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Hatching performance of Japanese quail from eggs stored for different periods - a preliminary study

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Abstract

Background Due to the fluctuating nature of the poultry market, including the need for chicks, there is often a necessity to extend the storage time of hatching eggs. Commonly they are stored at the farm to reduce the cost of daily collection, while hatcheries collect a large number of eggs to fill the entire incubator at once. In the case of hen eggs, eggs stored for three to seven days are usually used for incubation. In the case of quail eggs, there are no specific recommendations in this respect, while the common perception is that they "age" differently from hen eggs and stay fresh longer. The study was aimed to determine the maximum eggs storage time after which it is possible to obtain full-quality chicks. seven hundred and fifty six eggs of Japanese quail hatching eggs were collected at 4-day intervals for 52 days dividing them into 14 groups of 54 eggs (6 replications in each). Consecutive batches of eggs were stored (at physiological zero conditions) for 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52 days, respectively; the control group consisted of fresh, unstored eggs. Fifty two days after the first eggs were collected, all groups, including the control one, were incubated under species-specific conditions. The water loss of eggs during the incubation as well as hatching results were determined. The weight and quality of hatchlings were evaluated.

Results It was shown that storage time significantly affected the egg weight loss and hatchability of chicks, which were not obtained from eggs stored longer than 32 days. There was no effect of the experimental factor on hatchability from eggs stored for up to 20 days, after longer storage the ratio gradually decreased. With time passing, a decrease in early embryo viability and hatchability while an increase in embryo mortality was observed, mainly in the late incubation phase. Interestingly, the duration of egg storage did not differentiate the weight of the chicks obtained but affected their quality.

Conclusions The results of the study confirmed the negative effect of long-term Japanese quail eggs storage on the hatching performance; however, the viability of Japanese quail embryos is noteworthy. The loss of egg weight during the incubation was directly proportional to the change in this trait before incubation due to the storage time. The prolonged egg storage may have negatively affected chick hatching by limiting the embryo's access to essential nutrients and oxygen. These results have important practical implications for small-scale poultry producers, as they

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suggest that storage times of hatching quail eggs can be extended, but not beyond 24 days, which is still three times longer than usually recommended seven days for hen eggs.

Keywords Egg storage, Hatching eggs, Incubation, Quality of chicks

Background

The quality and number of chicks are the most important factors affecting the subsequent economic effect of poultry production, regardless of the species of birds kept or their utility type (meat, laying). However, it is not easy to maintain, as it is determined by many factors, from genetic ones like the degree of homozygosity of the parent flock [1], although environmental factors affecting the parent flock like the condition and welfare of the birds [2], feed nutritional value [3] and mineral-vitamin balance [4], zoohygienic conditions [5] and veterinary prophylaxis [6], or egg morphological quality [7], to those influencing the eggs after they are laid. The latter group, which includes egg handling before incubation [8], their disinfection [9, 10], location in the setter chamber [11] and incubation conditions [12–14], appears to be crucial for satisfactory brood performance.

According to breeding practice, eggs which are characterised by the correct weight and structure, as well as a suitable shape index, are intended for incubation. The handling of hatching eggs prior to incubation, including the time of storage, is also important in this regard. Regardless of the intended use of the eggs (consumption, hatching), qualitative changes occur in them with time. The cuticle covering the shell becomes desiccated, the pores of the shell unseal and gas exchange between the egg contents and the external environment increases [15], which can result in the weight loss. In addition to water vapour, carbon dioxide from the dissociation of carbonic acid is released from the eggs, a process that leads to alkalinisation of the albumen [16], and this change results in the breakdown of electrostatic bonds within the ovomucin-lysozyme complex [17] and loosening of the structure of this element, including the chalazae. Due to the osmotic migration of water from the albumen to the yolk, the yolk increases in size while the tension of the vitelline membrane is weakened [18]. In the context of hatching eggs, the aforementioned changes, especially water loss, may result in embryo desiccation during the incubation. The negative correlation between egg weight loss during storage and the amount of water and oxygen available to the embryos during incubation may also contribute to increased mortality during the early hatching phase [19]. Albumen dilution may increase yolk sphere motility, but the need to maintain its alkaline pH is primarily indicated by the fact that optimal gastrulation occurs at pH 8.2 [20]. Oxidation of the yolk lipids of stored eggs, can lead to toxin formation [21, 22], which, as the yolk is a reservoir of nutrients for the developing embryo, can result in embryogenesis disorders.

Therefore, for optimum hatching results, egg collection for laying should be limited to 7–10 days and the eggs stored under temperature conditions of so-called physiological zero, i.e. a temperature that will not stimulate embryonic development. The temperature range reported in the available literature is 21–28 °C. In contrast, storage at lower temperatures for longer than 7 days may lead to an increase in embryo mortality [23], as well as a significant "widening" of the hatching window and a deterioration in the quality of the chicks obtained [24]. Based on microscopic analysis of embryonic development, it was found that storage of fertile eggs at 14 °C for varying lengths of time (0–21 days) completely inhibited embryonic development [25]. Interestingly, it is not recommended to apply to hatching eggs on the day of laying due to the high stiffness of the dense protein, which impedes the flow of nutrients to the blastoderm and gas exchange between the embryo and the environment [26].

