Effects of breeder age on mineral contents and weight of yolk sac, embryo development, and hatchability in Pekin ducks

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ABSTRACT The current study was carried out to investigate the effects of breeder age on egg composition, changes of embryo, yolk sac, and yolk minerals during incubation and hatchability in Pekin ducks. A total of 495 freshly laid eggs were obtained from the same flock of Pekin ducks, aged 28, 34, and 40 wk, and were reared in accordance with the management guide of the duck breeders (Star 53-Grimaud Freres). At each breeder age, egg measurements were made on a random subsample of unincubated eggs. Embryo and yolk sac measurements were made on embryonic day (E) 12, E16, E20, and E25. On d 28 of incubation, the healthy ducklings were removed and sex of chicks was determined. All chicks were weighed and hatching results were determined. Egg weight and yolk percentages increased; however, albumen percentages, shell thickness, and yolk index decreased as the flock aged. Shell percentages, shell breaking strength, albumen index, and haugh units were not affected by breeder age. Also, breeder age affected the Mg, P, K, Ca, Cu, and Zn levels in the yolk, except for Na level on day of setting, and breeder age affected the mineral consumed by embryo during incubation. However, on E25, the levels of examined minerals, except for P level in the yolk sac, were not statistically different in duck breeder age groups. Relative yolk sac and embryo weights of eggs obtained from different breeder ages varied from E16 to E25; however, embryo length was different in breeder age groups from E12 to E20. Hatching weight was affected by breeder age and sex. Hatching results were not different among breeder age groups. This study indicates that breeder age is important for some egg characteristics, relative yolk sac weight, some contents of minerals in the yolk, embryonic growth during incubation, and duckling weight.

Key words: Pekin duck, breeder age, yolk, mineral, embryo development

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INTRODUCTION

The poultry embryo derives all its nutrient requirements during incubation from the albumen and yolk (Uni et al., 2012). These nutrients are used to build new tissue, maintain existing tissue and muscular activity, and sustain development through hatching (Vleck, 1991). In Pekin duck eggs, albumen represents about 53.0 to 55.6% of the egg's total content and consists of approximately 85.7 to 88.1% water and 10.9 to 13.1% protein. The yolk represents about 31.3 to 33.9% of the egg's total content and consists of approximately 41.7 to 44.1% water, 17.3 to 17.8% protein, and 35.5 to 38.4% lipid (Onbaşılar et al., 2011). Yolk is also the major storage area of the most minerals. The hen deposits minerals in the yolk via the ovary (Yair and Uni, 2011). Minerals are important for the growth and development of embryos and mineral deficiency can cause skeletal, immune, and cardiovascular system disorders, as well as reduced hatchability and increased mortality (Uni et al., 2012). Therefore, egg contents primarily influence embryo weight, body measurements, and hatchability.

Flock age is a major factor that determines the albumen and yolk content in eggs (O'Sullivan et al., 1991; Nangsuay et al., 2011). Egg weight follows a curvilinear function in relation to flock age and it reaches a plateau toward the end of the laying cycle (French and Tullet, 1991; Nangsuay et al., 2011). Yolk and albumen weights change with hen age. A larger increment of yolk relative to albumen occurs as the hen gets older (O'Sullivan et al., 1991; Peebles et al., 2000).

However, information regarding embryo development and the yolk and mineral absorption of ducklings origi-

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nating from different breeder ages of Pekin duck are very limited. The purpose of this study was to examine the effects of breeder age (28, 34, and 40 wk) on the development of embryos, the levels of yolk sac, and several minerals (Na, Mg, P, K, Ca, Cu, and Zn) in the fertile egg yolk of Pekin ducks on the day of setting [embryonic day (**E**) 0] and during the incubation period (E12, E16, E20, and E25). Likewise, egg quality and hatching results were determined under these conditions.

