Egg quality, hatchability, gosling quality, and amino acid profile in albumen and newly-hatched goslings' serum as affected by egg storage

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ABSTRACT In modern poultry husbandry, storing fertilized eggs is a common measure to cope with the variable demands of the market and the maximum hatching capacity of the hatchery. However, this measure is harmful to the hatchability of eggs and the quality of newly hatched birds. Knowledge about the effects of storing fertilized eggs on the performance of goslings is still limited. The objective of this study was to investigate the effects of storing fertilized eggs on egg quality, hatchability, gosling quality, hatching weight, post-hatching growth performance, and amino acid profile in albumen and newly hatched goslings' serum. A total of 1,080 fertilized goose eggs (Jilin White goose) with a similar egg weight (126.56 ± 0.66 g) were used in this study. All eggs were distributed into 3 groups with 24 replicates per group and 15 eggs per replicate. The differences between groups were the storage duration of eggs (0, 7, or 14 d). We found that the Haugh unit, yolk weight, and eggshell weight decreased linearly, whereas the albumen pH increased linearly, with storage duration. Prolonging storage duration had negative effects on hatchability, hatching weight, post-hatching growth performance parameters, and gosling quality in a time-dependent manner. The analysis of the amino acid profile in albumen and newly-hatched goslings' serum showed that the amino acid content increased linearly with storage duration. Additionally, eggs stored for 14 d had the worst performance for all measured parameters. Therefore, we concluded that the storage of fertilized eggs negatively affects egg quality and post-hatching gosling quality. To produce high-quality goslings, it is necessary to shorten the storage duration for fertilized eggs.

Key words: storage duration, gosling, egg quality, amino acid profile, hatchability

2023 Poultry Science 102:102367
https://doi.org/10.1016/j.psj.2022.102367

INTRODUCTION

In modern poultry husbandry, storing fertilized eggs is a common measure to cope with the variable market demand for newly hatched birds and the maximum hatching capacity of the hatchery. However, the quality of fertilized eggs will deteriorate with storage duration (Gao et al., 2017). During the storage, the carbon dioxide ($\mathbf{CO_2}$) in the eggs will be gradually lost through the pores of the eggshells (Jin et al., 2010; Akter et al., 2014), which will result in a high pH of egg contents (Quan and Benjakul, 2018). The increase of pH in egg contents will further lead to the decrease of Haugh unit (Quan and Benjakul, 2019), the flattening of yolk (Gao et al., 2017), and/or the hydrolysis of albumen protein (Liu et al., 2018). Changes in the quality of fertilized eggs will result in low hatchability and poor post-hatching growth performance for birds (Reijrink et al., 2010).

In the process of incubation, albumen proteins move into the amniotic fluid and are swallowed by the embryo for further utilization (Tona et al., 2003). Proteolysis induced by storage causes changes in internal amino acid contents (Liu et al., 2018). It is self-evident that amino acids are important for animal development. The changes in amino acid content will affect the development of newly hatched birds to a certain extent (Tona et al., 2003).

Studies on the hatchability and post-hatching growth performance as affected by storing fertilized eggs have been investigated in broiler chicks (Tona et al., 2004), ducks (Quan and Benjakul, 2018), turkeys

Received October 24, 2022.
Accepted November 27, 2022.

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(Stępińska et al., 2017), and pheasants (Demirel and Kırıkçı, 2009). However, knowledge about geese is limited. For goose eggs, the common storage strategy to ensure favorable hatchability in modern poultry husbandry is less than 7 d (https://www.thepoultrysite.com/articles/care-and-incubation-of-hatching-eggs). Additionally, some people think that storing fertilized eggs for more than 7 d is also acceptable (https://www.cnpoultry.com/380.html). The current storage strategy for goose eggs is formulated according to the studies on chicken eggs. However, the effects of storing fertilized eggs on the changes in egg quality are different in different poultry species (Qiu et al., 2012; Quan and Benjakul, 2018, 2019). Compared with other poultry species, goose eggs are characterized by low hatchability and high embryo mortality (Tai et al., 2001; Rosinski et al., 2006a,b). It is expected that the storage of fertilized eggs will have a greater effect on the hatching performance of goslings than other poultry species.

