## Effects of delayed access to feed on growth performance, yolk absorption and gastrointestinal tract histological changes of neonate Japanese quail

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#### Abstract

The hypothesis of this study was to investigate how Japanese quail chicks in post-hatching period can response to delayed feeding through their growth performance and gastrointestinal tract development. One hundred and twenty as hatch Japanese quail chicks were randomly assigned to 12 pens. Four replicate groups of 10 chicks were randomly assigned to each of the three treatments. Experimental treatments included; chicks early feeding (EF) for 3h, and delayed feeding (DF) for 24 and 48h after hatching. After that, all birds were fed a mash diet ad-libitum until 15 days of age. At 1, 3, 6, 9, 12, and 15d of ages, one bird of each replicate (four birds of each treatment) was randomly selected, weighted and euthanized. Samples from the middle part of jejunum dissected free and fixed in 10% formaldehyde solution. Sections were stained with Hematoxylin and Eosin, Alcian blue-van Gieson and Periodic acid-Schiff stain. Morphometric indices were included villus height (VH), crypt depth (CD), villus width (VW), tunica muscular thickness (MT) and villus surface area (VSA). Birds subjected to treatments showed no significant differences in feed intake and residual yolk weight. In the EF birds the 15d live body weight and weight gain during the 1-15d of ages were significantly higher, but feed conversion ratio (FCR) was significantly lower than DF birds. Similarly, in the EF birds, the VH and VSA were significantly higher than DF birds. In conclusion, delayed access to feed after hatch has adverse effects on Japanese quail chick growth and gastrointestinal tract development.

Key words: Quail, Delayed feeding, Histology, Jejunum

#### Introduction

Because of its small size, inexpensive rearing requirement, rapid maturation and high adaptability to a wide range of environmental conditions quail chicks have been used as newly poultry activity in Iran (Ebrahimi et al, 2017). In commercial conditions, because of hatchery processing, the chicks remain without food for more than 24-36h after removal from the incubator until placement in the farm (Noy et al, 2001; Gangali et al, 2015). This delay access to feed after hatching results in a lowering of development and function of the gastrointestinal tract thereby undesirable bird performance such as reducing growth, decreasing immune

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response, retarding growth and increasing mortality (Latour et al, 1994; Gonzales, 2003; Gangali et al, 2015; Lilburn and Loeffler, 2015). Reported, post-hatch deprivation of feed and water for 48-72h reduced body weight by 7.8% compared to those fed immediately after hatch (Noy et al, 2001). Early access to food and water stimulates gastrointestinal tract (GIT) absorptive capacity, development and system, yolk chick's immune suck utilization and subsequent bird's growth performance (Pinchasov and Noy, 1993; Bigot, 2003; Gangali et al, 2015).

In the previous study, attention has been given to the effect of feeding on the performance of birds during post-hatch period, but the physiological basis remains completely to be elucidated (Noy and Pinchasov, 1993; Noy & Sklan, 1998b; Sklan and Noy, 2000). Therefore, our objective was to investigate the effects of delayed access to feed in post-hatching period on growth performance, gastrointestinal tract development and small intestine morphology in Japanese quail.

## **Materials and Methods**

One hundred twenty hatched Japanese quail chicks were obtained from a local hatchery and divided into 3 treatments; each group was further divided into 4 replicates (pens) of 10 birds each. The lighting program was applied 23:1h light: dark for the overall of the experiment. Birds in each pen had free access to food and water from completely handing instruments. Α randomized design (CRD) experiment of 3 used. Experimental treatments was treatments included; chicks early feeding (EF) for 3h, and delayed feeding (DF) for 24 and 48h after hatching. An experimental diet (Table 1) was formulated in a minimum equation by user-friendly cost feed formulation done again (UFFDA, 1992) software, University of Georgia, Athens, GA, United States, according to the recommended requirement for Japanese quail (NRC, 1994).