MATERIALS AND METHODS

Experimental Design

Eggs were obtained from the same flock of Pekin ducks reared in accordance with the management guide of the duck breeders (Star 53-Grimaud Freres). Eggs were collected at 28, 34, and 40 wk of breeder age. At each of these ages, 165 freshly laid eggs from each age (a total of 495 eggs) were collected at the farm between 1000 and 1100 h on each day of the collection. Eggs were weighed and numbered. Fifteen eggs for each age were taken to determine egg characteristics. Egg internal quality and shell quality analysis were completed within 24 h of the eggs being collected. Egg shell breaking strength was measured by using an egg breaking tester (static compression device, Dr.-Ing. Georg Wazau Mess- und Prufsysteme GmbH, Berlin, Germany). The egg content was broken out onto a glass-top table. Shells were washed and dried. Egg shell thickness was measured in 3 different parts (upper and lower ends and middle) using a micrometer (Mitutoya, no. 1044N, 0.01–5 mm; Kawasaki, Japan). Then the height of the albumen and the yolk was measured with a tripod micrometer (Mitutoya, no. 2050–08, 0.01–20 mm). The length and width of the albumen and the diameter of the yolk were measured using a digital caliper. By using these values, yolk index, albumen index, and Haugh units were calculated (Onbaşılar et al., 2011).

Albumen and yolk were separated and percentages of albumen, yolk weights, and the ratio of yolk to albumen were determined. For mineral analyses, samples from each yolk were weighed and digested with a mixture of 2 mL of 30% H_2O_2 and 4 mL of 70% HNO_3 inside a 50-mL plastic tube for 6 h in a 95°C bath. The digested samples were analyzed for their mineral content (Na, Mg, P, K, Ca, Cu, and Zn) using an inductively coupled plasma atomic emission spectroscopy (Yair and Uni, 2011).

Eggs were fumigated with formaldehyde gas at a temperature of 24°C and 75% RH for 20 min and then stored for 3 d at 17°C and 75% RH. Eggs were incubated in the same incubator (I168, Petersime, Olsene, Belgium) at 37.5°C and 60% RH and were turned from 1 to 25 d of incubation. At E12, E16, E20, and E25, 5 eggs were randomly selected, weighed, and embryos were removed and euthanatized by decapitation. The yolk sacs and embryo were weighed and the levels of yolk minerals were determined on examined incubation days. Embryo length was defined as the length from the tip of the beak to the toe.

On E25, all eggs were transferred to the hatcher (H168, Petersime). The hatcher was operated at 37.0°C and 72% RH. On E28, the healthy ducklings were removed and the sex of chicks was determined. All chicks were weighed. Eggs that failed to hatch were counted, opened, and visually evaluated to determine fertility and embryonic dead. Fertility rate for each breeder age group was determined as a percentage of fertile eggs to total eggs. Early, middle, and late embryonic mortalities during incubation for each group were calculated as a percentage of dead embryos in each term out of the total number of fertile eggs. Hatchability of fertile eggs for each treatment group was calculated as hatched ducklings out of the total number of fertile eggs. Hatchability of total eggs for each treatment group was determined as hatched ducklings out of the total number of eggs.

Statistical Analysis

Statistical analyses were performed using the software package SPSS for Windows (SPSS Inc., Chicago, IL). Data were tested for distribution normality and homogeneity of variance. One-way ANOVA was used to determine the differences among breeder age on egg characteristics, yolk minerals, relative yolk sac weight, relative embryo weight, and length. Two-way ANOVA was used to determine the differences between breeder age and sex on hatching weight. Significances of differences in hatching results were determined using the Chi-squared test (Dawson and Trapp, 2001). When a significant difference was found among groups for post hoc multiple comparisons, Duncan's test was used. Statistical significance was taken at $P \leq 0.05$.

RESULTS AND DISCUSSION

As expected, eggs from the 40-wk-old breeder hens were heavier than those from the 28- and 34-wk-old hens (Table 1). Percentages of yolk weight increased ($P < 0.01$) and percentages of albumen weight decreased ($P < 0.01$) with age of Pekin duck breeders. The observed egg weight, yolk, and albumen ratios are in agreement with previous studies in different poultry species (Applegate et al., 1998; Vieira and Moran, 1998; Hamidu et al., 2007; Nangsuay et al., 2011). Shell quality determines gas exchange and moisture loss during incubation, and poor shell quality has been related to a higher egg moisture loss during incubation (Reis et al., 1997; Peebles et al., 2001). Percentages of shell weight were 9.97, 9.72, and 9.97% obtained from the 28-, 34-, and 40-wk-old breeder hens, respectively ($P > 0.05$). Shell breaking strength and shell thickness decreased with breeder age but only shell thickness was found to be statistically significant ($P < 0.01$) among examined breeder ages. Okruszek et al. (2006) reported that duck egg shell weight, shell thickness, and breaking strength

