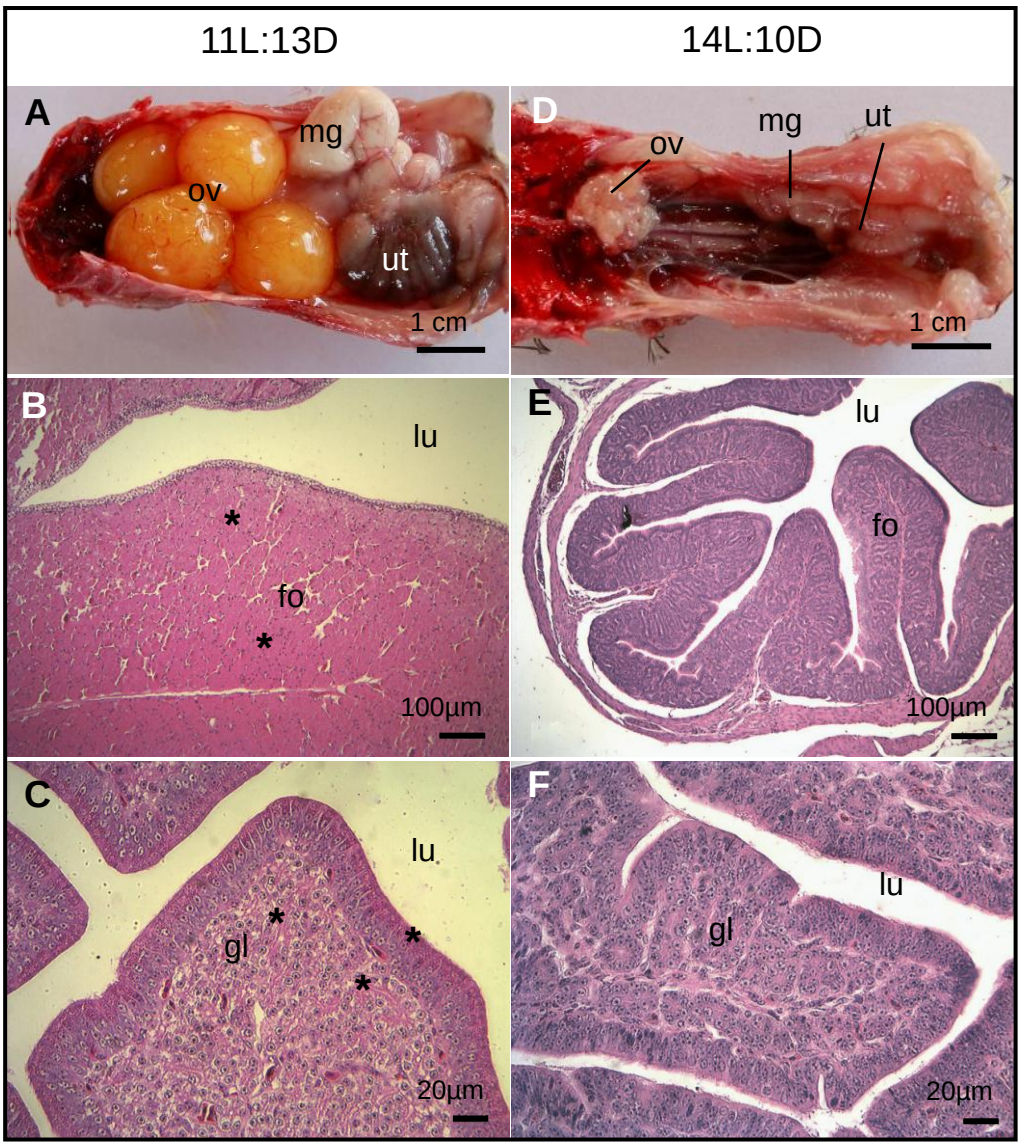

FIGURE 3 Macro and microscopic aspects of genital organs in quails at 49 days submitted to two different light programs. A, D) Macroscopical view of ovary (ov) and oviduct *in situ*. This maturation difference is also observed histologically. Both, magnum (mg) and uterus (ut) from treatment 11L:13D showed developed folds with glandular mucosa full of secretion (*). While in treatment 14L:10D. magnum has smaller diameter with low folds and uterus has no glandular secretion activity. lu: lumen. B-F) HE. Scale bar: A. D) 1 cm; B.E) 100 µm; C.F) 20 µm.

Histological analysis of the magnum and uterus confirmed the macroscopic observations where magnum presented high folds with glands filled with secretion and uterus with active glands and secretory epithelium (Figure 3).

Blood analysis

Table 5 shows the blood analyzes, where albumin and alkaline phosphatase had a significant effect on the interaction between the light program and age. Albumin increased with age and was higher in the 11L:13D light program at 31 and 42 days.

Alkaline phosphatase had a difference between treatments at 25 days, being higher in the 14L:10D light program. However, it can be observed that the highest averages occurred at the initial ages, 21 days in the light program 11L:13D and 25 days in the 14L:10D.

Regarding the age, the total calcium was higher in birds with 17 days, reducing up to 35 days, while it increased again up to 70 days. This increase occurred first in birds under 11D:13D light program at 42 days with 5.45 mg/dL, and then at 49 days in 14L:10D light program with 6.40 mg/dL. Phosphorus was similar to calcium, as it presented higher averages at 17 days, with a drop between 25 and 28 days and thereafter increasing gradually up to 70 days. There was no effect of the light program in serum levels of protein and ionic calcium, but only effect was observed for the age of the birds increasing values up to 70 days.

