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Effects of chitosan coating and different storage periods of broiler breeder eggs on growth performance and carcass characteristics

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ABSTRACT - This study was carried out to determine the effects of coating with chitosan film and storing at different periods (7, 14, and 21 days) of broiler breeder eggs on growth performance and carcass characteristics of the chicks. The present study was arranged as three different storage periods (7, 14, and 21 days) and coating or not the eggs with chitosan film. In total, 1800 hatching fertilized eggs were used. These eggs were divided into six groups with 100 eggs in each and 600 eggs in each replication. A total of 751 chicks obtained from the hatching were used as material in this study. As a result, all chicks in the coated chitosan groups were alive during the 42-day growth period. The average hatching weight was determined as 42.7±0.1 g. The mean body weight (BW) of chicks on the 42nd day was determined as 2541.8±12.3 g in all groups. The effect of repetition on weekly BW and body weight gain (BWG) was found to be significant in the growth period. The differences between the groups for the BW were significant on day 1. While the weekly BWG varied, the growth performance was similar in the growth period in all groups. The differences between the groups in terms of slaughter weight and carcass characteristics were insignificant. It was determined that coating broiler eggs with chitosan and storing them in different periods does not have significantly negative effect on growth performance and carcass characteristics.

Keywords: broiler, carcass quality, egg, fattening

1. Introduction

Poultry production at all scales of operation is wholly dependent on the supply of day-old chicks. Fertility and hatchability are two major parameters that highly influence the supply of day-old chicks. Fertility refers to the percentage of incubated eggs that are fertile while hatchability is the percentage of fertile eggs that hatch. It is therefore important to understand the factors that influence the fertility and hatchability of eggs (King'ori, 2011).

Hatchability performance is related to the survival skill of the embryo in the poultry sector. This condition is closely related to factors such as number of cells of the blastoderm, storing condition and duration of the egg before hatching, type of egg, age and breed of the chick from which the egg was obtained, individual variations, weight of the egg, position of the egg, egg gathering model, environmental humidity and temperature, and rates of environmental gases (Brake et al., 1997).

Morphological changes develop in the blastoderm due to long-term storage of the eggs before hatching, and cellular necrosis in the embryo leads to malformations (Brake et al., 1997).

There are significant interactions between egg quality and both storage temperature and period. Haugh unit (HU) is adversely affected by the length of the storage period and increased storage temperature. Interaction effects between storage temperature and time are also significant in terms of internal and external egg quality properties (Jin et al., 2011; Perić et al., 2017). Embryo development is correlated to egg weight, and chick weight at hatching corresponds to 70.9% of initial egg weight (Schmidt et al., 2009).

The storage time of the hatching eggs was reported as short (1-7 days), normal (8-14 days), and long (15-21 days) (Butcher, 2004). Fertile eggs should not be stored for longer than 10-14 days; after 14 days of storage, hatchability begins to decline significantly (King'ori, 2011). Decreased hatchability leads to significant economic losses. In some studies, embryo development was negatively affected, abnormal embryo formation occurred, late-period deaths increased and hatching efficiency decreased in cases of storage for longer than 8-10 days (Altan et al., 2002; Schmidt et al., 2009; Nasri et al., 2020). However, the storage time may exceed one week depending on the market demand and the capacity of the incubator in hatching farms. The quality of life during the growing period was also reported as being impaired due to prolonged storage time. Moreover, it has been stated that a long storage period negatively affects the growth performance of chicks obtained from a young breeder (Shiranjang et al., 2018).

Some applications are used today to preserve the internal quality of the eggs by coating them with different materials to prevent the development of negative effects due to the long-term storage of those eggs (Caner, 2005). It was determined that the eggs covered with sugared water did not have an incubation ability, and the effects of the other materials were found to be minimal in a study investigating the effects of long-term storage of breeder eggs by coating them with sugared water (diluted 1:1), gelatinous water (3%), and stretch (Durmuş et al., 2009). On the other hand, chitin-based films play an important role among materials used for coating the surfaces of the eggs (Wu, 2003).

Chitosan, which is obtained from the de-acetylation of chitin, found in the shells of some beetles (23.5%), crabs (17%), and shrimps (32%), is one of the most important films (Demir and Seventekin, 2009). It is also known as non-toxic, harmless to the environment, and easily bio-degradable (Berger et al., 2004). Commercial eggs coated with chitosan and stored at 25 °C were found to have a lower weight loss and 2-3 weeks longer shelf life in the study of No et al. (2005).