Table 1. Effect of breeder age on egg characteristics (n = 15)

Variable	Breeder age (wk)			*P*-value
	28	34	40	
Egg weight (g)	75.66 ± 0.53[c]	83.61 ± 1.18[b]	87.62 ± 1.42[a]	0.001
Yolk weight (%)	28.80 ± 0.48[b]	29.54 ± 0.50[b]	31.78 ± 0.58[a]	0.001
Albumen weight (%)	61.24 ± 0.60[a]	60.74 ± 0.60[a]	58.25 ± 0.58[b]	0.001
Shell weight (%)	9.97 ± 0.19	9.72 ± 0.17	9.97 ± 0.10	NS
Eggshell breaking strength (kg/cm^2)	3.04 ± 0.16	2.93 ± 0.14	2.88 ± 0.07	NS
Shell thickness (μm)	46.76 ± 0.67[a]	44.27 ± 0.58[b]	43.51 ± 0.49[b]	0.001
Yolk index	43.46 ± 0.67[a]	41.84 ± 0.63[a]	40.11 ± 0.49[b]	0.001
Albumen index	9.37 ± 0.33	9.66 ± 0.37	8.75 ± 0.27	NS
Haugh unit (%)	81.45 ± 1.30	81.72 ± 1.63	78.50 ± 1.27	NS

[a–c]Means within a row with different superscript letters differ.

decreased from wk 6 to 22 of the laying period, and these decreases were found to be statistically significant ($P \leq 0.05$). The yolk index of eggs was lower for 40-wk-old hens than the others ($P < 0.001$). This was likely because of a higher yolk diameter and lower yolk height in this group. However, hen age did not affect the albumen index or Haugh units.

Minerals in the yolk are important for the growth and development of embryos. The hen deposits minerals in the egg via 2 routes: the ovary to the yolk and oviduct to the albumen, shell, and shell membrane for proper development of the embryo depending on the correct deposition of minerals by the hen into the egg (Wilson, 1997). Yair and Uni (2011) reported that the yolk is the major storage compartment for P, Fe, Zn, Cu, and Mn in hatching eggs. Table 2 presents the examined mineral distribution in the yolk on day of setting, E12, E16, E20, and E25. The levels of Na on day of setting were 55.8, 51.0, and 51.9 mg/yolk, which then decreased to 27.0, 25.1, and 22.8 mg/yolk on E25 for 28-, 34-, and 40-wk-old ducks, respectively. The differences were not significant among breeder age groups on examined days. On day of setting, the yolk contained 5.5, 4.5, and 4.7 mg/yolk Mg obtained from 28-, 34-, and 40-wk-old ducks, respectively; the levels then decreased to 2.5 mg/yolk in all breeder age groups on E25. Yolk Mg levels were only different on day of setting and on E16. On these days, Mg level of yolk ob-

Table 2. Effect of breeder age on amount of specific minerals in the yolk on day of settings and during incubation