Mineral Bones analysis

In the femur bone analysis there was interaction of medullary bone weight, with a gradual increase over time and higher weight in 11L:13D light program at 35 and 56 days, but the weight at 70 days was similar in the 2 light programs. Total and cortical bone weights increased along the age. There was isolated effect of treatment on femur cortical bone in ashes percents with 31.13% and 29.44% in the 11L:13D and 14L:10D program, respectively. While to the ashes percentage of the femur medullary bone, analyzed from 49 days, there was no effect of treatment with overall average 17.17% (Table 6). It can be observed that the ashes content of cortical femur increased until 42 and 49 days in the 11L:13D and 14L:10D light programs, respectively, with the peak occurring at 63 days in both treatments.

Calcium and phosphorus content of the femur medullary bone (Table 7) showed interaction between the light programs and ages. In quails under 11L:13D light program there was more calcium in the ashes of medullary bone at 49 and 56 days, while in birds in the 14L:10D light program, this effect was observed at 63 days, with increase of the percentage of calcium and phosphorus percentage from 17 up to 70 days. The

phosphorus content increased significantly at 49 days in both treatments but was higher in 14L:10D light program.

All variables had an age effect increase with it , with the exception of the cortical Ca:P ratio that decreases reaching 0.93 and 0.91 at 70 days. It shows that in the femur bone, both in the cortical and in the medullary portion, the calcium content is higher and phosphorus is lower in younger quails.

There was an interaction between light programs and age in the percentage of ashes in tibiotarsus medullary bone, wherein the 11L:13D light program showed higher ashes content, especially in 49 and 56 days, compared to the 14L:10D light program. However, it was not significant for age between 49 and 70 days. The cortical, medullary weights and cortical ashes percentage had significant effects isolated from the light programs and age. The weight of cortical bone was higher in 14L:10D light program while to the medullary bone was lower. The ashes percentage was higher in the 11L:13D light program and there was difference between treatments at 17 and 35 days. The absolute weight of medullary bone was higher at 31 and 56 days in the 11L:13D light program. The tibiotarsus total weight had significant effect for age exhibited a gradual increase at 49 and 56 days, 14L:10D and 11L:13D light programs, respectively, remaining stable until 70 days of age (Table 8).

Table 5. Serum biochemistry in Japanese quail of 17 to 70 days under two light programs.

	Albumin (g/dL)		Phosphorus (mg/L)		Proteins (g/dL)		Alkaline phosphatase (U/L)		Total calcium (mg/dL)		Ionic Calcium (mg/dL)	
Age (days)	A	B	A	B	A	B	A	B	A	B	A	B
17	1.04^{d}	0.96^{cde}	5.76^{abc}	5.08^{ab}	2.99^{ab}	3.51^{ab}	802.78^{abc}	499.60^{b}	4.70^{bcde}	6.22^{abc}	3.62^{ab}	4.81^{ab}
21	0.78^{fd}	0.82^{de}	4.41^{bc}	4.57^{ab}	2.59^{ab}	1.23^{c}	1173.93^{a}	1021.76^{ab}	3.68^{cde}	2.18^{cd}	2.93^{ab}	1.82^{b}
25	1.25^{cd}	1.29^{abcde}	3.53^{c}	4.57^{ab}	1.90^{b}	1.92^{bc}	541.13^{bcB}	1217.82^{aA}	3.21^{de}	2.28^{cd}	2.48^{ab}	1.72^{b}
28	1.05^{d}	0.62^{e}	3.88^{bc}	3.71^{b}	2.56^{ab}	2.56^{abc}	591.65^{abc}	625.40^{ab}	2.99^{de}	3.82^{bcd}	3.25^{ab}	3.15^{ab}
31	1.94^{abcA}	1.18^{bcdeB}	4.54^{bc}	4.55^{ab}	2.36^{ab}	2.69^{abc}	480.01^{bc}	789.58^{ab}	3.08^{e}	2.27^{cd}	2.17^{b}	1.68^{b}
35	1.39^{bcd}	1.63^{abcd}	4.42^{bc}	4.46^{ab}	3.18^{ab}	3.03^{abc}	541.28^{bc}	726.75^{ab}	2.57^{e}	1.92^{d}	2.44^{ab}	1.27^{b}
42	1.95^{abcA}	1.01^{bcdeB}	6.61^{abc}	4.52^{ab}	2.10^{ab}	1.76^{bc}	325.54^{c}	387.14^{b}	5.45^{bcdeA}	2.32^{bcdB}	3.74^{ab}	1.81^{b}
49	1.54^{bcd}	1.82^{abc}	7.40^{ab}	6.20^{ab}	3.48^{ab}	2.81^{abc}	810.47^{abc}	529.77^{b}	6.90^{abcd}	6.4^{ab}	4.91^{ab}	4.44^{ab}
56	2.01^{abc}	1.74^{abcd}	8.84^{a}	5.97^{ab}	3.88^{a}	2.70^{abc}	539.28^{bc}	561.56^{ab}	7.72^{abc}	5.17^{abcd}	5.31^{a}	4.41^{ab}
63	2.10^{ab}	2.20^{a}	7.44^{ab}	7.86^{a}	3.87^{ab}	2.63^{abc}	969.55^{ab}	794.00^{ab}	8.10^{ab}	7.99^{a}	5.43^{a}	5.40^{a}
70	2.45^{a}	1.95^{ab}	5.94^{abc}	6.24^{ab}	3.59^{ab}	4.28^{a}	591.65^{abc}	570.31^{b}	10.11^{a}	8.07^{a}	5.49^{a}	5.43a
Means	1.59^{A}	1.38^{B}	5.70	5.28	2.95	2.65	670	702	5.32^{A}	4.42^{B}	3.80	3.27
SEM	0.04		0.17		0.09		28.23		0.18		0.15	
CV (%)	29.96		34.16		37.09		44.82		40.31		45.96	
Light	0.011		0.172		0.112		0.495		0.024		0.106	
Age	<0.001		<0.001		<0.001		<0.001		<0.001		<0.001	
Light *vs* Age	0.024		0.422		0.243		0.019		0.254		0.764	

Means with different small letters within the same column and capital letters within same line differ significantly in Tukey test ($p < 0.05$).
A: 11L:13D; B: 14L:10D

Table 6. Means of femur weights (g) and ashes (%) (cortical and medullary bone) in Japanese quail of 17 to 70 days under two light programs.

