An Acad Bras Cienc (2023) 95(4): e20191188 DOI 10.1590/0001-3765202320191188
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

ANIMAL SCIENCE

Productive performance, breast growth and digestive system development in European quail subjected to post-hatch fasting for different periods

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Abstract: This study assessed the effect of different periods of post-hatch fasting on animal performance and breast and digestive system growth in European quail. Quail chicks were distributed in a completely randomized design, with four fasting periods (0, 24, 36, and 48 hs) and four replications of 40 birds per treatment. In 1 to 14-day-old chicks, weight gain decreased with increasing fasting time. Compensatory gain was observed from 15 days of age onward. Fasted quail had a lower length and relative weight of the digestive system than fed animals for up to 14 days. Histologically, the duodenal villus height was significantly lower in 3-day-old quail fasted for 36 hs than in those fasted for 48 hs, but this effect was not observed at 7 days. Scanning electron microscopy showed no differences in the small intestinal mucosa between fasted and fed birds at 3 days of age. Post-hatch fasting reduced the relative weight of the breast in quail aged 1 to 14 days but did not affect type IIa and IIb fiber diameter at 35 days. On the basis of these results, it is recommended that European quail raised for meat should not be fasted for more than 48 hs post-hatch.

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

INTRODUCTION

Quail are important members of the order Galliformes and the family Phasianidae, the largest and most varied family of gallinaceous birds (Chang et al. 2007). Two of the most common commercial species are the Japanese quail (*Coturnix coturnix japonica*), originated in Asia and used mainly for egg production, and the European quail (*Coturnix coturnix coturnix*), used for both egg and meat production. European quail produce larger eggs but are less efficient than Japanese quail (Bertechini 2010). European quail also grow faster than Japanese quail, reaching a mean body weight of 200 g at 35 days, which is 25 times higher than the body weight at 1 day of age (about 8 g) (Silva et al. 2012).

In terms of animal productivity, quail have competitive advantages over other bird species, including early sexual maturation, short interval between generations, rapid growth rate, and high reproductive capacity (Narinc et al. 2013). Quail meat is considered exotic and can be sold for high market prices because of its limited supply. This market niche shows important growth potential, as quail meat has quality characteristics that are highly accepted by the general population, such as pleasant aroma, rich flavor, and tenderness (Murakami et al. 2007).

Newly hatched chicks are subjected to sexing, vaccination, and conditioning before being transported to the farm. Depending on the distance between the hatchery and the farm, chicks may undergo fasting for 24 to 72 hs. Such stressful events can cause weight losses of up to 10% (Cançado & Baião 2002). The negative effects of post-hatch fasting are directly related to changes in physiological processes that affect the development of the digestive system. Soon after hatching, most of the nutrient demand is directed toward the development of digestive organs, as they will support the growth of other tissues by supplying nutrients. For this reason, rapid access to exogenous food is fundamental during the transition from the embryonic to the post-hatch phase (Noy & Sklan 1999, Cançado & Baião 2002).

Previous studies have shown that fasting impairs intestinal development (Uni et al. 1998, Corless & Sell 1999, Maiorka et al. 2003), nutrient absorption from the yolk sac (Moran & Reinhart 1980, Noy et al. 1996, Noy & Sklan 2001, Pedroso et al. 2006), organ weight gain (Maiorka et al. 2003), and bird performance. Nir & Levanon (1993) observed that post-hatch fasting for 24 and 48 hs delayed broiler growth (weight gain) by 1 and 2 days, respectively.

Various studies have been conducted on the effect of fasting on quail, but their focus lies on nutritional aspects of layer quail. Physiological aspects of fasting, particularly those related to incubation, are usually studied in broiler chickens and extrapolated to meat quail. However, these species show distinct growth curves and characteristics. For instance, quail reach sexual maturity in 40–45 days. Regardless of their particularities, quail are an excellent animal model for biological and medicinal studies and for comparative analyses between poultry species (Minvielle 2004). Thus, quail can be used to determine the best post-hatch fasting period for reduced growth impairment. Considering the importance of early feeding in the development and maturation of organs of the digestive system and their impacts on performance indices, this study aimed to assess the effect of different periods of post-hatch fasting on animal performance, digestive system development, and breast growth in 1- to 35-day-old European quail.