Tabe1: Ingredients and nutrients composition of								
experimental diet								

Ingredients	(%)									
Corn	49.5									
Soybean meal	44.7									
Soybean oil	2.7									
Limestone	1.3									
Dicalcium phosphate	0.7									
Sodium chloride	0.4									
Vitamin premix <sup>1</sup>	0.25									
Mineral premix <sup>2</sup>	0.25									
L-Lysine-HCl	0									
DL-Methionine	0.1									
L-Threonine	0.1									
Calculated nutrients composition,	as-fed basis									
Metabolizable energy, kcal/kg	2900									
Crude protein, %	24									
Calcium, %	0.80									
Available phosphorus, %	0.30									
Sodium, %	0.15									
lysine, %	1.33									
Methionine, %	0.49									
Sulphur amino acids, %	0.87									
Threonine, %	1.01									

<sup>1</sup>Vitamin premix supplied the followings per kilogram of diet: vitamin A (all-trans-retinol), 11000 IU; vitamin D3 (cholecalciferol), 1800 IU; vitamin E ( $\alpha$ tocopherol), 36 mg; vitamin K3 (menadione), 5 mg; vitamin B12 (cyanocobalamin), 1.6 mg; B1(thiamine), 1.53 mg; B2 (riboflavin), 7.5 mg; B3 (niacin), 30 mg; B6 (pyridoxine), 1.53 mg; H2 (biotin), 0.03 mg; B9 (folic acid), 1 mg; B5 (pantothenic acid), 12.24 mg; choline chloride, 1100 mg; etoxycoin, 0.125 mg. <sup>2</sup>Mineral premix supplied the followings per kilogram of diet: Zn (zinc sulfate), 84 mg; Mn (manganese sulfate), 160 mg; Cu (copper sulfate), 20 mg; Se (Sodium Selenite), 0.2 mg; I (calcium iodate), 1.6 mg; Fe (iron sulfate), 250 mg.

Daily weight gain, daily feed intake and feed conversion ratio were calculated during the 1-15d of ages. One bird/replicate (four birds/treatment), with a weight near to the pen mean weight was selected, weighed and slaughtered at 1, 3, 6, 9, 12 and 15 days of age. The gastrointestinal tract organs were excised and weighed. The gastrointestinal organ weight expressed to body weight. Then, sections were taken from the middle part of jejunum (about 0.5 cm in length), then all samples were fixed in 10% buffered formalin solution.

After processing, all samples were sectioned at 6µm thickness using a microtome (Leica RM 2145-Japan). Intestinal sample section was stained by

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Hematoxylin and Eosin (H&E), Alcian blue- van gieson and Periodic acid-Schiff stain. Histomorphometric measurements were performed on 9 vertical villi from samples. Morphometric indices include crypt depth (CD), villus height (VH), villus width and tunica muscular thickness (MT) (Geyra et al, 2001; Allahdo et al, 2018). Villus surface area (VSA) was calculated by the formula:  $\left[\frac{VW}{2} \times VH \times 2\pi\right]$  (Solis de los Santos et al, 2007).

All percentage data were transformed to arc-sin before statistical analysis. Analysis of variance performed using a randomized complete design experiment. All data were analyzed by ANOVA using the GLM procedure of the SAS 9.4 software (SAS 2014). Duncan multiple range test (P<0.05) was used for comparison of the means.

## Results

The growth performance indices during the 1-15 days of age period include; final live body weight (LBW), daily weight gain (WG), daily feed intake (FI) and feed conversion ratio (FCR) are shown in Table 2. The 15d LBW and WG significantly decreased (P<0.05) and FCR significantly increased (P<0.05) by increasing the fasting time, but FI was not affected by experimental treatment (P>0.05).