To explore the influences of chitosan coating and structure changes during storage on egg preservation, eggs coated by chitosan solution for a single time (CS1), two times (CS2), and three times (CS3) were prepared separately and stored with untreated eggs (CK1), eggs washed in water (CK2) and eggs treated by acetic acid solution (CK3). Eggs in CS2 and CS3 presented the lowest weight loss, the highest HU and yolk index, a stabilized pH, and the highest thickness of chitosan coating layers (>2 μm) among all the groups, which extended egg shelf life for 20 days longer as compared with CK1 and CK2. The results demonstrated that eggs with the thickest coating showed the best qualities during storage, while the destruction of coating layers led to a quality drop in the eggs (Xu et al., 2018).

In another study, the weight loss, HU, and yolk index values suggested that the coating of eggs with shrimp α-chitosan increased the shelf life of eggs by almost four weeks at 22±1 °C and three weeks at 32±1 °C compared with the controls (non-chitosan coated and acetic acid-coated eggs) (Suresh et al., 2015). In that same study, the three-time repeated coating was more effective in preserving the internal quality and preventing weight loss than a single-time coating of chitosan on the egg. Therefore, a three-time coating of eggs with 2206 mPa.S chitosan offers a protective barrier for preserving the internal quality of eggs stored at tropical room conditions and concomitantly prevents contamination with microorganisms.

The shelf life of the eggs was determined to be prolonged, and the internal and external quality was determined to improve when commercial eggs were coated with chitosan film. To the best of our knowledge, no studies have been found in the literature investigating the effects of that type of application on hatching, living potential after hatching, growth performance, and carcass characteristics of chicks.

Therefore, the present study was carried out to determine the effects of storing broiler breeder eggs in different storage periods and coating them with chitosan film on the survival, growth performance, and carcass characteristics of chicks.

2. Material and Methods

The present study was conducted in Malatya province of the East Anatolia Region of Turkey. The area of Malatya province is 12.313 km^2 and is located between 35°54' and 39°03' North latitude and 38°45'and 39°08' East longitude, and altitude of 964 m. The research was approved by the institutional ethics committee (case number: 2017/A-15).

A total of 1800 daily fertilized Ross 308 broiler breeder eggs, aged between 36-40 weeks and a mean weight of 59.9±0.1 g, was obtained from a commercial company and included in the study.

The present study was arranged in a 3×2 factorial design: three different storage periods (7, 14, and 21 days) and administration or not of coating of the eggs with chitosan film. A total of 1800 hatching fertilized eggs were divided into six groups with 100 eggs in each group and 600 eggs at every independent repeat (three times) as follows:

Groups 1, 3, and 5: 7, 14, and 21 days of storing, respectively, and coated with chitosan film; groups 2, 4, and 6: 7, 14, and 21 days of storing, respectively (Table 1).

This study was repeated three times independently with the same incubator at different times to minimize environmental conditions (such as in-machine conditions, climate, and personnel-induced effects) that may affect the output performance of the incubator.

In this study, six groups during the 1-42-day growth period of chicks hatched from incubation were formed based on the grouping during incubation. A total of 751 chicks, 12 of which were in the chitosan film-applied groups 1, 3, and 5 (n: 2, 9, and 1, respectively) and 739 in groups 2, 4, and 6 (n: 256, 248, and 235, respectively), constituted the study material.

High-molecular-weight chitosan (310000-375000 Da, Poliglusam; Deacetylchitin; Poly-(D) glucosamine; Sigma-Aldrich: CAS 9012-76-4) was used to coat the eggs in the study groups. To increase the solubility and efficacy of the coating material (chitosan), 1% acetic acid (Glacial acetic acid; CH_3CO_2H) of 60.05 molecular weight was used. Also, glycerol (0,25 mL/g chitosan) was used as a plasticizer to avoid the fragility of the coating and to improve the film layer formation. Chitosan film was diluted with distilled water at 1% concentration, and homogenized via a magnetic vortex; 1% acetic acid was added to improve the solubility and efficacy. This solution was mixed using a magnetic vortex for 45 min at low temperature, and plasticizer was added to the solution to avoid fragility of the coating and to strengthen the film layer. The solution was mixed using a magnetic vortex for 15 min at low temperature (Bhale et al., 2003; Caner and Cansiz, 2007). The eggs were immersed into the freshly prepared solution for 1 min, air-dried for 15 min, and immersed again into the solution for 1 min (Xie et al., 2002). Chitosan-covered