Goose is one of the important commercial meat sources, which is rich in protein and a variety of trace elements (Xu et al., 2018). Goose, as a nutritious and healthy food resource, has been widely raised around the world (Kumbar et al., 2016). The future development of goose husbandry will benefit from knowing more about the effects of storing fertilized eggs on egg quality and post-hatching gosling quality. According to the results of investigations on the effects of storing fertilized eggs on hatching performance in other species of poultry, we hypothesized that the storage of fertilized goose eggs had negative effects on egg quality and post-hatching gosling quality. The objective of this study was to investigate the effect of storing fertilized eggs on egg quality, hatchability, gosling quality, hatching weight, post-hatching growth performance, and amino acid profile in albumen and newly hatched goslings' serum.

MATERIALS AND METHODS

The protocol and implementation procedure were supervised by the Animal Care and Use Committee of Jilin Agricultural University (Changchun, China)

Experimental Design and Animals

A total of 1,200 fertilized eggs were collected from a commercial breeder flock (Jilin White goose). The age of the breeder flock was 3 yr old. All eggs are purchased from Dekun Poultry Food Co., Ltd (Meihekou, Jilin) in 3 different batches, of which the first batch of eggs was freshly laid and was considered as control, whereas the second batch and the third batch of eggs were laid 7 d or 14 d prior to the freshly laid egg, respectively.

The number of fertilized eggs selected from each batch was 400 at first. The storage duration was determined according to production practice, in which fertilized eggs are commonly stored for less than 7 d before moving into incubators, but storage for longer than 7 d is avoided. Additionally, since egg weight is a dominant factor affecting the quality of newly-hatched birds (Nowaczewski et al., 2022). Three different batches of eggs used in this study had similar weights (126.56 ± 0.66 g). Eggs in the storage group were stored at 15°C and 75% relative humidity. All eggs from different groups were put into the incubator at the same time.

Before moving the eggs into the incubator, unfertilized eggs were removed by candling. Fertilized eggs were then disinfected (37% formaldehyde and potassium permanganate in a ratio of 2:1) and pre-heated (30°C for 12 h). Subsequently, a total of 360 fertilized eggs were selected, numbered, weighed, placed on incubation trays (90 eggs per tray; 6 × 15), and moved into egg hatching incubator (Keyu microcomputer automatic incubator, Dezhou, Shandong). Each column of the tray was considered as a replicate (15 eggs). There were 24 replicate columns in each group. The incubation period consisted of 3 stages: stage 1, d 1 to 14 (the temperature was 38°C and the humidity was 65%); stage 2, d 15 to 28 (the temperature was 37.5°C and the humidity was 55%); stage 3, d 29 to 31 (the temperature was 37.2°C and the humidity was 70%). All eggs were automatically turned once per 2 h for 180 s. An automatic ventilation program equipped in the incubator ensures that the concentration of CO_2 was kept below 0.10%.

After hatching, hatched birds were moved to a temperature-controlled room and assigned to cages according to the replicate. Goslings were raised in plastic-floored cages. All birds had free access to receive feed and water via one-side installed feeder and nipple drinker. Feeds (Table 1) were provided immediately as birds were distributed into the cage. All of the goslings were raised until d 14 of age. The lighting program was 24 h a day for the first 3 d and then reduced to 16 h of light and 8 h of dark. The room temperature was kept at 30°C for the first 3 d and then decreased by 2°C per week. The relative humidity of the room was around 65%.

Parameters Analysis

Egg Quality Analysis. Before moving the eggs into the incubator, 3 eggs with a similar weight to the average weight were selected from each replicate column to measure the egg quality. A dial pipe gauge (Ozaki MFG. Co., Ltd., Tokyo, Japan) was used to measure eggshell thickness, which excluded the inner membrane and was determined on the average thickness of the sharp, equatorial, and blunt end of the egg. An egg multitester (Touhoku Rhythm Co., Ltd., Tokyo, Japan) was used to measure the Haugh unit, yolk weight, and eggshell weight. A pH meter (Fisher Scientific, Pittsburgh, PA) was used to measure the pH in albumen and yolk. The albumen was frozen for further analysis.