Item	Breeder age (wk)	On day of settings[1]	Days of incubation[2]			
			E12	E16	E20	E25
Na (mg/yolk)	28	55.8 ± 2.0	49.3 ± 3.4	47.8 ± 3.7	39.9 ± 2.5	27.0 ± 3.3
	34	51.0 ± 1.0	50.5 ± 1.5	49.5 ± 1.4	37.7 ± 3.0	25.1 ± 2.1
	40	51.9 ± 1.6	48.1 ± 3.0	47.5 ± 2.0	39.3 ± 3.1	22.8 ± 2.8
P-value		NS	NS	NS	NS	NS
Mg (mg/yolk)	28	5.5 ± 0.4[a]	5.0 ± 0.5	4.7 ± 0.1[a]	3.2 ± 0.3	2.5 ± 0.3
	34	4.5 ± 0.2[b]	4.3 ± 0.4	3.1 ± 0.3[b]	2.7 ± 0.3	2.5 ± 0.3
	40	4.7 ± 0.2[b]	4.4 ± 0.4	2.8 ± 0.3[b]	2.6 ± 0.2	2.5 ± 0.1
P-value		0.032	NS	<0.001	NS	NS
P (mg/yolk)	28	133.1 ± 4.8[a]	102.1 ± 4.1[a]	51.9 ± 4.3	30.5 ± 2.7	20.5 ± 1.0[a]
	34	126.1 ± 4.5[ab]	93.0 ± 2.8[ab]	46.3 ± 1.6	24.6 ± 1.5	17.2 ± 1.4[a]
	40	116.5 ± 3.0[b]	84.9 ± 1.6[b]	41.4 ± 2.1	25.5 ± 1.5	12.0 ± 1.5[b]
P-value		0.026	0.006	NS	NS	0.002
K (mg/yolk)	28	97.7 ± 1.7[a]	96.4 ± 4.6[a]	82.7 ± 6.5	70.1 ± 4.5[a]	54.2 ± 4.5
	34	82.3 ± 2.7[b]	76.5 ± 3.8[b]	70.8 ± 5.7	56.2 ± 4.4[b]	44.5 ± 5.2
	40	78.1 ± 1.4[b]	74.7 ± 4.2[b]	66.9 ± 4.3	54.4 ± 3.2[b]	36.7 ± 4.2
P-value		<0.001	0.003	NS	0.037	NS
Ca (mg/yolk)	28	36.8 ± 0.9[a]	26.5 ± 2.0	25.7 ± 1.8[a]	23.2 ± 1.3[a]	21.2 ± 1.6
	34	28.9 ± 1.1[b]	25.2 ± 1.6	18.8 ± 1.8[b]	17.8 ± 1.8[b]	15.8 ± 1.8
	40	30.2 ± 1.1[b]	26.0 ± 1.8	23.3 ± 1.7[ab]	22.0 ± 0.8[a]	17.9 ± 2.3
P-value		<0.001	NS	0.047	0.036	NS
Cu (μg/yolk)	28	24.6 ± 1.1[a]	20.1 ± 0.6[a]	11.2 ± 1.5	8.9 ± 0.4	6.2 ± 0.8
	34	17.3 ± 0.6[b]	14.8 ± 1.2[b]	11.6 ± 1.8	9.3 ± 0.6	5.9 ± 1.1
	40	16.1 ± 0.6[b]	13.3 ± 1.8[b]	11.1 ± 1.2	9.2 ± 0.9	5.3 ± 1.1
P-value		<0.001	0.007	NS	NS	NS
Zn (mg/yolk)	28	0.62 ± 0.02[a]	0.50 ± 0.01[a]	0.29 ± 0.04	0.22 ± 0.03	0.14 ± 0.02
	34	0.54 ± 0.03[b]	0.38 ± 0.04[b]	0.28 ± 0.02	0.19 ± 0.02	0.10 ± 0.02
	40	0.48 ± 0.02[b]	0.36 ± 0.04[b]	0.21 ± 0.04	0.15 ± 0.03	0.09 ± 0.01
P-value		<0.001	0.015	NS	NS	NS

[a,b]Means within a column with different superscript letters differ.

[1]n = 15.

[2]n = 5. E = embryonic day.

Table 3. Effect of breeder age on relative yolk sac weight, relative embryo weight, and embryo length during incubation (n = 5)

Item	Breeder age (wk)	Days of incubation[1]			
		E12	E16	E20	E25
Relative yolk sac weight[2] (%)	28	26.95 ± 0.67	24.78 ± 0.99[a]	21.02 ± 0.44[a]	13.13 ± 0.82[a]
	34	25.74 ± 0.67	21.40 ± 0.88[b]	18.23 ± 0.53[b]	12.09 ± 0.57[b]
	40	25.45 ± 0.70	20.18 ± 0.88[b]	15.83 ± 1.07[c]	10.33 ± 0.24[c]
P-value		NS	0.011	0.001	0.018
Relative embryo weight[3] (%)	28	4.27 ± 0.13	12.92 ± 0.77[b]	32.99 ± 0.72[c]	49.73 ± 0.65[c]
	34	4.70 ± 0.66	15.68 ± 0.90[a]	36.92 ± 0.85[b]	52.46 ± 0.52[b]
	40	5.18 ± 0.41	17.40 ± 0.61[a]	40.99 ± 1.04[a]	54.92 ± 0.64[a]
P-value		NS	0.005	<0.001	<0.001
Embryo length (mm)	28	53.07 ± 1.22[b]	76.10 ± 3.94[b]	99.07 ± 1.24[b]	112.24 ± 1.99
	34	56.62 ± 0.67[a]	88.15 ± 1.32[a]	106.08 ± 2.09[a]	113.96 ± 1.12
	40	57.04 ± 0.42[a]	89.33 ± 1.06[a]	105.68 ± 1.79[a]	114.31 ± 2.12
P-value		0.011	0.005	0.025	NS

[a–c]Means within a column with different superscript letters differ.