Table 1 - Experimental design of the study[1]

Storage period (day)	Chitosan film coating		Number of eggs used in three repetitions
	Situation[2]	Treatment group	
7	A	1	300
	B	2	300
14	A	3	300
	B	4	300
21	A	5	300
	B	6	300
Total			1800

[1] The study was independently repeated three times.
[2] A: chitosan film coating (Group 1, 3, and 5); B: no chitosan film coating (Group 2, 4, and 6).

eggs were kept at proper room conditions for drying and were stored in the same storage conditions as those of the control groups until they were transferred into the hatching pre-development machine.

The weights of the eggs (59.9±0.1 g) were recorded before storage, at the beginning of incubation, and on the 18th day of incubation. The eggs in the study groups with no chitosan cover (groups 2, 4, and 6) were kept at 12-18 °C and 70-75% relative humidity. For placing all the eggs in the same pre-development machine at the same time, and for follow-up, the eggs were brought from the hatching company when the eggs were one day old and stored according to a schedule. The eggs stored for seven days were transferred to the hatching machine. A week later, 14-day-eggs were transferred, and two weeks later, the two-day eggs were transferred. All eggs were weighed for a second time before being transferred into the machine.

In this study, eggs were incubated in an incubator ÇİMUKA™, with a capacity of 2400 chicken eggs and with automatic regulation with temperature and relative humidity. The eggs were automatically turned every 4 h. On the 6th, 8th, and 18th days of incubation in the hatching machine (37.8 °C, 55-60% moisture), fertilization control was carried out with light, and those that were not fertilized were removed from the machine. The dead embryos were categorized as early (< six days), mid-term (7-14 days), and late (15-18 days + no hatching) embryo deaths.

Hatched chicks were placed in separate compartments, each with their group. These compartments were laid with 5-10-cm thick sawdust, and the ambient temperature was 33-35 °C and 55-60% humidity. The chicks were housed on 1-5, 6-10, 11-16, 17 days, and above at 40-50, 25-40, 20-25, and 15-20 chicks/m^2, respectively. The ambient temperatures were decreased linearly every day from 33 °C at one day of age to reach 22 °C, by 21 days, and continued through 42 days of age.

The chicks were given 8.5-10% sugared water to meet energy requirements and for meconium discharge. They were also given fresh water at 17-22 °C (15-20 chicks/dropper) and fed (50-60 chicks/feedbox)

Table 2 - Ingredients and nutrient composition of the basal diet

Item	Starter (Days 1-14)	Grower (Days 15-37)	Finisher (Days 38-42)
Ingredient (%)			
Corn	42.42	44.84	45.72
Soybean meal, 44% CP	10.57	8.91	7.96
Full-fat soybean	28.9	27.3	27.0
Meat-chicken meal	3.3	3.5	3.5
Meat-bone meal	2.7	2.7	2.7
Wheat	8.0	8.0	8.0
Soy oil	1.28	1.9	2.35
Salt	0.29	0.20	0.185
DL-methionine	0.31	0.358	0.315
Limestone	0.73	0.56	0.48
Dicalcium phosphate	1.2	1.35	1.39
Vitamin premix[1]	0.2	0.2	0.2
Mineral premix[2]	0.2	0.2	0.2
Nutrient composition (%, dry matter basis)			
Metabolizable energy (kcal/kg)[3]	3100	3180	3220
Crude protein	24	22	19.5
Calcium	1.01	0.97	0.97
Available phosphorus	0.49	0.48	0.48
Methionine[3]	0.65	0.65	0.62
Lysine[3]	1.45	1.42	1.39