Hatchability and Gosling Quality Analysis. After hatching, the number of hatched goslings was

Table 1. Composition and nutrient levels of the experimental basal diet, (%, as-fed basis).

Ingredients, %	
Corn	60.00
Soybean meal	29.11
Wheat bran	6.00
Fish meal	2.00
L-Lysine-HCl	0.20
DL-Methionine	0.23
Dicalcium phosphate	0.84
Limestone	0.82
Sodium chloride	0.30
Vitamin and trace mineral premix[1]	0.50
Total	100.00
Analyzed value, %	
Metabolizable energy, MJ/kg	11.67
Available phosphorus	0.40
Crude protein	19.78
Methionine	0.50
Total sulfur amino acid	0.77
Lysine	1.08
Calcium	0.78
Crude fiber	0.31
Neutral detergent fiber	1.09
Acid detergent fiber	0.35

[1]Provided per kilogram of complete diet: vitamin D_3, 200 IU; vitamin A (retinyl acetate), 5×10^6 IU; vitamin E (DL-α-tocopheryl acetate), 27.8 IU; vitamin K_3, 1.5 mg; thiamine, 2.2 mg; riboflavin, 5 mg; nicotinic acid, 65 mg; folic acid, 1 mg; pantothenic acid, 15 mg; pyridoxine, 2 mg; biotin, 0.2 mg; choline, 1,000 mg; Fe (ferrous sulfate), 90 mg; Cu (copper sulfate), 6 mg; Mn (manganese oxide), 85 mg; Zn (zinc oxide), 85 mg; I (potassium iodide), 0.42 mg; Se (sodium selenite), 0.3 mg; Co (cobalt chloride), 2.5 mg.

recorded to determine the hatchability with the following formula:

$$Hatchability = \frac{No.\ of\ hatched\ eggs}{No.\ of\ fertile\ eggs\ after\ sampling} \times\ 100$$

Subsequently, each gosling was weighed and macroscopically examined to score them according to the criteria proposed in the study of Tona et al. (2003). The criteria were adjusted to make them applicable to this study. Parameters in the criteria consist of activity, down and appearance, retracted yolk, eyes, legs, navel area, remaining membrane, and remaining yolk. The total score of these criteria was 100. The quality score for a gosling was defined as the sum of the scores quoted for all characteristics.

Post-hatching Growth Performance Analysis. Goslings were raised until d 14 of age. The body weight of goslings was checked on hatching day and d 14 of age based on the replicate cage to determine average daily gain (**ADG**). Replicate cage-based feed intake was measured daily to determine average daily feed intake (**ADFI**). The feed conversion ratio (**FCR**) was calculated according to the values of ADG and ADFI. The formulas for measuring ADG, ADFI, and FCR were shown below:

$$ADG = \frac{Final\ body\ weight - Initial\ body\ weight}{Experimental\ period}$$

$$ADFI = \frac{Total\ feed\ intake}{Experimental\ period}$$

$$FCR = \frac{ADFI}{ADG}$$

Amino Acid Profile Analysis in Albumen and Newly Hatched Goslings' Serum. On hatching day, 3 birds were randomly selected from each replicate for blood collection from the wing vein using a sterile syringe. Blood samples and frozen albumen samples were used for amino acid profile analysis using Hitachi 835-50 amino acid automatic analyzer and ammonia concentration analysis by Dimension Vista Ammonia method (Cha et al., 2018). Samples were homogenized and then moved to a sealed tube with 10 mL of 6 mol/L HCl. Samples were then frozen with liquid nitrogen for 5 min and later vacuumed-dried. After post-column derivatization with ninhydrin, samples were hydrolyzed with 0.1% phenol in a thermostated container (110°C) for 22 h. After the sample was cooled to room temperature, the solution was filtered into a volumetric flask (50 mL) and the volume was fixed with distilled water. Then, 1 mL of the filtrate was moved into a tube and dried under reduced pressure (50°C). The residue was dissolved in 2 mL of water and dried under reduced pressure again. Subsequently, samples were dissolved in 2 mL of sodium citrate solution (pH of 2.2). Samples were homogenized and filtered with a 0.22-μm filter. The filtrate was injected into the automatic amino acid analyzer for analysis. Each analysis was conducted in duplicate. The amino acid concentration in the solution was calculated by peak area. For ammonia concentration analysis, samples were centrifuged (3,000 × *g*) for 15 min. The supernate was analyzed by Dimension Vista system (Siemens, Munich, Germany). Monitoring the change of absorbance induced by the oxidation of the reduced cofactor at 340/700 nm to calculate the ammonia concentration.

