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The effects of hatching time and feed access time on chick quality, organ development, blood parameters, and intestinal morphology in broilers

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Abstract: The current study was performed to investigate the effects of hatching time and feed access time on chick quality, organ development, blood parameters, and intestinal morphology in broilers. A total of 1935 hatching eggs were incubated at 37.8 °C with 55 to 60% RH conditions. Chicks were classified according to the hatching time (HT) as follows: chicks hatched before 482 h (early HT), between 482–496 h (medium HT) and 496–510 h (late HT). The hatched chicks were randomly divided into two groups to create two feed access (FA) time: early FA and late FA. Chicks with early FA started to consume feed at the end of each HT, whereas chicks with late FA started to feed at the end of the hatching period (at 510 h). Higher chick weight was observed in chicks in early HT and medium HT groups ($P = 0.002$). The chicks with late FA were heavier and taller than other chicks with FA. A higher Tona score (86.2) was observed for the chicks in medium HT ($P = 0.001$). Chicks in late HT group had a higher residual yolk weight, a lower yolk absorption, and a lower yolk free body mass. The chicks with late FA had a lower residual yolk weight, higher yolk absorption and, yolk free body mass ($P < 0.01$). Development of gizzard, heart, small intestine, and bursa of Fabricius were affected by both hatching time and feed access time. The lowest concentration of glucose was observed in chicks obtained from early HT with late FA ($P = 0.002$). Villus morphometric parameters in duodenum, jejunum, and ileum were affected by HT and FA time ($P < 0.05$). The current findings showed that the HT of chicks with regard to FA time markedly affected the development and growth pattern of chicks.

Key words: Chick quality, feed access, glucose, hatching time, villus morphometry

1. Introduction

In hatchery practice, the "spread of hatch" is the time difference between the first and last hatched chicks, also defined as the hatch window [1, 2]. Under commercial conditions, the spread of hatch can vary from 24 to 48 h [3, 4]. Due to variation in this period, the age of chicks at pulling time differ, therefore biological age (BA) can vary in one batch [4, 5]. Therefore, many chicks that hatch earlier stay in the hatcher without feed and water from hatching time until to the placing in broiler house [5].

There is a growing interest in gastrointestinal development and feed efficiency in broilers. Time in the hatcher after hatching has critical importance for yolk absorption, nutrient utilization, and development of the gastrointestinal system [6, 7]. During the early days after hatching, prolonged times in the hatcher without feed and water could negatively affect the development and maturation of the gastrointestinal tract, and finally chick hatching weight and broiler growth performance [5, 8, 9, 10, 11].

The physiological status (glucose, thyroid hormones, immunoglobulins, heterophils, and lymphocytes) and developmental traits (intestinal morphology and organ development) of chicks during the early posthatch period are important for a healthy starting for the growing period [5, 6, 11]. Also, understanding these physiological transitions is important for improving industrial practices including new hatching concepts and early nutrition alternatives, such as access to feed and water in the hatcher or hatching system on a farm. The current study aimed to describe the effects of hatching time (HT) and feed access (FA) time on chick quality, organ development, blood parameters, and intestinal morphology in broilers.

2. Material and methods

The care and use of animals followed the laws and regulations of Turkey and was approved by the ethics committee of Uludağ University (License Number 2014-17/08).

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A total of 1935 hatching eggs (58.0–62.0 g) were obtained from a commercial Ross 308 broiler breeder parent stock at 36 weeks of age. The breeder flock received a broiler breeder diet (2750 kcal of ME/kg and 14.50% CP) and was kept under management conditions according to the breeding company's recommendations[1].

Eggs were stored at 18 °C and 65% RH for three days and then warmed to room temperature (21°C) for 8 h before setting. To calculate yolk absorption, a total of 15 eggs were randomly sampled before incubation to measure the fresh egg weight and initial yolk weight. The hatching eggs were weighed with ± 0.1 precision and placed in 80-egg incubator trays (n = 24 trays). The trays were placed in an incubator (T2400 C, Cimuka Inc., Ankara, Turkey), and incubated at 37.8 °C with 55%–60% RH during the first 18 days of incubation.