[1] Vitamin premix provides the following per kilogram: vitamin A, 12,000 IU; vitamin D3, 2400 IU; vitamin E, 32 mg; vitamin K3, 4 mg; vitamin B1, 2.4 mg; vitamin B2, 4.8 mg; vitamin B6, 4 mg; B12, 0.016 mg; niacin, 16 mg; calcium-D-pantothenate, 8 mg; folic acid, 1.2 mg; biotin, 0.06 mg; and choline chloride, 360 mg.
[2] Mineral premix provides the following per kilogram: manganese, 80 mg; iron, 60 mg; copper, 8 mg; cobalt, 0.5 mg; and iodine, 2 mg.
[3] Calculated value according to tabular values listed for the feed ingredients.

ad libitum throughout the experimental period. Illuminance was adjusted as 3-5 watt/m^2 for the first three weeks and 2-2.5 watt thereafter. The illumination program was applied as a cycle of 23 h of light and 1 h of dark. The chicks fed a starter diet from days 1 to 14, a grower diet from days 15 to 37, and a finisher diet from days 38 to 42 (NRC, 1994; Table 2). The commercial feed was obtained from a commercial feed factory (Seher Tavukçuluk, Malatya, Turkey). All environmental conditions and management procedures were performed by the standard Ross regulations for chicks in all groups.

The animals in the experiment were weighed every week (days 7, 14, 21, 28, 35, and 42) with a 1-g precision scale, and their mean body weight (BW) and weekly BW were determined. The weekly total feed intake of the animals was recorded until the end of the experiment, and the feed conversion ratio (FCR; feed consumed in g: weight gained in g) of the animals was calculated. Also, the number of animals that died from each group was recorded and survival (%) was calculated at the end of the trial.

Viability during days 1-42 was determined. The slaughter weight was detected for all chicks obtained in three repeats from the chitosan-coated film for the detection of carcass characteristics. In the other groups, 18 chicks were selected at every repeat with six in each, and a total of 54 chicks in three repeats were randomly selected to represent the mean weight on day 42. Slaughter weight was measured and then the chicks were sent for slaughter, with the number of males and females in balance. The chicks were selected to be equal in terms of female and male sexes to determine the slaughter and carcass characteristics in groups with no chitosan cover (groups 2, 4, and 6).

Before slaughter, chicks were subjected to a total feed withdrawal of 8-12 h. The previously weighed chicks were cut using the cervical dislocation method and then were plucked and eviscerated. Post-slaughter hot and cold carcass weights were measured, and the carcass characteristics were determined. For that, the carcass cuts weights (breast, leg, wing, neck, and back) were determined, and carcass yields were calculated (Cömert et al., 2016). The hot carcass weight was obtained by removing the head, neck, shanks, and abdominal fat from bled, plucked, and eviscerated chicks. The cold carcass weights were determined by waiting at +4 °C for 24 h. The weights of the cuts were determined using a 1-g scale.

Chemical analyses of the basal diet for crude protein (#988.05), ether extract (#932.06), crude fiber (#962.09), crude ash (#936.07), Ca (#968.08), and P (#965.17) were conducted in triplicate using methods described by the AOAC (1990). Metabolizable energy, methionine, and lysine ingredients were computed from tabular values arranged for the feedstuffs.

The descriptive characteristics for all factors were calculated using the obtained data. In this study, data were analyzed by two-way ANOVA using the General Linear Model (GLM) procedure (SPSS, 2015). The 3 (storage periods) × 2 (coating or not the eggs with chitosan film) factorial research design was used to investigate the effects of the factors (storage period and coating with chitosan film) on the weekly BW, weekly body weight gain (BWG), and slaughter and carcass characteristics of the chicks during 1-42 days. The factors and the effects of the interactions between the factors were analyzed using the GLM procedure with the following mathematical model:

$$Y_{ijk} = \mu + a_i + b_j + (ab)_{ij} + e_{ijk},$$

in which Y_{ijk} = observed value of the investigated parameter, μ = population mean, a_i = effect of storage period (i = 7, 14, and 21 days), b_j = chitosan film coating [j = A (chitosan film coating) and B (no chitosan coating film)], $(ab)_{ij}$ = effects of interaction between storage period and chitosan film coating, and e_{ijk} = random errors in observing Y_{ijk}.

Duncan multiple comparison tests were used for the inter-group comparisons concerning the related parameters for the statistically significant factors ($P<0.05$). The Chi-square test was used for analysis of the proportional values of vitality and some carcass characteristics of the chicks (Akgül, 2005; Özdamar, 2003; Snedecor and Cochran, 1980). SPSS version 22.0 was used for all calculations and analyses (SPSS, 2015).