Statistical Analysis

Before the analysis, all the percentage data were transformed by arcsine transformations. Data were then subjected to statistical analysis in a randomized complete block design using the General Linear Models procedures (SAS Institute, Cary, NC). The normality of data was examined by the Shapiro-Wilk test and QQ plots. The replicate cage (n = 24) served as the experimental unit. Orthogonal polynomials were used to assess the linear and quadratic effects of storage duration. Since no quadratic effect has been observed on the effects of storage duration on the measured parameters, we did not present the *P*-value of the quadratic effect in the Table. Differences among groups were evaluated by the one-way ANOVA for multiple comparisons. Variability in the data was expressed as the pooled standard error of means (**SEM**). $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

It is generally believed that storing fertilized eggs will damage the quality of eggs. The storage of eggs will

change their physical properties, such as decreasing Haugh unit (Quan and Benjakul, 2019), flattening yolk (Gao et al., 2017), and/or increasing pH in albumen and yolk (Quan and Benjakul, 2019). In the present study, we observed that the Haugh unit ($P = 0.032$), yolk weight ($P = 0.004$), and eggshell weight ($P = 0.030$) decreased linearly, whereas albumen pH ($P < 0.001$) increased linearly, with storage duration. However, yolk pH and eggshell thickness did not differ among groups. Additionally, eggs stored for 14 d had the worst Haugh unit ($P = 0.047$), yolk weight ($P = 0.016$), and eggshell weight ($P = 0.047$), and the highest albumen pH ($P < 0.001$; Table 2). The Haugh unit is a measure of egg protein quality based on the height of its albumen (Jones and Musgrove, 2005; Samli et al., 2005). The decrease in the Haugh unit is related to the deterioration of protein quality in albumen (Akter et al., 2014). The pH is an important factor affecting the net charge of proteins (Quan and Benjakul, 2019), so its increase is considered to be the main reason for changing the physicochemical properties of protein in albumen and yolk (Omana et al., 2011; Eke et al., 2013; Gao et al., 2017). The variation of egg contents' pH is related to the loss of internal CO_2. During the storage, the CO_2 in the eggs will be gradually lost through the pores of the eggshells (Jin et al., 2010; Akter et al., 2014), which will result in a high pH of egg contents (Quan and Benjakul, 2018). The increase in egg contents' pH will cause chain reactions, such as the weakening of the yolk membrane (Karoui et al., 2006; Belitz et al., 2009). The yolk membrane plays a key role in determining the diffusion rate of substances from the yolk to the albumen. The weakening of the yolk membrane will increase the permeability of the vitelline membrane, and therefore, lead to the migration of inclusions from the yolk to the albumen (Berardinelli et al., 2008; Al-Hajo et al., 2012; Réhault-Godbert et al., 2014; Gao et al., 2017). Therefore, we considered that the reduction of yolk weight caused by storing fertilized eggs was related to the increase of albumen pH. Additionally, the storage of eggs is also reported to be capable to impair the eggshell quality. de Abreu Fernandes and Litz (2017) observed that storing fertilized eggs will decrease the eggshell thickness and eggshell strength. The mechanism of eggshell quality damage caused by storage is still unknown. It is speculated that the release of CO_2 forced the embryo to obtain carbonate ions from the eggshell. However, in this study, the storage of fertilized eggs had no significant effects on eggshell thickness, but decreased eggshell weight. Storing fertilized eggs may destroy the tight structure of eggshells, thus reducing the weight of eggshells without affecting the thickness. However, we did not measure the strength of eggshells. More study is needed to verify the above conjecture. We can still conclude that the storage of fertilized eggs had negative effects on the egg quality of geese.