At day 18 of incubation, eggs were transferred to a hatcher (T2400 H, Cimuka Inc.,) at 36.7 °C and 58% RH. The hatching process was monitored every 2 h from 468 h (19 days, 12 h) of incubation, and the process was finished at 510 h (21 days, 6 h). Chicks were classified according to the hatching time (HT) as follows: chicks hatched before 482 h (early HT), between 482–496 h (medium HT) and 496–510 h (late HT). The hatched chicks were weighed individually and randomly divided into 2 groups to create two feed access (FA) time as early feed access (early FA) and late feed access (late FA). The FA group had direct feed access at the end of each hatching interval as mentioned above. These chicks were placed in experimental pens and provided feed according to the broiler commercial guide[2]. The late FA group was restricted for feed until the hatching process was completed at 510 h, regardless of their hatching time. Therefore, these chicks stayed in the hatcher until the end of hatching.

A total of 60 chicks from each experimental group were randomly selected to measure chick quality according to HT and FA groups. Chick quality was determined using the Tona score, as previously reported by Tona et al. [12] with scoring activity, feathering and appearance, condition of eyes, conformation of legs, condition of navel area, remaining yolk sac and status of the yolk membranes. It was expressed as a hedonic scale, and the quality score was calculated by summing up the scores for these characteristics.

A total of 20 chicks from each experimental group were measured for chick body weight and length with ±0.01 g and cm precision [13]. Chicks were killed by cervical dislocation to determine residual yolk weight, yolk free body mass, and organ development (heart, gizzard, liver, bursa Fabricius weight, small intestine weight, and length). Yolk absorption was calculated by subtracting residual yolk weight from initial yolk weight [14]. Small intestinal measurements were done after emptied and the length of the small intestine was measured with a digital caliper (Mitutoyo Europe GmbH, Neuss, Germany). Organ weights were expressed as a percentage of chick weight.

The blood sampling was performed by the punctuation of the jugular vein from 10 birds in each experimental group (HT and FA groups) and the samples were collected in heparinized tubes, and then centrifuged at 3,000 rpm for 15 min; the plasma was removed into vacutainer tubes. Plasma levels of glucose (mg/dL), triiodothyronine (T_3, ng/mL), thyroxine (T_4, µg/dL), and immunoglobulins (IgA, IgG, and IgM; mg/dL) were determined by a commercial kit (Roche Cobas 6000 series E601, Roche Diagnostics Corp., Indianapolis, IN, USA) [15]. One drop of blood was smeared on a slide and stained using the May Grunwald and Giemsa method for counting heterophils and lymphocytes and calculation of H:L ratio [16].

Five chicks from each experimental group (HT and FA groups) were euthanized and dissected to remove the small intestine. Samples of the small intestine were emptied using a 10% buffered formaldehyde solution and fragmentized as 3 segments as duodenum, jejunum, and ileum [8]. Samples were trimmed 2 cm from the midpoints of the intestinal segments and fixed in a 10% buffered formaldehyde solution. Sampled tissue slices were left in the "Bouins fluid" for a day to processing of dehydration using an alcohol gradient of 70%–100% [17]. These slices were embedded in paraffin, and paraffin blocks were sectioned into 5 µm thick pieces by using a microtome (Leica RM2155, Leica Biosystems Nussloch GmbH, Nussloch, Germany). These sections were placed on a glass slide and stained using a hematoxylin and eosin staining method for microscopic evaluation [17]. To evaluate the small intestinal morphometric variables as villus height, villus width, villus apparent surface area, and crypt depth were measured using a light microscope (Leica, DM-500, Leica Microsystems, Heerbrugg, Switzerland) with a computerized image system (Leica Application Suite, LAS Version 3.7.0, Leica Microsystems). Fifteen to 20 villus and crypt were measured for each intestinal segment of the chicks.

In the present study, data for chick quality, organ development, serum biochemical parameters and intestinal morphology at hatching were subjected to mixed model analysis using the PROC MIXED procedure in SAS [18] according to the following model: Yijk = BAi + FAj +

[1] Ross PS Management Handbook (2018). Ross 308 [online]. Website http://en.aviagen.com/assets/ Tech_Center/Ross_PS/RossPSHandBook2018.pdf [accessed 25 January 2018].

[2] Ross 308 Broiler Management Handbook (2018). Ross 308 [online]. Website http://en.aviagen.com/ assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf [accessed 15 February 2018].

interaction terms + eijk, where BAi is the hatching time of chicks (early, medium and late HT), FAj is feed access (early FA, late FA), and eijk is the residual error term. For statistical analysis, the experimental unit was the individual chick. Statistically insignificant interactions were not given in the tables, except for small intestinal morphology parameters. In all cases, a difference was considered significant at $P < 0.05$. Analysis for percentage data was conducted after arcsine transformation of the data. Significant differences among the treatment means were determined using least-squares means with Duncan's adjustment for multiple comparisons. The effects of the 3 hatching times (early, medium, and late HTs) were determined by the contrast analysis of the GLM procedure. Orthogonal polynomial contrasts were also applied to determine the linear and quadratic responses to different hatching times in early FA and late FA.