Unfortunately, due to the fluctuating nature of the poultry raw material market, including the need for chicks, there is often a necessity to extend the storage time of hatching eggs. They are often stored at the stage of production farm to reduce the cost of daily collection, while hatcheries collect a large number of eggs to fill the entire incubator at once. In the case of hen eggs, eggs stored for three to seven days are usually used for incubation. In the case of quail eggs, there are no specific recommendations in this respect, while the common perception is that they "age" differently from hen eggs and stay fresh longer.

This study aimed to assess the effect of the storage time of Japanese quail hatching eggs before incubation on hatching performance and the quality of the chicks obtained. An attempt was also made to determine after what time of egg storage it is possible to obtain full-quality chicks.

Results

Table 1 summarises the hatching results of Japanese quail according to the storage time of the eggs before laying. It was found that increasing the storage time of quail eggs, even under physiological zero conditions (17 °C), significantly affected the hatching results. A storage time of 32 days was the limit beyond which no chicks of normal quality were obtained, so the analysis of the main results from this experiment was limited to this period. The oldest eggs in which embryonic development was observed were stored for up to 52 days. In 25 eggs stored between

Table 1 The hatching results of Japanese quails depending on the duration of the egg storage time before incubation

Trait		Days of storage								SEM	F-test (*p*-value)
		0	4	8	12	16	20	24	28	32			
Fertility (%)		85.19^{a}	79.63^{a}	81.48^{a}	81.48^{a}	79.63^{a}	74.07^{ab}	61.11^{ab}	59.26^{ab}	46.30^{b}	2.67	*0.002*
Hatchability (%) of	set eggs	81.48^{a}	75.93^{a}	74.07^{a}	77.78^{a}	62.96^{ab}	66.67^{ab}	38.89^{bc}	24.07^{c}	12.96^{c}	3.97	*<0.001*
	fertile eggs	95.14^{a}	97.55^{a}	90.08^{a}	95.83^{a}	80.86^{a}	87.92^{a}	64.13^{ab}	40.83^{bc}	22.08^{c}	4.32	*<0.001*
	crippled chicks	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-
Mortality (%) 0–14 days	set eggs	0.00	0.00	1.85	0.00	7.41	0.00	7.41	1.85	3.70	0,76	*0.052*
	fertile eggs	0.00	0.00	2.38	0.00	7.94	0.00	11.11	2.78	11.67	2.09	*0.127*
Mortality (%) 15-17.5 days	set eggs	3.70^{c}	7.41^{b}	3.70^{c}	3.70^{c}	9.26^{b}	9.26^{b}	14.81^{b}	33.33^{a}	29.63^{a}	1.32	*<0.001*
	fertile eggs	4.86^{c}	10.78^{c}	4.76^{c}	4.17^{c}	11.20^{c}	14.46^{c}	24.76^{bc}	56.39^{ab}	66.25^{a}	3.80	*<0.001*
Total mortality (%)	set eggs	3.70^{c}	7.41^{c}	5.56^{c}	3.70^{c}	16.67^{abc}	9.26^{bc}	22.22^{abc}	35.19^{a}	33.33^{ab}	2.28	*<0.001*
	fertile eggs	4.86^{c}	10.78^{c}	7.14^{c}	4.17^{c}	19.14^{c}	14.46^{c}	35.87^{b}	59.17^{ab}	77.92^{a}	4.22	*<0.001*

^{a,b,c,d} – means marked with different letters differ significantly at $P \leq 0.05$

36 and 52 days, embryonic development was observed, with embryonic death occurring in each case above day 40th of storage, with death occurring by day 5th of incubation. The percentage of chicks hatched from fertile eggs set in the incubator is considered an objective indicator of hatching quality, with fertility in our experiment at an average of 72% and significantly lower for longer-stored eggs, but this was probably due to the difficulty in assessing their fertility, usually, as a result of embryo death at a very early stage of development. The highest hatchability from fertile eggs (97.55%) was obtained from eggs stored for 4 days (Table 1). At the same time, the eggs lost their hatchability value with storage time beyond the 28 days. After 32 days of storage, hatchability was only 22.08%. It was also observed that while egg storage time did not significantly affect embryo mortality between days 1st and 14th of incubation, it significantly differentiated this parameter between day 15th of incubation and the hatching of chicks (after candling and eliminating eggs that died during the first incubation period). As a result, total embryo mortality was significantly associated with egg storage time and reached a maximum value (77.92%) for eggs stored for 32 days. A detailed analysis of the timing of embryo mortality showed that significantly the highest percentage of embryos (28%) died early in development up to day 5th of incubation and on day 16th of incubation due to incorrect positioning of the chick before hatching (35%) (Table 2). Data were analysed according to groups determined by the time the eggs were stored before incubation, but due to the relatively small numbers of material, relationships were not statistically confirmed in every case.

It was also found that the length of time the eggs were stored prior to incubation had a significant effect on their weight loss at the different stages of the experiment (Table 3; Fig. 1). Table 1S and 2S show the exact average weights of the eggs weighed at the different dates of the testing procedures according to the storage time before incubation, as well as the descriptive statistics of the different quantitative traits analysed. For infertile eggs, these were the periods from egg collection (oviposition day) to start of incubation (WL1) and from collection to candling on day 14th of incubation (WL2). In general, a positive correlation was found between the storage time of eggs before incubation (under physiological zero conditions) and their weight loss (Table 4). For eggs fertile and died up to day 14th, there were no significant differences between subgroups in terms of weight loss at any of the periods analysed. A significant change in egg weight was observed as a function of storage time for eggs in which embryos died after day 14th in the period from collection to start of incubation (WL1) and from collection to candling at day 14th (WL2). A similar trend was observed for hatched eggs, although in the period between setting