3. Results

When viability during 1-42 days was analyzed, only 12 chicks (groups 1, 3, and 5) were determined to hatch from eggs coated with chitosan film and all survived (100%) during the growing period. The total number and rate of chick mortality in all groups were found to be 32 and 4.4%, respectively. The differences among the treatment groups were determined to be significant (P<0.000) (Table 3).

In addition, 256, 248, and 235 chicks were determined to hatch in the no chitosan film groups (2, 4, and 6), and the mortality rates were determined as 4.7, 2.4, and 6.0% in these groups, respectively. The mortality rate was found to be highest on days 1-7 (Table 3).

The effect of repeats on the cumulative BW and weekly BWG was found to be significant in all periods (P<0.05). The effects of interaction between the factors were not significant for both the cumulative BW (g) (P>0.05) and the weekly BWG of chicks (g) (P>0.05).

The differences between the treatment groups were found to be significant concerning BW on day 1 (P<0.05) (Table 4). When groups were analyzed in terms of cumulative BW, the chicks in group 2 (on days 7, 21, 28, and 35), group 4 (on day 14), and group 6 (on day 42) were determined to reach the maximum value (Table 4). On the other hand, when the weekly BWG of the chicks was examined, group 1 (on days 15-21, 36-42 and 1-42), group 2 (on days 1-7), group 3 (on days 22-28), group 4 (on days 8-14), and group 6 (on days 15-21 and 29-35) obtained the maximum values (Table 5).

While the weekly BWG varied between the groups during the growing period, the growing performance was found to be similar in all groups and reached a similar BW on day 42.

When groups were analyzed in terms of cumulative feed intake, group 2 (on days 1-7, 1-21 and 1-28), group 4 (on days 1-14), and group 6 (on days 1-35 and 1-42) had the maximum intake. Likewise, when the 1-42 days were evaluated at the end of the feeding, the maximum feed intake was determined in group 1 (4601 g), and the minimum was determined in group 5 (3663 g) (Table 6). When the groups were evaluated at the end of feeding (on days 1-42), FCR was found to vary between 1.65 and 1.75, and the best value was determined in group 5 (1.65) (Table 7).

The present study revealed that repeat does not affect both slaughter and carcass characteristics (P>0.05), and the effects of interaction between the factors were not significant for slaughter and carcass characteristics (P>0.05).

The average slaughter weight of chicks was found to be 2545.7±34.5 g, and the average hot carcass weight was determined to be 1927.6±26.1 g. The average cold carcass weight was obtained as 1888.1±25.8 g, and the average waiting loss of the carcass weight was 39.5±1.8 g (Table 8).

Overall, hot carcass performance (%) was determined as 75.7±0.3 and cold carcass performance (%) was determined as 74.2±0.2 (Table 9). Additionally, the average waiting loss of the carcass was 1.5±0.1% (Table 9).

Table 3 - Chick mortality

Treatment group	Total number of chick mortality		Day 1 (total number of hatching chicks; n)	Chick mortality in different fattening periods (n)					
	n	%		Days 1-7	Days 8-14	Days 15-21	Days 22-28	Days 29-35	Days 36-42
1	-	0.0A	2	-	-	-	-	-	-
2	12	4.7B	256	3	1	4	3	1	-
3	-	0.0A	9	-	-	-	-	-	-
4	6	2.4B	248	6	-	-	-	-	-
5	-	0.0A	1	-	-	-	-	-	-
6	14	6.0B	235	7	3	-	1	-	3
P		0.000							
Total	32	4.4	751	16	4	4	4	1	3

A, B - Values followed by different letters in the same column are significant (P<0.05).