MATERIALS AND METHODS

This research was approved by the Animal Ethics Committee of the State University of Maringá, Paraná, Brazil (protocol no. 8793250615). The experiment was conducted at the poultry farm (Iguatemi Experimental Farm) of the State University of Maringá.

Animals and incubation

European quail breeders aged 15 weeks were standardized by weight (female = 292 g, male = 251 g) and egg production (90 ± 5%) and distributed in galvanized wire cages (25 × 39 cm) at a ratio of 2 females to 1 male. The animals were kept under a 17L:7D photoperiod and had *ad libitum* access to water and feed. Eggs were collected during 3 days, selected to obtain a variation of up to 5% in weight (11.80 ± 0.59 g), and stored in a refrigerated room at 20°C.

Selected eggs were incubated in an automatic vertical incubator (Petersime®, model Labo 13, capacity of 3,978 quail eggs) at 60% relative humidity and 37.6°C. After 348 hs of incubation, the eggs were transferred to a hatcher (Petersime®, model Labo 9) at 70% relative humidity and 37.0°C, and the hatching process was monitored hourly. Chicks hatched during the peak (4 hs) of the hatching period were separated, weighed (9.0 ± 0.64 g) and immediately distributed in pens according to the experimental design.

Experimental design

Quails (*n* = 640) were distributed in a completely randomized design, with four fasting periods (0, 24, 36, and 48 hs) and four replications of 40 (mixed-sex) birds per treatment. Chicks were reared at an initial density of 16 birds/m^2 in pens (2.5 × 1 m) equipped with heating lamps and lined with a bed of rice straw and corrugated paper. Constant light (a 24 h light photoperiod) was maintained until the chicks were 35 days old, in accordance with recommendations for quail meat production. Control quail were provided access to water and food immediately after being placed in the pens. For fasted birds, the first procedure adopted at the end of the fasting period was to provide water for about 30 min, until the quail were well hydrated and did not show any interest in the drinker. After this period, the birds were given solid feed and water *ad libitum*.

Quail diets were based on corn and soybean meal, formulated according to quail requirements and chemical composition values reported by Silva & Costa (2009). The starter diet (1 to 21 days of age) contained 25% crude protein and provided 2,900 kcal/kg of metabolizable energy, whereas the grower diet (22 to 35 days of age) contained 21% crude protein and provided 3,050 kcal/kg of metabolizable energy.

Productive performance

Body weight and the amounts of feed provided and leftovers were analyzed weekly per pen. Weight gain (g), feed intake (g), and feed conversion ratio (g/g) were determined, and the results were analyzed by age interval (1–15, 15–35, and 1 to 35 days). Each pen was treated as an experimental unit (*n* = 4 per treatment).

Morphometric analysis of digestive organs and breast

On days 2, 3, 7, 14, 21, 28, and 35 post-hatch, two quail per pen (*n* = 8 per treatment) were weighed, anesthetized intraperitoneally (25 mg/kg thiopental + 10 mg/kg lidocaine), and sacrificed by cervical dislocation. These birds were used for morphometric analysis of digestive organs and breast and then subjected to additional evaluations, as will be detailed below.

The following morphometric variables were analyzed: live body weight (g), relative weight (%) and length (cm) of the digestive system and digestive organs, and breast weight (g). The breast was dissected with the skin and bones and weighed. Digestive organs were dissected, separated, emptied of their contents, and weighed. Because of size limitations, it was not possible to accurately determine the weight and length of digestive organs in chicks aged less than 7 days; thus, chicks aged 2 to 3 days were only evaluated for total weight and length of the digestive system. Birds aged 7 days and older were analyzed for length of the duodenum and jejuno-ileum and relative weight of the proventriculus, gastric ventricle, duodenum, and jejuno-ileum; digestive organ weights were used to calculate the total weight of the digestive system. Relative weights were calculated by dividing the organ weight by the live body weight and multiplying by 100.

