UNIVERSIDADE ESTADUAL DE MARINGÁ
CENTRO DE CIÊNCIAS AGRÁRIAS

MORFOLOGIA DE ÓRGÃOS DO SISTEMA DIGESTÓRIO EM CODORNAS E OS EFEITOS DO JEJUM PÓS-ECLOSÃO

Autora: Flavia Kleszcz da Cruz
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Tese apresentada como parte das exigências para a obtenção do título de DOUTORA EM ZOOTECNIA, no Programa de Pós-graduação em Zootecnia da Universidade Estadual de Maringá- Área de concentração Produção Animal.

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TITULAÇÃO: Doutora em Zootecnia - Área de Concentração Produção Animal

APROVADA em 23 de fevereiro de 2018.

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Orientadora

“Aqueles que semeiam com lágrimas, com cantos de alegria colherão. Aquele que sai chorando enquanto lança a semente, voltará com cantos de alegria, trazendo os seus feixes” (Salmos 126:5,6).

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nos momentos mais difíceis.

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BIOGRAFIA

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Em dezembro de 2010, concluiu a graduação em Zootecnia pela Universidade de Mato Grosso do Sul.

Em março de 2011, iniciou no Curso de Pós-Graduação em Zootecnia, em nível de mestrado, pela mesma instituição, submetendo-se aos exames finais de defesa de dissertação em fevereiro de 2013.

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III. Development and growth of digestive system organs of European and Japanese quails at 14 days post-hatch

RESUMO

Foram realizados dois experimentos com o objetivo de avaliar o desenvolvimento e o crescimento de órgãos do sistema digestório, do 11º dia de incubação até os 14 dias de vida pós-eclosão e a influência do jejum pós-eclosão sobre o desempenho zootécnico e o desenvolvimento de órgãos do sistema digestório de codornas. No experimento I, objetivou-se avaliar o desenvolvimento e crescimento de órgãos do sistema digestório, do 11º dia de incubação até os 14 dias de vida pós-eclosão em codornas europeias e japonesas. Ovos das matrizes de codornas foram selecionados por peso (europeia 11,80±0,59 e japonesa 9,79±0,49g) e incubados em incubadora automática com 60% de umidade e a 37,6ºC, com viragem automática. Nos dias 11, 13 e 15 de incubação, na eclosão e aos 4, 7, 10 e 14 dias pós-eclosão, foram coletados embriões ou pintainhos de codornas europeias e japonesas (n=6). As características estudadas foram analisadas por meio de Inferência Bayesiana. O peso corporal foi significativamente maior aos 15 dias de incubação e após quatro dias nas codornas europeias. O peso do sistema digestório aumentou progressivamente com o tempo e foi semelhante entre as codornas europeias e japonesas aos 11, 13 e 15 dias de incubação e aos 10 dias pós-eclosão, nos demais períodos as codornas europeias apresentaram maior peso. No entanto, ao analisar os dados de peso relativo, apenas aos 15 dias de incubação essa diferença foi observada, mostrando que o sistema digestório se desenvolve proporcionalmente de forma semelhante entre os tipos de codornas. Para o peso relativo do intestino delgado + pâncreas, o peso do proventrículo e do ventrículo gástrico aumentou significativamente entre as idades analisadas em ambos os tipos de codornas, e aos 14 dias após a incubação de codornas europeias teve maior peso para esses órgãos. Houve diferença

entre os tipos de codornas para a altura de vilosidade do duodeno aos 14 dias, sendo mais altas em codornas europeias e para altura das vilosidades no jejuno aos 10 dias, maior nas codornas japonesas. Conclui-se que o desenvolvimento e o crescimento dos diferentes órgãos do sistema digestório até aos 14 dias de idade, é semelhante entre codornas europeias e japonesas, apresentando peso relativo equivalente. Em codornas o duodeno aumenta as vilosidades até 14 dias, enquanto o jejuno e o íleo até os 10 e 4 dias, respectivamente. No experimento II, objetivou-se avaliar o efeito de diferentes períodos de jejum pós-eclosão, sobre o desempenho zootécnico e o desenvolvimento de órgãos do sistema digestório de codornas europeias até os 35 dias de idade. Para isso, foram utilizados pintainhos de codornas nascidos no pico de eclosão, e distribuídos em delineamento inteiramente casualizado, com quatro tratamentos (controle e três períodos de jejum de 24, 36 e 48 horas) e quatro repetições de 40 aves por unidade experimental. As características estudadas foram analisadas por meio de Inferência Bayesiana. No período de 1-14 dias, os pintainhos submetidos a jejum pós-eclosão apresentaram redução do ganho de peso à medida que se intensificou a restrição alimentar. Todavia, a partir dos 15 dias de idade as codornas europeias apresentaram ganho compensatório. Para o peso relativo e o comprimento do sistema digestório, as aves submetidas ao jejum apresentaram menores valores do que as alimentadas. No entanto, aos 14 dias, houve recuperação do digestório em geral. A altura dos vilos do duodeno apresentou efeito significativo aos 3 dias de idade com alturas reduzidas a partir de 36 horas de jejum, apresentando recuperação a partir dos 7 dias. Já a altura dos vilos do jejuno e do íleo não foi influenciada em função dos períodos de jejum estudados. Conclui-se que o período de até 48 horas de jejum pós-eclosão influencia o desenvolvimento dos órgãos do sistema digestório de codornas europeias até os 7 dias de idade, porém, esse apresenta ganho compensatório a partir deste período. Já o ganho de peso, a conversão alimentar durante o período de 1-35 dias de idade, a integridade do epitélio da mucosa intestinal e o desenvolvimento muscular não são influenciados pelo jejum pós-eclosão.

Palavras-chave: ganho compensatório, incubação, intestino, peso da ave, restrição alimentar.

ABSTRACT

Two experiments were carried out in order to evaluate the digestive system organs development and growth at 11th day of incubation up to 14 days post-hatch and the influence of post-hatch fasting on the performance and development of quails digestive system organs. In the experiment I, the objective was to evaluate the development and growth of digestive system organs, from the 11th day of incubation until the 14 days post-hatch in European and Japanese quail. Eggs from quails' breeders were selected by weight (European 11.80±0.59 and Japanese 9.79±0.49g) and incubated in an automatic incubator with 60% humidity and 37.6 °C, with automatic turning. On days 11, 13 and 15 of incubation, at hatch and at 4, 7, 10 and 14 days post-hatch, embryos or chicks of European and Japanese quails (n=6) were analyzed. The evaluated characteristic were analyzed by means of Bayesian Inference. The body weight was significantly heavier at 15 days incubation and after four days in European quails. The digestive system weight progressively increased with time and was similar between European and Japanese quails at 11, 13 and 15 days of incubation and 10 days post-hatch, in the others periods the European quails had higher weight. However, when analyzing the relative weight data, only at 15 days of incubation this difference was observed, showing that the digestive system develops proportionally similarly between quail types. For relative weight of the small intestine + pancreas, the weight of the proventriculus and gastric ventricle increased among ages analyzed in both types of quails, and at 14 days post-hatch European quails had higher weight of these organs. There were differences between quail types for duodenal villi height at 14 days, being higher in European and for villi height in jejunum at 10 days it was higher in Japanese quails. It is concluded

that the development and growth of different digestive system organs up to 14 days of age is similar between European and Japanese quails, presenting equivalent relative weight. In quail the duodenum increases the villi up to 14 days, while the jejunum and ileum up to 10 and 4 days, respectively. In experiment II, the objective was to evaluate the effect of different periods of post-hatch fasting on animal performance and organ development of the European quail digestive system up to 35 days of age. For this, quail chicks born at the peak hatching and distributed in completely randomized design were used, with four treatments (control and three fasting periods of 24, 36 and 48 hours) and four replicates of 40 birds per experimental unit. The evaluated characteristics were analyzed by means of Bayesian Inference. In the period of 1-14 days, chicks submitted to post-hatch fasting periods had a reduction in weight gain as the feed restriction intensified. However, from the age of 15 days European quails showed a compensatory gain. For relative weight and length of the digestive system, the birds submitted to fasting had lower values than that fed. However, at 14 days, there was a recovery of the digestive system. The height of the duodenum villi showed a significant effect at 3 days of age with reduced heights from 36 hours of fasting, presenting recovery from 7 days. However, the height of the jejunum and ileum villi was not influenced by the fasting periods studied. It is concluded that the fasting period of up to 48 hours post-hatch influences the organs development of the European quail digestive system up to 7 days of age, but this one presents a compensatory gain from this period. Weight gain, feed conversion during the period of 1-35 days of age, intestinal mucosal epithelial integrity and muscle development are not influenced by post-hatch fasting period.

Keywords: compensatory gain, incubation, intestine, body weight, feed restriction.

I. INTRODUÇÃO

A coturnicultura apresentou grande desenvolvimento adequando-se às novas tecnologias de produção, nas quais a atividade tida como de subsistência, passou a ocupar um cenário de atividade altamente tecnificada, com resultados promissores aos investidores.

Dois tipos de codornas têm sido utilizados comercialmente, as japonesas (*Coturnix coturnix japonica*), de origem asiática, com aptidão para produção de ovos, e as europeias (*Coturnix coturnix coturnix*), que são destinadas tanto para produção de ovos quanto para a produção de carne. As codornas europeias apresentam crescimento mais rápido quando comparadas as japonesas, atingindo aos 35 dias em média 200g, ou seja, cerca de 25 vezes o peso da codorna com 1 dia de idade (~8g) (Silva et al., 2012).

Vários fatores que têm contribuído para o aumento da criação de codornas, entre eles se destacam: o rápido crescimento, a precocidade na maturidade sexual (35 a 42 dias), a alta produtividade (média de 300 ovos/ano), a longevidade em alta produção (14 a 18 meses), a necessidade de pequenos espaços para grandes populações, o baixo investimento e o rápido retorno financeiro (Murakami e Ariki, 1998; Oliveira et al., 2002; Albino e Barreto, 2003). E também, por ser considerada uma excelente alternativa para alimentação humana, podendo ser utilizada tanto para a produção de ovos quanto para a produção de carne, sendo aceita universalmente por ser um produto de excelente qualidade e rica em aminoácidos essenciais.

Com o avanço na cortunicultura, demandaram-se maiores estudos no setor, a fim de melhorar o desenvolvimento dos animais. Para isso, diversas pesquisas estão sendo

desenvolvidas desde o período de incubação até o abate. As aves domésticas, como por exemplo, frangos e codornas de corte, passam de 30 a 40% de sua vida útil dentro do ovo (Hulet et al., 2007). Dessa forma, tudo que comprometa o desenvolvimento embrionário pode prejudicar o desempenho e a saúde pós-eclosão.

Um fator importante para o desempenho das aves é o desenvolvimento intestinal durante e após a incubação. Ao eclodir os pintainhos apresentam o sistema digestório anatomicamente completo, mas ainda imaturo para o aproveitamento dos nutrientes provenientes de uma dieta exógena (Uni e Ferket, 2004). Após a eclosão, os pintainhos apresentam reserva pancreática de lipase, pois o ovo é rico em lipídeos, mas é pobre em amilase. A ingestão de alimentos permite que o pâncreas e as atividades das enzimas do intestino delgado apresentem mudanças para se adaptar às novas condições, sendo que as maiores mudanças do sistema digestório ocorrem nesse período (Nitsan et al., 1995).

Diante disso, este trabalho foi desenvolvido com o objetivo de avaliar o desenvolvimento e crescimento de órgãos do sistema digestório, do 11º dia de incubação até os 14 dias de vida pós-eclosão e a influência do jejum pós-eclosão sobre o desempenho zootécnico e o e desenvolvimento de órgãos do sistema digestório de codornas.

1.1. Desenvolvimento Embrionário de Codornas

O desenvolvimento embrionário é dividido em cinco fases. A primeira é a morfogênese, cujos processos envolvem a origem de forma especifica. A fase seguinte é a organogênese, em que acontece nova série de alterações morfogenéticas, seguidas pelas iniciação da atividade funcional, quando cada órgão atinge um ponto ou estágio na organogênese, começando a perceber sinais de atividade funcional. Do 10º ao 13º dia de incubação é o período definido como o período de integração funcional, quando os órgãos já não funcionam de forma independente, mas estão interligados na sua atividade funcional. A fase final, a partir do 14º dia de incubação, é quando ocorre a maturação embrionária (Willier, 1954).

O período de incubação é diferente entre as espécies aviárias, caracterizando-se por diferenças nos tempos de formação e diferenciação de tecidos e órgãos. Diante disso, Ainsworth et al. (2010) e Hamburger e Hamilton (1951) descreveram o

desenvolvimento de codornas e frangos de corte, respectivamente, em 46 etapas (Tabela 1).

Os estágios iniciais das codornas (estágios 4-28) correspondem diretamente aos estádios do frango de corte e, portanto, as descrições e os tempos de incubação são idênticos. A partir do estágio 29 até o 35, como os embriões de codornas atingem cada etapa mais rapidamente, não é mais possível atribuir estágios equivalentes a ambas as espécies com base nos tempos de incubação. De forma que, as codornas se desenvolvem a uma taxa acelerada atingindo o estágio 46 aproximadamente 100 horas antes que o frango de corte.

Outras características gerais no desenvolvimento de codornas podem ser observadas na Tabela 2 e na Figura 1.

Tabela 1. Comparação dos estágios de desenvolvimento de embriões de codornas japonesas e frangos de corte.

Estágios	Codornas japonesas (Ainsworth et al., 2010)	Frangos de corte (Hamburger e Hamilton, 1951)
4	18–19 h	18–19 h
5	19–22 h	19–22 h
6	23–25 h	23–25 h
7	23–26 h	23–26 h
8	26–29 h	26–29 h
9	29–33 h	29–33 h
10	33–38 h	33–38 h
11	40–45 h	40–45 h
12	45–49 h	45–49 h
13	48–52 h	48–52 h
14	50–53 h	50–53 h
15	50–55 h	50–55 h
16	51–56 h	51–56 h
17	52–64 h	52–64 h
18	72 h	72 h
19	3 dias	3 – 3,5 dias
20	3,5 dias	3 – 3,5 dias
21	3,5 dias	3,5 dias
22	4 dias	3,5 – 4 dias
23	4 dias	4 dias
24	4 dias	4 dias
25	4,5 dias	4,5 dias
26	4,5 – 5 dias	4,5 – 5 dias
27	5 dias	5 dias
28	5,5 dias	5,5 dias
29	5,5 – 6 dias	6 dias
30	6 – 6,5 dias	6,5 dias
31	6,5 dias	7 dias
32	7 dias	7,5 dias
33	7 dias	7,5 – 8 dias
34	7,5 dias	8 dias
35	8 – 8,5 dias	8 – 9 dias
36	8 – 9 dias	10 dias
37	9,5 dias	11 dias
38	9,5 – 10 dias	12 dias
39	10,5 – 11 dias	13 dias
40	11 dias	14 dias
41	11,5 dias	15 dias
42	12 – 13 dias	16 dias
43	14 dias	17 dias
44	15 – 16 dias	18 dias
45	16 – 16,5 dias	19 – 20 dias
46	16,5 dias (eclosão)	20 – 21 dias (eclosão)

Fonte: Adaptado de Ainsworth et al. (2010).

Tabela 2. Principais características no desenvolvimento de codornas.