Table 4 - Cumulative body weight (BW) of chicks (g)

Treatment group	Day 1		Day 7		Day 14		Day 21		Day 28		Day 35		Day 42	
	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$
1	2	45.0±3.0	2	156.0±0.1	2	390.0±10.0	2	728.0±32.0	2	1300.6±198.7	2	1625.8±248.3	2	2669.3±112.0
2	256	43.6±0.2B	253	187.1±1.8	252	555.9±11.4	248	902.0±11.4	245	1364.8±9.9	244	1769.3±15.4	244	2537.3±22.3
3	9	45.7±2.3	9	150.0±13.5	9	450.3±76.2	9	717.8±88.1	9	1302.6±54.6	9	1628.3±68.3	9	2557.4±108.4
4	248	42.2±0.2A	242	184.6±1.5	242	566.4±10.5	242	893.5±10.6	242	1330.5±11.2	242	1720.7±14.6	242	2513.9±20.0
5	1	42.0	1	168	1	320.0	1	536.0	1	1156.1	1	1445.1	1	2258.6
6	235	42.3±0.2A	228	183.8±1.8	225	548.5±12.1	225	892.8±12.1	224	1349.7±10.8	224	1758.5±15.4	221	2576.7±22.5
Total	751	42.7±0.1	735	184.7±1.0	731	555.0±6.5	727	893.0±6.6	723	1347.4±6.1	722	1747.0±8.7	719	2541.8±12.3
P[1]		0.000		0.475		0.544		0.835		0.083		0.079		0.126

[1] These groups were not included in the analysis since there were not enough data in the 1st, 3rd, and 5th groups.
A, B - Values followed by different letters in the same column are significant (P<0.05).

Table 5 - Weekly body weight gain of chicks (g)

Treatment group	Days 1-7		Days 8-14		Days 15-21		Days 22-28		Days 29-35		Days 36-42		Days 1-42	
	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$	n	$\bar{X}\pm S_{\bar{X}}$
1	2	111.0±3.0	2	234.0±10.0	2	338.0±22.0	2	572.6±166.7	2	325.1±49.6	2	1043.5±360.3	2	2624.3±109.0
2	253	143.2±1.9	252	368.7±10.3	248	340.2±5.8	245	458.1±13.8	244	405.0±11.4	244	768.0±26.6	244	2493.7±22.6
3	9	104.2±12.0	9	300.3±63.6	9	267.5±32.1	9	584.8±106.8	9	325.6±13.6	9	929.0±126.3	9	2511.7±108.0
4	242	142.7±1.5	242	381.8±10.2	242	327.0±5.9	242	436.9±14.9	242	390.2±10.5	242	793.1±26.2	242	2471.7±20.1
5	1	126.0	1	152.0	1	216.0	1	620.0	1	289.0	1	813.5	1	2216.6
6	228	141.3±1.8	225	364.3±11.2	225	344.2±5.8	224	455.2±14.8	224	408.7±11.5	221	814.8±27.8	221	2534.4±22.5
Total	735	141.9±1.0	731	370.2±6.0	727	336.0±3.3	723	452.3±8.3	722	399.8±6.3	719	793.7±15.3	719	2499.1±12.4
P[1]		0.756		0.494		0.107		0.543		0.497		0.473		0.548

[1] These groups were not included in the analysis since there were not enough data in the 1st, 3rd, and 5th groups.

Table 6 - Cumulative feed intake of chicks (g)

Treatment group	Days 1-7		Days 1-14		Days 1-21		Days 1-28		Days 1-35		Days 1-42	
	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$
1	2	133	2	431	2	890	2	1816	2	2411	2	4601
2	249	167	248	643	244	1142	241	1901	240	2622	240	4221
3	9	128	9	501	9	862	9	1809	9	2453	9	4249
4	240	165	240	652	240	1137	240	1853	240	2603	240	4225
5	1	139	1	340	1	618	1	1601	1	2151	1	3663
6	227	164	224	641	224	1136	224	1873	224	2629	221	4412
Total	728	164	724	643	720	1138	717	1877	716	2621	713	4233

Table 7 - Feed conversion ratio of chicks

Treatment group	Days 1-7		Days 1-14		Days 1-21		Days 1-28		Days 1-35		Days 1-42	
	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$
1	2	1.20	2	1.25	2	1.30	2	1.45	2	1.53	2	1.75
2	249	1.17	248	1.25	244	1.33	241	1.44	240	1.52	240	1.69
3	9	1.23	9	1.24	9	1.28	9	1.44	9	1.55	9	1.69
4	240	1.16	240	1.24	240	1.33	240	1.44	240	1.55	240	1.71
5	1	1.10	1	1.22	1	1.25	1	1.44	1	1.53	1	1.65
6	227	1.16	224	1.26	224	1.33	224	1.43	224	1.53	221	1.74
Total	728	1.16	724	1.25	720	1.34	717	1.44	716	1.54	713	1.69