3. Results

In the present study, the HT of chicks with early FA and late FA affected parameters including chick quality, some serum biochemical parameters, and intestinal morphology at hatch. Average egg weight, albumen, and yolk weights were 62.4, 38.4, and 19.0 g, respectively. The overall hatchability of total and fertile eggs were 88.7% and 92.4%, respectively.

The spread of hatch is presented in Figure 1. The hatching spread was 19.1, 55.6, and 25.2% for chicks in early HT, medium HT, and late HT groups respectively. Chick hatching weight was similar with values of 43.2, 44.5, and 43.1 g for chicks with early HT, medium HT, and late HT (Figure 2, $P > 0.05$).

The effects of HT of chicks and FA time on chick quality parameters at hatch are presented in Table 1. Chick weight measured at 510 h was affected by HT and FA times time ($P < 0.05$). Higher chick weight was observed in chicks in early HT and medium HT groups compared with chicks in the late HT group ($P = 0.002$). Interestingly, chicks with late FA were heavier and taller than other chicks with FA ($P = 0.001$). In the late FA group, HT of chicks linearly affected the chick weight ($P = 0.015$), whereas chick length was quadratically affected by HT in the early FA group ($P = 0.020$). On the other hand, chicks in medium HT had a higher Tona score (86.2) than the other chicks in early HT and late HT groups (74.0 and 74.0 scores respectively, $P = 0.001$). Differences in HT caused an increase in residual yolk weight (g and %) and a reduction in yolk absorption (g and %) and yolk free body mass. Chicks in late HT group had a higher residual yolk weight, a lower yolk absorption, and a lower yolk free body mass. In contrast, chicks with late FA stimulated yolk absorption until completion of hatching, compared to the other HT groups. The chicks with late FA had a lower residual yolk weight, higher yolk absorption, and yolk free body mass ($P < 0.01$). In the late FA group, HT linearly affected both the residual yolk weight ($P = 0.034$) and the relative residual yolk weight ($P = 0.037$). Yolk free bodyweight was linearly affected by HT ($P = 0.043$), yolk absorption, and relative yolk absorption were linearly affected in the late FA group ($P < 0.05$).

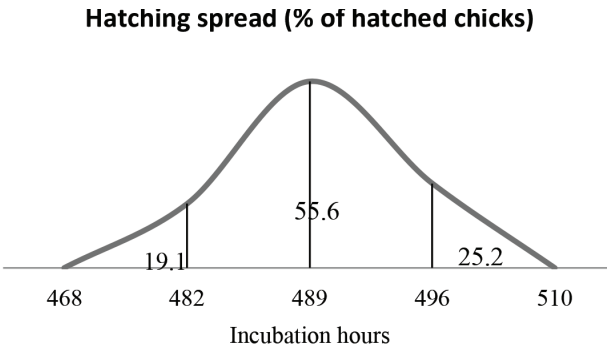

Figure 1. Spread of hatch during hatching period.

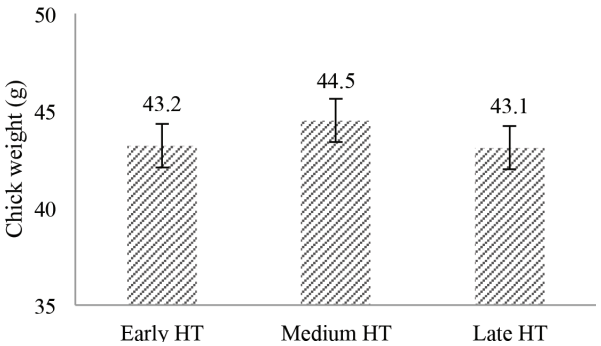

Figure 2. Chick hatching weight according to the hatching times (SE ± 1.4; $P > 0.05$).

The effects of HT and FA time on organ development at hatching were presented in Table 2. The weight and relative weight of gizzard were found to be lower in chicks at the late HT group and also in the early FA group. Gizzard weight and relative weight of gizzard were linearly decreased in the early FA group ($P = 0.005$ and $P = 0.012$), whereas a quadratic decline was observed for gizzard weight in the late FA group ($P = 0.040$), according to the HT groups. Heart development was only affected by FA time, and the heart weight and relative weight of heart were found to be higher in chicks with late FA groups. In the early FA group, a linear increment trend was observed for heart development ($P < 0.05$). Liver weight was higher in chicks with late FA (1.2 g) than for chicks with early FA (1.0 g, $P < 0.01$). Chicks with late FA had a higher small intestine weight and relative weight of small intestine than other chicks with early FA ($P = 0.001$). The weight and relative weight of the bursa of Fabricius were higher in

Table 1. Effects of the hatching time and feed access time on chick quality parameters at hatch.