Estágios	Tempo de incubação	Principais características
4	18–19 h	A linha primitiva está totalmente alongada
5	19–22 h	Notocorda é visível
6	23–25 h	A dobra cefálica é aparente
7	23–26 h	Um somito evidente
8	26–29 h	Quatro somitos evidentes
9	29–33 h	Sete somitos evidentes
10	33–38 h	10 somitos evidentes
11	40–45 h	13 somitos evidentes, coração inclinado a direita
12	45–49 h	16 somitos evidentes e haste ótica evidente
13	48–52 h	19 somitos evidentes
14	50–53 h	22 somitos evidentes. Primeira e segunda fendas branquiais visíveis
15	50–55 h	24-17 somitos evidentes. A terceira fenda branquial é definida
16	51–56 h	26-28 somitos evidentes. Uma crista espessada define a primeira aparência dos brotos das asas. Ainda não existe evidência dos brotos das pernas nesta fase
17	52–64 h	29-32 somitos evidentes. O brotos das pernas são visíveis e os brotos das asas aumentaram ligeiramente
18	72 h	O alantoide é o primeiro anexo aparente. O âmnion geralmente fechado
19	3 dias	O processo maxilar torna-se distinto. Os olhos ainda não são pigmentados
20	3,5 dias	Alantoide se torna vesicular e mais visível. Olhos levemente pigmentados
21	3,5 dias	O processo maxilar excede o processo mandibular em comprimento
22	4 dias	É visível a pigmentação dos olhos
23	4 dias	Os brotos dos membros são iguais em largura e comprimento
24	4 dias	Os brotos dos membros são maiores em comprimento do que em largura
25	4,5 dias	As articulações de cotovelo e joelho são visíveis
26	4,5 – 5 dias	Demarcação de dedos do pé
27	5 dias	A região pressuposta do bico pode ser identificada
28	5,5 dias	O crescimento do bico é nítido
29	5,5 – 6 dias	A curvatura da asa é visível. O dente do bico ainda não é visível
30	6 – 6,5 dias	São visíveis uma a duas papilas esclerais. O dente do bico é visível
31	6,5 dias	São evidentes 6 papilas esclerais
32	7 dias	São evidentes de 6-8 papilas esclerais. Os dedos do pé se alongaram e tornaram-se mais visíveis
33	7 dias	13 papilas esclerais evidentes
34	7,5 dias	Crescimento diferencial do segundo e terceiro dedos do pé. 13-14 papilas esclerais
35	8 – 8,5 dias	As pálpebras começam a sobrepor a superfície do globo ocular
36	8 – 9 dias	A pigmentação preta e marrom começa a ser visível. Comprimento do bico = 1,2 mm, comprimento do terceiro dedo = 3,2 mm
37	9,5 dias	A área de pigmentação preta expandiu-se para incluir a testa e a coroa. A pigmentação marrom já está presente na região lombo-sacral. Comprimento do bico = 1,5 mm, comprimento do terceiro dedo = 4,1 mm
38	9,5 – 10 dias	Pigmentação preta visível nas laterais do crânio. Listras distintas de pigmentação marrom na região lombo-sacral. Comprimento do bico = 1,5 mm, comprimento do terceiro dedo = 4,7 mm
39	10,5 – 11 dias	Aumento significativo no comprimento de todos os folículos de penas pigmentadas. Padrões de pigmentação expandidos na asa e pigmentação visível microscopicamente em torno das articulações intertarsais. Comprimento do bico = 2,0 mm, comprimento do terceiro dedo = 6,0 mm
40	11 dias	Folículos de penas pigmentadas estão presentes na região periocular. Pigmentação agora evidente nos pés. Comprimento do bico = 2,0 mm, comprimento do terceiro dedo = 6,1 mm
41	11,5 dias	Os folículos de penas brancas são evidentes ao longo do comprimento do embrião e proeminentes ao redor do olho. Comprimento do bico = 2,0 mm, comprimento do terceiro dedo = 6,1 mm
42	12 – 13 dias	Pigmentação visível nos dedos dos pés. Comprimento do bico = 2,3 mm, comprimento do terceiro dedo = 8,6 mm
43	14 dias	Comprimento do bico = 2,6 mm, comprimento do terceiro dedo = 9,4 mm
44	15 – 16 dias	Comprimento do bico = 3,0 mm, comprimento do terceiro dedo = 10,8 mm
45	16 – 16,5 dias	Comprimento do bico = 3,5 mm, comprimento do terceiro dedo = 11,9 mm
46	16,5 dias	Eclosão

Fonte: Adaptado de Ainsworth et al. (2010).

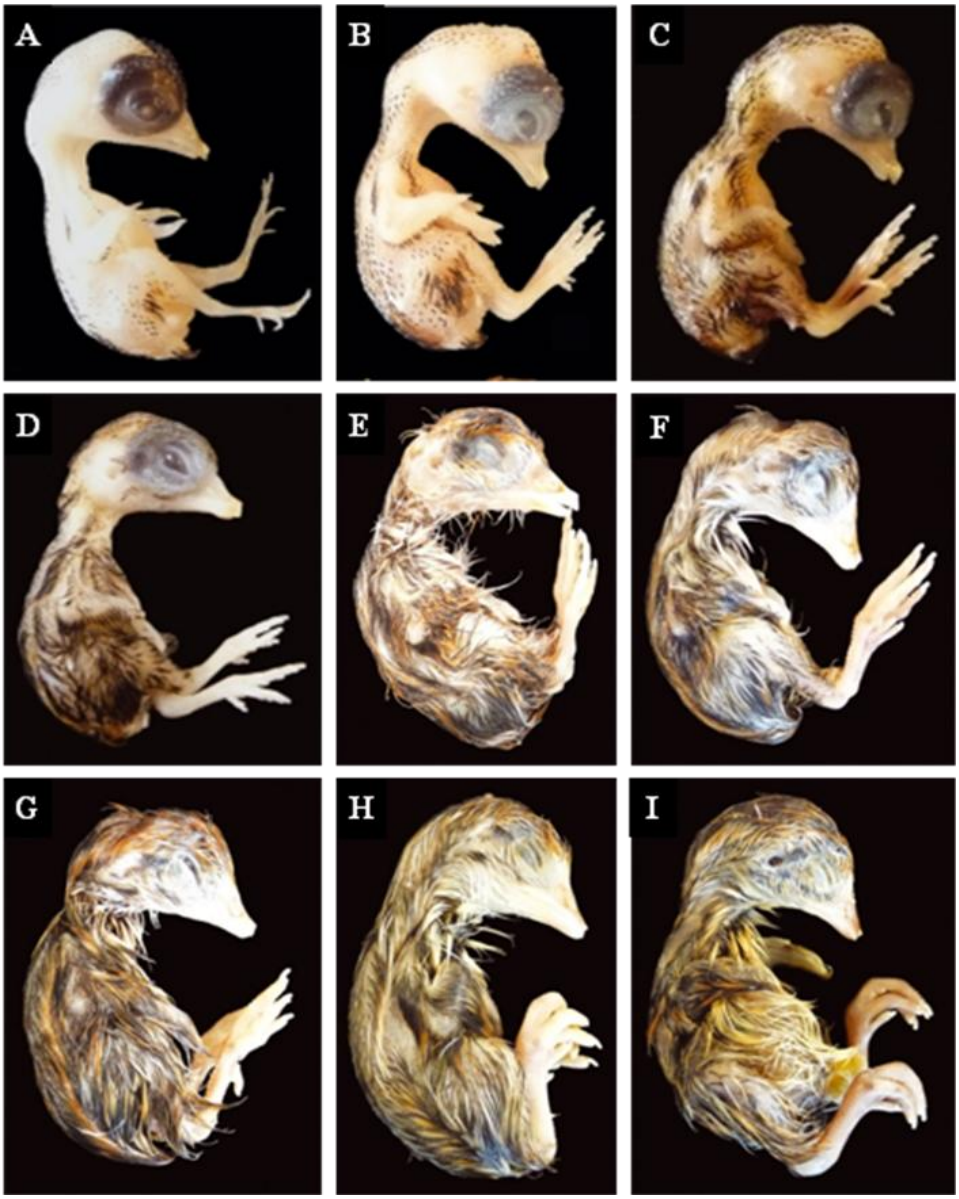

Figura 1. Estágios de desenvolvimento de embriões de codornas. A) Estágio 37 (9,5 dias). B) Estágio 38 (9,5-10 dias). C) Estágio 39 (10,5-11 dias). D) Estágio 40 (11 dias). E) Estágio 41 (11,5 dias). F) Estágio 42 (12-13 dias). G) Estágio 43 (14 dias). H) Estágio 44 (15-16 dias). I) Estágio 45 (16-16,5 dias). Fonte: Adaptado de Ainsworth et al. (2010).

1.2. Sistema Digestório de Aves

O sistema digestório consiste de cavidade oral, esôfago, papo (inglúvio), proventrículo, ventrículo gástrico (moela), intestino delgado (duodeno, jejuno e íleo) e

intestino grosso (ceco, cólon e reto). A ele também estão conectadas duas glândulas anexas, o fígado e o pâncreas (Getty, 1986; Dyce et al., 1997; Macari et al., 2002).

As características estruturais da cavidade oral têm uma relação estreita com o processo de apreensão, escolha e ingestão do alimento pela ave. Consiste em: bico, língua, glândulas salivares e faringe.

O esôfago e o papo respondem pela condução do alimento ingerido da faringe até o proventrículo e pela reserva ou estocagem do mesmo. O esôfago é um tubo relativamente longo, com grande capacidade de distensão, e tem por função conduzir o bolo alimentar da orofaringe para o proventrículo. Há presença de glândulas mucosas que secretam muco para amolecer os alimentos (Dyce, 1997).

Na entrada torácica sua parede ventral, expande-se e forma um divertículo sacular ímpar, denominado papo ou inglúvio. O papo serve como órgão de estocagem que regula parcialmente a entrada do alimento ingerido na moela. No caso das aves domesticadas, principalmente as utilizadas na produção de carne e ovos, tal estrutura passou a ter importância secundária, já que as mesmas recebem ração à vontade, não precisando estocar o alimento. Todavia, durante todo o período de alimentação o papo permanece cheio. Ele possui grande capacidade de dilatação, podendo, quando cheio, atingir tamanho bem maior que quando cheio (Macari et al., 2002).

Para que o alimento seja absorvido e incorporado ao corpo ou metabolizado para fornecer energia, as grandes e complexas moléculas de alimento (proteínas, lipídios, carboidratos, etc) devem ser quebradas em subunidades menores. Em aves o processo de digestão tem início no estômago, que é dividido em duas partes funcionalmente distintas: o proventrículo (estômago glandular) e o ventrículo gástrico ou moela (estômago muscular) (Getty, 1986; Macari et al., 2002; Zaher et al., 2012).

O proventrículo é formado por glândulas multilobulares constituídas de células oxintopepticas, secretoras de pepsinogênio (precursor ativo de pepsina) e ácido clorídrico, os quais são drenados para um ducto secundário e, confluindo com outros, formam o ducto primário que desemboca nas papilas secretoras (Macari et al., 2002).

Do proventrículo, o alimento é impulsionado para o ventrículo gástrico, um órgão muscular que apresenta musculatura circular altamente desenvolvida, cujas contrações rítmicas e fortes são responsáveis pela trituração do alimento ingerido (Macari et al., 1994; Macari et al., 2002).

O intestino delgado é a porção mais longa do sistema digestório, responsável pela digestão final do alimento e absorção dos nutrientes, e é dividido em três regiões: duodeno, jejuno e íleo (Frandson et al., 2005).

O duodeno consiste na alça intestinal localizada logo após o proventrículo e constituída de uma porção proximal descendente e uma distal ascendente, entre as quais fica localizado o pâncreas. Na sua porção ascendente, abrem-se os ductos biliares e pancreático, que conduzem os sucos biliar e pancreático para o interior da região anterior do intestino. O jejuno é a parte mais longa do intestino delgado e encontra-se disposto em várias alças (Macari et al., 2002).

O íleo continua a partir do jejuno, sem delimitação definida, sendo invariavelmente descrito como iniciando no divertículo vitelino, ou oposto aos ápices dos cecos e delimitado posteriormente pelo ponto de ligação ceco-cólico. O intestino grosso compreende os cecos e o cólon e reto (Macari et al., 2002). E o intestino grosso compreende os cecos, cólon e reto.

1.2.1. Morfologia do proventrículo e do ventrículo gástrico

O proventrículo participa de forma determinante na dissolução dos minerais (dependente do ácido clorídrico) assim como na digestão pepsínica sobre as proteínas. A organização da parede do proventrículo de codornas caracteriza-se por túnica mucosa, submucosa, muscular e serosa. O epitélio é revestido por células secretoras de mucina, uma glicoproteina ácida com papel chave para evitar ou minimizar as injúrias locais (Macari et al., 2002). O sistema de ducto das glândulas proventriculares é revestido com epitélio colunar com núcleos ovais ou vesiculares. A superfície mucosa proventricular é coberta por várias projeções ou papilas em diferentes níveis, dando a aparência pseudostratificada do epitélio (Ahmed et al., 2011).

A parede do ventrículo gástrico também possui as quatro túnicas: mucosa, submucosa, muscular e serosa. O epitélio de revestimento da superfície é do tipo simples e em forma de coluna, com núcleo basal arredondado a oval e citoplasma basofílico.

Um dos aspectos característicos deste segmento é a presença de um revestimento interno, uma camada secretora das glândulas, acima da túnica mucosa, conhecida como membrana de coilina.

As glândulas localizadas dentro dos sulcos da mucosa são menos ramificadas e apresentavam aparência regular e reta, enquanto as localizadas nas depressões das superfícies mucosas são mais ramificadas. As glândulas tubulares são revestidas com células baixas ou coloidais com núcleo basal vesicular arredondado e citoplasma basófilo.

A túnica muscular é bem desenvolvida, formando a maior parte da parede do ventrículo gástrico, representada pelas fibras musculares lisas dispostas principalmente de maneira circular (Ahmed et al., 2011).

1.2.2. Morfologia Intestinal

A barreira intestinal é composta por uma única camada de células epiteliais colunares unidas por junções intercelulares, servindo como a primeira linha de defesa do corpo contra micro-organismos e antígenos potencialmente nocivos que residem no interior do lúmen intestinal (Moeser et al., 2007). Qualquer alteração nesta barreira protetora pode alterar a permeabilidade intestinal deixando o animal mais susceptível a colonização por agentes patogênicos entéricos, levando a processos inflamatórios crônicos na mucosa intestinal e desta forma, prejudicando concomitantemente a absorção dos nutrientes da dieta e modificando o metabolismo (Durant el al., 1999; Pelicano et al., 2005).

No epitélio intestinal se encontram principalmente três tipos de células, com funções específicas: os enterócitos, as células caliciformes e as células enteroendócrinas. Os enterócitos são as células mais comuns do epitélio intestinal, responsáveis pelo transporte transepitelial dos nutrientes e pela digestão final do alimento, a partir do lúmen. São caracterizadas como células colunares altas, com uma proeminente borda em escova, composta de microvilosidades, que é sítio de diferentes enzimas que garantem o processo de digestão (Gurtler et al., 1987; Maiorka, 2004).

Posicionadas entre os enterócitos se encontram as células caliciformes, estas células se distribuem gradualmente na superfície dos vilos e criptas, aumentado sua

proporção gradativamente. São compostas por numerosos grânulos mucosos, localizados entre os núcleos basais e o polo celular apical, em sua maioria liberam seus produtos na luz do intestino de forma intermitente, estes produtos são denominados mucinas, glicoproteínas altamente glicosiladas que têm como uma de suas funções proteger o epitélio intestinal da ação de enzimas digestivas e dos efeitos abrasivos da digesta (Gurtler et al., 1987; Smirnov et al., 2005).

O desenvolvimento das células caliciformes no intestino delgado em pintainhos de frangos de corte ocorre no período final de incubação e imediatamente após a eclosão. Estas são observadas aos 17 dias de incubação e secretam somente mucina ácida. Até a primeira semana, após a eclosão, o intestino delgado contém proporções iguais de células caliciformes, produzindo mucina ácida e mucina neutra. Um aumento nas concentrações de células caliciformes pode ser observado ao longo do duodeno (Uni et al., 2003).

As células enteroendócrinas estão distribuídas pelo epitélio dos vilos, criptas e glândulas intestinais, e são produtoras de hormônios peptídicos (gastrina, secretina, colecistoquinina, entre outros), que possuem uma variedade de funções, incluindo efeitos na proliferação de células epiteliais, inflamação e, consequentemente, integridade de barreira intestinal. Um destes hormônios é o peptídeo 2 semelhante ao glucagon (GLP-2), um hormônio que é importante na manutenção da integridade epitelial. Estas também produzem monoaminas biogênicas, substâncias essas que participam na regulação da digestão, absorção e utilização dos nutrientes. A ativação dessas células ocorre por meio de vários sinais luminais, incluindo sinais bacterianos (Maiorka, 2004).

Em relação ao desenvolvimento e crescimento, o trato gastrintestinal é um dos órgãos que mais cresce de tamanho e proporção, durante o desenvolvimento embrionário, compondo cerca de 1% do peso do embrião aos 18 dias de incubação e 3,5% no momento da eclosão, este o aumento do peso intestinal, é devido ao desenvolvimento dos vilos. No entanto, além disso, no final do período de incubação, já é possível observar aumento da atividade de enzimas da borda em escova, sendo que aos 19 e 21 dias, observa-se atividade das enzimas sucrase-isomaltase e aminopeptidase e dos transportadores Na+K ATPase e Na-Glicose transportador-1(Uni et al., 2003).

Apesar dessas características, na eclosão o sistema digestório não se encontra em sua total capacidade funcional. Em frangos de corte, o desenvolvimento bioquímico e morfológico, e a consequente maturação do intestino delgado, ocorrem nos primeiros 10 dias de vida e a área e o tamanho dos vilos continuam a aumentar rapidamente entre um e dois dias de idade. Embora a taxa de crescimento intestinal diminua gradualmente, atingindo um patamar entre cinco e 10 dias pós-eclosão (Uni et al., 1996).

Uni et al. (1998), ao avaliarem o desenvolvimento pós-eclosão da mucosa do intestino delgado de frangos de corte, constataram que o desenvolvimento completo dos vilos duodenais ocorreu até os sete dias de idade; nos vilos do jejuno e íleo, continuou até aos 14 dias de idade.

Uma das características mais importantes do intestino é a de ser o órgão com maior *turnover* de todos os tecidos do corpo, dependendo do equilíbrio entre proliferação, migração celular e apoptose. Este aspecto é pautado por dois eventos citológicos associados: renovação celular (proliferação e diferenciação das células totepotentes localizadas na cripta e ao longo dos vilos) e, perda de células por descamação, que ocorre naturalmente no ápice dos vilos. O equilíbrio entre os dois processos (perda e proliferação celular) determina o *turnover* (proliferação – migração – extrusão) e assegura a manutenção do número de células e da capacidade funcional do epitélio. Quando o intestino responde a algum agente estimulador de um desses eventos, deverá ocorrer modificação na altura dos vilos (Macari et al., 2002).

O processo de renovação intestinal ocorre através das células tronco localizadas na região das criptas, que se diferenciam e migram para o topo dos vilos, sendo então extrusadas do ápice para o lúmen intestinal (Uni et al., 2001). No entanto, em frangos a proliferação de enterócitos não é restrita à região da cripta, mas também ocorre ao longo dos vilos. Há aumento no volume das vilosidades e na profundidade das criptas entre 04 e 21 dias de idade. Apesar de detectar-se pouca alteração na densidade dos enterócitos com o avançar da idade nos frangos de corte (Uni et al., 1998).