[1]E = embryonic day.

[2]Relative yolk sac weight = (yolk sac weight/egg weight at set) × 100.

[3]Relative embryo weight = (yolk free embryo weight/egg weight at set) × 100.

tained from 28-wk-old breeders was significantly higher than the other breeder ages ($P < 0.05$). The P levels on day of setting were 133.1, 126.1, and 116.5 mg/yolk and decreased to 20.5, 17.2, and 12.0 mg/yolk on E25 for 28-, 34-, and 40-wk-old breeders, respectively. Yolk P levels were only different on day of setting, E12 and E25. On these days, yolk P level obtained from 28-wk-old breeders was significantly higher than that from 40-wk-old breeders ($P < 0.05$). The K levels on day of setting were 97.7, 82.3, and 78.1 mg/yolk, which decreased to 54.2, 44.5, and 36.7 mg/yolk in the 28-, 34-, and 40-wk-old ducks, respectively, on E25. On E16 and E25, no significant differences in K levels occurred among breeder age groups. The Ca levels were 36.8, 28.9, and 30.2 mg/yolk on day of setting and 21.2, 15.8, and 17.9 mg/yolk on E25 obtained from 28-, 34-, and 40-wk-old ducks, respectively. On E12 and E25, no significant differences in Ca levels occurred among breeder age groups. The Cu levels on day of setting were 24.6, 17.3, and 16.1 μg/yolk, which decreased to 6.2, 5.9, and 5.3 μg/yolk obtained from 28-, 34-, and 40-wk-old ducks, respectively, on E25. On day of setting and E12, Cu level in yolk obtained from 28-wk-old breeders was significantly higher than that from 34- and 40-wk-old breeders ($P < 0.01$). The Zn levels on day of setting were 0.62, 0.54, and 0.48 mg/yolk and decreased to 0.14, 0.10, and 0.09 mg/yolk on E25. Yolk Zn level was higher in yolk sac obtained from 28-wk-old breeders than that obtained from other breeder ages on day of setting and E12 ($P < 0.05$). These findings showed that breeder age affected the mineral levels, except for Na level, in the yolk on day setting. Likewise, breeder age changed the level of mineral consumed by embryo during incubation. However, on E25 the levels of examined minerals, except for P level in the yolk sac, were not statistically different among duck breeder age groups. Only P level in yolk sac obtained from 40-wk-old breeders was significantly lower ($P < 0.01$) than that obtained from the other breeder ages on E25. Phosphorus is associated with most bone abnormalities (Dibner et al., 2007); therefore, ovo enrichment with P in eggs obtained from 40-wk-old breeder ducks may be beneficial.

Table 4. Effects of breeder age and sex on hatching weight and relative hatching weight (% of egg weight at set)

Breeder age (wk)	Sex	n	Hatching weight (g)	Relative hatching weight (%)
28	Female	47	40.86 ± 0.46	55.16 ± 0.48
	Male	44	41.72 ± 0.47	55.70 ± 0.49
34	Female	56	46.70 ± 0.42	56.48 ± 0.44
	Male	43	47.22 ± 0.48	57.10 ± 0.50
40	Female	63	50.77 ± 0.40	59.55 ± 0.41
	Male	39	52.22 ± 0.50	60.74 ± 0.52
28		91	41.29 ± 0.33[c]	55.43 ± 0.34[c]
34		99	46.96 ± 0.32[b]	56.79 ± 0.33[b]
40		102	51.49 ± 0.32[a]	60.14 ± 0.33[a]
	Female	166	46.11 ± 0.25	57.06 ± 0.26
	Male	126	47.05 ± 0.28	57.85 ± 0.29
P-value				
Breeder age			<0.001	<0.001
Sex			0.012	0.043
Breeder age × Sex			NS	NS

[a–c]Means within a column with different superscript letters differ.