Item	Chick weight (g) [1]	Chick length (cm) [1]	Tona score	RYW (g) [1]	Relative RYW (%)	YFBM (g) [1]	Yolk absorption (g) [1]	Relative yolk absorption (%)
Hatching time (HT)								
Early HT	49.2[a]	19.3	74.0[b]	5.6[b]	11.4[b]	43.6[a]	13.5[a]	71.0[a]
Medium HT	47.8[a]	19.1	86.2[a]	4.9[b]	10.3[b]	42.9[a]	14.4[a]	75.5[a]
Late HT	45.9[b]	19.1	74.0[b]	7.4[a]	16.1[a]	38.5[b]	11.7[b]	61.7[b]
SEM	1.06	0.18	3.35	0.63	1.24	1.52	0.66	3.47
Feed access (FA)								
Early FA	46.8[b]	18.9[b]	76.0	7.9[a]	16.9[a]	38.9[b]	11.3[b]	59.6[b]
Late FA	50.1[a]	19.5[a]	80.2	4.0[b]	8.0[b]	46. [a]	15.1[a]	79.3[a]
SEM	0.98	0.15	2.73	0.52	1.01	0.42	0.53	2.83
Source of variation		Significance						
HT	0.002	0.402	0.001	0.002	0.001	0.031	0.002	0.002
FA	0.001	0.001	0.130	0.001	0.001	0.006	0.001	0.001
HTxFA	0.055	0.705	0.798	0.428	0.512	0.064	0.432	0.432
Contrast analysis								
Early FA								
Linear	0.420	0.741	0.986	0.254	0.144	0.043	0.305	0.325
Quadratic	0.044	0.020	0.605	0.598	0.217	0.587	0.382	0.342
Late FA								
Linear	0.015	0.103	0.984	0.034	0.037	0.750	0.042	0.048
Quadratic	0.976	0.447	0.576	0.580	0.699	0.870	0.840	0.785

[1]For chick weight, length, and yolk absorption measurements, 20 chicks per experimental group were sampled at the end of the hatching period.
[2]For the Tona score, 60 chicks per experimental group were sampled.
RYW: Residual yolk weight, Relative RYW: (RYW/chick weight) × 100, YFBM: Yolk free body mass, Yolk absorption: Initial yolk weight – RYS, Relative yolk absorption: (Yolk absorption/Initial yolk weight) × 100.
[a,b]Differences in letters within columns indicate significant differences among the experimental groups.

chicks of early HT group (0.09 g and 0.18% respectively, $P < 0.01$). In the late FA group, these parameters showed a linear increment ($P < 0.05$). Furthermore, the length of the small intestine was found to be the longest with a value of 39.8 cm ($P = 0.001$), whereas it was also the longest in the late FA group (39.8 cm, $P = 0.001$). The length of the small intestine showed a linearly decline in the early FA group ($P = 0.004$).

The effects of HT of chicks and FA time on blood parameters at hatching were presented in Table 3. A significant interaction (BA × FT) was observed for glucose concentration in the study (Figure 3). The lowest concentration of glucose was observed in chicks obtained from early HT with late FA ($P = 0.002$), whereas glucose concentration showed a linear increment in late FA groups according to the HT ($P < 0.001$). Plasma T_3 level was higher in chicks at early HT and medium HT groups ($P = 0.001$), and also higher in chicks with early FA ($P = 0.042$). Also, a linear increment was observed in the late FA group ($P = 0.004$). However, a higher plasma T_4 level was observed for chicks in early HT and medium HT groups with a value of 1.2 µg/dL ($P = 0.003$). The level of plasma T_4 was linearly affected by HT in the early FA group ($P = 0.002$). Furthermore, chicks with early FA had a higher level of plasma IgA level than the chicks with FD (35.9 mg/dL vs. 33.1 mg/dL, $P < 0.01$).

The effects of HT of chicks and FA time on villus morphometry in the duodenum were presented in Table 4. Villus height, villus width, and the ratio of villus height/crypt depth in the duodenum were only affected by FA time. The interaction (HT × FA) was significant for villus apparent surface area ($P = 0.011$) and crypt depth ($P = 0.013$) in the duodenum.