Abaixo do epitélio, estão presentes muitos tipos de células, que em conjunto vão formar a lâmina própria da mucosa intestinal. Entre essas células estão incluídos, os fibroblastos e as células endoteliais. Células imunes também se encontram presentes na mucosa, formando tecidos linfóides, entre as células. Todas as células não epiteliais são sensíveis a sinais luminais, incluindo as células enteroendócrinas, desta forma, a

inflamação, a função intestinal e a integridade da barreira intestinal, são influenciadas por sinais luminais, muitos deles produzidos pela microbiota intestinal (Havenaar, 2011).

O fato do epitélio de revestimento intestinal agir como uma interface entre qualquer substância (ração e microbiota intestinal) e o intestino animal, faz com que apesar de na maioria das vezes os materiais que entram em contato com a mucosa não serem patogênicos, eles estimulem uma resposta imune por parte da ave (Hughes, 2005). Que também poderão causar impactos negativos na eficiência alimentar, por serem energeticamente dispendiosas e desviarem os nutrientes da produção (Yegani e Korver, 2008). Pertubações induzidas por qualquer tipo de estresse, como o jejum pós eclosão, reduzem a integridade da microbiota intestinal normal ou a integridade do epitélio intestinal pela diminuição de agentes de proteção inatos e aumentando o potencial de agentes patogênicos (Burkholder et al., 2008).

No animal maduro, o epitélio do intestino delgado é continuamente renovado pela proliferação de células da cripta que migram em direção as vilosidades. Estas células diferenciam e as funções microvílicas apicais e absortivas desenvolvem-se durante o este movimento da cripta para as vilosidades. No entanto, o mesmo não ocorre em pintos submetidos a uma situação de jejum pós-eclosão, bem como em mamíferos neonatais, em que todas as células epiteliais intestinais estão se proliferando na eclosão, sendo que dentro de um curto período, a proliferação é restrita, principalmente às criptas intestinais (Uni et al., 2000). Porém, em algumas situações a proliferação celular, tanto nas criptas quanto ao longo dos vilos, é sensível à falta de alimento, sendo que no período de jejum, poucas células se mostram disponíveis ao crescimento das criptas e das vilosidades (Geyra et al., 2001).

1.3. Período Pós-eclosão das Aves

No final da incubação, a gema residual internalizada dentro da cavidade abdominal é a única fonte de nutriente que a ave possui até que consiga uma fonte gena de alimento. Nessa fase, o vitelo residual está constituído de aproximadamente 46% de água, 20% de proteína e 34% de lipídios e compõe cerca de 10% do peso da ave pós-

eclosão, contribuindo dessa forma para a manutenção do intestino nas primeiras 48 horas (Sklan e Noy, 2000; Noy e Sklan., 2001).

Ao considerar a nutrição de aves, tem-se como desafio a mudança do tipo de nutrientes fornecidos, que passa do fornecimento exclusivo de proteínas e gorduras oriundas do vitelo, para uma dieta composta predominantemente por carboidratos (Sklan, 2001; Longo, 2005). Essa transição é acompanhada por um rápido desenvolvimento físico e funcional do trato gastrointestinal (Uni et al., 1999).

O conteúdo do saco vitelino é utilizado por transferência direta de nutrientes para a circulação, ou por meio do transporte de nutrientes para o lúmen intestinal. A transferência do conteúdo das porções distais, em que é secretado, para as porções proximais do intestino delgado, em que ocorre a ação de enzimas se dá por meio de movimentos peristálticos. No entanto, após 48 horas, essa transferência começa a reduzir, pela obstrução do pedúnculo vitelínico por células linfoides, que se completa cerca de 4 dias após a eclosão (Noy e Sklan, 1998).

A capacidade de utilizar carboidratos na dieta pode ser detectada no embrião de frango com 18 dias de incubação, mas uma capacidade significativa só é observada dias após a incubação. A enzima pancreática alfa-amilase também é detectada aos 18 dias de incubação, no entanto sua atividade mais específica só é confirmada 4 dias após a eclosão. A capacidade de digerir amido é 85% completa após 4 dias da eclosão sem grandes melhorias evidentes após esse período (Vieira e Moran, 1999; Longo, 2005).

As dietas ricas em carboidratos logo após a eclosão aumentam a concentração e diminuem a atividade da glicose-6-fosfato hepática, que indica redução na gliconeogênese. Sob condições comerciais, o período de jejum e o, consequente acesso das aves aos carboidratos, pode demorar um tempo considerável após a eclosão, aumentando o risco de cetose e desidratação (Viera e Moran, 1999).

Murakami et al. (1988) mostraram que a alimentação apenas com as reservas do saco vitelino nas primeiras horas de vida pós-eclosão não foi suficiente para otimizar o desempenho, pois estas reservas correspondem a apenas 50% da exigência de energia e 45% da exigência proteica exigida pela ave no seu primeiro dia de vida. Este fato demonstra a importância da alimentação desde o primeiro dia pós-eclosão, pois sem suprimento de nutrientes o pintainho certamente irá perder peso, pelo balanço negativo de energia (Dibner et al., 2005).

À medida que se aumenta a idade das aves o peso do saco vitelino diminui gradativamente, indicando sua absorção e utilização dos nutrientes (Riccardi et al., 2009). Diversos estudos evidenciaram que essa velocidade de absorção independe do período de jejum entre o nascimento e o alojamento (Gonzales et al., 2003; Maiorka et al., 2003).

Resultados contraditórios a estes foram encontrados por Vieira e Moran (1999), que avaliando o saco vitelino 24 horas após o jejum e 24 horas após a alimentação, verificaram que o peso do saco vitelino foi 49,5% menor para os pintainhos submetidos ao jejum.

1.4. Período de Jejum Pós-Eclosão das Aves

1.4.1. Influência do período de jejum pós-eclosão sobre o sistema digestório

A ausência de sincronismo na eclosão e o tempo utilizado para sexagem, vacinação e acondicionamento, submetem os pintos de frangos de corte recém-nascidos ao período de jejum que pode durar até 72 horas, dependendo da distância entre o incubatório e a granja (Hager e Beane, 1983; Baião e Cançado, 1999).

Nesse período de jejum alimentar e hídrico, os pintainhos podem sofrer um processo de desidratação (Agostinho et al., 2012), aumento das taxas de mortalidade (Pedroso et al., 2006), redução no peso do fígado, pâncreas, intestino, dificuldade de absorção do saco vitelino (Almeida et al., 2006) e queda nos índices de desempenho zootécnico aos 42 dias de idade (Carvalho et al., 2013).

Durante as primeiras horas de vida da ave, a maior parte da demanda de nutrientes se direciona para o crescimento dos órgãos do trato gastrointestinal, pois são esses órgãos que darão suporte para o crescimento de outros tecidos com fornecimento de nutrientes (Noy e Sklan, 1999; Cançado e Baião, 2002; Maiorka et al., 2003).

Noy e Sklan (2000) observaram aumento de 200% no peso do intestino delgado já nas primeiras 48 horas de vida, contra um aumento de apenas 60% em pintainhos desprovidos de alimento. Os mesmos autores, em outro estudo, observaram aumento de 600% no peso do intestino delgado com o estímulo de alimento nos primeiros sete dias de vida (Noy e Sklan, 2001).

Resultados semelhantes foram encontrados em estudo com perus, em que foi observado que atrasos no acesso à alimentação e à água retardaram o crescimento do sistema digestório e limitaram a capacidade de uso de nutrientes na dieta, resultando em redução do peso corporal (Corless e Sell, 1999).

A restrição alimentar atua diretamente na altura das vilosidades intestinais (Uni et al., 1998; Gonzales et al., 2003), reduz a área superficial das microvilosidades do jejuno (Geyra et al., 2001), causa diminuição do número de enterócitos e aumento da densidade das células caliciformes no jejuno (Uni et al., 2003).

Pedroso et al. (2005), estudando o efeito do jejum de 24 e 48 horas sobre o peso relativo do intestino delgado, pâncreas, proventrículo + moela e saco vitelino, em frangos de corte, observaram maior peso para aves que receberam água e alimento precocemente.

Maiorka et al. (2003) observaram que o peso do fígado foi influenciado negativamente pela privação de água e ração por 24 horas pós-eclosão, indicando que o metabolismo e desenvolvimento desse órgão após a eclosão, provavelmente estão associados aos substratos provenientes da absorção intestinal. Resultados semelhantes foram observados por Riccardi et al. (2009), em que o peso do fígado com 48 e 72 horas de vida foi maior para as aves que receberam água e ração *ad libtum*.

Em relação à produção enzimática a presença do alimento no trato gastrointestinal é importante, pois estimula principalmente a secreção de lípase, que é extremamente necessária para as aves pós-eclodidas, pois o lipídio da gema é a principal fonte energética (Palo et al., 1995). Após o nascimento, com a mudança de alimentação endógena para exógena, as reservas embrionárias de lipase são consumidas. O pâncreas ainda está imaturo e não é capaz de produzir quantidades suficientes dessa enzima para atender as demandas do organismo, observando-se diminuição de sua atividade nos cinco primeiros dias de vida do animal (Krogdahl e Sell, 1989).

Quanto mais cedo o pintainho for alimentado, maiores serão as sínteses e secreção enzimáticas do pâncreas, estimulando o aproveitamento dos nutrientes da ração. As enzimas pancreáticas se esgotam facilmente porque a síntese no período inicial é menor que a demanda para secreção no intestino para manter a concentração inicial (Nitsan et al., 1991).

1.4.2. Influência do período de jejum pós-eclosão sobre o desempenho zootécnico

Pintainhos que tiveram acesso imediato à alimentação em comparação aqueles com restrição alimentar de 48 horas pós-eclosão, apresentaram aumento significativo no seu peso vivo do primeiro ao sexto dia pós-eclosão (Uni et al., 2003). Noy e Sklan (2001) relataram que aves submetidas a 48 horas de jejum pesaram 11 gramas a menos que aquelas com acesso imediato ao alimento.

Resultados semelhantes foram obtidos por Pinchasov e Noy (1993); Baião e Cançado (1999); Almeida et al. (2006); Pedroso et al. (2006); El-Husseiny et al. (2008); Riccardi et al. (2009) e Carvalho et al. (2013) que observaram diminuição do peso corporal das aves submetidas à restrição alimentar pós-eclosão.

Nir e Levanon (1993) observaram atraso de 1 e 2 dias de ganho de peso corporal, respectivamente, em frangos submetidos a 24 e 48 horas de jejum pós-eclosão. No entanto, Oliveira (2012) e Carvalho et al. (2013) demonstraram que apesar da influência do jejum pós-eclosão na fase inicial, os índices zootécnicos (peso corporal, consumo de ração, conversão alimentar e viabilidade) se igualam ao longo da vida, indicando recuperação das aves e equiparação das características de desempenho analisadas.

O jejum prolongado também provoca alterações no sistema imunológico pelo aumento dos níveis de corticosterona, prejudicando a resposta imune celular (Klasing, 1998; Yi et al., 2005) e origina atrofia muscular pela mobilização dos aminoácidos estruturais do músculo peitoral para a gliconeogênese (Kornasio et al., 2011).

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II. OBJETIVOS GERAIS

Avaliar o desenvolvimento e o crescimento de órgãos do sistema digestório, do 11º dia de incubação até os 14 dias de vida pós-eclosão e a influência do jejum pós-eclosão sobre o desempenho zootécnico e o e desenvolvimento de órgãos do sistema digestório de codornas.

2.1. Objetivos Específicos

Experimento I:

- Avaliar a diferença no crescimento e no desenvolvimento de órgãos do sistema digestório, do 11º dia de incubação até os 14 dias pós-eclosão para codornas europeias e japonesas;
- Descrever a morfologia macro e microscópica dos órgãos do sistema digestório de codornas europeias e japonesas: proventrículo, ventrículo gástrico, duodeno, jejuno e íleo.

Experimento II:

- Evidenciar os efeitos do período de jejum pós-eclosão no desempenho zootécnico de codornas europeias, por meio do consumo de ração, ganho de peso e conversão alimentar;
- Identificar os efeitos do período de jejum pós-eclosão sobre o peso vivo e desenvolvimento morfométrico dos órgãos do sistema digestório (proventrículo,

ventrículo gástrico, duodeno, jejuno e íleo) de codornas europeias até os 35 dias de idade;

- Avaliar os efeitos do período de jejum pós-eclosão sobre a morfologia do intestino delgado (duodeno, jejuno e íleo) por meio da microscopia de luz e eletrônica de varredura;
- Classificar as fibras musculares através da técnica histoquímica de NADH-TR, determinar a quantidade por feixe muscular e o diâmetro no músculo peitoral de codornas europeias aos 35 dias de idade, submetidas a diferentes períodos de jejum pós-eclosão.

III. Development and growth of digestive system organs of European and Japanese quails at 14 days post-hatch

ABSTRACT: The objective of this study was to evaluate the development and growth of the digestive system organs, from the eleventh day of incubation until the 14 days of life post-hatch in European and Japanese quails. On days 11, 13 and 15 of incubation, at hatch and at 4, 7, 10 and 14 days post-hatch, embryos or chicks of European and Japanese quails (n=6) were analyzed. The body weight was significantly heavier at 15 days of incubation and from four days in European quails. The digestive system weight progressively increased with time and was similar between European and Japanese quails at 11, 13 and 15 days of incubation and 10 days post-hatch, European quail analyzed showed higher overall weight in the other periods. However, when analyzing the relative weight data, only at 15 days of incubation this difference was observed, showing that the digestive system develops proportionally similarly between quail types. For relative weight of the small intestine + pancreas, the weight of the proventriculus and of the gastric ventricle increased significant by among ages analyzed in both types of quails, and at 14 days post-hatch European quails had greater weight for these organs. There were differences between quail types for duodenal villi height at 14 days, being higher in European and villi height in jejunum at 10 days was higher in Japanese quails. It is concluded that the development and growth of different organs of the digestive system up to 14 days of age is similar between European and Japanese quails, presenting equivalent relative weight. In quail the duodenum increases the villi up to 14 days, while the jejunum and ileum up to 10 and 4 days, respectively.
Key words: *Coturnix coturnix*, *Coturnix japonica*, incubation, small intestine, villi.

INTRODUCTION

In the last few years, coturniculture had a higher development, no longer being considered a subsistence activity, occupying a highly technical activity scenario, adapting to new production technologies as well as providing investors with promising results. In Brazil, according to data from the Brazilian Institute of Geography and Statistics (IBGE, 2016), the number of quails in 2016 was 15.1 million.

Two types of quail have been used commercially, the Japanese (*Coturnix coturnix japonica*), of Asian origin, with capacity for egg production and average birth weight of 7 grams, and the European ones (*Coturnix coturnix coturnix*), which are destined for both egg production and meat production, with initial weight around 9 grams. European quails show a faster growth when compared to Japanese, reaching 35 days on average 200g, that is, about 25 times the weight of quail at 1 day of age (~ 8g) (Silva et al., 2012).

Several factors contribute to the increase of quail production, as: rapid growth, precocity at sexual maturity (35 to 42 days), high productivity (average of 300 eggs/year), longevity at high production (14 to 18 months), the need for small spaces for large populations, low investment, and rapid financial returns (Murakami and Ariki, 1998; Oliveira et al., 2002; Albino and Barreto, 2003).

In parallel to the development of the coturniculture, the scientific research in the sector advances, in order to allow the best physiological and metabolic understanding of these animals, from the incubation period until slaughter. Considering that poultry production, such as broilers and quails, spend 30 to 40% of their useful life inside the egg (Hulet et al., 2007), so factors that interfere with embryo development can modify the performance and post-hatch health in birds in general.

An important factor for bird performance is the development of the digestive system. The organs that make up this system have an important development in the embryonic final phase in broilers, at 18 days of incubation it makes up about 1% of the embryo weight, and in hatch, they already represent 3.5% of the total weight, this increase of the intestinal weight is attributed to the development of intestinal villi (Uni et al., 2003b).

At hatch, the bird's digestive system is anatomically complete but its functional capacity is not, thus the gastrointestinal tract undergoes major morphological and physiological changes even in the post-hatch period, which will provide greater efficiency in the digestion and absorption processes (Nitsan et al., 1995; Uni and Ferket, 2004).

The present work was developed with the objective of evaluating the development and growth of the digestive system organs, from the eleventh day of incubation until the 14 days of life post-hatch in European and Japanese quails.

MATERIAL AND METHODS

This research was approved by the Committee of Ethical Conduct on the Use of Animals in Experimentation of the State University of Maringá, protocol number 8793250615. The experiment was conducted in the Poultry Farm Sector of the Experimental Farm of Iguatemi (FEI) of the State University of Maringá (UEM).

Animals and tissue colection

A total of 900 quails breeders, 450 European quails and 450 Japanese quails were used in peak of posture, standardized by weight and egg production. The birds were distributed in galvanized wire cages (25 x 39 cm), in the ratio of 2 females to 1 male, receiving water and feed *ad libitum*. Eggs from the quails breeders were selected by weight (European 11.80 ± 0.59g and Japanese 9.79 ± 0.49g) and incubated in an automatic incubator with 60% humidity and 37.6°C, with automatic turning. After 348 hours of incubation, the eggs were transferred to the hatch chamber, with a temperature of 37.0°C and 70% humidity. The chicks hatched were housed in boxes in the coturniculture sector with 2.5 x 1 m, with bedding shaving, covered with corrugated paper, ring bell for heating. Water and feed were supplied *ad libitum* in baby feeders and drinking fountains.