Table 2 The time of embryonic death depending on the duration of the egg storage time before incubation

Day of storage	*N*	Time of embryonic death (day)												χ^2-test (*P*-value)
		< 5	6	7	8	9	10	11	12	13	14	15	16	
0	1												1	*0.999*
4	2									1		1		*0.327*
8	3		1		1								1	*0.006*
12	2	1											1	*1.000*
16	9	2				1		1				2	3	*0.868*
20	6	2										2	2	*0.966*
24	11	7		1					1				2	*0.614*
28	19	2							1		3	3	10	*0.606*
32	17	2		1					3			2	9	*0.129*
36	12	4	1				1	1		3			2	*0.446*
40	9	2		1		1		2					3	*0.790*
44	3	3												*0.996*
48	1	1												*0.996*
52	1	1												*0.999*
Frequency (%)		28	2	3	1	2	1	4	5	4	3	10	35	*< 0.001*

N - numbers

and candling and egg transfer to the hatching chamber (WL4), the weight loss was inversely proportional to the storage period (Table 3). The loss of water conductance of eggs with different storage periods was also investigated (Table 5) and a tendency for conductance to decrease with increasing storage period was observed.

Although chicks obtained from the oldest eggs (24–32 days) showed significantly lower activity, poorer down quality and limb malformations, this did not significantly affect the differences in their overall evaluation. Similarly, the proportion of chick body weight in the initial egg weight did not change significantly according to the length of egg storage (Table 6).

Spearman's rank correlations between selected traits (Table 4), confirm a high positive correlation (0.886) between egg storage length and egg weight loss in the period from collection to egg laying in the incubator (WL1). Egg weight loss was also significantly correlated at some stages of the experiment (WL1-WL4). A non-significant trend (-0.183) of decreasing egg weight loss in longer-stored eggs between 14 and 17.5 days was also observed (WL5). The change in conductance between collection and set (K1) was also negatively correlated (-0.207) with storage time and initial egg weight (-0.145) and positively correlated with the WL1 and WL2 periods. The correlation between conductance and egg weight loss during periods WL4 and WL5 was highly significant. The egg shape index was generally not significantly correlated with indices illustrating changes in egg weight during storage and/or incubation.

Discussion

Hatching eggs prior to setting should be stored at a temperature referred to as 'physiological zero', i.e. a temperature at which embryonic development is significantly slowed down, thus limiting the negative effects of the passage of time on hatching performance and the quality of the chicks obtained. However, it seems that the determination of an optimal, but also uniform, storage temperature is not easy. Fasenko et al. [25] found that the fertile eggs storage at 14 °C completely inhibited embryonic development in hen eggs, which was confirmed using microscopic techniques. Seker et al. [27] stored quail eggs at 9–12 °C for up to 15 days using egg turning during this time and further dividing the material by initial weight. It was shown that storage already beyond 6 days resulted in a decrease in hatchability, the best hatching results were obtained from eggs laid 3 days after laying, and the optimal eggs for incubation were considered to be those weighing 11.51–12.50 g. The average egg weight in our study was 11.5 ± 0.87 g, but no worse hatchability was observed from the smallest or largest eggs in the sample (Table S1). Lacin et al. [28] stored quail eggs at 21 °C and 75% relative humidity for 14 days, but with different materials (hay, perlite) that can reduce weight loss, and an interaction between storage time and the protective material used was noted for this trait. Hassan and Alsattar [29] found no significant differences in hatching performance from eggs of different utility types of Japanese quail stored at 7 and 20 °C for 14 days, although deteriorated hatchability was observed from eggs stored at lower temperatures, therefore, the quail eggs storage for 7–10 days at room temperature (20 °C) was considered optimal, the authors also point to a possible genetic-environmental interaction between the colour variety of

Table 3 The weight loss (WL, %) of eggs in particular experimental periods depending on the duration of the egg storage time before incubation

Trait		Days of storage								SEM	*F-test (P-value)*
		0	4	8	12	16	20	24	28	32		
Infertile	WL 1	-	0.88^{de}	1.29^{cde}	1.69^{cde}	3.12^{bcd}	3.40^{bcd}	3.75^{abc}	4.16^{ab}	6.19^{a}	0.16	*< 0.001*
	WL 2	8.60^{b}	9.52^{b}	12.02^{ab}	12.35^{ab}	12.92^{ab}	13.45^{a}	13.44^{ab}	13.12^{ab}	14.78^{a}	0.19	*0.002*
	WL 4	8.60	8.72	10.88	10.86	12.18	12.48	10.07	8.92	12.39	0.13	*0.837*
Fertile and died up to 14th day	WL 1	-	-	2.07	3.00	4.41	2.20	4.77	3.98	2.89	0.30	*0.578*
	WL 2	-	-	11.64	11.00	20.70	12.07	18.16	12.22	12.28	0.77	*0.622*
	WL 4	-	-	9.77	8.25	17.28	10.08	14.16	8.58	9.67	0.69	*0.626*
Fertile and died after 14th day	WL 1	-	0.87^{cd}	0.41^{d}	1.90^{bcd}	2.95^{abc}	2.99^{abc}	3.62^{ab}	4.08^{ab}	5.15^{a}	0.20	*< 0.001*
	WL 2	8.55^{ab}	8.88^{ab}	5.01^{b}	8.57^{ab}	11.43^{a}	11.79^{a}	12.23^{a}	13.30^{a}	13.07^{a}	0.31	*< 0.001*
	WL 3	17.09	10.67	17.19	7.62	13.71	12.72	13.56	16.61	14.44	0.38	*0.076*
	WL 4	8.55	8.08	4.63	6.78	8.75	9.09	8.93	9.61	8.34	0.22	*0.064*
	WL 5	9.35^{a}	8.97^{a}	8.33^{a}	4.19^{ab}	2.59^{a}	3.73^{a}	1.52^{a}	3.77^{a}	2.81^{a}	0.27	*0.002*
Hatched	WL 1	-	0.85^{e}	0.92^{e}	1.93^{d}	2.74^{c}	2.85^{c}	3.39^{c}	4.16^{b}	5.57^{a}	0.09	*< 0.001*
	WL 2	9.42^{d}	9.44^{d}	9.49^{cd}	10.28^{bcd}	11.22^{abcd}	11.84^{ab}	11.45^{abc}	11.72^{ab}	12.59^{a}	0.14	*< 0.001*
	WL 4	9.42^{a}	8.67^{ab}	8.64^{ab}	8.51^{ab}	8.73^{ab}	9.27^{ab}	8.34^{ab}	7.89^{ab}	7.44^{b}	0.12	*0.049*