 Table 2: Effect of delayed access to feed of post-hatch on live body weight, weight gain, feed intake and feed conversion ratio of quail chicks during 1-15d of age

Teeu conversion ratio of quan enecks during 1-150 of age												
Fasted hours	Live body weight (15d)	Weight gain	Feed intake	Feed conversion ratio								
Fasted nours	(g/b)	(g/t	p/d)									
3 (EF)	104.85 <sup>a</sup>	6.55 <sup>a</sup>	12.01	1.83 <sup>b</sup>								
24 (DF)	94.89 <sup>b</sup>	5.93 <sup>b</sup>	11.83	1.99 <sup>ab</sup>								
48 (DF)	94.91 <sup>b</sup>	5.93 <sup>b</sup>	12.81	2.16 <sup>a</sup>								
SEM	3.33	0.21	0.67	0.09								

<sup>a, b</sup> Means with no common superscripts in each column are significantly different (P < 0.05). EF: Early feeding; DF: Delayed feeding.

The effect of delayed access to feed on changes trends of live body weight, gastro intestinal tract relative weight, small intestine relative weight, and yolk relative weight of quail chicks from hatching to 15 d of age were shown in Figure 4, 5, 6 and 7. As expected, growth rate of the EF chicks was much greater than those of the 24 and 48h ones. In the EF, 24 and 48 h DF treatment's chicks, the relative gastro intestinal tract and small intestine weight attained peaks 21.32 and 8.5%, 18.5 and 7.7%, and 19.93 and 7.9% of live body weight at d 3 of age, respectively. The relative gastro intestinal tract and small intestine weight in the EF chicks were significantly higher than DF chicks at d 2 and 3. Thereafter, the relative gastro intestinal tract and small intestine weight

decreased gradually to attain 10 and 4.5% of live body weight at the age of 12 d in all three treatments. The residual yolk relative weight was not affected by access feed time. It was approximately 12% of body weight after hatching, about 5-7% at d 3 and negligible (about 0.75-1.09%) on d 6. There were not significant differences in GIT, Crop, Pro-ventriculus, Duodenum, Jejunum, Ileum and large intestine as percentage of LBW of birds DF.

The jejunum histologically measurement results are shown in Table 3. The VW, CD, MT and VSA were significantly (P<0.01) lower in the DF than EF birds at d 3 and 6 of age. The tissue structure of jejunum includes villi, enterocyte cell, Goblet cell, Brush border was normal in the three staining environments (Figure 1, 2, and 3).

	Fasted hours	3d				6d				15 d						
		VH	VW	CD	MT	VSA	VH	VW	CD	MT	VSA	VH	VW	CD	MT	VSA
	3 (EF)	132	39ª	35 <sup>a</sup>	27 <sup>b</sup>	16.8 <sup>a</sup>	160	46 <sup>a</sup>	38	37ª	23.2ª	281	55	45	32 <sup>b</sup>	48.5
	24 (DF)	116	30 <sup>b</sup>	29 <sup>b</sup>	35 <sup>a</sup>	11.5 <sup>b</sup>	149	40 <sup>ab</sup>	37	34 <sup>ab</sup>	18.1 <sup>b</sup>	285	47	44	42 <sup>a</sup>	43.3
	48 (DF)	111	30 <sup>b</sup>	28 <sup>b</sup>	37 <sup>a</sup>	10.8 <sup>b</sup>	143	37 <sup>b</sup>	34	31 <sup>b</sup>	17.7 <sup>b</sup>	279	50	44	41 <sup>a</sup>	44.4
	SEM	7.5	1.3	1.4	1.7	1.3	6.7	2.3	2.7	2.1	1.5	16.8	2.6	2.2	2.6	3.3

 Table 3: Effect of delayed access to feed of post-hatch on jejunum morphology parameter of quail chicks measured at 3, 6, and 15d of age

<sup>a, b</sup> Means with no common superscripts in each column are significantly different (P < 0.05),

EF: Early feeding; DF: Delayed feeding; VH: Villus height ( $\mu$ m); VW: Villus width ( $\mu$ m); CD: Crypt depth ( $\mu$ m); MT: Muscular thickness ( $\mu$ m); VSA: Villus surface area (1000  $\mu$ m<sup>2</sup>).