Table 8 - Slauhgter, carcass, and some carcass cuts weights of chicks

Treatment group	n	Slaughter weight (g)	Hot carcass weight (g)	Cold carcass weight (g)	Breast weight (g)	Leg weight (g)	Wing weight (g)	Neck and back weight (g)	Waiting loss of carcass weight (g)
		$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$
1	2	2490.0±10.0	1920.0±99.0	1889.0±83.0	648.9±6.3	558.0±39.0	196.0±14.0	515.2±47.2	31.0±17.0
2	18	2512.5±73.5	1927.7±58.9	1886.6±57.9	646.9±20.8	562.2±19.5	194.4±6.1	497.2±18.4	41.1±2.5
3	9	2546.6±95.6	1924.4±73.7	1889.5±74.0	659.6±30.5	558.8±21.9	193.7±7.2	482.5±19.3	34.8±5.5
4	18	2551.3±53.3	1924.4±40.8	1882.5±40.8	656.3±16.1	551.7±13.0	197.1±4.4	498.2±14.5	41.8±4.1
5	1	2540.0	1820.0	1806.0	655.2	519.0	182.0	468.0	14.0
6	18	2579.2±78.5	1939.2±55.2	1899.0±54.6	666.1±21.0	570.7±16.5	189.7±5.2	464.5±17.3	40.2±3.1
Total	66	2545.7±34.5	1927.6±26.1	1888.1±25.8	656.6±9.8	560.4±8.2	193.6±2.6	486.6±8.4	39.5±1.8
P[1]		0.972	0.994	0.996	0.976	0.919	0.878	0.547	0.711

[1] These groups were not included in the analysis since there were not enough data in the 1st and 5th groups.

Table 9 - Slaughter, carcass, and some carcass cuts ratios of chicks

Treatment group	n	Hot carcass (%)	Cold carcass (%)	Breast (%)	Leg (%)	Wing (%)	Neck and back (%)	Waiting loss of carcass (%)
		$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$	$\bar{X}\pm S_{\bar{x}}$
1	2	77.1±4.3	75.8±3.6	34.4±1.8	29.5±0.7	10.3±0.2	27.2±1.3	1.2±0.6
2	18	76.6±0.3	75.0±0.3	34.2±0.4	29.7±0.2	10.3±0.1	26.4±0.6	1.6±0.1
3	9	75.6±1.1	74.2±1.0	34.8±0.4	29.5±0.3	10.2±0.1	25.5±0.6	1.3±0.2
4	18	75.4±0.2	73.7±0.2	34.8±0.3	29.3±0.2	10.4±0.1	26.4±0.5	1.6±0.1
5	1	71.6	71.1	36.2	28.7	10.0	25.9	0.5
6	18	75.3±0.7	73.7±0.7	35.0±0.3	30.0±0.2	10.0±0.1	24.4±0.5	1.5±0.1
Total	66	75.7±0.3	74.2±0.2	34.7±0.2	29.6±0.1	10.2±0.1	25.7±0.3	1.5±0.1
P[1]		0.199	0.231	0.626	0.305	0.248	0.057	0.697

[1] These groups were not included in the analysis since there were not enough data in the 1st and 5th groups.

4. Discussion

The present study was conducted to investigate the effect of different storage periods and coating with chitosan film on the BW, viability, BWG, feed intake, FCR, and carcass characteristics of chicks.

Statistically significant differences were found between the uncoated and coated egg groups with chitosan film in terms of the viability of the chicks ($P<0.05$). The chicks hatched from chitosan-coated eggs had 100% viability until slaughter. This suggests that very few chicks that hatched from film-coated eggs were already heavy, healthy, durable, and strong, so they had a high chance of survival, and this condition continued during the growth period. Given that only 12 chicks were hatched from the chitosan film-coated eggs, a total of 739 chicks (265, 248, and 235) was found to have hatched from the other groups (2, 4, and 6, respectively); the differences between the groups concerning viability may be reasonable.

No difference was found in the in-group comparisons of the chitosan-coated and uncoated groups in terms of viability ($P>0.05$). The mean mortality rate was found to be 4.4% and was highest during the first week in the eggs of groups that had different storage periods and that were not treated with chitosan (Table 3). The greatest mortality rate was found in the chicks with 21 days of storage. Similar to the results of the present study, many studies in the literature report poorer vitality depending on prolonged storage time (Fasenko et al., 1992; Altan et al., 2002; Nasri et al., 2020).