The effects of HT of chicks and FA time on villus morphometry in the jejunum were presented in Table 5. The significant interaction (HT × FA) was observed for villus height ($P = 0.001$) and the ratio of villus height/crypt

Table 2. Effects of the hatching time and feed access time on organ development parameters at hatch.

	Weight (g)					Small intestine length (cm)	Relative weight (%)				
Item	Gizzard	Heart	Liver	Small intestine	Bursa of Fabricius		Gizzard	Heart	Liver	Small intestine	Bursa of Fabricius
Hatching time (HT)											
Early HT	2.6[a]	0.3	1.1	1.8	0.09 [a]	39.8[a]	5.3[a]	0.61	2.2	3.7	0.18[a]
Medium HT	2.7[a]	0.4	1.1	1.8	0.05 [b]	36.6[b]	5.6[a]	0.83	2.3	3.8	0.10[b]
Late HT	2.3[b]	0.4	1.1	1.6	0.05 [b]	34.7[b]	5.0[b]	0.87	2.4	3.5	0.11[b]
SEM	0.12	0.02	0.05	0.12	0.007	1.21	0.30	0.05	0.10	0.30	0.01
Feed access (FA)											
Early FA	2.2[b]	0.3[b]	1.0[b]	1.5[b]	0.06[b]	34.3[b]	4.7[b]	0.64[b]	2.1	3.2[b]	0.13[b]
Late FA	2.8[a]	0.4[a]	1.2[a]	1.9[a]	0.10[a]	39.8[a]	5.6[a]	0.79[a]	2.4	3.8[a]	0.20[a]
SEM	0.10	0.01	0.04	0.10	0.005	0.98	0.24	0.04	0.2	0.20	0.01
Source of variation		Significance									
P values											
HT	0.006	0.198	0.865	0.123	0.006	0.001	0.003	0.249	0.230	0.079	0.005
FA	0.001	0.004	0.001	0.001	0.001	0.001	0.001	0.001	0.993	0.001	0.001
HTxFA	0.523	0.152	0.449	0.925	0.170	0.528	0.809	0.063	0.406	0.806	0.095
Contrast analysis											
Early FA											
Linear	0.005	0.050	0.272	0.242	0.228	0.004	0.012	0.047	0.109	0.374	0.218
Quadratic	0.204	0.670	0.968	0.232	0.879	0.647	0.667	0.617	0.883	0.198	0.937
Late FA											
Linear	0.264	0.652	0.547	0.175	0.029	0.175	0.116	0.276	0.970	0.079	0.018
Quadratic	0.040	0.886	0.421	0.453	0.787	0.453	0.326	0.288	0.834	0.835	0.870

For organ development measurements, 20 chicks per experimental group were sampled.
Relative organ weight: (Organ weight/chick weight) × 100
[a,b]Differences in letters within columns indicate significant differences among the experimental groups.

depth (P = 0.015). Villus width and villus apparent surface area were only affected by the HT, whereas crypt depth was affected by FA time. The effects of HT and FA time on villus morphometry in the ileum were presented in Table 6. The interaction (HT × FA) was significant for villus height, villus width, villus apparent surface area, and crypt depth (P < 0.05).

4. Discussion

The age of chicks and the moment of the first feed consumption are important for early chick development [4, 7, 19]. Surprisingly, a higher chick weight observed in chicks in early HT and medium HT (at 510 h) could be related to a higher yolk absorption and a lower residual yolk weight of the chicks. Also, chicks with late FA stayed in the hatcher until 510 h of incubation and had more controlled environmental conditions (temperature, relative humidity, and air quality) than chicks with early FA that were placed experimental pens. Contrarily to these findings, some authors concluded that chicks fed immediately during the posthatch period caused an increment in body weight with a percentage of 10.5% due to some mechanical stimulating effects for gut development [20, 21].

The quality of day-old chicks is vitally important for the uniform growth of chicks and the final performance of broilers [22]. In the study, chick length was greater in chicks with late FA, whereas the Tona score was higher in chicks from the early HT group. These findings show that both HT and FA time affect development, growth pattern, and chick quality, due to variations in yolk absorption and yolk free body mass. Chicks exposed to late FA had a higher yolk absorption than the chicks with early FA. This could be explained by the residual yolk source was absorbed more quickly by fasted chicks.

Table 3. Effects of the hatching time and feed access time on blood parameters at hatch.