Table 5. Effect of breeder age on fertility; early, middle, and late embryonic dead; and hatchability of fertile and total eggs

Item	Fertility (%)	Early embryonic dead (%)	Middle embryonic dead (%)	Late embryonic dead (%)	Hatchability of fertile eggs (%)	Hatchability of total eggs (%)
Breeder age (wk)						
28	88.5	5.2	6.6	7.9	79.1	70.0
34	92.3	6.1	4.2	7.0	82.5	76.1
40	95.4	6.3	4.3	8.1	82.3	78.5
X^2	4.3	0.1	0.9	0.1	0.6	2.6
P-value	NS	NS	NS	NS	NS	NS

Although yolk percentages of eggs from the 40-wk-old breeder hens were higher than those from the young breeder flock, no significant differences in relative yolk sac weight were seen on E12 (Table 3). These data showed that yolk sac from the older breeder flock was consumed rapidly by the embryos during the first days of incubation. After E12, percentages of residual yolk sac weights in eggs from 40-wk-old breeders were less than those from the 34- and 28-wk-old breeders. Also, relative embryo weight was increased as the flocks aged from 28 to 40 wk. On E16, E20, and E25, relative embryo weights were significantly higher in eggs obtained from 40-wk-old breeders than those obtained from 28-wk-old breeders. These findings suggest an influence of yolk amount and yolk absorption on embryonic growth in Pekin ducks. Gous (2010) proposed that yolk sac utilization could be one of the limiting factors for embryo development. Similarly Hamidu et al. (2007) reported that percentages of yolk sacs generally increased as the flocks aged. They also reported that embryonic metabolism was highest in embryos from old and very old breeder flocks. Applegate et al. (1998) suggested that the weight of the relative yolk sac to egg weight at set was significantly increased at 24 d of incubation in eggs from 36-wk-old hens ($P \leq 0.001$) as compared with eggs from 26- and 31-wk-old hens. Although they observed this trend at 20 and 22 d, it was not significant. Embryo length was affected by the breeder age at E12, E16, and E20 ($P < 0.05$). On these incubation days, embryo lengths from the young breeder age were shortest in the examined breeder age groups. However, embryo lengths on E25 were similar among groups. We did not determine the internal egg temperature in the current study; as all the eggs are incubated by the same temperature and RH, it can be assumed that the bigger eggs of the older flocks will have an increased internal temperature, and this may be reflected in the lack of increase in chick length on E25 among breeder age groups. Nangsuay et al. (2011) reported that embryo length eggs from young breeders was shorter than for old breeders at 14 and 18 d of incubation, but these differences were not statistically significant. Hamidu et al. (2007) reported that a very old flock had longer chicks compared with the peak and post peak flocks, however they did not determine the lengths of embryos during incubation in their study.

Hatching weight and relative hatching weight (Table 4) were significantly affected by breeder age and sex ($P < 0.05$). Hatching weight and relative hatching weight increased with breeder age. Knizetova et al. (1992) determined that Pekin duck hatch weight is positively correlated with early posthatch growth. Faster growth rate among Pekin ducklings from larger eggs may be related to faster reabsorption of the larger yolk. Hatching weights of male ducklings were higher than those of female ducklings. Fertility, early, middle, and late embryonic dead, hatchability of fertile eggs, and hatchability of total eggs (Table 5) were not different among breeder age groups ($P > 0.05$). El-Hanoun et al. (2012) reported that the higher embryonic mortality of eggs from young parents may be because their thicker shell could negatively affect hatching process. In our study, embryonic mortality was higher in eggs obtained from 28-wk-old breeders, but this finding was not statistically different. Hatchability of fertile eggs was found to be low; this low level may be due to the all eggs in different breeder age groups being incubated at the same temperature and RH. Similarly, El-Hanoun et al. (2012) reported that hatchability of fertile eggs in Pekin ducks ranged from 73.5 to 86.1% according to breeder age and incubation humidity. They also suggested that duck eggs produced within a specific breeder age period require a specific incubation RH to attain the best hatchability.

In summary, breeder age significantly affected some egg characteristics, relative yolk sac weight, some contents of minerals in the yolk, embryonic growth during incubation, and duckling weight. The findings from the current study will contribute to the poultry sector and the new studies that will be carried out.

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