	Weights (g)						Ash (%)			
Age	Total		Cortical		Medullary		Cortical		Medullary	
(days)	A	B	A	B	A	B	A	B	A	B
17	0.229^{e}	0.221^{e}	0.215^{d}	0.205^{c}	0.014^{g}	0.015^{d}	22.34^{d}	22.99^{e}	-	-
21	0.303^{de}	0.328^{d}	0.281^{cd}	0.303^{b}	0.022^{fg}	0.024^{d}	22.36^{d}	21.15^{e}	-	-
25	0.332^{cd}	0.336^{d}	0.298^{bc}	0.302^{b}	0.036^{efg}	0.034^{cd}	24.64^{cd}	20.48^{e}	-	-
28	0.353^{cd}	0.345^{cd}	0.323^{abc}	0.322^{b}	0.030^{efg}	0.022^{d}	24.07^{cd}	23.44^{e}	-	-
31	0.340^{cd}	0.370^{cd}	0.293^{bc}	0.331^{b}	0.047^{def}	0.039^{cd}	26.74^{cd}	24.44^{e}	-	-
35	0.392^{bc}	0.374^{bcd}	0.336^{abc}	0.336^{ab}	0.056^{cdeA}	0.037^{cdB}	29.49^{c}	27.18^{de}	-	-
42	0.408^{abc}	0.396^{abcd}	0.355^{ab}	0.340^{ab}	0.054^{cde}	0.055^{bc}	37.20^{ab}	34.18^{bc}	-	-
49	0.442^{ab}	0.469^{a}	0.362^{ab}	0.371^{ab}	0.080^{abc}	0.084^{ab}	40.24^{ab}	39.72^{ab}	16.87	21.27
56	0.482^{a}	0.484^{a}	0.378^{a}	0.413^{a}	0.104^{aA}	0.071^{abB}	37.21^{abA}	32.41^{cdB}	20.70	-
63	0.399^{bc}	0.430^{abc}	0.328^{abc}	0.347^{ab}	0.071^{bcd}	0.083^{ab}	42.56^{a}	41.01^{a}	22.31	18.49
70	0.450^{ab}	0.460^{ab}	0.353^{ab}	0.364^{ab}	0.097^{ab}	0.096^{a}	35.62^{b}	36.88^{abc}	13.35	13.19
Means	0.375	0.383	0.32	0.33	0.06	0.05	31.13^{A}	29.44^{B}	18.31	17.65
SEM	0.004		0.003		0.001		0.293		1.189	
CV (%)	11.23		11.59		27.28		10.41		39.55	
Light	0.323		0.128		0.066		0.004		0.658	
Age	<0.001		<0.001		<0.001		<0.001		0.101	
Light *vs* Age	0.890		0.850		0.049		0.570		0.491	

Means with different small letters within the same column and capital letters within same line differ significantly in Tukey test ($p < 0.05$).
A: 11L:13D; B: 14L:10D

Table 7. Analysis of mineral content of the femur medullary and cortical bone in Japanese quail from 17 to 70 days of age under two light programs.

	Calcium (%)				Phosphorus (%)				Ca:P			
Age	Cortical		Medullary		Cortical		Medullary		Cortical		Medullary	
(days)	A	B	A	B	A	B	A	B	A	B	A	B
17	9.253^{c}	9.60^{d}	2.47^{b}	1.15^{d}	8.68^{b}	9.77^{ab}	0.96^{c}	0.57^{c}	2.02^{ab}	1.64^{abc}	1.09^{b}	1.02^{b}
21	10.01^{bc}	9.98^{cd}	1.76^{b}	1.92^{cd}	8.54^{b}	7.28^{b}	0.84^{c}	0.86^{c}	1.87^{abc}	2.05^{ab}	1.19^{ab}	1.40^{ab}
25	12.56^{bA}	10.29^{bcdB}	1.46^{b}	2.71^{cd}	9.28^{ab}	7.43^{b}	0.92^{c}	1.05^{c}	1.94^{ab}	2.43^{a}	1.36^{ab}	1.39^{ab}
28	12.74^{ab}	12.73^{abcd}	3.60^{b}	4.73^{bc}	9.40^{ab}	9.90^{ab}	2.24^{c}	1.88^{bc}	1.76^{abcd}	2.27^{a}	1.32^{ab}	1.17^{ab}
31	13.96^{ab}	13.57^{ab}	2.86^{b}	2.05^{cd}	10.48^{ab}	11.33^{a}	1.61^{c}	0.95^{c}	2.00^{a}	1.78^{abc}	1.25^{ab}	1.22^{ab}
35	14.54^{aA}	11.97^{abcdB}	1.84^{b}	2.45^{cd}	11.23^{ab}	9.81^{ab}	1.70^{c}	2.29^{bc}	1.06^{cde}	1.05^{bcd}	1.24^{ab}	1.33^{ab}
42	13.38^{ab}	13.01^{abc}	1.67^{b}	1.33^{cd}	10.38^{ab}	9.85^{ab}	2.77^{c}	2.33^{bc}	0.77^{e}	0.57^{d}	1.29^{ab}	1.33^{ab}
49	14.99^{a}	14.04^{a}	10.64^{aA}	1.04^{cdB}	11.45^{ab}	11.23^{a}	5.73^{bB}	7.64^{aA}	1.19^{abcdeA}	0.53^{dB}	1.25^{ab}	1.32^{ab}
56	14.03^{a}	12.88^{abcd}	9.76^{aA}	2.22^{cdB}	9.78^{ab}	9.68^{ab}	8.81^{a}	3.68^{b}	1.11^{bcde}	0.57^{d}	1.44^{a}	1.33^{ab}
63	14.59^{a}	16.07^{a}	10.42^{a}	8.96^{a}	12.15^{a}	10.28^{ab}	8.93^{a}	8.73^{a}	1.17^{abcde}	1.01^{cd}	1.22^{ab}	1.30^{ab}
70	15.15^{a}	15.01^{a}	7.21^{a}	7.31^{ab}	10.90^{ab}	10.86^{ab}	8.03^{a}	8.49^{a}	0.93^{de}	0.91^{cd}	1.31^{ab}	1.42^{a}
Means	13.20	12.65	4.88^{A}	3.26^{B}	10.21	9.77	3.87	3.50	1.28	1.28	1.44	1.34
SEM	0.15		0.16		0.16		0.09		0.043		0.017	
CV (%)	12.62		40.90		17.11		27.01		32.71		14.19	
Light	0.083		<0.001		0.134		0.058		0.050		0.900	
Age	<0.001		<0.001		<0.001		<0.001		<0.001		0.006	
Light *vs* Age	0.322		<0.001		0.488		<0.001		0.088		0.599	