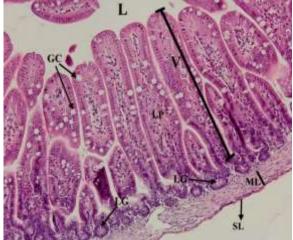


Figure 1: Jejunum in Japanese quail, L: Lumen, LG: Lieberkuhn gland, GC: Goblet Transvers section of cell, LP: Lamina propria, V: Villi, ML: Muscular layer, SL: Serosa layer, H&E Stain,× 200.



Figure 2: Transvers section of jejunum in Japanese quail, GC: Goblet cell, Periodic acidschiff Stain, ×200

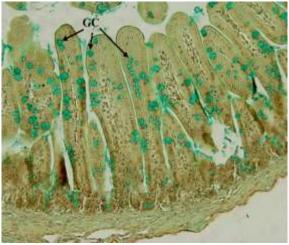


Figure 3: Transvers section of jejunum in Japanese quail, GC: Goblet cell, Alcian blue-Van gieson Stain, ×200

#### Discussion

The results obtained from this study showed a beneficial effect of EF versus DF Japanese quail chick's growth on performance during initial period (Table 2). The LBW at 15d and WG during 1-15d decreased and feed conversion ratio increased with increasing post-hatch DF. It confirmed the previous reports that birds with immediate post-hatch access to feed had improved growth performance (Noy and Sklan, 1998a; Noy et al, 2001; Batal and Parsons, 2002). The observed lower LBW and WG and higher FCR in DF chicks is consistent with another researcher (Latour et al, 1994; Saki, 2005), which reported DF decreased the broiler posthatch performance. The superior performance of birds on 3h (EF) is in agreement with findings that the nutrient utilization is dependent on digestion and absorption in the gastrointestinal tract. This

could also be due to an increased enzyme secretion from GIT. The effect of these enzymes is due to duration of food remain in GIT (Turner et al, 1999; Griffiths et al, 1977). The effects of DF observed in this study are in accordance with the previous observations pointing out that the common practice whereby food the first available to chicks one or more days post-hatch may depress subsequent development (Batal and Parsons, 2002). The poor FCR of birds for 48h is in line with earlier findings that showed that feeding broiler could affect growth, feed efficiency, uniformity and economic benefit (Batal and Parsons, 2002).

The results obtained from this study showed a beneficial effect of EF versus DF on Japanese quail chick's post-hatch yolk absorption. In agreement with the current study, the yolk utilization was stimulated by early feeding compared to delay feeding (Speake, 1998). The turnover of yolk in fed chicks was more rapid than in chicks without access to feed (Noy et al, 1996). This effect could also be inferred from studies reporting yolk size following foods in turkey's chick and in the earlier studies with broiler chicks (Moran and Reinhart, 1980). It seems that the presence of exogenous material in the gastrointestinal tract stimulates release of yolk through the yolk stalk. Additionally, reported GIT contained transfer proximally toward the gizzard by anti-peristaltic movements which resulted in increased amounts of yolk content in the proximal small intestine after hatching (Noy and Sklan, 1998b). The peristaltic movements of the intestine may also enhance yolk secretion into the intestine. Furthermore, the presence of bulk

within the intestine increased the physical pressure within the abdominal cavity on the yolk sac, which enhances yolk secretion into the intestine. Results indicate that an early feeding may retard degrading of the yolk leading to yolk sac inflammation (Uni and Ferket, 2004).