Histological analysis of the small intestine

Birds sacrificed on days 3, 7, and 14 were used for histological analysis (*n* = 4 per treatment). Sections (± 5 mm thickness) of the ascending duodenum, the cranial portion of the jejunum (before the vitelline diverticulum), and the distal portion of the ileum (before the entry to the cecum) were fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS) (pH 7.4). Fixed samples were

embedded in paraffin, cut to 3 µm thickness, and stained with hematoxylin–eosin. Digital images were captured using a light microscope coupled to a digital camera (Motican, China Group Co. Ltd., Xiamen, China) and analyzed using Motic Image Plus 2.0 software. Villus height and width, crypt depth, and villus/crypt ratio of the duodenum, jejunum, and ileum were determined. For each variable, 10 measurements were performed in at least three semi-serial cuts per bird.

Scanning electron microscopy (SEM)

Small intestine specimens from 3-day-old quail (n = 4 per treatment) were examined by SEM to investigate the morphological characteristics of the villus surface. Sections of the duodenum, jejunum, and ileum (collected as described above for histological analysis) were fixed in 2.5% glutaraldehyde in 0.1 M PBS (pH 7.4) and stored at 4°C until use. Samples were washed in 0.1 M PBS, dehydrated for 20 min in increasing concentrations of ethanol (70, 80, 90, 100, 100, and 100%), and critical-point dried (CPD 030, Bal-Tec, United States of America) using carbon dioxide. The materials were then mounted on metal stubs, sputter-coated with a 30 nm layer of gold (SCD 050, Bal-Tec), and analyzed on a Shimadzu SS-550 Superscan microscope (Japan). SEM images were analyzed qualitatively and descriptively.

Morphological analysis of the pectoral muscle

On day 35 post-hatch, fragments of the middle portion of the *Musculus pectoralis* were collected from four birds (n = 4 per treatment). A cross-sectional cut was made perpendicular to muscle fiber orientation (Scheuermann et al. 2004). Samples were immediately frozen in a cooled n-hexane solution (Chayen et al. 1969) and stored in liquid nitrogen. Frozen muscle sections were cross-sectioned to 10 µm thickness at –26°C using a cryostat microtome.

Samples were stained with hematoxylin–eosin for morphological analysis of muscle fibers. For identification and classification of muscle fiber type according to oxidative–glycolytic metabolism, sections were prepared using a histoenzymological technique of nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) staining (Dubowitz & Brooke 1973). Fibers were classified as type IIa (fast/oxidative/glycolytic) or type IIb (fast/glycolytic).

Muscle fascicles (n = 10 per bird) of different cuts were assessed to count fiber types. Fiber diameter was obtained by measuring 100 fibers in each bird (n = 4 quail per treatment). Digital images were captured using a light microscope coupled to a digital camera (Motican 5 MP) and analyzed using Motic Image Plus 2.0 software (Motic China Group Co. Ltd., Xiamen, China).

Statistical analysis

Data were analyzed using Bayesian posterior probability. In this procedure, the response (j = 1, 2, 3, 4) (Y_{ij}) follows a normal distribution (Gaussian), that is, $Y_{ij} \sim N(m_j, s_j^2)$. The following non-informative prior distributions were considered for model parameters: $m \sim N(0,10^{-6})$ and $t \sim gamma(10^{-3},10^{-3})$ ($t = 1/s^2$, OpenBUGS parameterization) (Rossi 2011). Differences between posterior means ($D_{jk} = m_j - m_k$, $j \neq k$) of treatments were considered significant based on the presence or absence of zero within their 95% credible intervals.

Posterior marginal distributions were obtained for all parameters using the Brugs package of the R program (R Development Core Team 2017). After a burn-in of 2,000 iterations, a total of 20,000 iterations were generated by Markov chain Monte Carlo sampling. Chain convergence was assessed using the coda package of R and the criterion of Heidelberger & Welch (1983).

In classical (frequentist) methods of analyses of variance, assumptions need to be fulfilled (e.g., normality of residuals and homoscedasticity of variances), and sample size (replicates) must be large enough, according to the asymptotic theory; then, inferences and consistent decisions can be made. The use of Bayesian procedures is a more parsimonious alternative, as fulfillment of such assumptions is not an impediment to good statistical analysis of data.