On days 11, 13 and 15 of incubation, at hatch and at 4, 7, 10 and 14 days post-hatch, 6 embryos or chicks of European quails and 6 of Japanese quails were collected. The embryos were sacrificed by cervical dislocation and the chicks were anesthetized (thiopental 25 mg/kg + lidocaine 10 mg/kg intraperitoneally) and sacrificed by cervical dislocation (European quail n=48 and Japanese quail n=48).

In each analyzed period, the birds were weighed and the organs of the digestive system were dissected and weighed. The variables analyzed were body weight of the embryo/quail, yolk sac and the digestive system and relative weight of the digestive system in relation to embryo/quail weight. From 4 days post-hatch it was possible to divide the segments of the digestive system, being evaluated: relative weight of the proventriculus, gastric ventricle (gizzard), small intestine + pancreas and large intestine.

In the embryonic period (11, 13 and 15 days of incubation) and in hatch, the total digestive system was weighed, before the separation of the organs. In chicks (4 to 14

days post-hatch), the organs were dissected, isolated and the contents washed, and the total weight of the digestive tract was obtained by adding the weight of the organs and the intestinal segments, obtained separately.

At each age, fragments (± 5 mm) of the organs of 6 birds were fixed by immersion in 4% paraformaldehyde in 0.1M PBS pH 7.4 for light microscopy and 2.5% glutaraldehyde in 0.1M PBS pH 7.4, for scanning electron microscopy.

Light Microscopy

For the histomorphological evaluation of the digestive system, at 15 days of incubation, at hatch and at 4, 7, 10 and 14 days post-hatch. The fragments of each organ were processed in the histological routine, included in paraffin, cut with 3 micrometers of thickness and stained with hematoxylin-eosin (HE) (proventriculus and gastric ventricle) and in HE + Alcian Blue (AB) pH 2.5 (duodenum, jejunum and ileum). Digital images were captured in a light microscope coupled to a digital camera (Motican®, China Group Co. Ltd., Xiamen, China) for morphometric analysis.

The images were analyzed using Motic Image Plus 2.0 software (Motican®). In the proventriculus the total thickness of the wall was determined and in the gastric ventricle, the thickness of the tunica mucosa and the coilin membrane, and in the duodenum, jejunum and ileum the villi height, crypt depth, villi/crypt ratio and number of goblet cells/villi (10 integers). For each variable 10 measurements were performed on each bird in at least 3 semi-serial cuts. In embryos only intestinal highest villi were measured.

Scanning electron microscopy (SEM)

At 15 days of incubation, at hatch and at 4 and 7 days post-hatch, the duodenum, jejunum and ileum fragments were fixed in glutaraldehyde, stored at 4°C and then washed in 0.1M phosphate buffer and dehydrated in increasing concentrations of ethyl alcohol (70, 80, 90, 100 I, 100 II and 100 III%), 20 minutes at each concentration. After dehydration, the material passed through the critical point chamber (Bal-Tec CPD 030) by the use of carbon dioxide. The material was then mounted on metal stubs, covered with a 30 nm gold layer on Bal-Tec SCD 050 metallizer, and analyzed on a Shimadzu SS-550 Superscan scanning electron microscope.

Statistical analysis

The characteristics studied were analyzed by Bayesian Methodology. In this procedure, the response (Y_{ij}) follows a normal distribution (Gaussian), $Y_{ij} \sim N(\mu_j, \sigma_j^2)$. There were considered the prior noninformative distributions, for the model parameters, $\mu \sim N(0, 10^{-6})$ and $\tau \sim Gamma(10^{-3}, 10^{-3})$ ($\tau = 1/\sigma^2$, OpenBUGS parameterization) (Rossi, 2011). The significance of the differences between averages ($\Delta_{jk}=\mu_j-\mu_k$, $j \neq k$) a posterior of the treatments/groups considered was based on the presence or absence of zero in the respective 95% credibility intervals.

The posterior marginal distributions were obtained for all parameters by the Brugs package of the R program (R Development Core Team, 2017). A total of 40.000 values were generated in a Monte Carlo Markov Chain (MCMC), sampling discard of 10% of initial values. The convergence of the chains was verified through the coda package, program R, using the criterion of Heidelberger and Welch (Heidelberger and Welch, 1983).

RESULTS AND DISCUSSION

The development of organs of the digestive system was analyzed in European and Japanese quails during the final stage of embryonic development (11 to 15 days), at hatch and up to 14 days post-hatch. According to Bayesian posterior estimates and the respective, credibility intervals it was possible to verify that the body weight of the embryo/quail was different among all ages analyzed with significant continuous growth (Table 1). When comparing body weights between quail types, European quails were significantly heavier at 15 days of incubation and from four days. This is mainly due to differences in growth potential, since European quail have aptitude for meat production, being more efficient in feed conversion in lean tissue, weighing in adult stage 80-100% more than the Japanese (Silva et al., 2012; Grieser et al., 2015).

For the yolk sac, a weight reduction was observed as a function of the days of age, in both types of quail, with European quails having a heavier yolk sac (Table 1). Between 15 days of incubation and hatch the yolk sac absorption process was intense, with a reduction of its weight up to 74%. At hatch, the yolk sac represented on average 6.9% of the live weight of Japanese and European quail chicks.

In the final stage of incubation of the birds, the yolk sac is internalized into the abdominal cavity and serves as a source of nutrients until exogenous feeding is provided post-hatch (Noy et al., 1996; Noy e Sklan, 1997). In broilers at hatch time, the yolk sac comprises 20 to 25% of the body weight, which accounts for 50% of the energy and 43% of the protein required by the bird on its first day of life (Murakami et al., 1988; Noy and Sklan, 1997), 80% of the total fat present as yolk sac contents is used on the first day, while the protein is used more slowly (Nitsan et al., 1991).

In this work, the total absorption of the residual yolk occurred during the first week of life, and at four days it was still possible to identify residual yolk sac in some quails (2/6 birds), representing less than 1% of the weight. In broiler chickens, it was observed that the yolk size decreases exponentially post-hatch and exhibit 1g after 4 days of life (Noy et al., 1996).

In relation to the digestive system, the weight progressively increased with time and was similar between European and Japanese quails at 11, 13 and 15 days of incubation and 10 days post-hatch. European quail analyzed showed higher overall weight in the other periods. However, when analyzing the relative weight data, only at 15 days of incubation this difference was observed, showing that the digestive system develops proportionally similarly between quail types (Table 1). The highest relative weight was observed at 4 days post-hatch (18,62%), with reduction of this relation on consecutive days, due to the fact that body weight increases proportionally more than the digestive system.

From 11 days of incubation to 4 days post-hatch digestive system weight increased 18,4 folds to European and 20,2 folds to Japanese quails. In some period, body weight in increased 4 folds, suggesting the intense proliferative activity in digestive organs during this period.

Murakami et al. (1992), working with broiler chickens, observed that the growth of the digestive system has its peak between the third and seventh post-hatch days. This growth in the first days of birds life is of extremely important for the development of intestinal villi to occur, and, consequently, there is absorption of nutrients from the diet. The post-hatch accelerated rate of development is reflected in the several-fold elevation in numbers of enterocytes during first few days post-hatch, resulting from the dramatic increase in villi length (Geyra et al., 2001).

For the relative weight of the small intestine + pancreas, there was no difference between the types of quail in any of the ages studied (Table 1), according to the Bayesian posterior estimates and the respective intervals of credibility. In European quails, this ratio was similar when evaluated according to age days, whereas for Japanese quails, lower values were observed at 10 and 14 days, when compared to 4 days post-hatch. For relative weight of the large intestine, it was observed that at four days weight is higher in Japanese quails, due to their reduced weight in relation to European quails (Table 1).

The Bayesian estimates for the mean weight of the gastric proventriculus (glandular) and the gastric ventricle (muscular) are represented in Table 2, starting 4 days post-hatch, whereas the histological variables were analyzed from the incubation (15 days). The weight of the proventriculus and of the gastric ventricle increased among the ages analyzed in both types of quails, with significant difference between quail type, only at the 14 days post-hatch in which the European quails had greater weight for these organs (Table 2). For the relative weight, this difference was observed at 10 days with higher means for Japanese quails, also characterized by a reduction in this relation throughout the days of age, for both types of quail.

Histological analysis, in both types of quail, has shown that the proventriculus wall consists of tunica mucosa, muscularis and serosa. The tunica mucosa contained two types of gastric glands, the deep ones and, the superficial ones that are of the simple tubular type, composed by secreting mucous cells. The tunica muscularis was moderately thick, and consists of an inner circular layer and an outer longitudinal layer (Figures 1A-C). This morphological organization was common to other birds, such as the chicken described by King and McLelland (1979) and McLelland (1990) and described for quails by Ahmed et al. (2011) and Zaher et al. (2012).

The total wall thickness of the proventriculus, including the 3 layers, showed a significant effect, with European quails having a less thick proventriculus wall than Japanese quails at hatch. Growth with the advancement of bird age was also observed, with a greater thickness at 14 days post-hatch for both types of quail (Table 2).

In the gastric ventricle (gizzard), tunica mucosa was composed by simple tubular glands. The thickness of the tunica mucosa was significantly lower in the embryos at

15 days of incubation and post-hatch it remained with similar average thickness until the 14 days of life.

The glandular secretion formed an acellular, eosinophilic, adherent mucosal band, called the coilin membrane (Figures 1D-F). This coilin membrane was already observed in embryos with 15 days of incubation, becoming thicker at hatch and throughout the growth of quail. In the structural analysis, the tunica muscularis presented an internal circular layer of well developed smooth muscle layer, and a narrow outer longitudinal layer, covered by a tunica serosa (Figures 1D-F).

The proventriculus was smaller and disposed between the esophagus and the ventricle, similar to that described in birds in general (Getty, 1986; Macari et al., 2002; Zaher et al., 2012; Hamdi et al., 2013). According to Pough et al. (2008), the gastric ventricle has the capacity to store the food while continuing the chemical digestion that was initiated in the proventriculus. This structure has a highly developed musculature, whose rhythmic and strong contractions were responsible for the crushing of the ingested food (mechanical digestion) (Macari et al., 2002).

Segments of the small intestine were analyzed by light and SEM and morphometric data from villi and intestinal crypts were measured (Tables 3-4, Figures 2-4). There were differences between quail types for duodenal villi height at 14 days, being higher in European (992 μm) than in Japanese quails (697 μm), and villi height in jejunum at 10 days, with higher values in Japanese quails (391 μm).

The age influenced the height of the intestinal villi, which increased as a function of days in the three segments of the small intestine (Table 3). The segment in which this increment was larger was the duodenum. In the analysis between the segments in each period it was observed that in the jejunum the villi increased in height up to 10 days and in the ileum up to 4 days.

At 15 days of incubation the height of the villi was similar between the segments, with a mean of 80 μm. At this age, it was possible to observe, in all segments, different sizes of intestinal villi, having an elongated shape towards the intestinal lumen, covered by enterocytes and with the presence of goblet cells. The larger ones had the apical surface more rounded than the base and smaller ones had more elongated format and digitiform (Figures 2A-C, 3A-C, 4A-C). Mucosal secretion was observed in the intestinal villi, both in light and in SEM (Figures 2A, 4A-B).

At hatch, birds had intestinal villi larger than at 15 days of incubation, with mean values of villi height of 330 μm for duodenum, 161 μm for jejunum and 137 μm for ileum, independent of quail type. At this time, the duodenum villi occupied almost completely the lumen, being characterized as the segment with the largest villi for this age (Figures 2D-F, 3D-F and 4D-F).

At this stage, smaller villi interspersed with larger ones were still frequent in the different segments (Figures 2E, 3D-E and 4D-E), as well as in quails at 7 days (Figure 4G).

From the hatch the duodenum presented higher villi than jejunum and ileum until the 14 days analyzed. However, between the jejunum and the ileum, this difference was not significant, suggesting that villi in these two segments show similar growth from hatch to 14 days (Table 3, Figures 2-4).

Corroborating with these results, Otutumi et al. (2008), working with European quails up to 35 days, observed a greater development of duodenum villi when compared to the ileum. The same was found in a study with broiler chickens, which observed significant differences in villi length among segments at hatch, with duodenal villi longer than those in the jejunum and ileum (Geyra et al., 2001).

This greater development can be attributed, to the fact that it is the fastest cell renewal segment and also because it is the first segment of the small intestine to receive physical, chemical and hormonal stimuli triggered by the presence of nutrients in the lumen (Macari et al., 2002). Nutrients are stimulating factors for the growth of villi and crypts (Moran Junior, 1985), and this is really evident in differences observed in this research between hatch and 4 days.

In relation to the crypt depth and the villi: crypt ratio for the duodenum, jejunum and ileum were similar between quail types and between segments, but throughout the analyzed period it was observed that the crypts increased in size up to 14 days (Tables 3 and 4). At 15 days of incubation the segments of the small intestine presented 14.95 μm of crypt depth, in hatch 23.75 μm and at 14 days 90.80 μm.

The villi: crypt ratio of the duodenum is higher post-hatch and higher than the ratio observed in the jejunum and ileum. In the jejunum the villi: crypt ratio is similar between the periods analyzed and variable for the ileum (Table 4).

The development of the crypt is crucial in intestinal maturation. The morphology of the small intestine changes rapidly in the late stages of incubation, so that the increase in crypt size provides enterocytes to increase the intestinal absorption surface as the villi grows (Ozaydın et al., 2012), corroborating with the results obtained in this work.

The desirable relationship between villi and intestinal crypts occurs when the villi are high and the crypts are low, since higher the villi height: crypt depth ratio, the better will be the nutrient absorption and the lower the energy losses with cell renewal (Li et al., 1991).

The number of goblet cells was determined by villi in the segments of the small intestine. The amount of goblet cells from the villi of the small intestine segments were similar between the types of quails. At the 15-day incubation, a mean of 4.43 cells per villi was observed in all segments (Table 4, Figures 2-4). These cells are characterized by the presence of oval nucleus in the basal region and cytoplasmic granules filled with secretion of PAS+ (not shown) and AB+ (Figures 2B, 3B and 4B). When the glycoprotein secretion is released by the cell, it becomes highly hydrated, forming a layer of mucus on the surface of the intestinal epithelium.

In hatch the number of goblet cells increased significantly in all segments and from 7 days the results suggest that the number of these cells was similar. From 7 to 14 days, the duodenum presented a number of goblet cell per villi, superior to the jejunum and ileum (Table 4).

Goblet cells play an important role in the maintenance and development of the intestinal epithelium. These are secreting mucus, have the function of protecting the epithelium during digestion and lubricating power on solid foods. Another important role of mucus would be to protect against infection by acting as a protective barrier preventing the contact of microorganisms with epithelial cells (Uni et al., 2003a; Smirnov et al., 2005).

Filamentous bacteria were observed by scanning electron microscopy in the jejunum and ileum from 4 days on quails (Figure 4I). These bacteria were autochthonous microorganisms that colonize the jejunum and ileum by binding to the cells of the intestinal epithelium. These apathogenic bacteria stimulate the mucosal

immune system by elevating the number of lymphoid cells and the secretion of immunoglobulin A (Klaasen et al., 1993).

CONCLUSION

The development and growth of different organs of the digestive system up to 14 days of age was similar between European and Japanese quails, presenting equivalent relative weight. In quail the duodenum increases the villi up to 14 days, while the jejunum and ileum up to 10 and 4 days, respectively.

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Table 1. Bayesian estimates (mean (standard deviation)) for embryo/quail body weight, weight of yolk sac and digestive system, and relative weights of digestive system, small intestine + pancreas and large intestine of European and Japanese quails from 11 days of incubation (11e) to 14 days post-hatch (n=6).

	Age of embryo (e) in days				Post-hatch age in days (d)			
	11e	13e	15e	Hatch	4d	7d	10d	14d
Body weight (g)								
European	2.86 H (0.11)	4.55 G (0.10)	6.53 aF (0.21)	8.24 E (0.38)	13.02 aD (0.86)	28.38 aC (2.79)	38.92 aB (3.11)	73.15 aA (1.84)
Japanese	2.62 H (0.06)	4.05 G (0.03)	6.02 bF (0.14)	7.44 E (0.37)	9.65 bD (0.81)	17.23 bC (2.13)	29.20 bB (1.84)	38.73 bA (3.10)
Weight of the yolk sac (g)								
European	3.22 aA (0.05)	3.44 aA (0.13)	2.40 aB (0.19)	0.59 C (0.10)	0.03 D (0.03)	-	-	-
Japanese	2.80 bA (0.14)	2.65 bA (0.17)	1.86 bB (0.12)	0.50 C (0.10)	0.03 D (0.03)	-	-	-
Weight of the digestive system (g)								
European	0.13 G (0.02)	0.23 F (0.02)	0.39 E (0.03)	0.98 aD (0.08)	2.39 aC (0.22)	4.39 aB (0.29)	5.02 B (0.49)	8.85 aA (0.67)
Japanese	0.09 G (0.04)	0.22 F (0.02)	0.44 E (0.03)	0.78 bD (0.03)	1.82 bC (0.15)	2.90 bB (0.38)	4.04 A (0.26)	4.93 bA (0.39)
Relative weight of the digestive system (%)								
European	4.52 E (0.59)	5.00 E (0.31)	5.96 bD (0.28)	11.83 C (0.62)	18.30 A (0.57)	15.72 B (0.95)	12.92 C (0.89)	12.11 C (0.96)
Japanese	3.27 E (1.26)	5.57 E (0.39)	7.25 aD (0.40)	10.52 C (0.63)	18.93 A (1.08)	16.86 A (1.01)	13.83 B (0.38)	12.78 B (0.60)
Relative weight of the small intestine + pancreas (%)								
European	-	-	-	-	7.95 (0.85)	7.92 (0.75)	6.97 (0.58)	7.01 (0.38)
Japanese	-	-	-	-	7.77 A (0.32)	7.73 AB (0.58)	6.71 B (0.28)	6.50 B (0.46)
Relative weight of the large intestine (%)								
European	-	-	-	-	1.57 bA (0.12)	1.20 AB (0.27)	1.17 B (0.12)	1.30 AB (0.18)
Japanese	-	-	-	-	2.41 aA (0.14)	1.62 AB (0.42)	1.27 B (0.11)	1.20 B (0.10)

[a,b] Distinct lowercase letters in the column (European x Japanese), and [A,B] distinct uppercase letters in the row, indicate significant differences, through Bayesian comparisons at a 95% level of credibility.