a, b, c, d – means marked with different letters differ significantly at $P \leq 0.05$; WL1 – weight loss between EW0 (initial egg weight) and EW1 (egg weight at the start of incubation), WL2 - weight loss between EW0 and EW2 (egg weight after 14.5 days of incubation), WL3 - weight loss between EW0 and EW3 (egg weight at the end of incubation), WL4 - weight loss between EW1 and EW2, WL5 - weight loss between EW2 and EW3

birds and egg storage periods. The hatchability reported in our study is significantly higher than in the cited paper. It should also be noted that in most of the works cited, eggs were stored for a maximum of 14 days, while our experiment lasted 52 days, and a statistically significant deterioration in hatchability from set and fertile eggs was recorded after 16 and 24 days, respectively (Table 1). It should be also noted that hatchability from fresh eggs and those stored for 4 days did not differ significantly and these were the highest results compared to the other experimental groups, which does not confirm previous observations in the context of reduced gas exchange and exposure of embryos to hypoxia due to too dense structural albumen in the fresh egg (Özlü et al., 2018). The early embryo breathes through the blood vessels of the vitelline membrane, so their rate of development determines embryonic survival and may serve as a major factor in early embryonic selection (Meuer and Baumann, 1988).

Similar to Damaziak et al. [24], our study showed a significant relationship between the length of egg storage and embryonic mortality, furthermore, it was observed that no live chicks were obtained for eggs stored longer than 32 days, although it is worth noting that embryonic development was found even in eggs stored for 52 days, which may be an interesting starting point for further studies on long-term egg storage and its effects on embryonic development. The results of Lacin et al. [28] and Hassan and Alsattar [29] confirm a significant decrease in hatchability with increasing egg storage period before incubation. However, as already mentioned, their study showed that not only the storage period of the eggs but also the storage temperature can affect embryo mortality during incubation and the quality of the hatched chicks. In the study by Romao et al. [30], the storage temperature was 28 °C (room temperature) for 14 days and no more chicks were obtained from eggs stored for 10 days. In addition, it was noted that in longer-stored eggs, embryo death occurred more frequently in the first than in the second incubation phase. In our study, the data show the opposite trend (Table 2). Garip and Dere [31] analysed 3 levels of storage temperature for quail eggs before set in the incubator (11, 21 and 27 °C), and the weight loss they recorded prior to incubation was, evidently, highest in the longest-stored eggs. Moreover, the WL values obtained in our study were slightly higher compared to the cited work, regardless of temperature. In general, the available literature confirms the significant effect of storage time and temperature on hatching quality, indicating the need for appropriate management of hatching eggs in quail rearing.

Based on the analysis of the embryo death timing (Table 2), there were two critical periods of embryo development, the first being up to the 5th day and, the