RESULTS AND DISCUSSION

Productive performance

Bayesian estimates for weight gain, feed intake, and feed conversion ratio of European quail aged 1–14, 15–35, and 1–35 days are shown in Table I. As expected, in chicks aged 1–14 days, weight gain reduced with increasing post-hatch fasting time. However, when analyzing the entire experimental period (1–35 days), this effect was not significant, indicating that chicks had a compensatory weight gain after the first two weeks.

Compensatory growth represents a modified growth pattern. During the period of feed restriction, chicks show reduced body weight and have low maintenance requirements (Furlan et al. 2001). However, when feed is supplied, there is a greater efficiency of nutrient use for growth (Furlan et al. 2001), which explains the recovery in weight gain of birds subjected to fasting in relation to other treatments.

No differences in feed intake were observed between treatments during the growing phase (1–14 days). However, from 15 to 35 days, quail chicks subjected to 36 hs of post-hatch fasting consumed less feed than chicks subjected to 24 and 48 hs of fasting. Considering the entire experimental period (35 days), birds fasted for 36 hs consumed less feed than fed birds (control) and birds fasted for 24 hs.

Feed conversion ratio was not influenced by fasting (Table I). This result was similar to those reported by Leu et al. (2002) and Carvalho et al. (2013), who did not observe differences in the feed conversion of fed broilers and broilers fasted up to 36 hs. In contrast, Pedroso et al. (2006) observed an improved feed conversion

Table I. Bayesian estimates (mean and standard deviation) for weight gain, feed intake, and feed conversion ratio of 1- to 35-day-old European quail (*n* = 40) subjected to post-hatch fasting for different periods.

Age (days)	Fasting period (hs)			
	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 ratio (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} **Different superscript letters indicate significant differences between posterior means based on Bayesian statistics (95% credible intervals).**

ratio at 21 days of age in broilers subjected to fasting during the first 48 hs of life compared with fed broilers.

Morphometric parameters of digestive organs

The body weight and digestive organ growth of fasted and fed chicks aged 2 to 35 days were assessed. Some variables could only be determined after 7 days of age because of the small organ size. According to posterior means and their respective credible intervals, there was a significant effect of fasting on body weight (Table II). Each experimental unit had an initial mean body weight of 9.0 ± 0.64 g. At 2, 3, and 7 days of age, fed birds (control) had higher body weight than fasted birds. Chicks with acute metabolic needs may metabolize muscle tissue for survival (Uni et al. 2005, Fairchild et al. 2006). This fact explains the low body weight of quail chicks subjected to post-hatch fasting for 24, 36, and 48 hs.

At 14 days of age, only chicks fasted for 48 hs post-hatch were underweight, indicating the occurrence of compensatory weight gain in chicks fasted for 24 and 36 hs. At 21 days, all birds had the same body weight (mean of 123.08 g, Table II).

Many studies have investigated the effects of fasting on avian body weight. Gonzales et al. (2000, 2003, 2008) observed that delaying access to feed for 36 hs post-hatch negatively affected the body weight and weight gain of broiler chicks selected for fast growth. Recently, Ribeiro et al. (2018) observed this effect in broilers subjected to fasting for 48 and 72 hs; at 10 days of age, chicks had poorer body development. Carvalho et al. (2013) found that broilers had a loss of 5.7 g in the first 48 hs of fasting. Under fasting conditions, weight reductions in the first hours of life occur due to the greater use of yolk sac reserves and dehydration (Halevy et al. (2000).

Fasting did not exert significant effects on the weight of residual yolk sac at 2 and 3 days of age (Table II). This result shows that the amount of residual yolk sac after hatching was not affected by the absence or presence of feed in the digestive system, corroborating the results of studies on broilers carried out by Murakami et al. (1992), Bigot et al. (2003), Gonzales et al. (2008),

Table II. Bayesian estimates (mean and standard deviation) for live body weight and weight of the residual yolk sac in European quail (*n* = 8) subjected to post-hatch fasting for different periods.