Means with different small letters within the same column and capital letters within same row differ significantly in Tukey test ($p < 0.05$).
A: 11L:13D; B: 14L:10D

The results of tibiotarsus mineral analysis are shown in table 9. The phosphorus content and the Ca:P ratio of the medullary bone had a significant effect on the interaction between treatments and age. The phosphorus content of the medullary bone increased along the age with difference between treatments at 49 days, with 5.31 and 2.51% in the 11L:13D and 14L:10D light programs, respectively. The Ca:P ratio in medullary bone decreased along the age showing differences between treatments at 21 days, with 1.76 and 2:1 for 11L:13D and 14L:10D light programs, respectively.

All variables analyzed for tibiotarsus had a significant effect for age. Calcium in cortical bone increased along the age in both treatments, whereas the calcium in medullary bone of the11L:13D light program was lower at 25 days, whereas the 14L:10D light program had the lowest average at 42 days. The two treatments had the highest value of calcium in medullary bone at 63 days old. The phosphorus content of the tibiotarsus cortical region remained stable over the age in the 14L:10D light program and showed a gradual increase in the 11L:13D light program, almost doubling the concentration of 17 to 70 days.

Bone density

The bone density was higher in the cortical region of both bones (table 10). There was interaction effect between the treatments and age of the density in the medullary bone in the femur and tibiotarsus. The femur and the tibiotarsus medullary bone were dense in the 14L:10D light program at 25 days, reversing 11L:13D light program at 49 and 56 days of age. There was a significant density increase in the femur and tibiotarsus cortical region as the birds became older.

Bones strength

The bone strength variables of both bones were significant differences only for age. It presented a gradual increase until the 49 days of age, where the bones presented higher strength, with a discreet decrease until the 70 days. The time to first break was higher in younger birds, decreasing as were older. Seedor index was statistically different only for age, increasing gradually (table 11).

Table 8. Average of tibiotarsus weights (g) and ashes (%) (cortical and medullary bone) in Japanese quail of 17 to 70 days under two light programs.

	Weights (g)						Ash (%)			
Age	Total		Cortical		Medullary		Cortical		Medullary	
(days)	A	B	A	B	A	B	A	B	A	B
17	0.294^{d}	0.334^{c}	0.289^{c}	0.321^{c}	0.008^{e}	0.013^{c}	25.28^{eA}	21.18^{fB}	-	-
21	0.359^{cd}	0.434^{abc}	0.341^{bcB}	0.413^{abcA}	0.018^{de}	0.021^{c}	28.14^{e}	25.18^{ef}	-	-
25	0.408^{bc}	0.408^{bc}	0.379^{abc}	0.382^{bc}	0.029^{cde}	0.026^{c}	26.05^{e}	25.21^{ef}	-	-
28	0.411^{bc}	0.420^{abc}	0.380^{abc}	0.398^{abc}	0.030^{cde}	0.022^{c}	29.65^{de}	31.02^{cde}	-	-
31	0.428^{bc}	0.439^{ab}	0.376^{abc}	0.406^{abc}	0.052^{bcA}	0.034^{bcB}	29.56^{de}	30.58^{de}	-	-
35	0.433^{abc}	0.456^{ab}	0.393^{ab}	0.420^{abc}	0.040^{cd}	0.036^{bc}	34.65^{cdA}	29.94^{deB}	-	-
42	0.471^{ab}	0.478^{ab}	0.423^{ab}	0.418^{ab}	0.049^{c}	0.060^{ab}	38.50^{bc}	35.53^{bcd}	-	-
49	0.503^{ab}	0.518^{a}	0.426^{a}	0.426^{ab}	0.077^{ab}	0.072^{a}	40.57^{ab}	41.02^{ab}	14.35^{A}	4.70^{bB}
56	0.526^{a}	0.530^{a}	0.440^{a}	0.476^{a}	0.086^{aA}	0.062^{abB}	40.53^{ab}	37.49^{abc}	18.8^{A}	7.37^{abB}
63	0.460^{ab}	0.473^{ab}	0.382^{abc}	0.382^{abc}	0.078^{ab}	0.085^{a}	45.08^{a}	43.71^{a}	13.85	14.54^{a}
70	0.474^{ab}	0.493^{ab}	0.386^{ab}	0.410^{abc}	0.088^{a}	0.074^{a}	42.05^{ab}	42.87^{a}	12.29	8.28^{ab}
Means	0.433	0.453	0.383^{B}	0.405^{A}	0.05^{A}	0.045^{B}	34.55^{A}	33.06^{B}	14.82^{A}	8.72^{B}
SEM	0.005		0.08		0.001		0.28		0.80	
CV (%)	11.29		11.3		28.3		8.61		40.05	
Light	0.088		0.025		0.023		<0.001		0.001	
Age	<0.001		<0.001		<0.001		<0.001		0.119	
Light *vs* age	0.904		0.758		0.078		0.233		0.046	