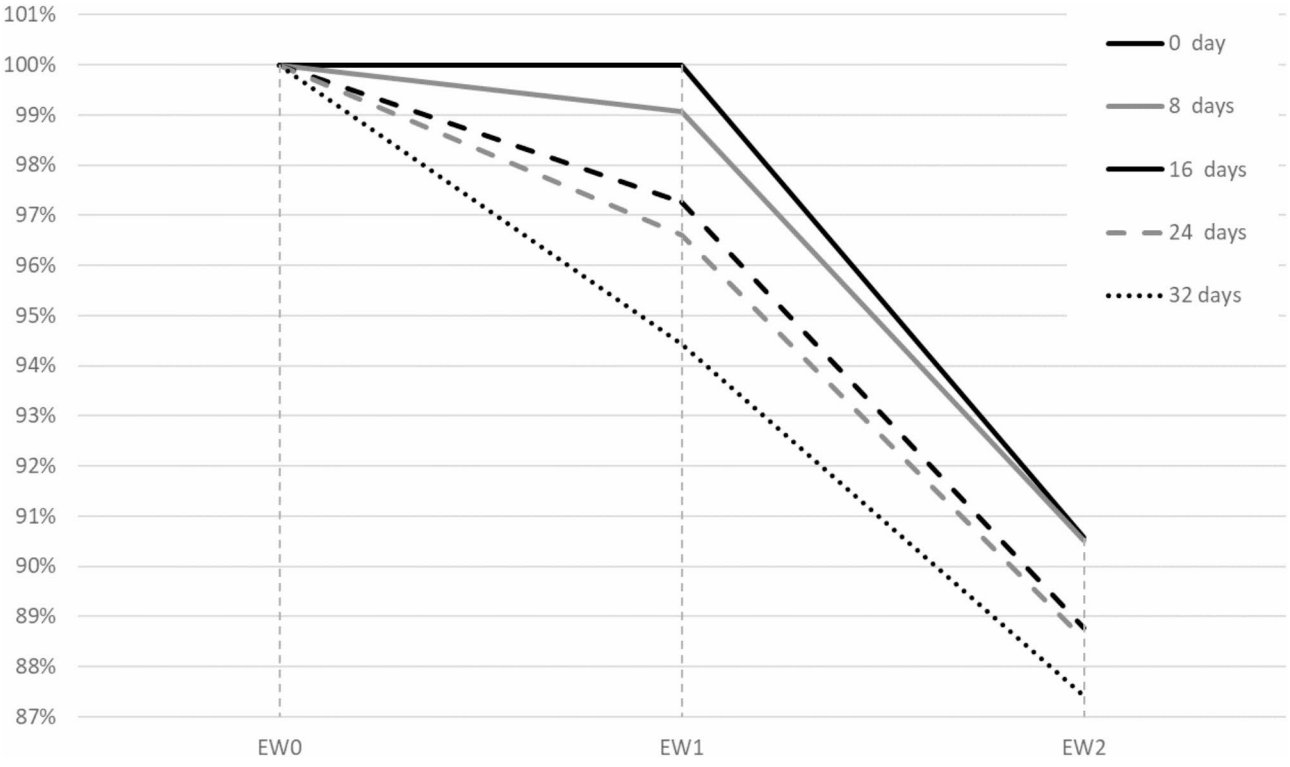

Fig. 1 Weight loss of eggs from which chicks were obtained as a function of storage time before the incubation (EW0 – initial egg weight; EW1 - egg weight at the start of incubation; EW2 - egg weight after 14.5 days of incubation)

Table 4 The coefficients of Spearman's correlation between particular analysed quality traits

	ST	SI	EW0	WL1	WL2	WL3	WL4	WL5	K1	K2
IK	0.065									
EW0	0.094	0.031								
WL1	0.886***	0.011	0.005							
WL2	0.604	-0.027	0.032	0.693***						
WL3	0.325*	0.204	0.125	0.441***	0.707***					
WL4	0.021	-0.029	-0.003	0.054	0.710***	0.588***				
WL5	-0.183	-0.008	-0.278*	-0.169	-0.100	0.313*	0.008			
K1	-0.207***	-0.099	-0.145***	0.305***	0.207***	0.051	0.020	-0.096		
K2	0.028	-0.027	0.000	0.062	0.710***	0.588***	0.999***	0.008	0.028	
K3	-0.221*	-0.014	-0.302*	-0.179	-0.073	0.325*	0.062	1.000***	-0.054	0.062

* – coefficients significant at $P \leq 0.05$; *** – coefficients significant at $P \leq 0.001$, ST – egg storage time, SI – shape index, EW0 – initial egg weight, WL1 – weight loss between EW0 (initial egg weight) and EW1 (egg weight at the start of incubation), WL2 - weight loss between EW0 and EW2 (egg weight after 14.5 days of incubation), WL3 - weight loss between EW0 and EW3 (egg weight at the end of incubation), WL4 - weight loss between EW1 and EW2, WL5 - weight loss between EW2 and EW3, K1 – egg shell conductance constant between EW0 and EW1, K2 - egg shell conductance constant between EW1 and EW2, K3 - egg shell conductance constant between EW2 and EW3

second, around the 16th day of incubation. Similarly, the periods of greatest embryonic mortality during incubation have been determined for hens [32], indicating that during the first eleven days of incubation, the (chicken) embryo is exposed to hypoxia, while the last ten days are a crucial phase for the compensatory response of the organs to the hypoxia phenomenon [33]. Therefore, during the initial incubation period, embryos are susceptible to changes in micro-environmental conditions [34], their circulatory system is just developing and taking up functioning respiratory functions [35], and in the final phase, when they are ready to hatch, they switch, not always successfully, to pulmonary respiration. During this period, the chick begins to breathe oxygen through the pores in the eggshell, and gas exchange between it and the environment is a key factor for its survival inside the egg. The chick takes its first breath from the air cell of the egg, at this stage a common cause of death has been poor positioning of the chick preventing the air cell from being pipped [36].

Table 5 The eggshell conductance constant (K) in particular experimental periods depending on the duration of the egg storage time before incubation