Table 2. Bayesian estimates (mean (standard deviation)) for weight, relative weight (n=6) and total wall thickness of the proventriculus (n=4), and weight, relative weight (n=6), thickness of the tunica mucosa and coilin membrane of the gastric ventricle (n=4) of European and Japanese quail from 15 days of incubation (15e) to 14 days post-hatch.

	Age of embryo (e) in days		Post-hatch age in days (d)			
	15e	Hatch	4d	7d	10d	14d
Weight of the proventriculus (g)						
European	-	-	0.15 C (0.03)	0.24 B (0.03)	0.30 B (0.03)	0.53 aA(0.04)
Japanese	-	-	0.12 C (0.02)	0.20 BC (0.03)	0.24 B (0.03)	0.37 bA (0.03)
Relative weight of the proventriculus (%)						
European	-	-	1.20 A (0.17)	0.90 AB (0.10)	0.76 B (0.06)	0.73 B (0.05)
Japanese	-	-	1.27 (0.17)	1.14 (0.11)	0.90 (0.10)	0.97 (0.06)
Total wall thickness of the proventriculus (µm)						
European	731.90 C (43.56)	771.10 bC (153.40)	1170.00 BC (201.30)	1549.00 B (142.90)	1826.00 B (265.30)	2514.00 A (110.70)
Japanese	772.10 C (63.57)	1136.00 aB (84.93)	1521.00 B (345.30)	1757.00 B (263.40)	1702.00 B (419.80)	2478.15 A (101.90)
Weight of the gastric ventricle (g)						
European	-	-	0.86 C (0.14)	1.49 B (0.11)	1.56 B (0.16)	2.58 aA (0.24)
Japanese	-	-	0.72 B (0.07)	1.11 AB (0.22)	1.38 A (0.14)	1.59 bA (0.19)
Relative weight of the gastric ventricle (g)						
European	-	-	7.07 A (0.63)	5.54 B (0.42)	4.03 bC (0.35)	3.56 C (0.33)
Japanese	-	-	7.48 A (0.76)	6.35AB (0.75)	5.06 aB (0.15)	4.10 C (0.29)
Tunica mucosa of the gastric ventricle (µm)						
European	178.30 B (14.35)	325.80 A (63.97)	236.10 A (59.30)	313.50 A (19.77)	276.90 A (53.52)	334.80 A (113.30)
Japanese	199.50 B (51.19)	316.30 A (30.37)	304.40 A (37.07)	315.90 A (61.82)	263.10 AB (70.08)	284.30 A (142.80)
Thickness coilin membrane of the gastric ventricle (µm)						
European	22.95 C (26.84)	125.40 B (10.06)	134.60 B (50.20)	205.50 A (6.12)	122.70 B (35.85)	242.60 A (45.36)
Japanese	22.47 C (29.30)	104.60 B (28.50)	125.50 AB (42.57)	148.20 AB (32.67)	169.20 A (13.15)	146.80 AB (51.97)

[a,b] Distinct lowercase letters in the column (European x Japanese), and [A,B] distinct uppercase letters in the row, indicate significant differences, through Bayesian comparisons at a 95% level of credibility.

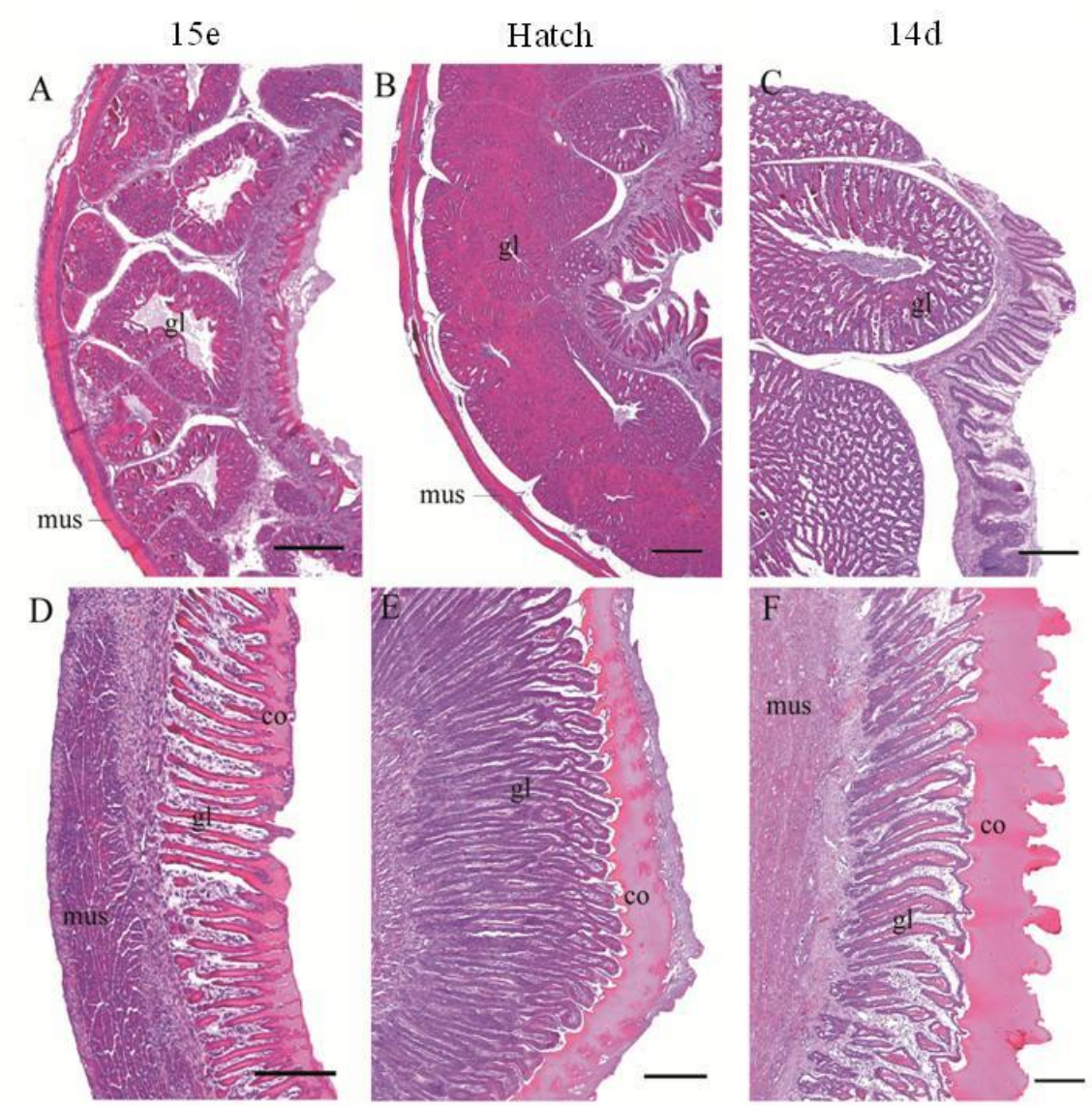

Figure 1. Proventriculus and gastric ventricle of European quails stained in HE. A-C) Proventriculus of European quails with 15 days of incubation (15e), hatch and 14 days post-hatch, respectively, evidencing the tunica muscularis (mus) and the glands (gl). D-F) Gastric ventricle, evidencing the tunica muscularis (mus), tubular glands (gl) and the eosinophilic coilin membrane (co). Scale bars: A-C) 200 μm; D-F) 100 μm.

Table 3. Bayesian estimates (mean (standard deviation)) for villi height and depth of crypt of the duodenum, jejunum and ileum of European and Japanese quails from 15 days of incubation (15e) to 14 days post-hatch (n=4).

	Age of embryo (e) in days		Post-hatch age in days (d)			
	15e	Hatch	4d	7d	10d	14d
			Villi height (µm)			
Duodenum						
European	101.70 E (19.48)	302.50 D (68.60) α	490.20 C (51.06) α	583.90 C (27.70) α	811.00 B (66.48) α	992.00 aA (38.55) α
Japanese	98.14 D (40.51)	357.10 C (28.94) α	501.80 B (36.75) α	599.30 AB (63.99) α	705.00 A (72.10) α	697.40 bA (74.29) α
Jejunum						
European	73.41 D (13.89)	160.00 C (49.00) β	243.80 BC (68.35) β	285.40 B (22.12) β	324.80 bAB (29.22) β	374.30 A (41.60) β
Japanese	72.74 D (10.57)	161.40 C (4.54) β	198.80 BC (25.66) β	250.10 B (53.78) β	391.10 aA (16.49) β	422.90 A (47.20) β
Ileum						
European	72.41 C (19.80)	128.40 B (19.18) β	220.10 A (33.76) β	313.90 A (50.97) β	333.30 A (60.51) β	340.30 A (74.01) β
Japanese	59.06 D (5.61)	144.80 C (55.95) β	199.30 BC (48.31) β	252.40 AB (18.32) β	302.00 AB (33.73) γ	338.50 A (54.22) β
			Crypt depth (µm)			
Duodenum						
European	17.46 C (1.43)	23.08 C (6.75)	43.09 B (2.50)	48.88 B (6.98)	60.52 B (16.21)	100.10 A (2.19)
Japanese	14.19 E (1.82)	23.79 D (1.77)	41.67 C (4.16)	54.02 BC (6.94)	70.90 B (6.45)	88.61 A (5.75)
Jejunum						
European	14.33 E (2.14)	24.93 D (0.98)	35.64 C (4.46)	52.96 B (20.97)	60.72 B (26.15)	101.10 A (11.30)
Japanese	14.20 E (2.11)	25.33 D (4.73)	41.84 C (5.47)	49.28 BC (5.27)	63.51 AB (9.41)	87.29 A (12.92)
Ileum						
European	15.02 D (6.90)	22.28 D (2.12)	38.29 C (1.34)	55.53 B (8.73)	60.93 AB (25.90)	87.09 A (9.42)
Japanese	14.51 D (1.52)	23.08 C (0.80)	40.85 B (0.81)	44.02 B (8.09)	68.89 A (10.75)	80.61 A (2.56)

[a,b] Distinct lowercase letters in the column (European x Japanese), and [A,B] distinct uppercase letters in the row, and [α.β] distinct Greek letters between the segments, indicate significant differences, through Bayesian comparisons at a 95% level of credibility.

Table 4. Bayesian estimates (mean (standard deviation)) for villi: crypt ratio and number of goblet cells/villi of the duodenum, jejunum and ileum of European and Japanese quail from 15 days of incubation to 14 days post-hatch (n=4).

	Age of embryo (e) in days		Post-hatch age in days (d)			
	15e	Hatch	4d	7d	10d	14d
			Villi: crypt (µm/µm)			
Duodenum						
European	5.84 B (1.15)	12.62 A (4.70) α	11.50 A (2.02) α	11.98 A (1.40) α	13.91 A (3.94) α	10.03 A (0.21) α
Japanese	7.01 B (3.93)	15.02 A (0.20) α	12.21 AB (1.86) α	11.21 B (1.04) α	9.87 B (3.63) α	7.72 B (1.67) α
Jejunum						
European	5.22 (1.09)	5.24 (1.77) β	6.75 (1.14) β	5.54 (1.79) β	5.86 (3.21) β	3.92 (2.32) β
Japanese	5.17 (1.82)	6.41 (1.20) β	4.89 (1.06) β	5.15 (2.87) β	6.25 (0.66) αβ	4.83 (0.97) αβ
Ileum						
European	5.01 (3.87)	5.79 (1.00) β	5.75 (1.02) β	5.68 (0.92) β	5.65 (1.71) β	3.58 (1.91) β
Japanese	4.09 B (0.95)	6.27 A (0.07) β	4.92 AB (1.30) β	5.76 A (0.76) β	4.42 B (0.96) β	4.14 B (1.03) β
			Goblet cells			
Duodenum						
European	4.38 D (1.36)	19.62 C (5.68)	36.84 BC (2.51)	129.60 A (29.38) α	69.35 AB (30.75) α	87.48 A (13.39) α
Japanese	4.14 C (2.09)	23.24 B (38.04)	45.19 B (13.47)	107.40 A (6.16) α	110.90 A (33.46) α	91.36 A (20.71) α
Jejunum						
European	4.84 C (1.41)	13.82 C (7.73)	30.79 B (12.58)	53.92 A (20.27) β	32.47 bB (10.58) β	51.22 A (8.33) β
Japanese	3.28 D (1.63)	14.25 C (29.10)	25.94 C (3.49)	49.01 B (7.13) β	76.43 aA (9.79) β	58.89 AB (15.65) β
Ileum						
European	4.91 C (4.13)	17.02 B (0.50)	32.74 AB (16.25)	58.52 A (18.70) β	31.42 AB (37.73) β	43.65 A (12.67) β
Japanese	5.00 C (2.16)	17.16 B (5.82)	26.68 B (4.20)	35.94 A (16.00) β	48.65 AB (21.61) β	61.74 A (9.25) β

[a,b] Distinct lowercase letters in the column (European x Japanese), and [A,B] distinct uppercase letters in the row, and [α,β] distinct Greek letters between the segments, indicate significant differences, through Bayesian comparisons at a 95% level of credibility.

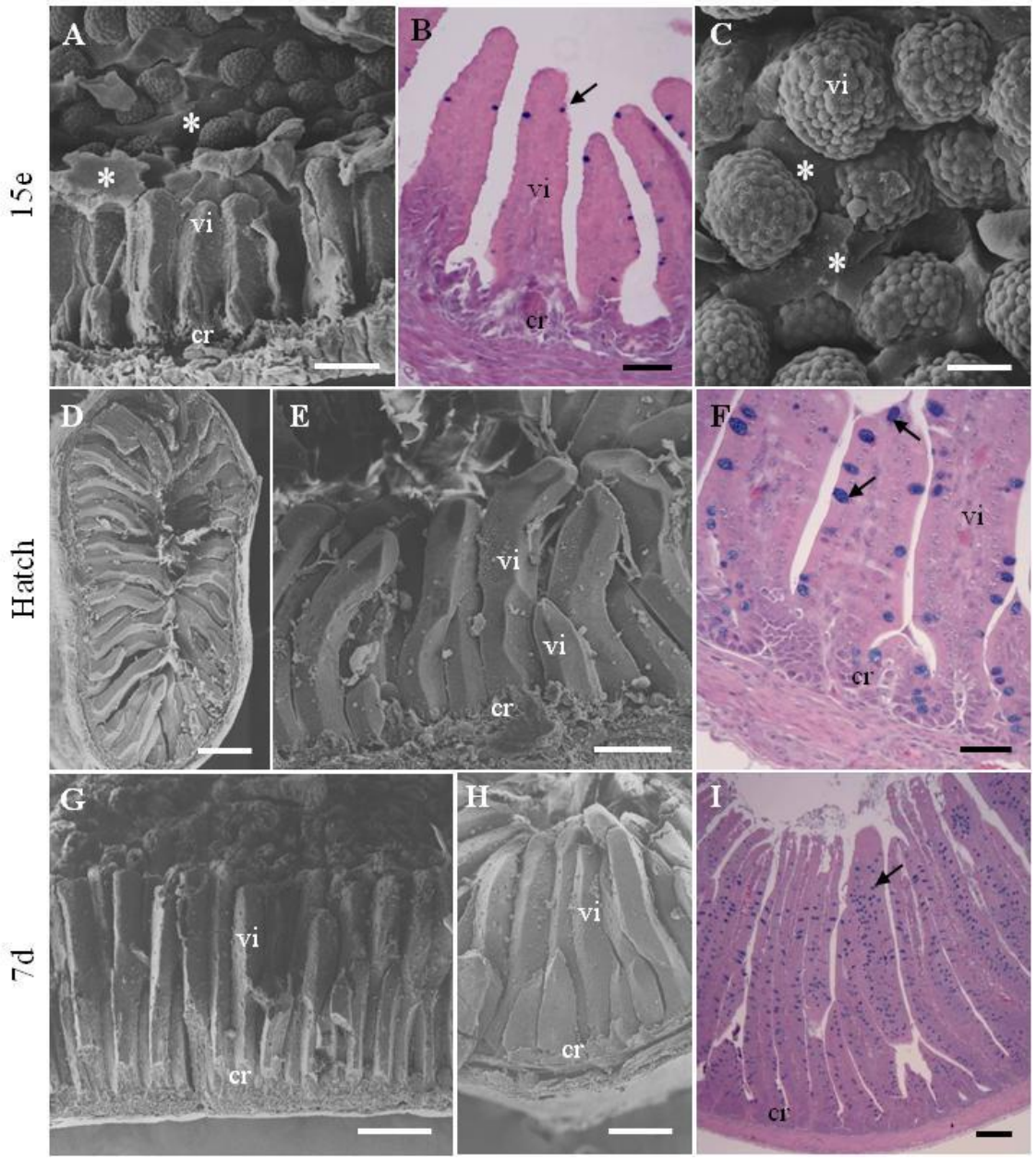

Figure 2. Intestinal villi of the duodenum of quail seen by SEM and by light microscopy. A-C) Japanese quails with 15 days of incubation. D-F) European quails at hatch. G-I) Japanese quails with 7 days post-hatch. Goblet cells (arrows) are already present in the duodenum at 15 days of incubation (B) marked by the blue stained cytoplasmic granules (AB+) in the crypts (cr) and along the villi (vi). The number of these cells and the height of the villi increased over time. Detect secretion around the villi (*) at 15 days of incubation and the appearance of the enterocytes covering the villi (C). Detect shorter villi in hatch. B, F, I) HE + Alcian Blue (pH 2.5). Scale bars: A) 50 μm; B, F) 30 μm; C) 20 μm; D, H) 200 μm; E, I) 100 μm; G) 250 μm.