Means with different small letters within the same column and capital letters within same line differ significantly in Tukey test ($p < 0.05$).
A: 11L:13D; B: 14L:10D

Table 9. Analysis of mineral content of the tibiotarsus medullary and cortical bone in Japanese quail from 17 to 70 days of age under two light programs

	Calcium (%)				Phosphorus (%)				Ca:P			
Age	Cortical		Medullary		Cortical		Medullary		Cortical		Medullary	
(days)	A	B	A	B	A	B	A	B	A	B	A	B
17	10.69^{c}	10.82^{c}	6.35^{ab}	5.08^{ab}	6.61^{c}	8.93	0.46^{e}	1.16^{bc}	1.46	1.24^{ab}	4.32^{a}	5.22^{a}
21	13.34^{abc}	12.68^{bc}	4.27^{c}	2.50^{b}	7.71^{bc}	7.97	1.39^{de}	0.52^{c}	1.56	1.62^{a}	2.80^{bB}	4.94^{aA}
25	13.40^{abc}	12.11^{bc}	2.25^{c}	4.43^{ab}	10.79^{ab}	9.13	1.01^{e}	2.63^{bc}	1.32	1.34^{ab}	2.71^{b}	2.27^{b}
28	15.18^{ab}	16.03^{a}	6.41^{ab}	5.70^{ab}	10.60^{ab}	11.29	3.03^{cde}	2.51^{bc}	1.46	1.37^{ab}	1.89^{c}	1.83^{bc}
31	15.45^{a}	16.04^{a}	3.87^{c}	6.26^{ab}	10.90^{ab}	11.89	1.73^{de}	3.19^{bc}	1.43	1.39^{ab}	1.61^{cde}	1.89^{bc}
35	15.96^{a}	14.84^{ab}	4.01^{c}	3.35^{ab}	12.28^{a}	10.63	2.33^{de}	1.85^{bc}	1.45	1.33^{ab}	1.76^{cd}	2.03^{b}
42	12.54^{bc}	11.04^{c}	3.89^{c}	1.43^{b}	10.29^{abc}	8.42	3.88^{cd}	2.88^{bc}	1.22	1.35^{ab}	0.63^{f}	0.70^{d}
49	12.62^{bc}	12.62^{bc}	4.95^{c}	3.48^{ab}	10.48^{abc}	9.95	5.31^{bcA}	2.51^{bcB}	1.22	1.32^{ab}	0.92^{ef}	0.79^{d}
56	12.59^{bc}	10.82^{c}	6.90^{ab}	1.99^{b}	10.38^{abc}	10.24	8.28^{a}	3.85^{b}	1.07	1.22^{b}	0.83^{f}	0.45^{d}
63	14.53^{ab}	14.39^{ab}	10.04^{a}	8.42^{a}	10.81^{ab}	11.48	9.10^{a}	6.95^{a}	1.30	1.24^{ab}	1.09^{def}	1.17^{cd}
70	13.60^{abc}	13.53^{abc}	6.76^{ab}	6.49^{ab}	11.13^{ab}	10.86	7.84^{ab}	8.48^{a}	1.23	1.27^{ab}	0.85^{f}	0.76^{d}
Means	13.63	13.17	5.43	4.47	10.18	10.07	4.03^{A}	3.32^{B}	1.35	1.33	1.76^{B}	2.00^{A}
SEM	0.13		0.23		0.18		0.12		0.020		0.037	
CV (%)	10.30		48.83		18.69		35.75		16.19		22.31	
Light	0.090		0.061		0.587		0.002		0.812		<0.001	
Age	<0.001		<0.001		<0.001		<0.001		0.003		<0.001	
Light *vs* age	0.428		0.073		0.307		<0.001		0.669		<0.001	

Means with different small letters within the same column and capital letters within same line differ significantly in Tukey test ($p < 0.05$).
A: 11L:13D; B: 14L:10D

Table 10. Bones density in Hounsefield Units (HU) in Japanese quail from 17 to 70 days under two light programs.

	Femur				Tibiotarsus			
Age	Cortical		Medullary		Cortical		Medullary	
(days)	A	B	A	B	A	B	A	B
17	235.68^{de}	209.01^{cd}	-313.67^{bc}	-295.89^{ab}	403.33^{bcd}	385.30^{b}	-206.36^{bc}	-183.54^{abc}
21	210.99^{de}	364.83^{abcd}	-386.25^{c}	-317.33^{ab}	332.13^{cd}	488.82^{ab}	-332.24^{cde}	-344.48^{c}
25	128.07^{e}	122.10^{d}	-628.44^{dB}	-336.70^{abA}	280.89^{d}	261.04^{b}	-457.00^{eB}	-301.73^{bcA}
28	211.07^{de}	232.16^{de}	-419.15^{cd}	-383.49^{b}	316.18^{d}	302.75^{b}	-422.23^{de}	-291.05^{bc}
31	278.24^{cde}	349.45^{abcd}	-413.80^{cd}	-436.13^{b}	331.48^{cd}	380.35^{b}	-254.98^{bcde}	-358.17^{c}
35	387.49^{bcd}	418.56^{abc}	-320.64^{abc}	-249.73^{ab}	399.53^{bcd}	451.40^{ab}	-254.85^{bcde}	-211.69^{abc}
42	479.68^{abc}	287.97^{bcd}	-291.09^{abc}	-341.25^{ab}	503.26^{bcd}	335.69^{b}	-245.06^{bcd}	-306.54^{c}
49	491.73^{abc}	509.20^{ab}	-203.30^{abc}	-254.60^{ab}	539.86^{abc}	481.54^{ab}	-79.30^{abA}	-217.67^{abcB}
56	612.71^{a}	493.21^{ab}	-130.70^{abA}	-317.11^{abB}	738.61^{a}	647.27^{a}	17.39^{aA}	-215.73^{abcB}
63	569.87^{ab}	561.24^{a}	-131.20^{ab}	-143.01^{a}	593.21^{ab}	618.39^{a}	-72.74^{ab}	-70.66^{ab}
70	560.09^{ab}	582.38^{a}	-91.95^{a}	-111.68^{a}	572.61^{ab}	674.45^{a}	-76.52^{ab}	-41.19^{a}
Means	378.69	375.46	-302.74	-289.72	455.55	457.00	-216.72	-231.13
SEM	10.41		10.47		10.25		10.07	
CV (%)	29.76		-38.74		23.96		-49.53	
Light	0.910		0.496		0.870		0.519	
Age	<0.001		<0.001		<0.001		<0.001	
Light *vs* Age	0.081		0.015		0.071		0.004	