In groups with 12 live chicks from hatching eggs covered with chitosan, almost all of the other embryos died in early embryonic period. Thus, very few embryos reached mid- or long-term embryonic development. Therefore, the mid- or long-term embryonic mortality was 2.41-6.89%. It is understood that chitosan film completely covers the surface of the egg, plugs the pores on the shell, and forms a barrier between the inner and outer environment of the egg, and thus, the weight loss of the egg is reduced (Köseman et al., 2020). However, when the findings in both studies are evaluated, it is understood that there is no negativity in the survival of chicks hatching from eggs covered with chitosan film.

Body weight is known to have significant effects on the performance of the chicks during the growth period, and chicks with heavier BW are known to exhibit better performance. When the mean BW of chicks was analyzed (Table 4), the chicks that hatched from the film-coated eggs had the highest values. The BW of the chicks that had been stored for different periods and hatched from the eggs that were not coated with the chitosan film was statistically significant, and the chicks that had hatched from the eggs stored for seven days were seen to be lighter than those that had hatched from the eggs stored for 14 and 21 days (Table 4). The results of the present study are consistent with those of Reis et al. (1997), who reported that BW losses may develop due to the incubation of eggs with a longer storage time together with eggs with a shorter storage time; however, this differs from the results of Tona et al. (2004), who reported no difference in BW. On the other hand, a previous study (Tona et al., 2002) reported that BWG and BW values in the first week decrease in chicks that are hatched from eggs stored in different periods, similar to the results of the present study (Tables 4 and 5).

A rapid increase in albumen pH is observed after just two days of storage, regardless of the storage temperature. Interaction effects between storage temperature and time were also significant in terms of albumen and yolk pH. Albumen pH is greatly influenced by the storage temperature and time (Jin et al., 2011). The chicks hatched from the 21-day stored eggs were found to have higher BW (Table 4) and were found to deteriorate (Table 7).

Broilers are poultry that gain high BW in a short time by consuming high amounts of feed. However, high feed intake and conversion of feed to BW depends on many factors. Whether the feed intake and FCR of chicks hatching from chitosan-coated eggs differ from those of chicks not covered with chitosan is closely related to the use of this method for long-term storage of hatching eggs.

Group 5 was comparatively the lowest in all weeks except week 1 in terms of cumulative feed intake, but had the highest FCR. The eggs in this group were covered with chitosan and stored for 21 days before

incubation. This situation is promising in terms of storing the hatching eggs covered with chitosan for a long time. Groups were similar in terms of cumulative feed intake (Table 6) and FCR (Table 7), suggesting that broiler breeder eggs can be stored for a long time if they are covered with chitosan, despite their low incubation rate.

In this study, the fact that there was no difference between groups in terms of slaughter, carcass, and some carcass cuts weights and ratios (Tables 8 and 9) is evaluated positively by covering the eggs with chitosan to preserve their internal and external quality and prolong their storage time.

Some researchers (Nillipour and Butcher, 1998) reported that the slaughter weight significantly decreased with increased storage time. The present study revealed no difference between the storage groups concerning slaughter weight ($P>0.05$) (Table 8). The results of our study are supported by Bowling and Howarth Jr. (1981), who reported no significant difference between the slaughter weights of chicks that are hatched from eggs with a long storage time.

5. Conclusions

All chicks that had hatched from the chitosan-coated eggs survived during days 1-42; however, the mortality rate varied between 2.4 and 6.0% in the other groups, and was highest between days 1-7. The chicks exhibited similar performances concerning growing and feed intake, and tolerated the BW differences with time. No significant difference was present when they had reached day 42, despite differences in the effects of repeat, treatment with chitosan film, and storage period. Therefore, coating the eggs with chitosan film and storing for different periods does not affect the carcass characteristics of chicks.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

Data curation: A. Köseman. Funding acquisition: F. Akdemir. Investigation: A. Köseman, F. Akdemir and İ. Şeker. Methodology: İ. Şeker. Project administration: F. Akdemir and İ. Şeker. Writing-original draft: A. Köseman and İ. Şeker.

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