Additionally, with the variation of egg contents' pH, the protein will hydrolyze to form amino acids (Liu et al., 2018). In this study, we observed that most albumen amino acids, such as aspartic acid, glutamic acid, glycine, alanine, methionine, tyrosine, and histidine increased linearly with storage duration ($P < 0.05$; Table 3). The contents of the above amino acid in eggs stored for 14 d were the highest ($P < 0.05$). The proteolysis in albumen caused by storing fertilized eggs was also observed in duck eggs (Liu et al., 2022). Liu et al. (2022) found that the storage of duck eggs causes increased leucine, tyrosine, arginine, tryptophan, aspartic acid, glutamic acid, histidine, serine, threonine, and phenylalanine contents in albumen. Therefore, we considered that the proteolysis in albumen occurred along with the storage. Moreover, it was worth noting that the ammonia content in albumen gradually increased with storage duration ($P = 0.004$; Table 3), which indicated that the storage led to the production of biogenic amines (Zhang et al., 2020). Biogenic amines are formed during the decomposition of proteins (Moy and Todd, 2014). Similarly, Min et al. (2007) observed the production of ammonia contents increased with the storage of animal products. Liu et al. (2022) also noted that a lot of ammonia and biogenic amines were produced during the storage of duck eggs. Therefore, we considered that the storage of fertilized eggs will cause proteolysis in albumen.

Any changes in the quality of fertilized eggs will inevitably affect their hatchability. It is well documented that the storage of fertilized eggs will impair their hatchability (Yassin et al., 2008; Reijrink et al., 2010; Khan et al., 2013). Yassin et al. (2008) reported that the hatchability was reduced by 0.2% for each extra day of storage before the 7th day and after the 7th day by 0.5%. Additionally, Khan et al. (2013) noted that the hatchability was not affected by eggs stored for 3 d, but significantly decreased by eggs stored for 4 to 8 d. In the present study, the hatchability decreased linearly with storage duration ($P = 0.020$; Table 4). As reported by Reijrink et al. (2010), the death of cells in embryos increased over time, which would decline the viability of the embryo. On the other hand, Elibol et al. (2002) suggested the conformation of shell membrane-formed protein changed with the storage of fertilized eggs. These changes will affect the way of interaction and development between the chorioallantoic membrane and the inner shell membrane. Growth of the chorioallantoic

Table 2. Effect of storage duration on egg quality parameters[1].

Items	Storage duration, day			SEM	*P*-value	
	0	7	14		Linear	ANOVA
Egg weight, g	127.54	126.13	126.02	0.658	0.106	0.197
Haugh unit	96.73[a]	92.82[ab]	87.96[b]	28.304	0.032	0.047
Yolk weight, g	58.50[a]	54.79[ab]	50.89[b]	1.810	0.004	0.016
Eggshell weight, g	18.03[a]	17.13[ab]	16.61[b]	0.452	0.030	0.047
Albumen pH	8.44[b]	8.53[b]	8.90[a]	0.074	<.001	<.001
Yolk pH	6.24	6.33	6.36	0.043	0.059	0.143
Eggshell thickness, mm	0.57	0.56	0.57	1.421	0.736	0.821

Abbreviation: SEM, standard error of the mean.

[1]Values represent the means of 24 replicates with 3 eggs per replicate per group (n = 24).

[a,b]Means in the same row with different superscript differ significantly ($P < 0.05$).

Table 3. Effect of storage duration on albumen amino acid profile[1].