Means with different small letters within the same column and capital letters within same line differ significantly in Tukey test ($p < 0.05$).
A: 11L:13D; B: 14L:10D

Table 11. Bone strenght of the femur and tibiotarso bones Japanese quail from 17 to 70 days of age under two light programs.

	Femur				Tibiotarsus			
	Hardness (kg)	Fracture (kg)	Time (s)	Seedor index (mg/mm)	Hardness (kg)	Fracture (kg)	Time (s)	Seedor index (mg/mm)
Light Program								
11L:13D	2.40	2.38	10.64	14.06	2.38	2.35	12.08	12.42
14L:10D	2.36	2.25	10.49	14.10	2.47	2.42	12.22	12.86
Age (days)								
17	1.51^{d}	1.50^{d}	10.48^{abc}	11.11^{e}	1.47^{e}	1.42^{e}	15.19^{a}	11.83^{b}
21	2.10^{cd}	2.10^{bcd}	10.63^{abc}	13.17^{d}	1.92^{de}	1.74^{de}	11.07^{b}	12.34^{b}
25	1.59^{d}	1.59^{d}	12.30^{abc}	13.43^{cd}	1.59^{e}	1.52^{e}	12.30^{ab}	12.28^{b}
28	1.93^{d}	1.92^{cd}	13.88^{a}	13.57^{cd}	2.01^{cde}	2.00^{cde}	14.02^{ab}	12.64^{ab}
31	2.05^{cd}	2.05^{bcd}	12.14^{abc}	13.28^{cd}	2.03^{cde}	2.03^{cde}	12.24^{ab}	12.70^{ab}
35	2.26^{bcd}	2.26^{bcd}	12.43^{ab}	13.24^{cd}	2.33^{bcd}	2.29^{bcd}	12.47^{ab}	11.70^{b}
42	2.85^{ab}	2.73^{ab}	9.62^{abc}	14.17^{bcd}	2.58^{bc}	2.51^{bc}	10.85^{b}	12.48^{b}
49	3.39^{a}	3.12^{a}	8.75^{d}	15.79^{ab}	3.39^{a}	3.39^{a}	11.58^{b}	12.96^{ab}
56	2.76^{abc}	2.74^{ab}	9.19^{cd}	16.24^{a}	3.24^{a}	3.23^{a}	12.13^{ab}	14.35^{a}
63	3.02^{ab}	2.76^{ab}	8.58^{d}	15.14^{abc}	3.21^{a}	3.21^{a}	10.92^{b}	12.79^{ab}
70	2.71^{abc}	2.67^{abc}	8.22^{d}	15.71^{ab}	2.94^{ab}	2.94^{ab}	10.88^{b}	12.92^{ab}
SEM	48.38	48.98	0.19	0.12	39.84	42.07	0.23	0.12
CV (%)	22.16	23.00	19.82	9.39	17.95	19.26	20.51	10.31
Light	0.472	0.146	0.792	0.913	0.317	0.486	0.716	0.093
Age	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001	0.001
Light *vs* Age	0.889	0.701	0.473	0.773	0.681	0.526	0.517	0.618

Means with different small letters within the same column differ significantly in Tukey test ($p < 0.05$).

DISCUSSION

Live performance

Regarding performance, Shit et al. (2014) observed that Japanese quails reaching maximum body weight at 57 days in the 14L:10D light program. Nishimura et al. (2007) observed a rapid increase in body weight up to 30 days of age. Finco et al. (2016), studying quails of the same lineage, observed that the maximum growth rate occurred at 16.27 days, where it observed weight at 21 and 70 days of 87.51 and 148.81g, whereas in this study it was 65.61 and 139.14g, respectively. The 3 hours more light than the program quails with 14L:10D program increased the feed intake between 1 and 28 days.

Quails with 28 and 35d were heavier in the 11L:13D than 14L:10D light program, probably because the genital organs had already started its development. This fact justified the higher body weight gain at the same ages. Broiler breeders with a higher body weight tends to delayed sexual maturity and worse egg production and quality (Rutz et al., 2007). In this study, the quails remained housed in groups of 50 birds in cages of 0.80 m^2, therefore, it should be taken into account that social competitiveness can cause stress by increasing blood levels of corticosteroids, changing the feed intake as well as inducing reproductive problems (Langen et al., 2017), including the hormonal imbalance that normally occurs at the beginning of sexual maturity stage (Shit et al., 2014). Besides, the laying falling observed in the last week was probably due to a technical problem with the timer around 66 days.

Several factors influence the age of sexual maturity of birds, including body weight, photoperiod and neuroendocrine stimulation (Shit et al., 2014). Posture data are similar with Langen et al. (2017) who observed the first egg at 41 days and at 58 days all quails had reached sexual maturity with a totally artificial of 14L:10D light program.

Genital organs and liver

In this study it was observed that Japanese quail ovary represented 0.11 to about 2.70%, of 35 to 70 days, respectively, while in broilers breeder the ovary represents about 0.02% of the body weight before sexual maturity and reaches 3% in the reproductive phase (Macari et al., 2005). Nunes et al. (2016) observed oviduct and ovary relative weights of 5.92 and 2.57%, respectively, in quails with 163.20g body

weight and 24.72 cm oviduct length at 57 days of age. At 64 days the values found were 4.14 and 3.86% for relative weight of oviduct and ovary, 148.53g body weight and 30.60cm for total oviduct length.