Trait		Days of storage								SEM	*F-test (P-value)*
		0	4	8	12	16	20	24	28	32		
Infertile	K1	-	0.188^{a}	0.142^{ab}	0.096^{b}	0.134^{ab}	0.082^{b}	0.107^{b}	0.117^{b}	0.133^{ab}	0.005	*0.002*
	K2	0.128	0.130	0.162	0.162	0.182	0.169	0.150	0.139	0.185	0.012	*0.937*
Fertile and died up to 14th day	K1	-	-	0.177	0.171	0.189	0.076	0.136	0.097	0.062	0.017	*0.484*
	K2	-	-	0.146	0.123	0.257	0.150	0.211	0.128	0.144	0.020	*0.626*
Fertile and died after 14th day	K1	-	0.150	0.145	0.109	0.126	0.102	0.103	0.101	0.108	0.004	*0.082*
	K2	0.127^{a}	0.120^{a}	0.040^{b}	0.101^{ab}	0.130^{a}	0.135^{a}	0.133^{a}	0.145^{a}	0.125^{a}	0.004	*0.004*
	K3	0.575^{a}	0.541^{a}	0.513^{a}	0.258^{ab}	0.199^{bc}	0.229^{b}	0.094^{c}	0.159^{c}	0.137^{c}	0.018	*0.002*
Hatched	K1	-	0.199^{a}	0.101^{b}	0.116^{b}	0.117^{b}	0.098^{b}	0.097^{b}	0.102^{b}	0.119^{b}	0.004	*< 0.001*
	K2	0.140	0.129	0.129	0.127	0.130	0.177	0.124	0.118	0.111	0.005	*0.226*

^{a,b,c,d} – means marked with different letters differ significantly at $P \leq 0.05$; K1 – egg shell conductance constant between EW0 (initial egg weight) and EW1 (egg weight at the start of incubation), K2 - egg shell conductance constant between EW1 and EW2 (egg weight after 14.5 days of incubation), K3 - egg shell conductance constant between EW2 and EW3 (egg weight at the end of incubation)

A relationship between egg storage time, egg weight loss and hatching quality was also reported in our study. The results of Lacin et al. [28], Petek et al. [37] and Seker et al. [27] suggest that egg weight loss due to storage significantly reduces the quality of hatching eggs. Fasenko [23] found that poorer hatching performance from eggs stored for longer periods is related to egg weight loss due to water transpiration, which consequently worsens the environmental conditions of the developing embryo. Furthermore, some researchers indicate that the duration of egg storage may be less important than the loss of egg mass itself, which may suggest the desirability of research into reducing water loss in stored eggs. Romao et al. [38] showed that the eggs storage for more than a week significantly increased the proportion of unhatched eggs, mainly as a result of embryo death even before incubation or in the first few days of incubation. Similarly, the study in question indicated a critical period up to the 5th day of egg incubation. It can also be speculated that the higher percentage of eggs classified as infertile may be due to so-called preincubation death.

Amos and Rahn [39] and Sparks and Board [40] also pointed to the role of eggshell pores in water permeability and gas exchange, which may have important consequences in terms of hatching performance. The loss of egg mass due to water evaporation can be linked to the findings of D'Alba et al. [41], who point to the already mentioned protective role of the cuticle, the organic layer covering the eggshell, in preventing water loss as well as protection in bacterial penetration. This is important due to the potential implications for hygiene and the health of the embryo. On the other hand, in the experiment performed, the rate of infected eggs was statistically insignificant (verification during biological analysis after incubation) regardless of the length of storage, which may be explained by the higher concentration of lysozyme in the protein, which protects the egg from harmful microbiological agents, compared to eggs of other poultry species [42].

The egg shell conductance constant is the resultant equation defining the functional characteristics of incubated eggs, which determines the energy and circulation of water resources [43]. Water conductance measures the functional properties of the egg shell [44], while the conductance constant allows functional properties to be measured in terms of physical properties (egg mass, incubation time). Loss of egg mass is an important indicator of incubation and hatching success. Too rapid a loss of moisture has a negative impact on the embryonic development of embryos and their metabolic status [45]. Knowledge of the variability in the ability of embryos to regulate water content and eggshell conductivity is essential to estimate the relationship between egg weight loss and embryo survival [46]. The water loss of an egg during

Table 6 The evaluation results of newly hatched chicks depending on the duration of the egg storage time before incubation

Trait		Days of storage									
		0	**4**	**8**	**12**	**16**	**20**	**24**	**28**	**32**	**χ^2-test (*P*-value)**
Number of hatched chicks (pcs)		44	42	40	42	34	36	21	12	7	-
Mean chick evaluation note (%)		99.84	99.48	99.25	98.76	97.32	98.00	86.33	86.08	96.14	*0.725*
Chick proportion in egg weight (%)		64.59	71.72	70.36	67.98	70.29	69.04	68.75	65.97	68.63	*0.388*
Activity	N	0	1	2	3	6	5	15	10	1	*111.495*
	F	0	0.02^{c}	0.05^{c}	0.07^{a}	0.18^{b}	0.14^{b}	0.71^{a}	0.83^{a}	0.14^{b}	*< 0.001*
Down	N	0	1	0	1	3	2	5	8	1	*78.530*
	F	0^{d}	0.02^{cd}	0^{d}	0.02^{cd}	0.09^{bc}	0.06^{c}	0.24^{ab}	0.67^{a}	0.14^{b}	*< 0.001*
Belly button	N	1	0	2	2	2	2	2	4	1	*21.906*
	F	0.02^{c}	0^{d}	0.05^{c}	0.05^{c}	0.06^{c}	0.06^{c}	0.1^{cd}	0.33^{a}	0.14^{b}	*0.005*
Yolk sac	N	0	0	0	0	0	0	0	0	0	*0.000*
	F	0	0	0	0	0	0	0	0	0	*1.000*
Eyes	N	0	0	0	0	0	0	2	0	0	*24.565*
	F	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0^{b}	0.10^{a}	0^{b}	0^{b}	*0.002*
Legs	N	0	1	1	1	5	3	9	6	2	*62.335*
	F	0^{d}	0.02^{d}	0.03^{d}	0.02^{d}	0.15^{bc}	0.08^{cd}	0.43^{a}	0.5^{a}	0.29^{b}	*< 0.001*
Membranes remaines	N	1	0	0	0	1	0	0	0	0	*5.266*
	F	0.02	0	0	0	0.03	0	0	0	0	*0.729*