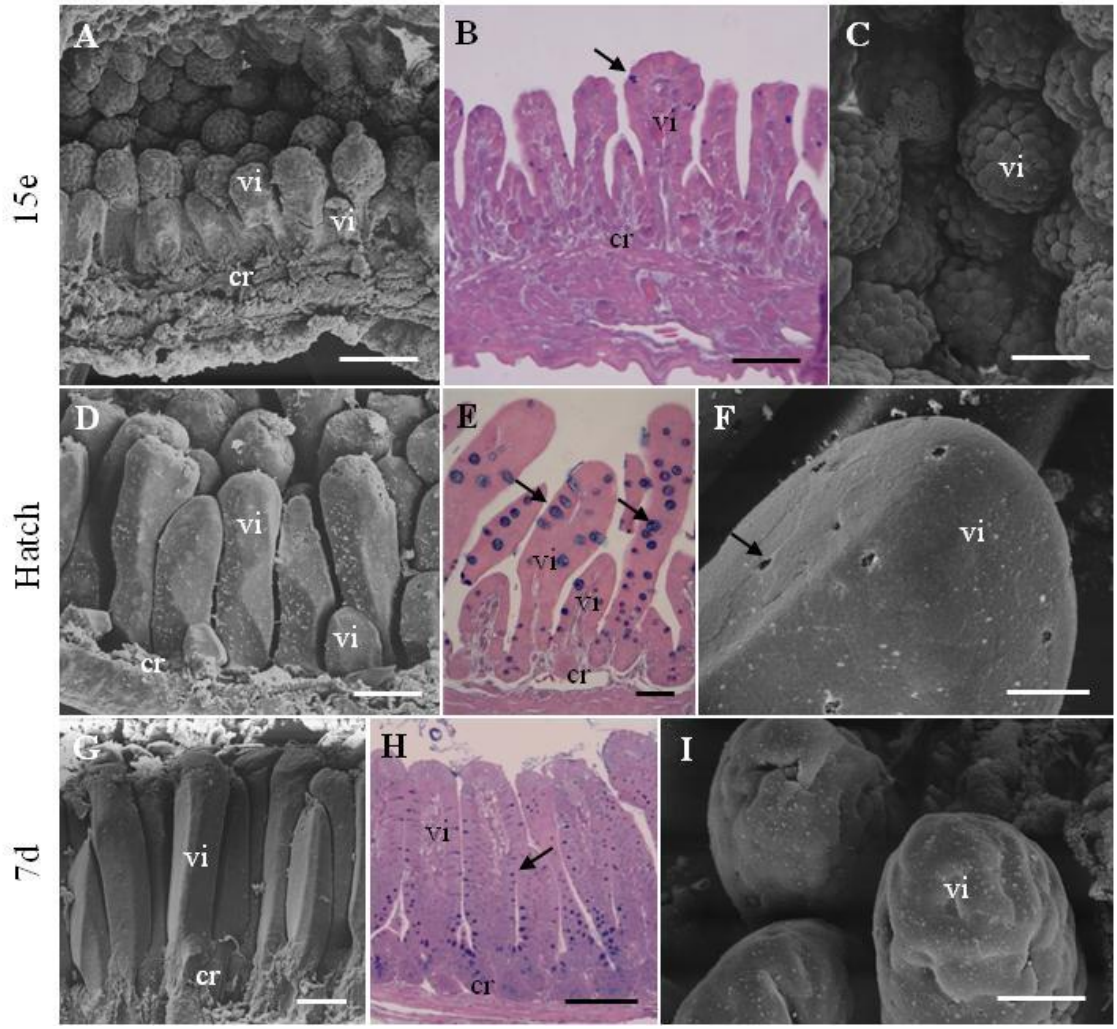

Figure 3. Intestinal villi of the quail jejunum seen by SEM and by light microscopy. A-C) Japanese quails with 15 days of incubation. D-F) Japanese quail in hatch. G-I) European quails with 7 days post-hatch. Goblet cells (arrows) are already present in the jejunum at 15 days of incubation (B) marked by cytoplasmic granules stained in blue (AB+) in the crypts (cr) and along the villi (vi). The number of these cells and the height of the villi increased over time. Detect shorter villi at 15 days of incubation and in the hatch. B, E, H) HE + Alcian Blue (pH 2.5). Scale bars: A, D, G) 50 μm; B, E) 30 μm; C, I) 20 μm; F) 10 μm; H) 100 μm.

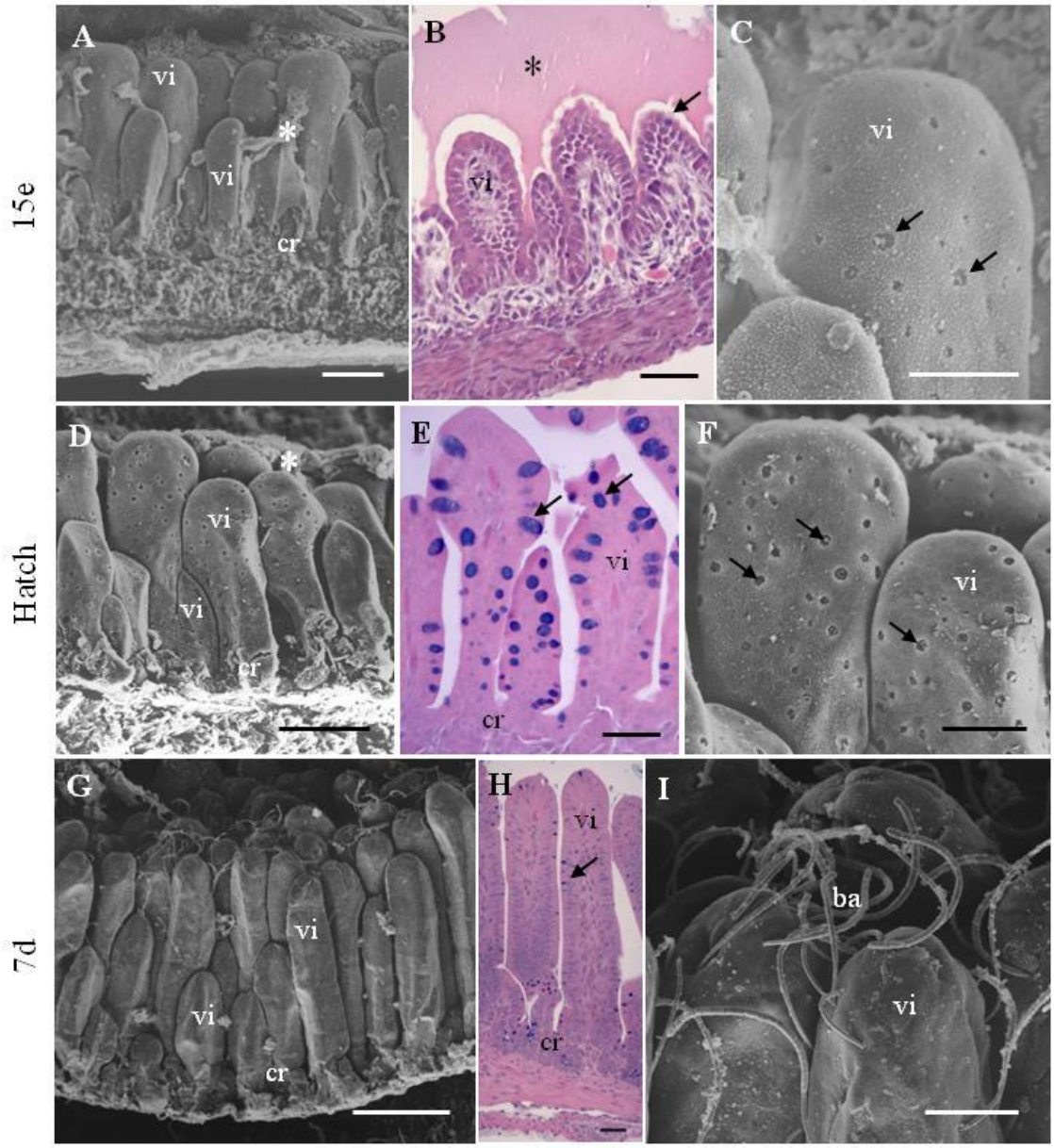

Figure 4. Intestinal villi of the quail ileum seen by SEM and by light microscopy. A-C) European quails with 15 days of incubation. D-F) European quails at hatch. G-I) Japanese quails with 7 days post-hatch. Goblet cells (arrows) are already present in the ileum at the 15 days of incubation (B) labeled by the cytoplasmic granules stained in blue (AB +) in the crypts (cr) and along of the villi (vi). The number of these cells and the height of the villi increased over time. Detected secretion around the villi (*) (A, B, D) and presence of bacteria (ba) (G, I). Detect interspersed shorter villi at all ages. B, E, H) HE + Alcian Blue (pH 2.5). Scale bars: A, G, H) 100 μm; B, E) 30 μm; C, F, I) 20 μm; D) 50 μm.

IV. Animal performance and development of the digestive system of European quails submitted to different post-hatch fasting periods

ABSTRACT: The objective of this study was to evaluate the effect of different post-hatch fasting periods on the animal performance and organs development of digestive system of European quail up to 35 days of age. Quail chicks born at the peak hatching were used. Chicks were distributed in completely randomized desing, with four treatments (control) and three fasting periods (24, 36 and 48 hours) and four replicates of 40 birds per experimental unit. The characteristics studied were analyzed by Bayesian Methodology. In the period of 1-14 days, chicks submitted to post-hatch fasting periods had a reduction in weight gain as the feed restriction intensified. However, from the age of 15 days European quails showed a compensatory gain. For relative weight and length of the digestive system, the birds submitted to fasting had lower values than those fed. However, at 14 days, there was a recovery of the digestive system. The height of the duodenum villi showed a significant effect at 3 days of age with reduced heights from 36 hours of fasting, presenting recovery from 7 days. Although, the height of the jejunum and ileum villi was not influenced by the fasting periods studied. It is concluded that the fasting period of up to 48 hours post-hatch influences the development of the organs of the European quail digestive system up to 7 days of age, but this one presents a compensatory gain from this period. Weight gain, feed conversion during the period of 1-35 days of age, intestinal mucosal epithelial integrity and muscle development were not influenced by post-hatch fasting period.

Key words: body weight, compensatory gain, *Coturnix coturnix*, food restriction, intestine.

INTRODUCTION

The negative effects of post-hatch fasting are directly related to changes in the physiological processes that affect the development of the avian digestive system. Soon after hatch, most of the nutrient demand is directed towards to the development of the digestive system organs, because these will support the growth of other tissues with nutrient supply. For this reason, rapid access to food is fundamental during the

transition phase between embryonic phase and the use of exogenous diets (Noy and Sklan, 1999; Cançado and Baião, 2002).

The operating factors of the hatchery, such as the sexing, vaccination and conditioning periods, associated with travel time to the farm, subject the newly hatched chicks to periods of fasting that can last from 24 to 72 hours, depending on the distance between the hatchery and the farm. All these factors can determine to the chick, up to 10% of weight losses (Cançado and Baião, 2002).

Researches showed that the fasting in birds impairs intestinal development (Uni et al., 1998; Corless and Sell, 1999; Maiorka et al., 2003), hinders the absorption of nutrients from the yolk sac (Moran and Reinhart, 1980; Noy et al., 1996; Noy and Sklan, 2001; Pedroso et al., 2006), reduces the weight of the organs (Maiorka et al., 2003) and causes losses in animal performance. Nir and Levanon (1993), working with broiler chickens, observed that the post-hatch fasting of 24 and 48 hours resulted in delayed growth of the bird, equivalent to one and two days of weight gain, respectively.

Considering the importance of early feeding in the development and maturation of organs of the digestive system and their impacts on perfomance indexes, this study was developed with the objective of evaluating the effect of different periods of post-hatch fasting on animal performance and organs development of digestive system of European quail up to 35 days of age.

MATERIAL AND METHODS

This research was approved by the Committee of Ethical Conduct on the Use of Animals in Experimentation of the State University of Maringá, protocol number 8793250615. The experiment was conducted in the Poultry Farm Sector of the Experimental Farm of Iguatemi (FEI) of the State University of Maringá (UEM).

A total of 450 European quails were used in peak laying, standardized by weight and egg production. The birds were distributed in galvanized wire cages (25 x 39 cm), in the ratio of 2 females to 1 male, receiving water and feed *ad libitum*.

Eggs from quail breeders were selected by weight with variation of up to 5% of the average weight, and incubated in an automatic incubator with 60% humidity and at

37.6ºC, with automatic turning. After 348 hours of incubation, the eggs were transferred to the hatch chamber, with a temperature of 37.0°C and humidity of 70%.

From the beginning of hatch the birth was monitored hourly, and chicks were selected in peak of hatch and discarding the birds born before and after this period. After four hours of the peak of birth, the animals were housed in boxes of 2.5 x 1 m, with bed of rice straw, covered with corrugated paper and brooder heating plate.

The quails were distributed in a completely randomized design, with four treatments, control and three fasting periods of 24, 36 and 48 hours, and four replications of 40 birds per experimental unit (mixed lots). Birds received water and food immediately in control treatment and only 24, 36 and 48 hours after lodging, according to each treatment. The first procedure adopted after fasting was the water supply (about 30 minutes), until all quails were hydrated and did not look for the drinker anymore, and after this period they received the feed.

The diet provided was based on corn and soybean meal, formulated according to the requirements and values of chemical composition of food according to Silva and Costa (2009). During the experimental period (1 to 35 days), weekly the birds and the leftovers were weighed and the variables of animal performance were determined: feed intake (g), weight gain (g) and feed conversion (g/g).

On days 02, 03, 07, 14, 21, 28 and 35, 08 chicks per treatment were anesthetized by thiopental 25 mg/kg + lidocaine 10 mg/kg (intraperitoneally) and, after anesthesia was detected due to loss of reflexes, they were sacrificed by cervical dislocation and destined for analysis (n=56 per treatment).

In each period, the organs of the digestive system were dissected, isolated, emptied of contents and weighed. The variables analyzed were: live weight (g), relative weight (%) and length of the digestive system (proventriculus, gastric ventricle, small and large intestine). The total weight of the digestive system was obtained before the separation of the organs in chicks of 02 and 03 days of age. In the larger chicks, the weight was the result of the sum of the isolated weight of the different analyzed organs.

From 7 days of age it was possible to divide the segments of the digestive system, being evaluated: relative weight of the proventriculus, gastric ventricle, duodenum and jejunum + ileum, and duodenum and jejunum + ileum length.

At 3, 7 and 14 days of age, fragments (± 5 mm) of the intestine were fixed by immersion in 4% paraformaldehyde in 0.1M PBS pH 7.4 for light microscopy and 2.5% glutaraldehyde in 0.1M PBS pH 7.4, at 3 days, for scanning electron microscopy.

Light Microscopy

For the histomorphological evaluation of the digestive system, at 3, 7 and 14 days of age (n=4). The fragments of each organ were processed in the histological routine, included in paraffin, cut with 3 micrometers of thickness and stained with hematoxylin-eosin (HE) + Alcian Blue (AB) pH 2.5 (intestine). Digital images were captured in a light microscope coupled to a digital camera (Motican®, China Group Co. Ltd., Xiamen, China) for morphometric analysis.

The images were analyzed using Motic Image Plus 2.0 software (Motic®). There was determined in the duodenum, jejunum and ileum the villi height, crypt depth, villi/crypt ratio and villi width. For each variable 10 measurements were performed on each bird in at least 3 semi-serial cuts.

Scanning electron microscopy (SEM)

At 3 days of age, the duodenum, jejunum and ileum fragments were fixed in glutaraldehyde, stored at 4°C and then washed in 0.1M phosphate buffer and dehydrated in increasing concentrations of ethyl alcohol (70, 80, 90, 100 I, 100 II and 100 III%), 20 minutes at each concentration. After dehydration, the material passed through the critical point chamber (Bal-Tec CPD 030) by the use of carbon dioxide. The m aterial was then mounted on metal stubs, covered with a 30 nm gold layer on Bal-Tec SCD 050 metallizer, and analyzed on a Shimadzu SS-550 Superscan scanning electron microscope.