The oviduct growth is stimulated by estrogen which causes cell differentiation of mucosal epithelium, resulting in tubular glandular cells, ciliated cells and globular cells, and progesterone is responsible for the production and secretion of ovalbumin and conalbumin (Macari et al., 2005; Rutz et al., 2007).

It is considered a mature and active ovary that has follicles of various sizes and in several stages (Sreesujatha et al., 2016). When a follicle reaches 6 to 8 mm, it enters the hierarchy causing atresia in other small ones (Rutz et al., 2007). Mattsson and Brunström (2017) reported that Japanese quails under a 16L: 8D light program already had visible left oviduct at 3 weeks of age. Shit et al. (2014) did not observe yellow follicles until the 39 days, only appearing some only with 42 days and a large amount of follicles at 57 days, in quails under a program with 14 hours of light.

The early development of the genital organs of birds occurs before the first egg and ends at the peak period (Finco et al., 2016). The first external signal is sexual differentiation by plumage which occurs around 29 days of age (Langen et al., 2017), that is, before puberty (Etches, 1996). Puberty bird is characterized by the development of the oviduct and medullary bone combined with increased absorption of calcium from the intestine into the egg production (Rutz et al., 2007, Bar 2009), considered at around 8 weeks old (Hosseini et al., 2016; Mattsson and Brunström, 2017). In sexual maturation phase, osteoblasts cease activity on cortical and trabecular bone, to develop medullary bone (Rodriguez-Navarro et al., 2018).

For Shit et al. (2014) the goal for laying quails is that the sooner the sexual maturity, the better will be the posture. In laying hens, it is recommended to initiate the light stimulus later when all the birds are in sexual maturity and thus to standardize the lot (Rutz et al., 2007). In addition, the gradual increase of the luminous stimulus avoids prolapse cases in laying hens (Etches, 1996), which was not observed in the quails of this study that underwent rapid luminous increase.

Usually in commercial laying hens, 17 hours of light (natural + artificial) are used (Borille et al., 2013). The luminous increase can be done from 1 to 2 hours per week up to 14L:10D for laying hens; however, if the increase is performed 15 minutes per week up to 18L:6D delays the refractory period (Etches, 1996). Jácome et al. (2012)

to evaluate different types of light source in Japanese quails, initiated a light increment of 30 minutes per week at 42 days of age, taking 8 weeks from 13 to 17 hours, ending at 50 weeks old. In this study, the two light programs 11L:13D and 14L:10D fixed to 35 days old was 30 minutes incremented every 3 days until reached 17 hours of light. This period lasted 33 days in the 11L: 13D light program and 15 days in the 14L: 10D program, when all birds reached a 17L: 7D light program at 69 days old.

Regarding liver data, the relative weight is associated with the increase of the body weight added to the reproductive organ, since the absolute values of liver weight showed a gradual increase from 17 to 70 days. Another fact to be considered is that during the sexual maturation stage, several metabolic events occur at the same time overloading the liver (Barbosa et al., 2010), besides the exchange of ration that occurred at 42 days with appropriate levels of calcium energy protein and phosphorus for laying. Another important role of the liver in laying birds is the production of vitellogenic content of the follicles (Morais et al., 2012) by stimulating estrogen (Etches, 1996; Rutz et al., 2007). The onset of laying increase the liver synthesis of follicular growth.

Through ultrasound studies, it was possible to identify ring-shaped alternate structures in the follicles in the layers of yolk deposition in the first follicles at the stage of sexual maturity (Hosseini et al., 2016) and white and yellow yolk alternated in the preovulatory follicles (Sreesujatha et al., 2016). The basal medium hypothalamus is responsible for the perception of the day-long and signals to the beginning of the genital organs development, through the release of GnRH and sex hormones (Goodson et al., 2005; Rutz et al., 2007; Hosseini et al., 2016; Srivastava and Chaturvedi 2016), which is influenced by body weight gain (Shit et al., 2014). In quails, the increase or decrease of the light stimulus is immediately perceived by the hypothalamus, even if it takes a few weeks for reproductive signals to be observed (Etches, 1996).

The release of GnRH is very sensitive to changes in the photoperiod, birds reach sexual maturity earlier when stimulated with a shorter light period in the rearing phase. On the other hand, in some birds, long periods of light can cause refractoriness to light-sensitive hormones (Rutz et al., 2007), because it is not possible to maintain the secretion of gonadotropins for a long time (Etches, 1996). Srivastava and Chaturvedi (2016) have suggested that the mechanism of hormonal stimulation to promote sexual maturation may be the same responsible for the circadian cycle in quails.

According to Etches (1996) there are two important signs to activate the circadian cycle of birds. First is to stimulate the photosensitive phase that occurs 11 hours after the beginning of the light stimulus, during two hours. Second is the light stimulus in the photosensitive phase, which activates the hypothalamus, to activate the release of hormones.

The day length causes more effect on the circadian cycle and behavior of birds than the light intensity (Blatchford et al., 2012), because 5 lux is enough to activate photoreceptors, although the difference of luminosity between the photophase (period of light) and scotophase (dark period) (10:1) is quite relevant (Etches, 1996).

Blood analysis

The follicle has about 12% protein, derived from plasma albumin, which explains the increase of albumin at 31 and 42 days in the quails under 11L:13D light program, that is, in the period before and after the first ovulation (Etches, 1996; Scholtz et al., 2009). The values found by Sedaghat Torshizi and Karimi (2017) were 3.29 g/dL of albumin and 5.57 g/dL of total protein in quails 42 days old, it is higher than the values described in table 6. However, similar values were obtained by Scholtz et al. (2009) in laying quails (Albumin 1.5 g/dL and total protein 3.65 g/dL).