Age (days)	Fasting period (hs)			
	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)
	Yolk sac weight (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} **Different superscript letters indicate significant differences between posterior means based on Bayesian statistics (95% credible intervals).**

and Ribeiro et al. (2018). The lack of differences in yolk sac weight between fasted and fed quail chicks might be related to the poorly developed digestive system, reduced growth, and, consequently, low metabolic requirements of fasted birds (Gonzales et al. 2003). Therefore, these findings indicate that feed is the main source of post-hatch nutrients. Quail chicks subjected to longer post-hatch fasting periods (36 and 48 hs) did not compensate feed restriction by rapidly absorbing the yolk sac. Different results were obtained with broilers. Moran & Reinhart (1980), Noy et al. (1996), Noy & Sklan (2001), and Pedroso et al. (2006) reported that yolk sac weight was higher in fed animals than in animals fasted for up to 72 hs post-hatch.

The relative weights of the digestive system and organs are presented in Table III. Fasted quail chicks had a lower relative weight of the digestive system than fed chicks up to 7 days of age. From the age of 14 days onward, no differences were observed between fasted and fed birds (Table III).

A lack of dietary nutrients may compromise the development of the digestive system and consequently the body weight of quail chicks. In the first days of life, digestive organs, particularly the intestine, increase substantially in size (Nitsan et al. 1991).

The relative weights of the proventriculus and gastric ventricle at 7 days were lower in fed than in fasted chicks because of the higher body weight of fed chicks (Table III). Maiorka et al. (2003) found that the relative weight of the proventriculus/gastric ventricle was lower in fed broilers than in broilers fasted for 72 hs post-hatch. Low relative proventriculus and gastric ventricle weights are due to an increase in body weight with feeding.

A similar effect was observed for the relative weight of the duodenum. At 7 days of age, fasted quail had a higher relative duodenum weight than fed quail (Table III). From 14 to 35 days, no effects were observed between treatments. Relative jejunoileum weight was not influenced by fasting, suggesting that the jejunoileum recovered more rapidly from nutrient restriction than the duodenum.

Posterior estimates and their respective credible intervals suggested a significant effect of fasting on relative digestive system length (proventriculus, gastric ventricle, and intestines) at 2–7 days and on duodenal length at 7 days (Table IV). At 2 days of age, fed European quail chicks had a 1.62-fold longer digestive system than chicks fasted for 48 hs post-hatch. At 7 days, the difference between these two treatments was reduced to 1.22-fold. No differences in digestive system length between treatments were observed at 14 days of age. Duodenal length was shorter in 7-day-old chicks fasted for 48 hs post-hatch than in the control (Table IV).

Similar results were found for turkey chicks; delayed access to food and water reduced the growth of the digestive system and limited nutrient use efficiency, resulting in lower body weight (Corless & Sell 1999). During the first hours of life of quail chicks, nutrient demand is mainly directed toward the development of the digestive system, as these organs will support the growth of other tissues with nutrient supply (Noy & Sklan 1999, Cançado & Baião 2002). In chickens, post-hatch feed and water restrictions negatively affect the intestinal development; thus, feed should be offered as soon as possible after hatching to avoid impaired digestive system development (Maiorka et al. 2003). Uni et al. (1998) found that 36 hs of post-hatch fasting impaired small intestine growth and that the duration of developmental delay differed between intestine segments. Recovery of the jejunum occurred after 11 days of age, and that of the duodenum and ileum, after 5 days.

Table III. Bayesian estimates (mean and standard deviation) for relative weights of the digestive system, proventriculus, gastric ventricle, duodenum, and jejunoileum of European quail (*n* = 8) subjected to post-hatch fasting for different periods.

Age (days)	Fasting period (hs)			
	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)	15.14^{b} (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 jejunoileum (%)			
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] **Different superscript letters indicate significant differences between posterior means based on Bayesian statistics (95% credible intervals).**

Table IV. Bayesian estimates (mean and standard deviation) for length of the digestive system, duodenum, and jejunoileum of European quail (*n* = 8) subjected to post-hatch fasting for different periods.

Age (days)	Fasting period (hs)			
	0	24	36	48
	Length of the 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)
	Length of the 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)
	Length of the jejunoileum (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] **Different superscript letters indicate significant differences between posterior means based on Bayesian statistics (95% credible intervals).**

Light microscopy and SEM

Morphometric analysis of intestinal villi was performed using a light microscope (Tables V to VII). Duodenal villus height was lower in 3-day-old birds fasted for 36 and 48 hs post-hatch than in fed birds. Recovery occurred after 7 days of age (Table V). The mean duodenal, jejunal, and ileal villus heights were 785.35 ± 65.63 µm, 309.70 ± 43.56 µm, and 289.45 ± 19.81 µm at 7 days and 829.78 ± 18.64 µm, 430.25 ± 48.99 µm, and 353.40 ± 69.17 µm at 14 days, respectively.