Characterization of types of muscle fiber

For histological analyzes at 35 days of age, pectoral muscle samples from 4 animals per treatment were collected by means of a transverse cross-section, perpendicular to the fibers orientation according to Scheuermann et al. (2004), in the middle portion of the muscle.

The samples were immediately frozen in n-Hexane, previously cooled to -70°C in liquid nitrogen (Chayen et al., 1969) and packed in liquid nitrogen. The frozen muscle fragments were cut in a cryostat microtome at -26ºC with 10 micrometers thickness, oriented in the transverse direction of the muscle fibers.

For the morphological analysis of the fibers, the sections were stained at HE and for the identification and classification of the muscle fiber type, the sections were prepared by histoenzymological technique of Nicotinamide adenine dinucleotide-Tetrazolium Reductase (NADH-TR), used to evaluate the oxidative-glycolytic metabolism (Dubowitz and Brooke, 1973). The fibers were classified as IIa (fast/oxidative/glycolytica) and IIb (fast/glycolytica). In order to count the fiber types, 10 muscle bundles were evaluated in each bird in different cuts, and the diameter was obtained by measuring 100 fibers of each type per bird. Digital images were captured in a light microscope coupled to a digital camera (Motican® 5MP), and analyzed using Motic Image Plus 2.0 software (Motic® China Group Co. Ltd., Xiamen, China).

Statistical analysis

The characteristics studied were analyzed by Bayesian Methodology. In this procedure, the response (j = 1, 2, 3, 4) (Y_{ij}) follows a normal distribution (Gaussian), $Y_{ij} \sim N(\mu_j,\sigma_j^2)$. There were considered the prior noninformative distributions, for the model parameters, $\mu \sim N\ (0,10^{-6})$ and $\tau \sim Gamma\ (10^{-3},10^{-3})$ ($\tau = 1/\sigma^2$, OpenBUGS parameterization) (Rossi, 2011). The significance of the differences between averages ($\Delta_{jk}=\mu_j-\mu_k$, $j\neq k$) the posterior of the treatments/groups considered was based on the presence or absence of zero in the respective 95% credibilty intervals.

The posterior marginal distributions were obtained for all parameters by the Brugs package of the R program (R Development Core Team, 2017). A total of 20.000 values were generated in a Monte Carlo Markov Chain (MCMC), sampling discard of 10% of initial values. The convergence of the chains was verified through the coda package, program R, using the criterion of Heidelberger and Welch (Heidelberger and Welch, 1983). For some variables the data were transformed (y_t= log(y)) prior to analysis.

RESULTS AND DISCUSSION

Productive performance

The Bayesian estimates for weight gain, feed intake and feed conversion in the periods 1-14, 15-35 and 1-35 days are shown in Table 1. As expected, over the period of 1-14 days, chicks submitted to post-hatching fasting had a reduction in weight gain as the feed restriction intensified. However, from 1-35 days of age this effect was no observed, showing that European quails presented a significant compensatory weight gain.

Compensatory growth is a phenomenon that modifies the growth pattern of birds, that is, during the period of restriction the bird has reduced body weight, and decreased its maintenance requirement (Furlan et al., 2001). However, when the feed is supplied, this modification determines a greater efficiency of the nutrient use for its growth (Furlan et al., 2001), which justifies the recovery in the weight gain and the equalization with the other treatments studied.

For feed intake, in the period of 1-14 days no difference was observed. However, from 15-35 days, birds that underwent 36 hours of post-hatch fasting consumed less when compared to those who had 24 and 48 hours of fasting. From 15-35 days, the birds that were submitted to 36 hours of fasting consumed less ration when compared to the control and 24 hours.

Feed conversion was not influenced by the evaluated treatments (Table 1). This result was similar to those found by Leu et al. (2002) and Carvalho et al. (2013), who did not observe differences in feed conversion of broilers submitted to fasting periods of up to 36 hours. However, Pedroso et al. (2006) reported improved in feed conversion at 21 days of age in broilers submitted to fasting in the first 48 hours of life, in relation to those who had immediate access to feed after hatch.

Morphometric data of organs of the digestive system

The weight of the bird and the organs development of the digestive system were evaluated from 2 to 35 days of age as a function of the fasting periods studied. Some variables could only be obtained separately after 7 days due to the small size of the viscera (Tables 2-3).

According to the Bayesian posterior estimates and the respective, credibility intervals it was possible to verify that there was significant effect among the treatments for the live weight of the bird (Table 2). At 2 days of age, the animals that were not submitted to post-hatching fasting had higher body weight in relation to the others. In the presence of acute metabolic needs, muscle tissue can be metabolized to meet the chick's basic survival needs (Uni et al., 2005; Fairchild et al., 2006), which could explain the low body weight observed by the fasting (24, 36 and 48 hours).

It can also be observed that the birds presented compensatory gain when fed, so that at 14 days of age only the animals that were submitted to 48 hours of fasting were kept underweight. However, at 21 days all were characterized by the same body development (Table 2).

Similar results were observed by Noy and Sklan (1999), where the higher initial growth rate in delayed feeding chicks, induced by feed intake after 2 days of starvation was insufficient to correct the consequences of delayed feeding on body weight at 6 days of age.

Carvalho et al. (2013), when working with broilers, found a loss of 5.7 g in the first 48 hours of fasting. This reduction, according to Halevy et al. (2000), is attributed to greater use of yolk sac reserves and dehydration, which are more pronounced at this stage.

For the weight of yolk sac at 2 and 3 days of age no significant effect was observed (Table 2). This shows that the amount of yolk sac present in the body of the animals after hatching was not interfered by the absence or presence of feed in the digestive system, corroborating with Murakami et al. (1992) and Bigot et al. (2003). This equality of yolk sac absorption between animals can be related to the low development of the digestive system of the fasting birds, and to the low metabolism and growth of these that, consequently, reduce their energetic need (Gonzales et al., 2003).

Therefore, these findings indicate that the feed was the main source of post-hatch nutrients, since the animals that stayed longer in the fasting (36 and 48 hours) did not have the accelerated absorption of the yolk sac to supply the unavailability of feed, which may have contributed to low body weight.

Disagreeing with the results obtained in this work, Moran and Reinhart (1980), Noy et al. (1996), Noy and Sklan (2001) and Pedroso et al. (2006) reported in broilers

that the weight of yolk sac is higher when the animal receives feed than when it is fasted.

For the relative weight of the digestive system (proventriculus, gastric ventricle and intestines), the birds submitted to fasting presented lower weight than those fed *ad libitum*. However, it was possible to observe this behavior until the 7th day, because from the 14th day, there was a recovery of the digestory weight (Table 3).

The absence of some nutrients from food may jeopardize the digestive system development and consequently body weight of the quails, since the initial high development is due to the increase of the viscera and especially of the intestine (Nitsan et al., 1991).

The weight of the proventriculus and of the gastric ventricle at 7 days presented a percentage increase in relation to the live weight, from 24 hours of fasting and recovery of this proportion from 14 to 35 days of age (Table 3).

Maiorka et al. (2003), worked with broiler chickens submitted to 72 hours of post-hatch fasting and observed that the relative weight of the proventriculus + gastric ventricle was lower in the birds that received feed.

The relative weight of the duodenum at 7 days of age was lower in the fasting period of 48 hours in relation to quails fed *ad libitum* (Table 3). However, after 14 days a weight recovery was observed. As for the relative weight of the jejunum + ileum, no significant effect was observed as a function of the treatments for any of the analyzed ages, suggesting that the jejunum and ileum recovered more rapidly than the duodenum in quail after prolonged post-hatch.

For the visceral length data, according to the Bayesian posterior estimates and the respective credibility intervals, a significant effect was observed for the length of the digestive system (proventriculus, gastric ventricle and intestines) from 2 to 7 days and from the duodenum to 7 days, according to the fasting periods (Table 4). At 2 days of age, the European quails fed *ad libitum* had a 1.62-fold greater length of the digestive system when compared to those that were submitted to 48 hours of post-hatch fasting, reducing to 1.22 fold at 7. At 14 days of age, treatments had similar digestive length. For the duodenum, a difference was observed at 7 days of age, and the fasting of 48 hours was significantly shorter than birds fed *ad libitum* (Table 4).

Similar results were found in a study with turkeys, which observed that delays in access to food and water slow the growth of the digestive system and limit the capacity of nutrients use in diet, resulting in reduction of body weight (Corless and Sell, 1999).

During the first hours of the bird life, most of the nutrient demand is directed towards to the growth of the digestive system organs, since them will support the growth of other tissues with nutrient supply (Noy and Sklan, 1999; Cançado and Baião, 2002).

Absence of feed and water shortly post-hatch negatively affects intestinal development in chickens and therefore feed should be offered as soon as possible after hatching to avoid delay in the development of the chick's digestive system (Maiorka et al., 2003).

Uni et al. (1998), working with broiler chickens, observed that 36-hour fasting impaired small intestine growth, but the duration of this developmental delay was not the same in different segments of the intestine. In the jejunum, recovery occurred after 11 days of age, as well as in the duodenum and ileum, the size was recovery after 5 days.

Light microscopy and SEM

Segments of the small intestine were analyzed by light microscopy and morphometric data of intestinal villi were measured. For the duodenum, the villi height showed significant effect at 3 days of age with reduced heights from 36 hours of fasting, showing recovery from 7 days (Table 5). The villi height of the jejunum and ileum was not influenced by the fasting periods studied in any of the ages (Tables 6 and 7).

The duodenum, in addition to having higher villi height, also has a higher turnover rate of the intestinal mucosa. This rapid duodenal turnover can be explained by the fact that this is an extremely important region for the digestive process, since it is where the release of biliary and pancreatic secretions occurs (Macari et al., 2002). It is the first segment of the intestine to receive physical, chemical and hormonal stimuli, triggered by the presence of nutrients in the lumen, being considered a stimulating factor for the growth of villi and crypts (Moran Junior, 1985).

The development of the crypt is crucial in intestinal maturation. The morphology of the small intestine changes rapidly in the late stages of incubation, so that the

increase in crypt size provides enterocytes to increase the intestinal absorption surface as the villi grows (Ozaydın et al., 2012). Thus, animals submitted to the post-hatch fasting showed smaller crypts to the duodenum (Table 5). For the jejunum, recovery can be observed after 7 days and the ileum did not present difference in any of the ages (Tables 6 and 7).

In tables 5-7, it can be observed that the higher the fasting period, the higher the villi height: depth of crypt ratio for duodenum, jejunum and ileum, respectively.

For the villi width of the duodenum and jejunum at 3 days, larger villi were observed in the birds that were not subjected to fasting periods when compared to the 48 hours restriction (Tables 5 and 6). For the ileum, no significant effect was observed as a function of the treatments for any of the ages analyzed, suggesting that the ileum recovered its development faster than the duodenum and jejunum in quail after prolonged post-hatch fasting (Table 7).

In the analyzes performed by SEM at three days of age, it is possible to observe a lower villi height in birds that were submitted to the fasting period. However, with intact villi without lesions at the extremities (Figure 1). At that age, in all segments (duodenum, jejunum and ileum) villi were characterized in different sizes, with an elongated shape towards the intestinal lumen, covered by enterocytes and with goblet cells dispersed throughout the them.

Soares et al. (2007) studied the influence of water and feed restriction during the pre-initial stage on the performance of broiler chickens up to 42 days of age, and report that it was possible to verify visual differences between the villi in the intestine. In birds raised with water *ad libitum*, the villi presented a smoother and rounder appearance, while increased restriction resulted in flattened and wrinkled villi.

Uni et al. (1998) stated that some microvillus clumping and abnormal crypt structure occur in birds with delayed access to feed. Yamauchi et al. (1996) demonstrated that, after long periods of fasting, epithelial cells begin to show large lysosomal autophagic vacuoles, which suggests that fasting can cause cell death. Cell death probably leads to increased extrusion rate and a consequent reduction in villi size, which have an important impact on intestinal area for digestion and absorption (Smith et al., 1990).

Breast muscle

According to the Bayesian posterior estimates and the respective intervals of credibility it was possible to verify that there was no significant effect between the treatments for weight and relative weight of the breast, the amount of fibers per muscle bundle and the diameter of fibers of the type IIa and IIb of the *pectoralis* muscle of European quails at 35 days of age (Table 8).

In the cross-sectional histological sections of the pectoral muscles samples of European quails, two types of fibers were identified: IIa and IIb. Type IIa fibers showed intense reaction (dark staining) in relation to type IIb fibers, characterized by high concentrations of mitochondrial enzyme NADH-TR (Figure 2). The fibers of smaller diameter (IIa) and of greater oxidative capacity were located in the center of the muscular bundles and the fibers of larger diameter (IIb) and smaller oxidative capacity in the periphery.

In birds, muscle development occurs in two distinct periods. The first occurs in the embryonic stage, where the number of muscle fibers is established when a large number of precursor cells is determined to express muscle-specific genes. In the second period of development, the post-hatch, occurs the addition of protein and nuclei originating from the fusion and proliferation of satellite cells (Christ and Brand-Saberi, 2002).

In the embryonic stage, the increase in the number of muscle fibers occurs by cell division (muscular hyperplasia) (Smith, 1963; Velleman, 2007). After hatch, these cells proliferate and fuse with existing muscle fibers, synthesizing specific proteins, which increase the volume through the formation of new sarcomeres (muscular hypertrophy) (Silva and Carvalho, 2007). Taking into account that in this work the amount of fibers was analyzed by muscle bundle, it was expected that this number did not present significant difference.

Pinchasov and Noy (1993) working with turkeys, also show that early post-hatch feed deprivation decreases growth, possibly programming the muscle to be smaller via decreased satellite cell mitotic activity.

CONCLUSION

It is concluded that the period of up to 48 hours of post-hatch fasting influences the digestive system organs development of the European quail up to 7 days of age, but this one presents a compensatory gain from this period. Weight gain, feed conversion during the period of 1-35 days of age, intestinal mucosal epithelial integrity and muscle development are not influenced by post-hatch fasting.

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Table 1. Bayesian estimates (mean (standard deviation)) for weight gain, feed intake and feed conversion of European quails from 1 to 35 days of age submitted to different periods of post-hatch fasting (n=40).

	Fasting hours			
Days	0	24	36	48
	Weight gain (g)			
1-14	58.34 a (0.69)	54.99 ab (1.26)	54.09 bc (2.73)	49.70 c (3.00)
15-35	129.20 (3.67)	133.80 (3.33)	134.50 (6.74)	130.20 (19.77)
1-35	187.50 (3.23)	188.70 (3.03)	188.60 (6.28)	179.90 (20.60)
	Feed intake (g)			
1-14	127.10 (12.88)	121.40 (5.71)	110.80 (1.76)	108.00 (18.64)
15-35	257.60 ab (7.35)	266.60 a (1.82)	250.20 b (5.09)	263.80 a (26.06)
1-35	384.80 a (10.98)	388.00 a (6.01)	361.00 b (6.04)	372.40 ab (9.83)
	Feed conversion (g/g)			
1-14	2.18 (0.23)	2.21 (0.10)	2.06 (0.11)	2.18 (0.48)
15-35	1.99 (0.03)	2.00 (0.05)	1.87 (0.13)	2.03 (0.13)
1-35	2.05 (0.07)	2.06 (0.05)	1.92 (0.09)	2.07 (0.23)

[a, b, c] Distinct letters in the row, indicate significant differences between the means of the treatments (fasting hours), through Bayesian comparisons at a 95% level of credibility.

Table 2. Bayesian estimates (mean (standard deviation)) for body weight and weight of yolk sac of European quails submitted to different periods of post-hatch fasting (n=8).

	Fasting hours			
Days	0	24	36	48
	Body weight (g)			
2	12.82 a (0.58)	10.25 b (0.56)	9.15 b (0.20)	7.25 c (0.44)
3	15.77 a (0.87)	12.87 b (0.59)	11.13 bc (0.81)	9.09 c (0.52)
7	31.22 a (1.93)	26.42 b (0.92)	24.34 b (1.34)	18.42 c (1.29)
14	68.27 a (4.84)	71.34 a (3.21)	65.45 a (4.59)	54.47 b (3.08)
21	122.10 (1.76)	128.80 (12.64)	122.30 (3.35)	119.10 (4.57)
28	169.60 (0.90)	158.20 (7.06)	159.90 (3.38)	156.80 (9.47)
35	202.20 (9.54)	200.90 (5.71)	205.60 (3.51)	193.80 (6.23)
	Weight of the yolk sac (g)			
2	0.10 (0.02)	0.11 (0.02)	0.11 (0.01)	0.14 (0.03)
3	0.07 (0.01)	0.10 (0.02)	0.09 (0.02)	0.09 (0.03)

[a, b, c] Distinct letters in the row, indicate significant differences between the means of the treatments (fasting hours), through Bayesian comparisons at a 95% level of credibility.

Table 3. Bayesian estimates (mean (standard deviation)) for relative weights of the digestive system, proventriculus, gastric ventricle, duodenum and jejunum + ileum of European quails submitted to different periods of post-hatch fasting (n=8).