N – number of appearance; F – frequency of appearance; $^{a, b, c, d}$ – means marked with different letters differ significantly at $P \leq 0.05$ (Dwass-Steel-Chritchlow-Fligner test)

incubation, when the continuity of the shell is still intact, depends primarily on the nominal water conductivity of the shell and the humidity in the incubator chamber. On the other hand, conductivity in relation to initial egg weight may be the basis for determining such incubation conditions so that egg weight loss is optimal [47]. It has also been suggested [48] that the embryo grows to a size commensurate with the available water supply in the allantoic fluid, while its rate of depletion depends on the water loss provoked by the humidity of the incubator chamber. In the present study, all eggs were incubated at the same time, in the same setter and hatcher, so the variation in shell permeability (K) and water circulation between the interior of the egg and the external environment was probably caused by the length of time the eggs were stored prior to incubation. The loss of water from the eggs due to storage also reduces the water conductivity of the eggs, leading to reduced egg quality [23]. Also, in this case, a decrease in water conductance was observed with increasing storage period (Table 3). It is also worth mentioning that reduced water conductance reduces the transport of oxygen and nutrients to the embryo, potentially negatively affecting its development [49]. Damaziak et al. [24] also confirmed the relationship between storage length and reduced water conductivity of eggs. Their results suggested that eggs with reduced conductivity were more susceptible to embryonic death, which they explained by limiting the embryo's access to essential nutrients and oxygen.

Studies by Nowaczewski et al. [50] and by Taha et al. [51] egg storage period on various egg quality parameters and the quality of hatched chicks. In both studies, significant changes were observed not only in egg weight but in the yolk and albumen content of the eggs, as well as in the quality of the hatched chicks, which is consistent with the observations of Brake et al. [26], who also showed a negative effect of long-term egg storage on the quality of the hatched chicks. Furthermore, the results presented in this study indicate that chicks hatched from eggs stored for longer than 12 days showed more frequent limb malformations, lower activity and poorer down quality. In our study, the overall quality score was very high even for chicks obtained from eggs stored for 32 days, despite the observed decrease in hatchability.

Conclusions

In conclusion, the results of the study confirmed the negative effect of long-term storage of Japanese quail eggs on hatching performance; however, the viability of Japanese quail embryos is noteworthy, as the hatching of full-quality chicks was recorded from eggs stored for 32 days prior to incubation.

With time passing, a decrease in early embryo viability lity and hatchability while an increase in embryo mortality was observed, mainly in the late incubation phase. The loss of egg weight during the incubation was directly proportional to the change in this trait before incubation due to the storage time. A reduction in eggshell conductance constant was noted as a result of prolonged egg storage, which may have negatively affected chick hatching by limiting the embryo's access to essential nutrients and oxygen. The primary egg characteristic, such as shape

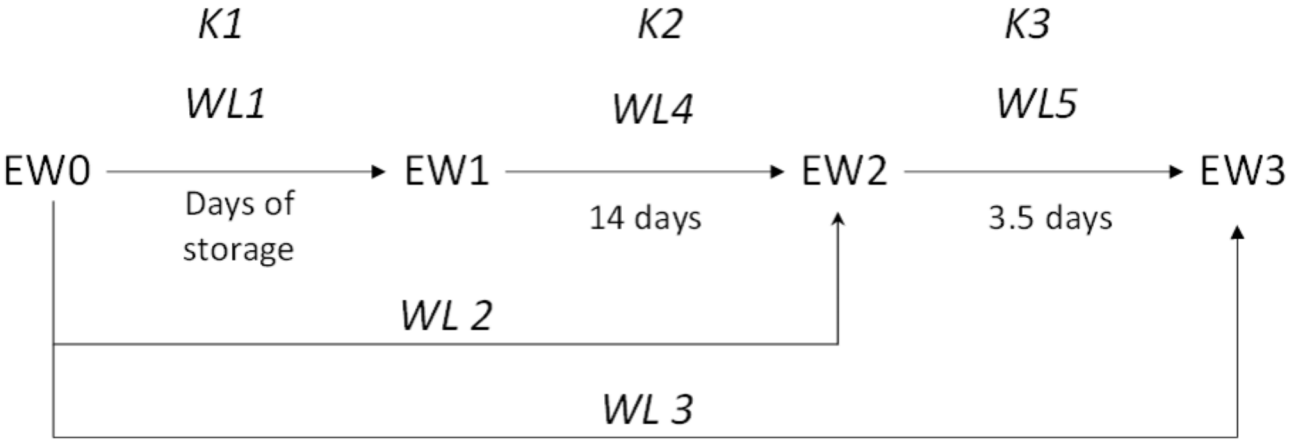

Fig. 2 The schema of egg weight loss estimation (EW – egg weight, WL – weight loss, K - eggshell conductance constant)

index, did not modify the subsequent weight changes in any way.

These results have important practical implications for poultry producers, especially small-scale ones, as they suggest that storage times of hatching quail eggs can be extended, but not beyond 24 days, which is still three times longer than usually recommended seven days for hen eggs.

Some additional research is required on the optimum storage time for quail eggs and on the mechanisms at the basis of the negative effects, the neutralisation of which may contribute to improving the efficiency of poultry production.