Item	Glucose (mg/dL)	T_3 (ng/mL)	T_4 (µg/dL)	IgA (mg/dL)	IgG (mg/dL)	IgM (mg/dL)	Heterophils (%)	Lymphocytes (%)	H/L
Hatching time (HT)									
Early HT	206.1[b]	1.3[a]	1.2[a]	34.1	118.1	6.1	33.4	48.0	0.69
Medium HT	210.7[b]	1.3[a]	1.2[a]	34.8	120.8	5.8	32.3	49.0	0.66
Late HT	231.8[a]	0.8[b]	0.9[b]	34.6	121.0	5.9	31.5	50.0	0.63
SEM	5.94	0.10	0.07	1.11	1.93	0.37	1.26	1.19	0.03
Feed access (FA)									
Early FA	235.8[a]	1.2[a]	1.1	35.9[a]	120.5	6.1	32.9	48.6	0.69
Late FA	196.6[b]	1.0[b]	1.1	33.1[b]	119.4	5.8	31.9	49.4	0.63
SEM	4.84	0.08	0.06	0.90	1.58	0.30	1.02	0.97	0.02
Source of variation		Significance							
HT	0.001	0.001	0.003	0.811	0.282	0.740	0.365	0.289	0.214
FA	0.001	0.042	0.974	0.007	0.517	0.382	0.314	0.065	0.056
HTxFA	0.002	0.122	0.064	0.051	0.060	0.095	0.102	0.366	0.148
Contrast analysis									
Early FA									
Linear	0.776	0.281	0.002	0.097	0.396	0.227	0.705	0.184	0.310
Quadratic	0.441	0.104	0.980	0.543	0.080	0.563	0.355	0.212	0.075
Late FA									
Linear	<0.001	0.004	0.497	0.096	0.238	0.295	0.224	0.416	0.221
Quadratic	0.975	0.877	0.082	0.735	0.366	0.713	0.941	0.451	0.971

For blood parameters, samples were collected from 10 chicks per experimental group.
[a,b]Differences in letters within columns indicate significant differences among the experimental groups.

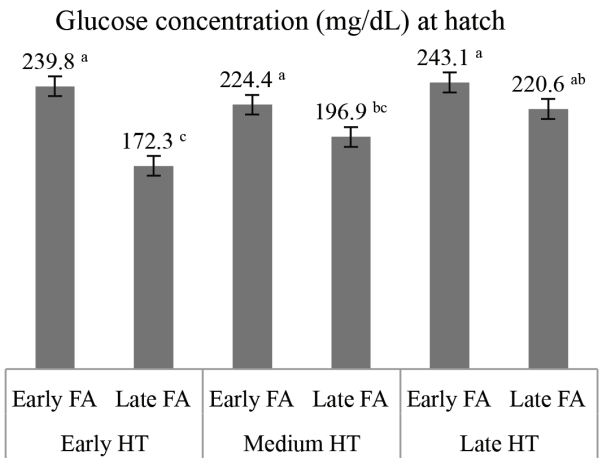

Figure 3. Glucose concentration of chicks by hatching time and feed access time at the end of the hatching period (SE ± 8.4; $P < 0.05$).

Current findings showed that the HT and FA time resulted in a different pattern of chick development and growth at hatch during the hatching period. Chicks from early HT group had a better growth of bursa of Fabricius, length of small intestine compared to the chicks from medium HT and late HT, whereas a higher value of gizzard was observed in early HT and medium HT groups when compared to the late HT group. Ricklefs [23] stated that organs had more time to be more mature with longer hatching time in a comparison among avian species. This could be also explained by a higher yolk absorption in these groups. Likewise, it was stated that labeled substances absorbed with yolk were identified in the intestines, blood, and gizzard of chicks after hatching [24]. However, an increase was observed for the weight and relative weight of gizzard, heart, small intestine, bursa of Fabricius, and small intestine length with late FA. The relative weight of bursa of Fabricius decreased by HT and FA time. In the present study, in addition to the relative weight of bursa of Fabricius, plasma Img-A level changed between early FA and late FD groups. Thus, the immunity of chicks could be affected by HT of chicks and FA time in the posthatch period.

In the present study, plasma glucose level increased in chicks with early FA, at all HTs. However, higher plasma

Table 4. Effects of the hatching time and feed access time on villus morphometry in duodenum at hatch.

