The effects of hen's age and egg storage duration on selected growth parameters of turkey embryos

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ABSTRACT The aim of this study was to determine the effects of hen's age (**A**) and egg storage duration (**T**) on selected growth parameters of turkey embryos. At 32, 38, 46, and 51 wk of hen's age, 1,512 eggs laid on one or 2 consecutive days were collected randomly and marked. At each sampling date, the eggs were randomly divided into 4 groups and were stored for various periods of time, that is, 7, 10, 13, and 17 d. All eggs were stored at a temperature of 15°C and relative air humidity of 76%. On d 9, 15, 21, and 24 of incubation, 5 eggs containing live embryos were randomly selected from each group for analysis of the following parameters: relative body weight (**RBW**) of embryos, relative weight of the yolk sac (**RWY**), relative weight of unused albumen (**RWA**). The effects of hen's age and egg storage duration on the RBW of embryos were observed on d 15, 21, and 24 of incubation ($P < 0.05$). The effects of hen's age and egg storage duration on RWY were noted on all analyzed days of incubation ($P < 0.05$). Embryos in eggs laid by younger hens (aged 32 and 38 wk) and stored for a shorter period were characterized by a faster rate of albumen utilization than embryos in eggs laid by older hens (aged 46 and 51 wk). The largest amount of unused albumen was found in eggs laid by hens in wk 51 of the laying season ($P < 0.05$), and stored for 17 d ($P < 0.05$). In conclusion, numerous interactions (**AxT**) between selected growth parameters of turkey embryos indicate that the quality of hatching eggs changes with hen's age, affecting their suitability for long-term storage under standard conditions. Therefore, eggs laid by younger breeders should not be stored for longer periods due to undesirable changes in RWY and RWA.

Key words: incubation, embryo body weight, yolk sac, albumen

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INTRODUCTION

The physical, morphological and mechanical characteristics of eggs play an important role in the processes of embryo development and hatching (Anandh et al., 2012; Ipek and Sozcu, 2014; Ghane et al., 2015). For logistical reasons, egg storage prior to incubation is a growing practice in the commercial turkey industry because it helps coordinate all actions and synchronize the hatching process (Bakst et al., 2016; Adriaensen et al., 2022). The results of many studies have shown that egg storage duration is negatively correlated with hatchability and poult quality (Fasenko et al., 2001; Silva et al., 2008; Dymond et al., 2013). Reijrink et al. (2008) found that when eggs are stored for prolonged periods (>7 d) under optimal conditions, the percentage of necrotic cells increases, contributing to early embryo mortality. Fasenko (2007) demonstrated that embryos from eggs stored for a long time are characterized by a slower growth rate than embryos from eggs stored for a short time. Bakst et al. (2016) reported that egg storage over increasing time periods resulted in a significant decrease in both embryo weights and developmental stage. Embryo mortality during storage is strongly correlated with egg weight loss, and it increases with storage time (Veira et al., 2005; Oblakova et al., 2008; Silva et al., 2008). It is hypothesized that particular embryonic developmental stages are better able to survive long-term storage (Christensen et al., 2003)

A previous study by Mróz et al. (2019), which investigated the interaction between hen's age and egg storage time on the frequency of occurrence of physical defects in turkey poults, revealed that the incidence of physical

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defects in poults was affected by hen's age rather than egg storage time. In turkeys, rapid growth and development of embryos is observed between d 9 and 25 of incubation (De Oliveira et al., 2008). Research shows that until d 22 of incubation, both the yolk and albumen are used as nutrient sources by turkey embryos, whereas the residual albumen, that is, the albumen left in the egg after the yolk had been internalized, is utilized until d 24, and the yolk sac is drawn into the body cavity from d 24 (Moran, 2007; De Oliveira et al., 2008). The optimization of egg storage time in view of hen's age is an important consideration in turkey reproduction because it affects embryogenesis and, consequently, poult quality. These 2 factors often interact with each other (Fasenko, 2007; Hamidu et al., 2011, Nasri et al., 2020)

The aim of this study was to determine the effects of hen's age and egg storage duration on selected growth parameters of turkey embryos.

MATERIALS AND METHODS

All procedures involving embryos were approved by the Local Ethics Committee at the University Warmia and Mazury in Olsztyn (No. 16/BŻ/2009). The experiment was conducted in Olsztyn, Poland (20°29′E, 53°47′N).