Jejunal and ileal villus heights at 3, 7, and 14 days of age were not influenced by fasting (Tables VI and VII). Quail chicks subjected to post-hatch fasting showed smaller duodenal (Table V) and jejunal (Table VI) crypt depths at 3 days of age, whereas ileal crypt depth was not influenced by fasting at any age (Table VII). Duodenal villus/crypt ratio was higher at 3 days of age in birds fasted for 48 hs. Jejunal and ileal villus/crypt ratio, on the other hand, was higher at 14 days in birds fasted for 24 hs. At 3 days of age, fed birds had larger duodenal and jejunal villus widths than birds fasted for 48 hs (Tables V and VI). Morphological parameters of the ileum did not differ between fasted and fed chicks at any age, suggesting that the ileum recovered more rapidly from the effects of post-hatch fasting than the duodenum and the jejunum (Table VII).

Table V. Bayesian estimates (mean and standard deviation) for villus height, crypt depth, villus/crypt ratio, and villus width in the duodenum of European quail (*n* = 4) subjected to post-hatch fasting for different periods.

Age (days)	Fasting period (hs)			
	0	24	36	48
	Duodenal villus 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)
	Duodenal 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)
	Duodenal villus/crypt ratio (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)
	Duodenal villus 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} Different superscript letters indicate significant differences between posterior means based on Bayesian statistics (95% credible intervals).
***Data were transformed to y_t = log(y) before analysis.**

The duodenum not only has a higher villus height but also a higher turnover rate of intestinal mucosa cells. The rapid turnover can be explained by the fact that the region is extremely important for the digestive process; the release of biliary and pancreatic secretions occurs in the duodenum (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, which are considered a stimulating factor for the growth of villi and crypts (Moran Junior 1985). Crypt development is crucial for intestinal maturation. The morphology of the small intestine changes rapidly in the late stages of egg incubation. The increase in crypt size enhances the availability of enterocytes for intestinal absorption (Ozaydın et al. 2012).

Small intestine segments of 3-day-old chicks were analyzed qualitative by SEM. Chicks had at least 24 h of access to feed prior to the analysis. Fasted chicks showed an apparent reduction in villus height (not measured), but villi were intact and showed no signs of lesions at the extremities (Figure 1). Villi from all intestinal segments had different sizes and an elongated shape toward the intestinal lumen and showed intact enterocytes interspersed with goblet cells.

Soares et al. (2007) studied the influence of water and feed restriction during the pre-starter phase on the performance of broilers up to 42 days of age. The authors observed apparent differences in intestinal villi between restricted and unrestricted birds. In birds raised with *ad libitum* water, the villus surface had a smoother and rounder appearance, whereas, in

Table VI. Bayesian estimates (mean and standard deviation) for villus height, crypt depth, villus/crypt ratio, and villus width in the jejunum of European quail (*n* = 4) subjected to post-hatch fasting for different periods.

Age (days)	Fasting period (hs)			
	0	24	36	48
	Jejunal villus 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)
	Jejunal 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)
	Jejunal villus/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)
	Jejunal villus 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} Different superscript letters indicate significant differences between posterior means based on Bayesian statistics (95% credible intervals).
***Data were transformed to $y_t = \log(y)$ before analysis.**

restricted birds, the villus surface was flattened and wrinkled.

Uni et al. (1998) observed crumped microvilli and abnormal crypt structure 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 can increase extrusion rate and consequently reduce villus size, which has an important impact on digestion and absorption (Smith et al. 1990).

Breast muscle

Breast growth was influenced by 48-h post-hatch fasting up to 14 days of age, according to Bayesian posterior estimates and their respective credible intervals. In birds fasted for 36 hs post-hatch, breast growth was affected up to 3 days of age. At 21, 28, and 35 days, treatments did not differ in relative breast weight (Table VIII). The number of fibers per muscle fascicle and the diameter of type IIa and IIb fibers of the pectoral muscle at 35 days were not influenced by post-hatch fasting (Table VIII). Overall, type IIb fibers were larger (43.31 ± 4.15 µm) than type IIa fibers (21.85 ± 0.18 µm) and were found to surround the smaller fibers, located at the center of muscle fascicles (Figure 2).