Item	Villus height (μm)	Villus width (μm)	Villus apparent surface area (μm²)	Crypt depth (μm)	VH/CD[1]
Hatching time (HT)					
Early HT	455.4	79.6	32062.8	53.9[b]	8.4
Medium HT	488.3	76.9	29236.5	61.8[a]	8.4
Late HT	440.9	73.9	30135.9	52.3[b]	8.0
SEM	28.46	5.45	1747	1.76	0.45
Feed access (FA)					
Early FA	384.1[b]	71.3 [b]	20017.0[b]	56.2	6.9[b]
Late FA	539.0[a]	82.3 [a]	40930.7[a]	55.8	9.6[a]
SEM	23.24	4.45	1426	1.43	0.37
HT ×FA					
Early HT × Early FA	349.8	71.1	18066.3[c]	52.8[b]	6.6
Early HT × Late FA	561.1	88.1	46059.3[a]	55.0[b]	10.2
Medium HT × Early FA	417.9	75.3	21450[c]	65.6[a]	6.4
Medium HT × Late FA	558.8	78.5	37023 [b]	57.9[ab]	9.6
Late HT × Early FA	384.6	67.4	20534.8[c]	50.2[b]	7.7
Late HT × Late FA	497.3	80.4	39737[ab]	54.4[b]	9.1
SEM	40.25	7.71	2471	2.49	0.64
Source of variation			Significance		
HT	0.272	0.593	0.292	0.001	0.627
FA	0.001	0.029	0.001	0.768	0.001
HT × FA	0.243	0.458	0.011	0.013	0.070
Conrast analysis					
Early FA					
Linear	0.335	0.613	0.259	0.708	0.141
Quadratic	0.155	0.191	0.264	0.318	0.319
Late FA					
Linear	0.225	0.374	0.134	0.807	0.123
Quadratic	0.973	0.328	0.337	0.181	0.722

[1] VH/CD: Villus height/crypt depth.
For villus morphometry, intestinal samples of the duodenum were collected from 5 chicks per experimental group.
[a,b,c]Differences in letters within columns indicate significant differences among the experimental groups.

glucose level was observed for chicks in late HT group. This can be explained by energy availability in this period [25]. It is known that the energy requirement for hatching is provided by glucose, which is obtained from glycogen in the liver and muscles [26]. As a result, the plasma glucose level increases between pipping and hatching [25, 26]. Therefore, chicks in late HT group had more plasma glucose until the end of the hatching period, due to less usage of glucose and lower metabolism, ultimately resulting in lower chick weight.

Thyroid hormones are crucially important for the regulation of hatching time and chick development during the posthatch period [27]. In the study, higher plasma T_3 and T_4 levels were found in chicks in early HT and medium HT and chicks with early FA. McNabb [28] reported that feed deprivation causes a reduction in the T_3 levels of chicks. Similar results were observed in the present study and another experiment by Van de Ven et al. [29]. It is reported that T_4 has a major contribution to chick body growth during the hatching period and the first 3 weeks of the posthatch period [30]. In the present study, a higher plasma T_4 level observed in early HT and medium HT groups, consistent with higher chick weights at hatch. The observed lower plasma thyroids levels in chicks in late

Table 5. Effects of the hatching time and feed access time on villus morphometry in jejunum at hatch.

Item	Villus height (μm)	Villus width (μm)	Villus apparent surface area (μm²)	Crypt depth (μm)	VH/CD[1]
Hatching time (HT)					
Early HT	269.3[c]	69.9[ab]	14458[b]	47.2	5.8
Medium HT	337.2[b]	64.2[b]	16510.5[ab]	51.9	6.8
Late HT	366.7[a]	78.1[a]	18128.5[a]	51.8	6.5
SEM	10.63	4.74	957.6	2.19	0.58
Feed access (FA)					
Early FA	309.6[b]	68.7	16406.9	56.6[a]	5.5[b]
Late FA	339.2[a]	72.7	16324.4	44.1[b]	7.2[a]
SEM	8.67	3.87	781.9	1.79	0.48
HT × FA					
Early HT × Early FA	280.7[b]	62.0	13943.3	51.7	5.5[b]
Early HT × Late FA	257.9[b]	77.8	14972.7	42.8	6.0[ab]
Medium HT × Early FA	281.5[b]	65.5	15760.6	59.3	4.8[b]
Medium HT × Late FA	392.9[a]	62.8	17260.4	44.6	8.8[a]
Late HT × Early FA	366.5[a]	78.5	19516.9	58.9	6.3[ab]
Late HT × Late FA	366.9[a]	77.6	16740.1	44.8	6.7[ab]
SEM	15.03	6.71	1354	3.10	0.83
Source of variation			Significance		
HT	0.001	0.038	0.008	0.089	0.241
FA	0.005	0.315	0.918	0.001	0.005
HT x FA	0.001	0.143	0.088	0.379	0.015
Contrast analysis					
Early FA					
Linear	0.010	0.044	0.005	0.120	0.299
Quadratic	0.760	0.773	0.799	0.661	0.490
Late FA					
Linear	0.022	0.983	0.222	0.306	0.667
Quadratic	0.019	0.117	0.559	0.269	0.134

[1] VH/CD: Villus height/crypt depth.
For villus morphometry, intestinal samples of jejunum were collected from 5 chicks per experimental group.
[a,b,c]Differences in letters within columns indicate significant differences among the experimental groups.