	Fasting hours			
Days	0	24	36	48
	Relative weight of the digestive system (%)			
2	17.04 a (0.61)	14.99 b (0.54)	14.06 b (0.65)	11.51 c (0.24)
3	17.05 a (0.34)	15.96 ab (0.89)	14.64 ab (0.79)	15.14b (0.22)
7	12.14 c (0.51)	13.45 bc (0.38)	15.37 a (0.99)	15.24 b (0.61)
14	9.98 (0.84)	10.07 (1.11)	10.16 (1.15)	10.41 (0.67)
21	7.70 (0.39)	8.07 (0.53)	8.30 (0.62)	7.85 (0.39)
28	6.99 (0.34)	7.14 (0.28)	7.50 (0.66)	7.04 (0.49)
35	6.88 (0.35)	6.13 (0.26)	6.15 (0.32)	6.25 (0.26)
	Relative weight of the proventriculus (%)			
7	0.85 b (0.10)	1.01 ab (0.05)	1.20 a (0.11)	1.15 a (0.05)
14	0.70 (0.08)	0.67 (0.05)	0.68 (0.08)	0.81 (0.06)
21	0.50 (0.06)	0.53 (0.02)	0.54 (0.05)	0.53 (0.07)
28	0.39 (0.02)	0.39 (0.02)	0.44 (0.03)	0.39 (0.02)
35	0.39 (0.02)	0.36 (0.02)	0.38 (0.02)	0.38 (0.01)
	Relative weight of the gastric ventricle (%)			
7	3.49 b (0.22)	4.46 ab (0.24)	4.96 a (0.46)	4.79 a (0.28)
14	3.02 (0.53)	3.21 (0.43)	3.23 (0.53)	3.46 (0.36)
21	2.71 (0.27)	2.79 (0.21)	2.62 (0.11)	2.54 (0.09)
28	2.21 (0.10)	2.43 (0.14)	2.45 (0.15)	2.34 (0.07)
35	0.39 (0.02)	0.36 (0.02)	0.38 (0.02)	0.38 (0.01)
	Relative weight of the duodenum (%)			
7	2.47 b (0.15)	2.50 ab (0.13)	2.94 a (0.07)	2.93 a (0.17)
14	1.66 (0.09)	1.87 (0.15)	1.90 (0.37)	1.92 (0.11)
21	1.21 (0.18)	1.21 (0.06)	1.43 (0.21)	1.23 (0.18)
28	1.11 (0.10)	1.15 (0.09)	1.26 (0.22)	1.13 (0.09)
35	1.09 (0.12)	0.93 (0.08)	0.94 (0.08)	0.94 (0.03)
	Relative weight of the jejunum + ileum (%)			
7	3.80 (0.30)	4.23 (0.21)	4.59 (0.36)	4.71 (0.41)
14	3.45 (0.46)	3.31 (0.44)	3.30 (0.33)	3.07 (0.31)
21	2.44 (0.18)	2.58 (0.29)	2.84 (0.30)	2.58 (0.10)
28	2.49 (0.12)	2.32 (0.08)	2.66 (0.20)	2.32 (0.26)
35	2.24 (0.18)	2.04 (0.14)	1.94 (0.16)	2.03 (0.12)

[a, b, c] Distinct letters in the row, indicate significant differences between the means of the treatments (fasting hours), through Bayesian comparisons at a 95% level of credibility.

Table 4. Bayesian estimates (mean (standard deviation)) for length of the digestive system and small intestine segments of European quails submitted to different periods of post-hatch fasting (n=8).

	Fasting hours			
Days	0	24	36	48
	Digestive system (cm)			
2	28.83 a (1.55)	23.22 b (0.55)	21.36 b (0.53)	17.78 c (0.88)
3	32.48 a (0.63)	26.77 b (1.92)	22.88 bc (1.13)	21.11 c (1.08)
7	40.26 a (2.15)	37.14 ab (1.62)	36.37 ab (0.91)	33.03 b (2.14)
14	51.70 (3.85)	51.34 (6.35)	47.90 (3.11)	45.26 (1.48)
21	52.54 (1.33)	57.50 (3.30)	59.85 (7.16)	56.69 (3.80)
28	64.67 (2.41)	57.82 (2.74)	63.11 (1.69)	58.35 (3.16)
35	64.53 (2.62)	62.15 (1.99)	65.62 (1.51)	61.22 (1.89)
	Duodenum (cm)			
7	8.56 a (0.24)	8.03 ab (0.20)	8.43 ab (0.19)	7.69 b (0.31)
14	9.74 (0.58)	10.45 (1.00)	10.41 (1.84)	8.96 (0.89)
21	10.00 (0.91)	11.15 (0.39)	11.48 (0.59)	10.94 (0.71)
28	11.97 (0.57)	11.64 (0.51)	12.80 (0.53)	11.76 (0.32)
35	12.09 (0.44)	11.89 (0.81)	11.87 (0.23)	10.70 (0.33)
	Jejunum + Ileum (cm)			
7	27.13 (2.01)	24.98 (1.57)	23.66 (0.94)	20.98 (1.84)
14	33.83 (3.57)	34.50 (9.97)	29.22 (4.29)	29.27 (2.86)
21	33.97 (2.35)	37.16(3.57)	39.24 (4.84)	36.99 (3.06)
28	40.13 (4.11)	38.85 (1.92)	41.92 (1.32)	39.33 (2.65)
35	43.78 (1.76)	42.06 (1.47)	44.96 (1.43)	42.26 (1.83)

[a, b, c] Distinct letters in the row, indicate significant differences between the means of the treatments (fasting hours), through Bayesian comparisons at a 95% level of credibility.

Table 5. Bayesian estimates (mean (standard deviation)) for villi height, crypt depth, villi: crypt ratio and villi width of the duodenum of European quail submitted to different periods of post-hatch fasting (n=4).

	Fasting hours			
Days	0	24	36	48
	Villi height (µm)			
3	619.30 a (55.36)	554.20 a (38.48)	413.70 bc (45.22)	428.50 b (40.76)
7	779.70 (52.84)	878.90 (68.98)	729.70 (72.91)	753.10 (104.10)
14	825.70 (216.80)	820.20 (89.96)	816.10 (106.30)	857.10 (65.86)
	Crypt depth (µm)			
3	48.76 a (3.07)	43.03 ab (4.76)	31.19 bc (7.94)	28.33 c (2.72)
7	48.57 (8.44)	56.62 (5.24)	71.20 (20.15)	43.87 (13.66)
14	99.62 a (13.61)	87.84 a (3.58)	60.60 bc (11.27)	52.25 c (17.20)
	Villi: crypt (µm/µm)			
3	12.80 b (1.66)	12.98 b (0.75)	14.00 ab (2.70)	15.16 a (0.68)
7	16.42 a (1.66)	15.85 ab (2.58)	10.35 b (4.78)	17.96 a (3.58)
14	8.62 b (2.66)	9.45 b (1.28)	14.68 a (5.51)	13.91 ab (2.64)
	Villi width (µm)			
3	93.18 a (6.32)	82.62 ab (8.32)	72.66 ab (9.60)	67.33 b (6.04)
7	133.90 (22.42)	111.90 (8.96)	108.50 (29.12)	122.70 (14.27)
14*	123.40 (33.54)	112.60 (19.32)	135.20 (13.02)	116.30 (44.00)

[a, b, c] Distinct letters in the row, indicate significant differences between the means of the treatments (fasting hours), through Bayesian comparisons at a 95% level of credibility.
*Analyzes were performed on transformed data (y_t= log(y)).

Table 6. Bayesian estimates (mean (standard deviation)) for villi height, crypt depth, villi: crypt ratio and villi width of the jejunum of European quail submitted to different periods of post-hatch fasting (n=4).

	Fasting hours			
Days	0	24	36	48
	Villi height (µm)			
3*	177.80 (32.96)	374.10 (131.50)	194.00 (18.32)	163.90 (50.63)
7*	334.70 (51.84)	250.90 (25.07)	349.40 (68.16)	303.80 (81.17)
14*	417.10 (27.22)	442.40 (87.69)	489.40 (67.37)	372.10 (394.60)
	Crypt depth (µm)			
3	36.02 a (5.02)	39.80 a (0.98)	31.91 ab (5.54)	21.13 b (3.96)
7*	52.90 (15.35)	74.58 (69.43)	73.61 (9.57)	32.30 (19.31)
14*	49.93 (8.73)	22.48 (5.65)	34.48 (16.98)	54.61 (120.90)
	Villi: crypt (µm/µm)			
3	4.95 (0.74)	9.66 (3.67)	6.24 (0.86)	8.09 (3.18)
7*	6.63 (1.14)	2.96 (22.83)	4.83 (1.01)	12.79 (5.88)
14*	8.53 b (1.11)	22.85 a (2.48)	17.07 ab (6.74)	7.32 b (35.53)
	Villi width (µm)			
3	69.44 a (6.43)	74.13 a (9.60)	52.87 b (3.82)	58.69 ab (5.74)
7	79.55 (13.06)	87.15 (17.14)	89.22 (8.22)	83.90 (8.26)
14*	106.50 (17.81)	84.56 (6.53)	96.29 (7.53)	80.00 (53.70)

[a, b, c] Distinct letters in the row, indicate significant differences between the means of the treatments (fasting hours), through Bayesian comparisons at a 95% level of credibility.
*Analyzes were performed on transformed data (y_t= log(y)).

Table 7. Bayesian estimates (mean (standard deviation)) for villi height, crypt depth, villi: crypt ratio and villi width of the ileum of European quail submitted to different periods of post-hatch fasting (n=4).

	Fasting hours			
Days	0	24	36	48
	Villi height (µm)			
3	138.70 (12.79)	179.50 (26.57)	144.80 (45.30)	200.20 (36.12)
7*	304.90 (59.11)	280.10 (53.46)	306.80 (27.75)	266.00 (109.00)
14*	397.20 (35.98)	405.00 (66.50)	356.80 (25.54)	254.60 (147.10)
	Crypt depth (µm)			
3	41.97 (11.27)	35.72 (1.51)	32.17 (5.30)	21.81 (3.80)
7*	44.15 (13.08)	37.86 (19.80)	53.19 (11.49)	26.38 (21.62)
14*	48.25 (12.26)	22.39 (9.00)	49.85 (25.63)	37.33 (20.48)
	Villi: crypt (µm/µm)			
3	3.55 b (0.94)	5.06 b (0.93)	4.42 b (0.89)	9.46 a (1.88)
7*	7.36 (2.41)	11.71 (7.44)	6.05 (1.40)	12.71 (17.31)
14	8.70 b (2.04)	18.55 a (5.94)	10.47 ab (5.98)	6.81 b (3.80)
	Villi width (µm)			
3	68.83 (3.34)	70.17 (5.63)	53.72 (10.24)	55.23 (2.90)
7*	88.74 (17.01)	74.55 (9.56)	85.69 (4.16)	87.12 (40.40)
14*	106.50 (17.81)	84.56 (6.53)	96.29 (7.53)	80.00 (153.70)

[a, b, c] Distinct letters in the row, indicate significant differences between the means of the treatments (fasting hours), through Bayesian comparisons at a 95% level of credibility.
*Analyzes were performed on transformed data (y_t= log(y)).

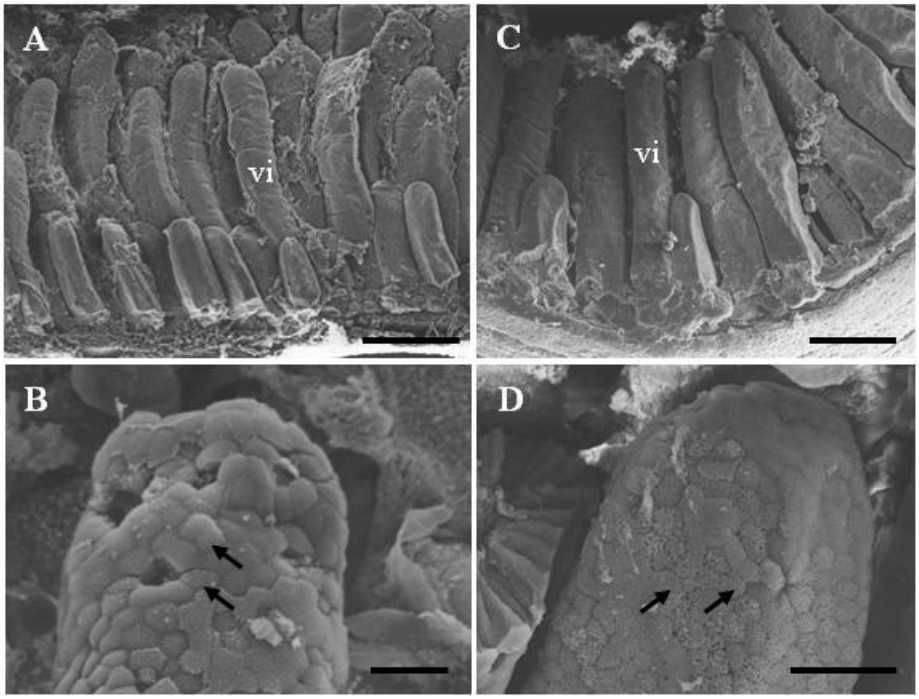

Figure 1. Intestinal villi of the duodenum of European quail seen by SEM. A, B) European quails submitted to 0 hours post-hatch fasting. C, D) European quails submitted to 48 hours post-hatch fasting. Note the difference in villus height between treatments 0hs (A) of fasting and 48hs (C). The fasting of 48 hours did not interfere in villi extremity morphology and enterocyte (arrows) are visible over villi tips. Scale bars: A) 200 μm; B) 20 μm; C) 100 μm; D) 10um.

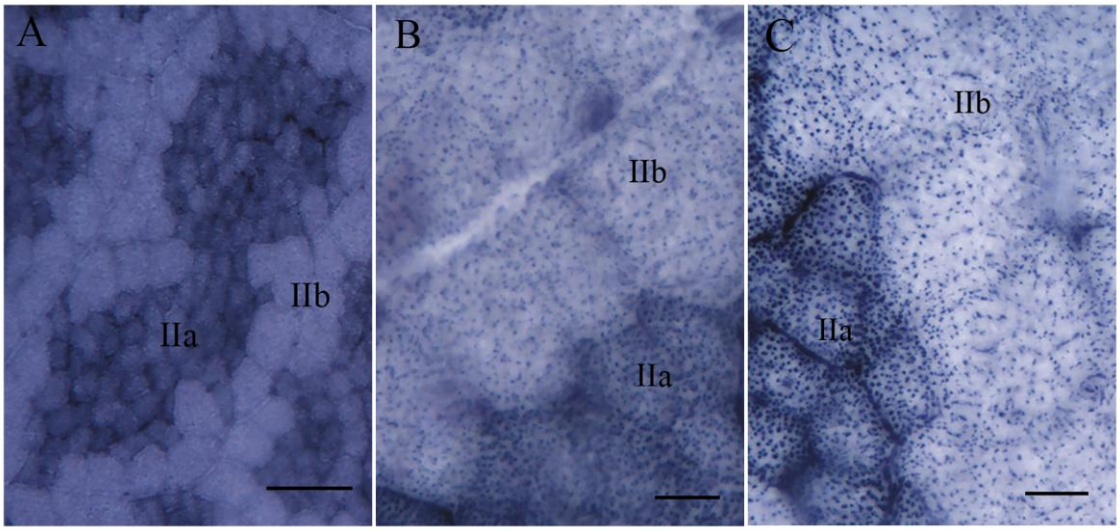

Figure 2. Muscle fibers of the pectoral muscle of European quails, stained in NADH-TR. A, B) European quails submitted to 0 hours post-hatch fasting. C) European quails submitted to 48 hours post-hatch fasting. Note the difference in the diameter of the fibers IIa and IIb and their arrangement in the muscle bundles with the central fibers IIa. Scale bars: A) 100 μm; B, C) 20um.

Table 8. Bayesian estimates (mean (standard deviation)) for weight and relative weights of the breast (n=8), number of fibers per bundle and diameter of fibers type IIa and IIb of pectoral muscle (n=4) European quails submitted to different periods of post-hatch fasting.

	Fasting hours			
	0	24	36	48
Breast (g)	56,90 (3,99)	57,14 (2,32)	58,90 (1,71)	55,76 (1,54)
Breast (%)	28,66 (0,78)	28,39 (0,47)	28,66 (0,89)	28,80 (0,46)
IIb	60,04 (12,15)	44,09 (9,57)	47,18 (1,83)	45,99 (12,98)
IIa	134,70 (12,66)	128,40 (22,07)	131,60 (23,87)	119,90 (8,84)
Diameter IIb	38,88 (4,52)	41,32 (4,60)	48,46 (4,77)	44,59 (4,75)
Diameter IIa	21,95 (1,86)	21,78 (0,75)	22,03 (0,97)	21,62 (1,83)

[a, b, c] Distinct letters in the row, indicate significant differences between the means of the treatments (fasting hours), through Bayesian comparisons at a 95% level of credibility.

V. CONSIDERAÇÕES FINAIS

Os achados contribuíram para novos estudos visando a compreensão de mecanismos fisiológicos envolvidos na incubação e no período pós-eclosão, bem como o fornecimento de subsídios para a compreensão dos processos fisiológicos do sistema digestório.

Os resultados obtidos possuem também impacto comercial ao estabelecer parâmetros para os produtores de pintainhos de codornas determinarem os prejuízos, que as aves expostas ao jejum pelo tempo do nascimento até a entrega e o fornecimento de ração nas granjas, podem causar em termos econômicos.

Existem ainda discrepâncias na literatura sobre o efeito do período de jejum, reforçando a necessidade de novas pesquisas com o intuito de avaliar o efeito fisiológico no sistema das aves, por meio de expressão gênica e de análises enzimáticas, como por exemplo, atividade enzimática no fígado (ALT, AST e do teor de proteína solúvel total) e no pâncreas (tripsina, quimiotripsina, amilase e lipase).