Sample Collection

Eggs were collected from female BUT 6 turkeys (8,100 females) raised in accordance with the Aviagen management guidelines for breeder turkeys (Aviagen turkeys, 2015). The hens started laying eggs at 30 wk of age. At 32, 38, 46, and 51 wk of hen's age, 1,512 eggs laid on one or 2 consecutive days (a total of 6,048 eggs) were collected randomly (nonprobability sampling method, in accordance with purposive sampling) and marked. At each sampling date, the eggs were randomly divided into four groups and were stored for various periods of time, that is, 7, 10, 13, and 17 d (4 groups differing in hen's age (**A**) x 4 groups differing in egg storage duration (**T**); 378 eggs per group). All eggs were stored at a temperature of 15°C and relative air humidity of 76%. Prior to incubation, eggs were transported from the storehouse to a hatchery where they were held for 8 h at an ambient temperature of 18°C and relative humidity of 60% to warm them up. Then the eggs were incubated in the Petersime P13 incubator. Incubation was carried out in line with the relevant technological standards for turkey eggs. Temperature and relative humidity were as follows: setting compartment−37.6°C and 58%, respectively; hatching compartment−37.2°C and 62%, 85% and 70%, respectively, on d 26, 27, and 28. In the setting compartment, eggs were turned automatically by 90° every hour. Eggs were candled on d 11 and 25, and they were transferred to hatching trays on d 25 of incubation.

Measurements of Embryo Quality Parameters

On days 9, 15, 21, and 24 of incubation, five eggs containing live embryos were randomly selected from each group (20 eggs × 4 storage periods × 4 age groups = 320 embryos) for analysis, which included:

- relative body weight (**RBW**) of an embryo −expressed as the ratio of embryo weight to egg weight × 100 (%),

$$\text{RBW} = \frac{\text{embryo weight}}{\text{egg weight}} * 100$$

- relative weight of the yolk sac (**RWY**) on d 9, 15, 21, and 24 of incubation (g)—expressed as the ratio of yolk sac weight to egg weight × 100 (%),

$$\text{RWY} = \frac{\text{yolk sac weight}}{\text{egg weight}} * 100$$

- relative weight of unused thick albumen (**RWA**) on d 24 of incubation—expressed as the ratio of albumen weight to egg weight x 100 (%)

$$\text{RWA} = \frac{\text{albumen weight}}{\text{egg weight}} * 100$$

Statistical Analysis

The results were processed statistically in the Statistica 13.0 PL program (StatSoft Inc., Tulsa, OK). Data were tested for normality by the Kołmogorow-Smirnow test ($P < 0.05$). Results were presented as mean ± standard error of the mean (**SEM**) and validated by 2-way (A × T) or one-way (A or T) ANOVA. Significant differences were determined by Tukey's HSD test with the level of significance set at $P < 0.05$

RESULTS AND DISCUSION

It is known that egg weight and size increase throughout the production cycle of turkeys (Anandh et al., 2012; Ghane et. al., 2015), which was also noted in the present study (Table 1). The weight of eggs set (weighed before incubation) increased with hen's age ($P < 0.001$), and decreased with egg storage duration ($P = 0.013$).

Rapid growth of turkey embryos is observed between d 15 and 24 of incubation (Christensen et al., 2003; Moran, 2007). The RBW of embryos in eggs laid by hens from all age groups was similar on d 9 of incubation. The effects of hen's age ($P < 0.001$) and egg storage duration on the RBW of embryos, and the interaction between both factors, were observed on d 15, 21 and 24 of incubation (Table 1). The interaction between hen's age and egg storage duration for RBW could validate the hypothesis proposed by Fasenko (2007) that not all embryos respond in the same way to different factors.

An analysis of the AxT interaction for the RBW of embryos on d 15 of incubation (Figure 1) revealed that changes in the RBW of embryos in eggs laid by hens aged 32, 38, and 51 wk followed a similar pattern—the highest value was noted in eggs stored for 7 d, and the lowest value was observed in eggs stored for 17 d ($P < 0.05$). In eggs laid by hens aged 46 wk, the RBW of embryos was highest in eggs stored for 17 d ($P < 0.05$).

Table 1. Main effects of egg weight before incubation (g) and relative body weight of turkey embryos (%) in eggs laid by hens aged 32−51 wk, stored for 7−17 d, on selected days of incubation (mean and SEM).

			Relative body weight of turkey embryos (%)			
			Incubation day			
Specification n = 20		Egg weight (g)	9	15	21	24
Hen's age (weeks)	32	87.40[a]	1.13	7.97	31.26	48.89
	38	94.22[b]	1.12	7.72	30.37	47.51
	46	97.45[c]	1.08	7.31	27.26	43.47
	51	98.05[d]	1.15	8.21	27.90	44.21
Storage time duration (days)	7	95.86[w]	1.21	8.67	31.23	48.68
	10	94.61[wx]	1.17	7.55	28.62	45.91
	13	93.60[x]	1.07	7.52	28.33	44.03
	17	93.06[y]	1.03	7.47	28.55	45.46
SEM		0.212	0.030	0.121	0.373	0.541
P-value						
Hen's age (A)		<0.001	0.211	<0.001	<0.001	<0.001
Storage time duration (T)		0.013	0.207	<0.001	0.003	0.004
Interaction (A x T)		0.419	0.107	<0.001	<0.001	<0.001

[a-d]−The mean values of hen's age (A) or
[w-y]Egg storage duration (T) followed by different letters differ significantly ($P < 0.05$).