	Storage duration, day				*P*-value	
Items, %	0	7	14	SEM	Linear	ANOVA
Aspartic acid	0.74[b]	1.13[b]	1.85[a]	0.218	0.001	0.004
Threonine	10.77	24.07	20.30	3.634	0.075	0.088
Serine	8.31	11.66	13.99	2.259	0.087	0.221
Glutamic acid	3.26[b]	4.19[ab]	5.50[a]	0.689	0.030	0.043
Glycine	3.20[b]	6.65[a]	8.05[a]	1.088	0.004	0.012
Alanine	6.34[b]	11.19[ab]	12.01[a]	1.808	0.035	0.048
Valine	6.14	5.54	5.67	1.616	0.841	0.963
Methionine	1.26[b]	1.41[b]	3.63[a]	0.630	0.013	0.022
Isoleucine	2.44	3.91	2.63	0.747	0.857	0.334
Leucine	3.07	6.22	4.34	1.022	0.385	0.109
Tyrosine	3.20[b]	6.10[a]	6.45[a]	0.986	0.028	0.038
Phenylalanine	2.09	4.51	3.72	0.667	0.095	0.074
Histidine	2.21[b]	4.82[a]	4.69[a]	0.733	0.024	0.030
Lysine	3.26	6.21	5.37	1.180	0.215	0.208
Arginine	7.59	17.07	14.24	2.535	0.075	0.053
Proline	4.95	5.24	5.63	1.314	0.717	0.935
Ammonia	0.65[b]	1.04[ab]	1.51[a]	0.192	0.004	0.014

Abbreviation: SEM, standard error of the mean.

[1]Values represent the means of 24 replicates with 3 eggs per replicate per group (n = 24).

[a,b]Means in the same row with different superscript differ significantly ($P < 0.05$).

Table 5. Effect of storage duration on gosling serum amino acid profile[1].

	Storage duration, day				*P*-value	
Items, %	0	7	14	SEM	Linear	ANOVA
Aspartic acid	0.49[c]	0.61[b]	0.75[a]	0.030	<0.001	<0.001
Threonine	0.31[c]	0.36[b]	0.43[a]	0.018	<0.001	<0.001
Serine	0.31[b]	0.34[b]	0.40[a]	0.017	<0.001	0.002
Glutamic acid	1.07[c]	1.27[b]	1.57[a]	0.063	<0.001	<0.001
Glycine	0.55[b]	0.62[ab]	0.72[a]	0.033	0.002	0.006
Alanine	0.48[c]	0.56[b]	0.68[a]	0.024	<0.001	<0.001
Valine	0.34[c]	0.41[b]	0.51[a]	0.018	<0.001	<0.001
Methionine	0.05[b]	0.11[a]	0.15[a]	0.016	<0.001	0.001
Isoleucine	0.30[c]	0.36[b]	0.46[a]	0.018	<0.001	<0.001
Leucine	0.53[c]	0.63[b]	0.78[a]	0.029	<0.001	<0.001
Tyrosine	0.21[c]	0.26[b]	0.31[a]	0.013	<0.001	<0.001
Phenylalanine	0.28[c]	0.34[b]	0.41[a]	0.015	<0.001	<0.001
Histidine	0.22[c]	0.27[b]	0.33[a]	0.012	<0.001	<0.001
Lysine	0.57[c]	0.68[b]	0.83[a]	0.033	<0.001	<0.001
Arginine	0.49[b]	0.56[b]	0.73[a]	0.030	<0.001	<0.001
Proline	0.40[b]	0.46[b]	0.54[a]	0.023	<0.001	0.001
Ammonia	0.12[b]	0.14[b]	0.18[a]	0.008	<0.001	<0.001

Abbreviation: SEM, standard error of the mean.

[1]Values represent the means of 24 replicates with 3 birds per replicate per group (n = 24).

[a,b,c]Means in the same row with different superscript differ significantly ($P < 0.05$).

membrane has been reported to be reduced by as much as 30% in the absence of proper turning during incubation (Tyrrell et al., 1954). Therefore, the normal development of the chorioallantoic membrane might be limited by storage, which may lead to the death of the embryo. To sum up, the storage had negative effects on the hatchability of fertilized eggs, also in goose eggs.