Methods

As the eggs were obtained from a commercial quail flock and, apart from storage, no research procedures compromising the welfare of the birds were performed, according to the legislation in force (Act of 15 January [52] on the protection of animals used for scientific or educational purposes; Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes [53]), ethics committee approval for the study was not required.

The material for the study consisted of hatching eggs from a commercial flock of Japanese quail (*Coturnix japonica*) maintained at the Laura Kaufman Didactic and Research Station of Small Animals, University of Life Sciences in Lublin. Birds in flocks of 1♂:4♀ were maintained in a windowless building in cages of 100 × 50 × 25 cm at a stocking density of 30 birds (6 flocks)/m^2. The temperature in the room was maintained at 21 °C and 65% humidity. Cold-coloured (6000 K) artificial LED lighting of 20 Lx in a sequence of 17 H daylight and 7 H night was used. Quails were fed with a balanced complete feed mixture for adult birds containing 2800 kcal, 21% protein and 3.5% fibre.

The 756 eggs were collected at 4-day intervals for 52 days. Thus, 14 groups (according to collection date) of 54 eggs were created by designating 6 replications in each group. Eggs were placed in extruders and stored at physiological zero conditions, i.e. 17 °C. Consecutive batches of eggs were stored for 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48 and 52 days, respectively; the control group consisted of fresh, unstored eggs.

On the collection day, the short and long axes were measured in all eggs to determine the shape index. All material was weighed on the day of collection, on setting day, during the transfer from the setting chamber to the hatching one, and after incubation was completed (unhatched). Based on egg weight measurements, egg weight loss was determined as a function of storage length. Percentage weight loss was estimated between the following dates: on the day of collection/oviposition (EW0, beginning of storage), on the day of setting eggs (EW2), on the day the eggs were transferred to the hatching chamber (EW3, 14.5 days of incubation) and on the day of hatching (EW4, after 17.5 days of incubation). A schematic of the variables determined is shown in Fig. 2.

After 52 days of the first eggs collection, all eggs, including fresh ones from the control group, were placed in an incubator (Jarson®, Gostyń, Poland) and incubated under species-specific standard conditions:

- setting chamber – 14 days, 37.6–38.0 °C, 50–65% humidity, 90° change of position every 1 h (24 times/day).
- hatching chamber – 3.5 days, 37.0–37.5 °C, 75–80% humidity.

Eggs were candled on the 14th day of incubation to verify the embryonic development. The number of infertile eggs and dead embryos was then determined. Eggs in which a viable embryo was found were then placed in hatching

nets (1 net = 1 replication) and transferred to the hatching chamber.

After 17.5 days of incubation, biological analysis of the set was performed, estimating the effective level of egg fertility [54], hatchability from set and fertile eggs, embryo mortality in both phases of incubation, shell water conductivity of infertile eggs, eggs with died embryos and eggs with normally developing embryos [55].

Based on the formula of Christensen et al. [55] the eggshell conductance constant (K) was determined as a criterion of egg weight loss separately for fertile, infertile and unhatched eggs.

$$K = \frac{G_{H_2O} \times I}{W}$$

Where:

G_{H_2O} - the conductance of the egg namely number of mg of water vapor that leave the egg in a 24-h period per mm of mercury,
I - the length of the incubation period of the egg (days),
W - the initial weight of the egg (g).

Assessment of chick quality was carried out according to a 100-point scale according to the methodology proposed by Tona et al. [56]. Chick activity, general appearance and down, eyes, legs, degree of navel healing and degree of yolk sac absorption, residuals of egg membranes and/or yolk were assessed. The chicks were also weighed.

The normal distribution of the data was verified using the Shapiro-Wilk test at $\alpha = 0.05$. One-way analysis of variance was then used, where the number of days of egg storage was a constant factor in the model. A Tukey's multiple comparisons test was used to verify statistical differences between groups, assuming a significance level of $\alpha = 0.05$. A Chi^2 Kruskal-Wallis test was performed to determine whether storage affects the percentage and weight of hatched chicks. A paired analysis of two-sided multiple comparisons was also performed using the Dwass, Steel and Critchlow-Fligner method to determine the significance of differences between the frequencies of occurrences of each trait. Relationships between traits were analysed using Spearman's correlation. Statistical calculations were performed using the academic SAS 9.4 package (SAS Institute Inc. Cary, NC).

Abbreviations

EW0	Initial egg weight
EW1	Egg weight at the start of incubation
EW2	Egg weight after 14.5 days of incubation
EW3	Egg weight of unhatched
K	Eggshell conductance constant
WL	Weight loss

Supplementary Information

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Supplementary Material 1

Author contributions

TP - conceptualization, methodology, software, formal analysis, project administration, writing - original draft; KK investigation, writing – review & editing, visualization; KD - conceptualization, methodology, writing – review & editing; AR – investigation, writing – review & editing; KW – investigation, writing – review & editing; JB - methodology, supervision, writing - original draft.

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Data availability

The data presented in this study are available on request from the corresponding author.

Declarations

Ethics approval and consent to participate

As the eggs were obtained from a commercial quail flock and, apart from storage, no research procedures compromising the welfare of the birds were performed, according to the legislation in force (Act of 15 January [52] on the protection of animals used for scientific or educational purposes; Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes [53]), ethics committee approval for the study was not required.

Consent for publication

Not applicable

Competing interests

The authors declare no competing interests.

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