The current study showed that in the Japanese quail the digestive apparatus growth at the initial period is faster than the body weight. These data agree with the previous studies reported for several avian species (Sell et al, 1991; Nir et al, 1993). Results of the present study confirm the previous research indicating that digestive processes are not fully developed in newly hatched chicks (Nitsan et al, 1991; Nir et al, 1993) and turkey poults (Sell et al, 1991). Additionally, this study proved a beneficial effect of EF versus DF on Japanese quail chick's post-hatch digestive apparatus development. As reported, decreased in the growth performance traits under the DF treatments could be attributed to poor development of the digestive tract (Saki et al, 1996). The fasting in early stages of bird's development, has a negative effect on initial performance due to an inadequate use of the yolk sac, which seemed to be caused by low stimulation of the gastrointestinal tract development (Noy and Sklan, 1998b). In EF the nutrients provided by feed are complementary to the yolk nutrients and this will trigger rapid growth rate in birds (Geyra, 2001). Although the digestive capacity begins to develop a few days before hatch, most of the development occurs in the post-hatch period when the neonatal chick begins consuming feed (Ferket and Uni, 2006).

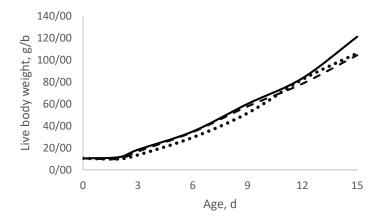


Figure 4: Effect of delayed access to feed 3 (\_\_), 24 (---) and 48 (....) hours of post-hatch on changes trend of live body weight of quail chicks from hatching to 15 d of age.

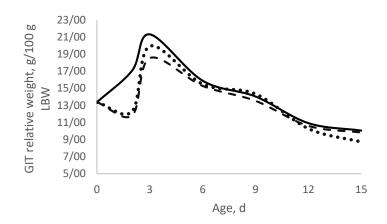


Figure 5: Effect of delayed access to feed 3 (\_\_), 24 (---) and 48 (....) hours of post-hatch on changes trend of gastro intestinal tract relative weight of quail chicks from hatching to 15 d of age.

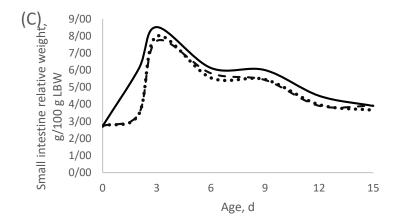


Figure 6: Effect of delayed access to feed 3 (\_\_), 24 (---) and 48 (....) hours of post-hatch on changes trend of small intestine relative weight of quail chicks from hatching to 15 d of age.

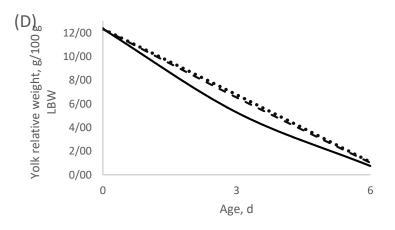


Figure 7: Effect of delayed access to feed 3 (\_\_), 24 (---) and 48 (....) hours of post-hatch on changes trend of yolk relative weight of quail chicks from hatching to 15 d of age.

Significantly higher VH, VW, CD and VSA at d 3 and 6 of age in the EF birds compared to DF birds indicate a tendency of better GIT development under immediately fed after hatching (Table 3). In agreement with this finding, the previous studies have shown that fast feeding after hatching accelerates the small intestine development (Yamauchi et al, 1996; Noy and Sklan, 1998a; Uni et al, 1998). The size and height of the villi are important for intestinal function (Yamauchi et al, 1996). The current study showed that the fine structure of intestinal epithelial cells is easily altered by post hatch fasting. Such basic morphological data for intestinal epithelial cells would be a useful index for judging the nutritional condition of the intestine in chickens fed with a normal diet. Fasting reduces the intestinal villi height and surface area for the absorption of nutrients and this might be the reason for the reduction in the overall growth performance of the DF birds (Shinde et al, 2015; Shinde et al, 2018; Shinde et al, 2010). Also, hyperplasia and atrophy were reported in the duodenum and jejunum of rats following 4 d fasting (Holt, 1986).

The results of the present study showed that up to 24h of DF has adverse effects on Japanese quail gastrointestinal tract development and performance. The yolk absorption rate in EF birds was more rapid than the DF. DF for 24 to 48h post-hatches decreased villus length and villus surface area in the jejunum. Therefore, the posthatch feeding of chicks should be done immediately after hatching.