Moreover, we also observed the changes in serum amino acid profile with storage duration. As shown in Table 5, the contents of serum aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, proline, and ammonia increased linearly with storage duration ($P < 0.05$). The content of the above parameters in eggs stored for 14 d was the highest ($P < 0.05$; Table 5). In the process of incubation, albumen proteins move into the amniotic fluid and are swallowed by the embryo for further utilization (Tona et al., 2003). Therefore, the albumen amino acid components decided the volume of utilizable amino acid ingredients for newly-hatched birds. As the albumen amino acid profile changed with storage duration, the changes in the serum amino acid profile can be expected.

Table 4. Effect of storage duration on hatchability, hatching weight, post-hatching growth performance, and gosling quality[1].

	Storage duration, day				*P*-value	
Items	0	7	14	SEM	Linear	ANOVA
Hatchability, %	87.13[a]	85.99[ab]	83.61[b]	1.051	0.020	0.049
Hatching weight, g	102.65[a]	99.84[a]	94.51[b]	1.558	<0.001	0.002
Body weight at day 14 of age, g	496.61[a]	478.87[a]	455.67[b]	7.794	<0.001	0.002
ADG, g	28.14[a]	27.07[ab]	25.80[b]	0.566	0.005	0.017
ADFI, g	48.08	45.73	45.21	1.396	0.151	0.308
FCR	1.78	1.69	1.77	0.082	0.967	0.722
Average score of all goslings	97.20[a]	92.20[a]	84.80[b]	2.065	<0.001	0.001

Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio; SEM, standard error of the mean.

[1]Values represent the means of 24 replicates per group (n = 24).

[a,b]Means in the same row with different superscript differ significantly ($P < 0.05$).

The egg quality and internal amino acid composition are closely related to the quality of newly hatched birds (Tona et al., 2003). Hatching weight and appearance quality scores of newly hatched birds are important parameters reflecting the quality of birds. The storage of fertilized eggs has been reported to reduce the proportion of high-quality chicks and the average score of chick quality (Tona et al., 2003; Reijrink et al., 2010). Reis et al. (1997) reported that chicks hatched from freshly laid eggs had higher hatching weight than those hatched from stored eggs. As reported by Tona et al. (2004), shortening storage duration or avoiding storage seems to be an effective way to increase the proportion of high-quality chicks. In this study, the hatching weight ($P < 0.001$) and average quality score ($P < 0.001$) of newly hatched gosling decreased linearly with storage duration (Table 4). Therefore, we considered that the storage of fertilized eggs had negative effects on the quality of newly-hatched goslings.

The quality of newly-hatched birds is an important indicator reflecting the growth potential of birds (Tona et al., 2003). In this study, we observed that the final body weight ($P < 0.001$) and ADG ($P = 0.005$) decreased linearly with storage duration (Table 4). Moreover, the worst growth performance parameters appeared in eggs stored for 14 d ($P < 0.05$; Table 4). The storage of fertilized eggs was reported to decrease the post-hatching growth performance of birds (Tona et al., 2003,2004; Khan et al., 2013). Tone et al. (2004) reported that up to the end of the third week, chicks hatched from freshly laid eggs were heavier than those hatched from eggs stored for 7 d. Tona et al. (2003) reported that the storage of fertilized eggs had an

adverse effect on the growth performance of chicks during the first week. Therefore, we considered that the post-hatching growth performance will benefit from the shortening of storage duration. Indeed, results observed by Demirel and Kırıkçı (2009) indicate the negative effects caused by storage will be aggravated when fertilized eggs were stored for more than 8 days. Other studies suggested that the storage duration of fertilized eggs should not exceed 7 d (Tona et al., 2003,2004; Reijrink et al., 2010). Results observed in this study also support the conventional production practice, that is, the storage duration should be avoided for more than 7 d.

In conclusion, the storage of fertilized eggs had negative effects on egg quality, and therefore impaired the hatchability, gosling quality, and post-hatching growth potential. The negative effects induced by storage were shown in a time-dependent manner, which indicated that high-quality gosling production would benefit from the shortening of storage duration. Therefore, based on the results obtained, fertilized eggs should be stored for less than 7 d.

DISCLOSURES

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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