Alkaline phosphatase participates only at the beginning of bone matrix mineralization and not its progress, that is, the activation of osteoblasts (Van De Velde et al., 1985). The mean values of alkaline phosphatase presented significant variation between ages and treatments, possibly because they are quails in growth, when many metabolic changes are occurring, such as bone and ovary growth that require more fromliver.

Therefore, liver enzymes show greater fluctuation in serum levels because the liver participates in the metabolism of all nutrients (Barbosa et al., 2010), which can lead to instability in results, in addition, commercial kits are tested for repeatability and reproducibility in specific analyzes for serum levels in humans. Calcium and phosphorus metabolism was more intense in young quails when bone mineralization is high at 17 days and in the sexual maturity when a higher absorption of calcium by the intestines occurred to form the medullary bone, and consequently the eggshell formation. Serum levels of calcium and phosphorus increased at 42 days probably by feed exchange.

Bone analysis

Studies with Japanese and European quail conducted by Figueroa (2016) concluded that the highest growth rate occurred at 14 and 21 days for femur and tibiotarsus bone respectively, besides that, observed that the ashes deposition in tibiotarso is almost nonexistent after the 21 days. Nishimura et al. (2007) observed that the increase in tibiotarsus length occurs up to 40 days old reaching 43mm, coinciding with the closure of the epiphyseal line, and in this study, it was observed at 49 days with 45.79 mm (unpublished data).

In this experiment, it was not possible to determine the ashes percentage of medullary bone between 17 and 42 days because they consisted of very small samples, but from that period it was observed that there was a gradual increase of the ashes until the 49 days in both treatments and it remained stable until the end of the experiment. The light program 14L:10D presented higher weight and lower ashes in the cortical bone as well as lower weight and ashes in the medullary bone, probably because its mineralization has been slower, showing little variation over time.

The quail presented a peak of mineralization of the femur and tibiotarsus cortical around 28 days, with slower deposition up to 49 days and remaining stable up to 70 days. Suggesting that recruitment of minerals, especially calcium, is also to form the medullary bone (Van De Velde et al., 1985). However, the bone density presented higher values only around 56 days. Nishimura et al. (2007) considered that bone strength is the density sum of the cortical and medullary bone. Seedor index observed were similar to those found by Figueroa (2016) of 12.84 and 11.48 for femur and tibiotarsus, respectively.

The observed increase in mineral content in analyzes of the femur bone marrow suggests the formation of medullary bone partially filling the cavity of the long bones in pelvic limbs, which is characterized by islets of apatite crystals and collagen fibers isolated and surrounded by osteoclasts (Van De Velde et al., 1985; Rodriguez-Navarro et al., 2018), making the bone cavity more radiopaque as observed in the imaging tests (Nishimura et al., 2007). In quails created with 11L:13D light program, the medullary bone seems to have developed up to 2 weeks before (49 days) than quails under the 14L:10D light program, reaching the same level at 70 days. In this tibiotarsus bone it occurred at 56 and 63 days in the 11L:13D and 14L:10D light programs, respectively.

Sexual maturity is represented not only by the genital organ development, but by the involvement of the endocrine system to activate the digestive and bone systems in providing calcium for egg formation. The development of medullary bone occurs in sexual maturation phase by the action of estrogen produced by the ovaries (Rutz et al., 2007, Hiyama et al., 2012), increasing calcium absorption capacity by the intestines in that phase.

The calcium content of the femur medullary bone presented the lowest value at 42 days, coinciding with the first laying, because the shell formation caused a depletion of calcium source of medullary bone. It can be assumed that the serum calcium level at 35 days decreased for medullary bone formation, since the quails were still with growth feed. However, as soon as the production feed was offered at 42 days, an increase of calcium in serum and bone levels was observed, being according to Van De Velde et al. (1984) that if absorption is low the bone formation will also be. The results demonstrate that the medullary bone formation occurred first in the femur, it is possible to observe that the metabolism of medullary bone formation is very fast and it is related only to the metabolism of eggshell formation (Van De Velde et al., 1985; Rodriguez-Navarro et al., 2018).

As Figueroa (2016) observed, the strength, Seedor Index and bone density increased as bone mineralization content increased along the growth. However, breakage time decreases over the age, suggesting that young quail bones are more flexible by having a lower degree of mineralization and the strength is imparted by the presence of collagen fibers and connective tissue (Nishimura et al., 2007). By contrast, although the mineralized bone supports more load, the breaking time is lower because it presented more abrupt fractures.

Many authors have pointed out that quails have similar metabolism and physiology with laying hens, justifying that the general management, even in the light program, is the same. However, since quails are more rustic and there is a lack of advancement in genetic technology such as chickens and laying hens, more research on quail farming is needed.

CONCLUSION

Japanese quail under 11L:13D light program began the posture at 40 days, and genital organs had started the development 1 week before those exposed to 14L:10D light program. At 63 days all birds analyzed for both lighting program presented ovary and oviduct developed. Medullary bone developed first in the femur bone cavity and actively participates in quails on calcium metabolism during eggshell formation.

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V. CONSIDERAÇÕES FINAIS

A literatura considera as codornas como modelo experimental por serem menores e de crescimento rápido, e acabam por diminuir muito o custo das pesquisas, considerando os mesmos manejos que galinhas e frangos. Contudo, as codornas são mais rústicas e ainda não têm a tecnologia genética tão desenvolvida.

Os resultados observados mostram entendimento melhor acerca do ciclo de formação do ovo, já que codornas realizam a ovoposição no período vespertino, sendo um ponto crucial na diferença entre as galinhas poedeiras, no que se refere ao metabolismo do cálcio.

O programa de luz testado possibilitou avaliar uma proposta diferente no manejo, visando agilizar e aproveitar as características das codornas para otimizar e rentabilizar a criação. Entretanto, faz-se necessário mais pesquisas para estabelecer referências no metabolismo e manejo de codornas japonesas.