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#### **Conflict of interest**

The authors declare that there is no conflict of interest.

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# تأثیر تأخیر در دسترسی به خوراک بر عملکرد رشد، جذب زرده و تغییرات بافتی دستگاه گوارش جوجه بلدرچین ژاپنی

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### چکیدہ

فرضیه این مطالعه بررسی پاسخ عملکرد رشد و توسعه دستگاه گوارش جوجه بلدرچین ژاپنی در دوره پس از هچ به تأخیری در تغذیه بود. ۱۲۰ قطعه جوجه بلدرچین ژاپنی تازه هچ شده، به صورت تصادفی در ۱۲ پن قرار گرفتند. چهار گروه ۱۰ تایی از جوجهها به طور تصادفی به هر یک از سه تیمار تقسیم شدند. تیمارهای آزمایشی شامل؛ تغذیه زود هنگام جوجهها (EF) به مدت ۳ ساعت، و تأخیر در تغذیه (DF) به مدت ۲۴ و ۲۸ ساعت پس از هچ بودند. پس از آن، همه پرندگان تا سن ۱۵ روزگی دسترسی آزاد با جیره آردی داشتند. تعذیه زر سنین ۱۰، ۲۰ ۳ و ۱۵ روزگی یک قطعه پرنده از هر تکرار (چهار پرنده از هر تیمار) به طور تصادفی انتخاب، وزن و کشتار شدند. نمونههایی بافتی از قسمت میانی ژژنوم تهیه و در محلول فرمالدئید ۱۰ درصد تثبیت شدند. اسلایدهای بافتی به روش رنگآمیزی بافتی و انوزین، آلسین بلو و ون گیسون و پریودیک اسید سنین ۱۰ درصد تثبیت شدند. اسلایدهای بافتی به روش رنگآمیزی باهماتوکسیلین و ائوزین، آلسین بلو و ون گیسون و پریودیک اسید شیف تهیه شدند. شاخصهای هیستومرفومتریک مورد بررسی شامل ارتفاع پرز (VH)، عمق کریپت (CD)، عرض پرزدگان تا سن ۱۵ درصد تثبیت شدند. اسلایدهای بافتی به روش رنگآمیزی زمونه ایمانی بافتی به در محلول فرمالدئید ۱۰ درصد تثبیت شدند. اسلایدهای بافتی به روش رنگآمیزی ارتفاع پرز (VH)، عمق کریپت (CD)، عرض پرزگان تا سن ۱۵ درصد تثبیت شدند. ساخصهای هیستومرفومتریک مورد بررسی شامل ارتفاع پرز (VH)، عمق کریپت (CD)، عرض پرز (VN) ، ضخامت لایه عضلانی (MT) و سطح پرز (VS) بودند. پرندگان تحت به اورن باقیمانده زرده نشان ندادند. در پرندگان SE وزن راد و زن باقیمانده زرده نشان ندادند. در پرندگان SE و افزایش در دوره سنی نداده، در پردگان TE وزن در دوره سای نداده در درم محرف کرد و این در تکاری و افزایش در معنو معنو در برد و روزن باقیمانده زرده نشان ندادند. در پرندگان کره و وزن باقیمانده زرده نشان ندادند. در پرندگان SE و افزایش در دوره سنی اسی در وزی به در معنو دورای و وزن باقیمانده زرده نشان ندادند. در پرندگان SE و وزن باقیمان در درد نشان ندادند. در پرندگان SE و وزن باقیمانده زرده نشان ندادند. در پرندگان SE و دا و وزن باقیمانده زرده نشان ندادند. در پرندگان SE و وزی باقیمانده زرده نشان ندادند. در پرندگان SE و دا و در در و در دورای در دوره ما و دا و در دورای و با و دا و در در و دورای و دا و در

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