Unlike chicken and turkeys, in which the pectoral muscles are entirely formed of glycolytic type fibers (white), quail have breast muscles composed of oxidative fibers (red). As a result, quail breast muscles are characterized by a lower rate of rigor mortis development, and,

Table VII. Bayesian estimates (mean and standard deviation) for villus height, crypt depth, villus/crypt ratio, and villus width in the ileum of European quail (*n* = 4) subjected to post-hatch fasting for different periods.

Age (days)	Fasting period (hs)			
	0	24	36	48
	Ileal villus 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)
	Ileal 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)
	Ileal villus/crypt ratio (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)
	Ileal villus 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] Different superscript letters indicate significant differences between posterior means based on Bayesian statistics (95% credible intervals).
***Data were transformed to $y_t = \log(y)$ before analysis.**

therefore, higher pH values and water holding capacity (Genchev et al. 2008).

In birds, muscle development occurs in two distinct periods. The first occurs in the embryonic stage, when the number of muscle fibers is established by precursor cell specification. The number of muscle fibers increases by cell division (muscular hyperplasia) (Smith 1963, Velleman 2007). After hatching, these cells proliferate and fuse with existing muscle fibers, synthesize specific proteins, and increase in volume through the formation of new sarcomeres, a process denominated muscular hypertrophy (Christ & Brand-Saberi 2002, Silva & Carvalho 2007). We analyzed the number of fibers per muscle fascicle, which was expected to not vary between treatments.

Pinchasov & Noy (1993) showed that early post-hatch feed restriction decreases the growth of turkey chicks and that muscles may become smaller because of the decreased mitotic activity of satellite cells. In the present study, post-hatch fasting did not affect pectoral muscle weight at a later age (35 days); pectoral muscle weight accounted for 28.6% of the live body weight.

In conclusion, weight gain, feed conversion ratio, and muscle development were not affected by up to 48 hs of post-hatch fasting in birds aged 1–35 days. However, digestive organ development was negatively influenced by fasting. Prolonged fasting (36 to 48 hs) affected breast weight up to 14 days of age, but compensatory growth was observed after this period. Because of the intense effect of post-hatch fasting on body weight and intestinal morphology during the first week of life, European quail chicks should not be subjected to more than 48 hs of fasting after hatching.

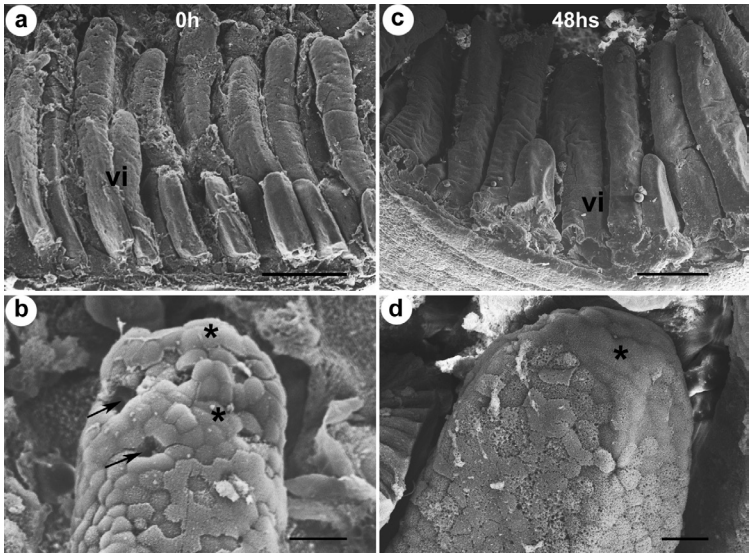

Figure 1. Scanning electron micrographs showing villi (vi) in the duodenum of European quail subjected to post-hatch fasting for 0 (a and b) and 48 hs (c and d). Note the difference in villus height between treatments. Post-hatch fasting for 48 hs did not affect surface morphology or associated enterocytes (*). Goblet cells are indicated by an arrow. Scale bars: (a) 200 µm, (b) 20 µm, (c) 100 µm, and (d) 10 µm.