HT group could be explained with a lower metabolism, as explained by Decuypere et al. [27].

Due to the prolonged time between hatching time and access to feed, chicks that hatch earlier go longer without feed and water in the hatcher [31]. During the holding time, chicks continue to consume their residual yolk via transport to the upper small intestine via their yolk stalk and, some physiological changes including maturation and development of the gastrointestinal tract occur [32]. During the immediate posthatch period, chicks consume lipid-rich yolk as a nutrient source by transition [33]. This consumption rapidly stimulates physiological changes, functional development, and maturation of the gastrointestinal tract, including villus length and surface area, as well as crypt depths [32, 34].

In the current study, increased villus height and villus apparent surface area in the duodenum were clearly associated with early FA and late FA. Villus height was higher in the late FA groups than the early FA groups. It could be concluded that chicks hatched at medium HT and late HT had an advantage in terms of villus height in the jejunum compared to chicks in early HT group. This may

Table 6. Effects of the hatching time and feed access time on villus morphometry in ileum at hatch.

Item	Villus height (μm)	Villus width (μm)	Villus apparent surface area (μm^2)	Crypt depth (μm)	VH/CD[1]
Hatching time (HT)					
Early HT	257.7	59.5^b	13587.8^b	50.6	5.1
Medium HT	240.2	57.2^b	13182.3^b	51.5	4.7
Late HT	235.6	71.1^a	16417.9^a	54.3	4.4
SEM	9.88	1.76	743.8	2.57	0.26
Feed access (FA)					
Early FA	249.2	68.9 a	15763.0^a	56.1^a	4.5^b
Late FA	239.8	56.3 b	13029.0^b	48.2^b	5.0^a
SEM	8.07	1.44	607.3	2.10	0.21
HT × FA					
Early HT × Early FA	246.4^{ab}	60.4^b	13119.2^b	50.1^{ab}	4.9
Early HT × Late FA	269.0^a	58.5^b	14056.5^a	51.2^{ab}	5.3
Medium HT × Early FA	269.2^a	61.3^b	17362.5^a	59.9^a	4.5
Medium HT × Late FA	211.2^b	53.1^b	9002.1^c	43.2^b	4.9
Late HT × Early FA	232.0^{ab}	85.0^a	16807.3^a	58.2^a	4.0
Late HT × Late FA	239.2^{ab}	57.2^b	116028.5^{ab}	50.3^{ab}	4.8
SEM	13.98	2.49	1052	3.64	0.36
Source of variation		Significance			
HT	0.101	0.001	0.002	0.373	0.056
FA	0.267	0.001	0.001	0.003	0.035
HT x FA	0.004	0.001	0.001	0.016	0.584
Contrast analysis					
Early FA					
Linear	0.402	0.003	0.035	0.064	0.025
Quadratic	0.531	0.929	0.341	0.194	0.505
Late FA					
Linear	0.262	0.682	0.506	0.865	0.012
Quadratic	0.622	0.513	0.100	0.087	0.092

[1] VH/CD: Villus height/crypt depth.
For villus morphometry, intestinal samples of ileum were collected from 5 chicks per experimental group.
[a,b,c]Differences in letters within columns indicate significant differences among the experimental groups.

be because the HT of chicks and FA time could stimulate the maturation and development of the gastrointestinal tract [31].

In conclusion, it is hypothesized that the HT of chicks with regard to FA time markedly affected physiological development during the hatching period, in the aspect of physiological developmental parameters of chicks. Results showed that chicks in late HT had a lower growth pattern at hatch compared with early HT and medium HT. These observed variations between one-day-old chicks could cause some limitations for posthatch growth and livability of broiler chicks. Therefore, in terms of commercial conditions, some innovator approaches could be improved for creating new hatching concepts and early feeding practices for producing high-quality one-day-old broiler chicks and subsequently profitable broiler production.

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Conflict of interest
The authors have no conflicts of interest to disclose.

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