Table VIII. Bayesian estimates (mean and standard deviation) for relative weight of the breast (*n* = 8) and number of fibers per fascicle (10 fascicles/quail) and type IIa and IIb fiber diameter (100 fibers/quail) in the *Musculus pectoralis* (*n* = 4) of European quail subjected to post-hatch fasting for different periods.

Age (days)	Fasting period (hs)			
	0	24	36	48
	Relative weight of the breast (%)			
2	5.02^{a} (0.23)	4.52^{ab} (0.30)	4.05^{bc} (0.19)	3.61^{c} (0.14)
3	6.17^{a} (0.27)	5.38^{ab} (0.25)	4.77^{bc} (0.32)	4.05^{c} (0.17)
7	11.28^{a} (1.08)	10.33^{ab} (0.55)	9.39^{ab} (0.70)	8.07^{b} (0.57)
14	19.83^{a} (0.43)	20.10^{a} (0.89)	19.46^{ab} (1.29)	17.21^{b} (0.67)
21	23.74 (0.78)	23.45 (0.59)	22.27 (1.41)	23.32 (0.87)
28	26.50 (0.80)	25.91 (0.57)	25.67 (0.36)	25.14 (0.36)
35	28.66 (0.78)	28.39 (0.47)	28.66 (0.89)	28.80 (0.46)
	***Musculus pectoralis* fiber parameters (35 days of age)**			
Number of type IIa fibers	134.70 (12.66)	128.40 (22.07)	131.60 (23.87)	119.90 (8.84)
Number of type IIb fibers	60.04 (12.15)	44.09 (9.57)	47.18 (1.83)	45.99 (12.98)
Diameter of type IIa fibers	21.95 (1.86)	21.78 (0.75)	22.03 (0.97)	21.62 (1.83)
Diameter of type IIb fibers	38.88 (4.52)	41.32 (4.60)	48.46 (4.77)	44.59 (4.75)

[a,b, c] **Different superscript letters indicate significant differences between posterior means based on Bayesian statistics (95% credible intervals).**

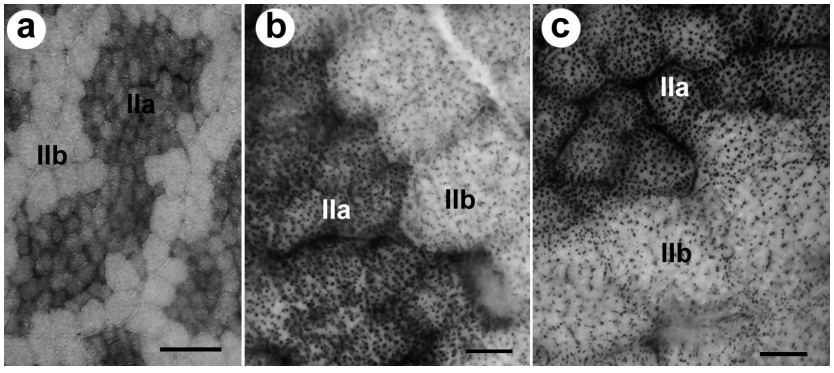

Figure 2. NADH-TR staining of muscle fibers in the pectoral muscle of European quail subjected to post-hatch fasting for 0 (a and b) and 48 hs (c). Note the typical morphological arrangement of quail pectoral fascicles composed of central type IIa fibers (dark color) surrounded by large external type IIb fibers (light color). Scale bars: (a) 100 µm and (b and c) 20 µm.

Acknowledgments

The authors are grateful for the financial support provided by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001).

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How to cite

CRUZ LK, FIGUEROA CDN, ANDRADE KG, KANEKO IN, BONAGURIO LP, BEZERRA JÚNIOR JS, ROSSI RM, MURAKAMI AE & SANTOS TC. 2023. Productive performance, breast growth and digestive system development in European quail subjected to post-hatch fasting for different periods. An Acad Bras Cienc 95: e20191188. DOI 10.1590/0001-3765202320191188.

Manuscript received on September 27, 2019;
accepted for publication on August 13, 2020

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