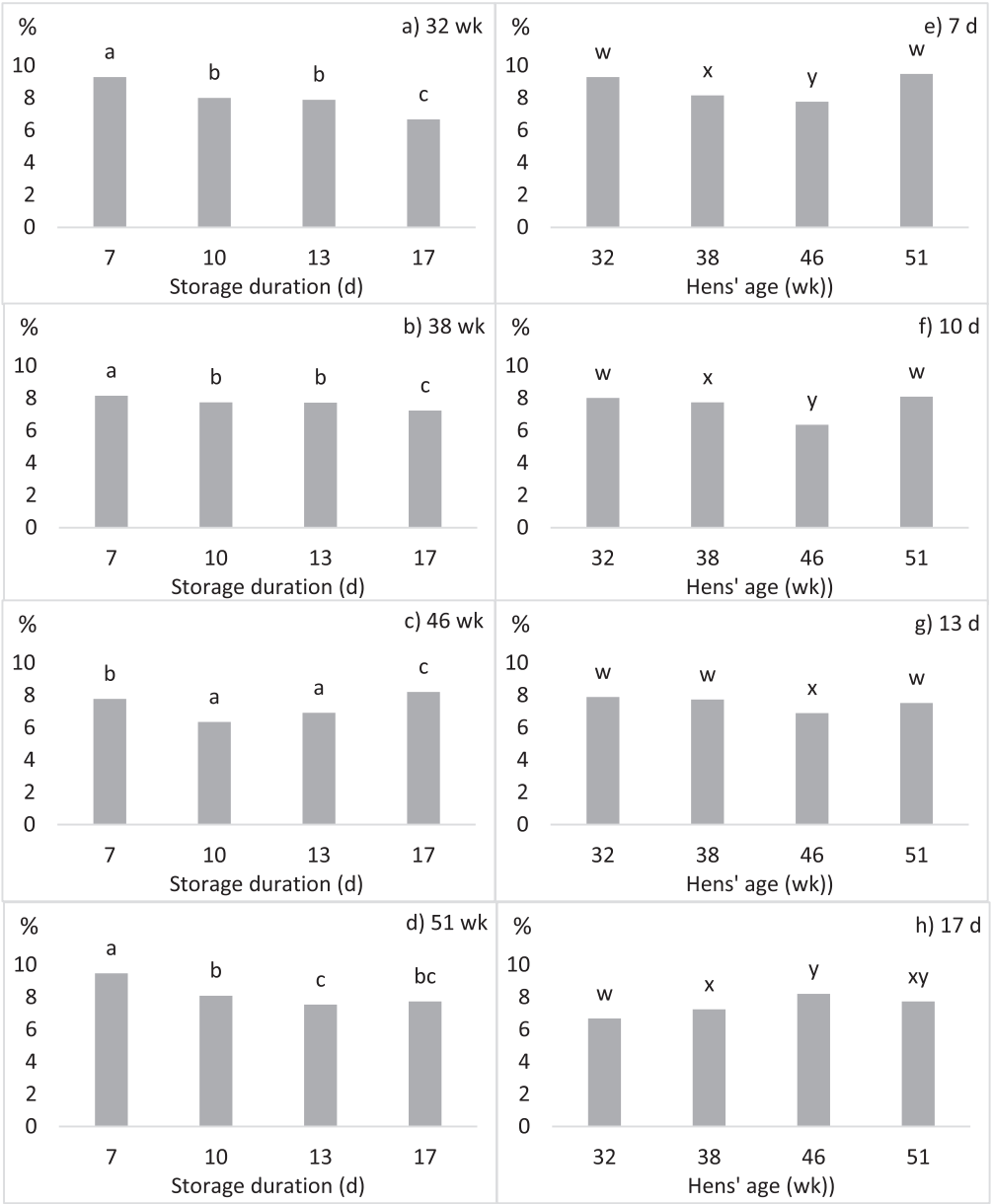

Figure 1. Relative body weight of turkey embryos (%) in eggs laid by hens aged 32−51 wk, stored for 7−17 d, on 15th day of incubation—interaction (means for subgroups). a-c- means for subgroups values followed by different letters differ significantly; ($P < 0.05$). w-z- means for subgroups values followed by different letters differ significantly ($P < 0.05$).

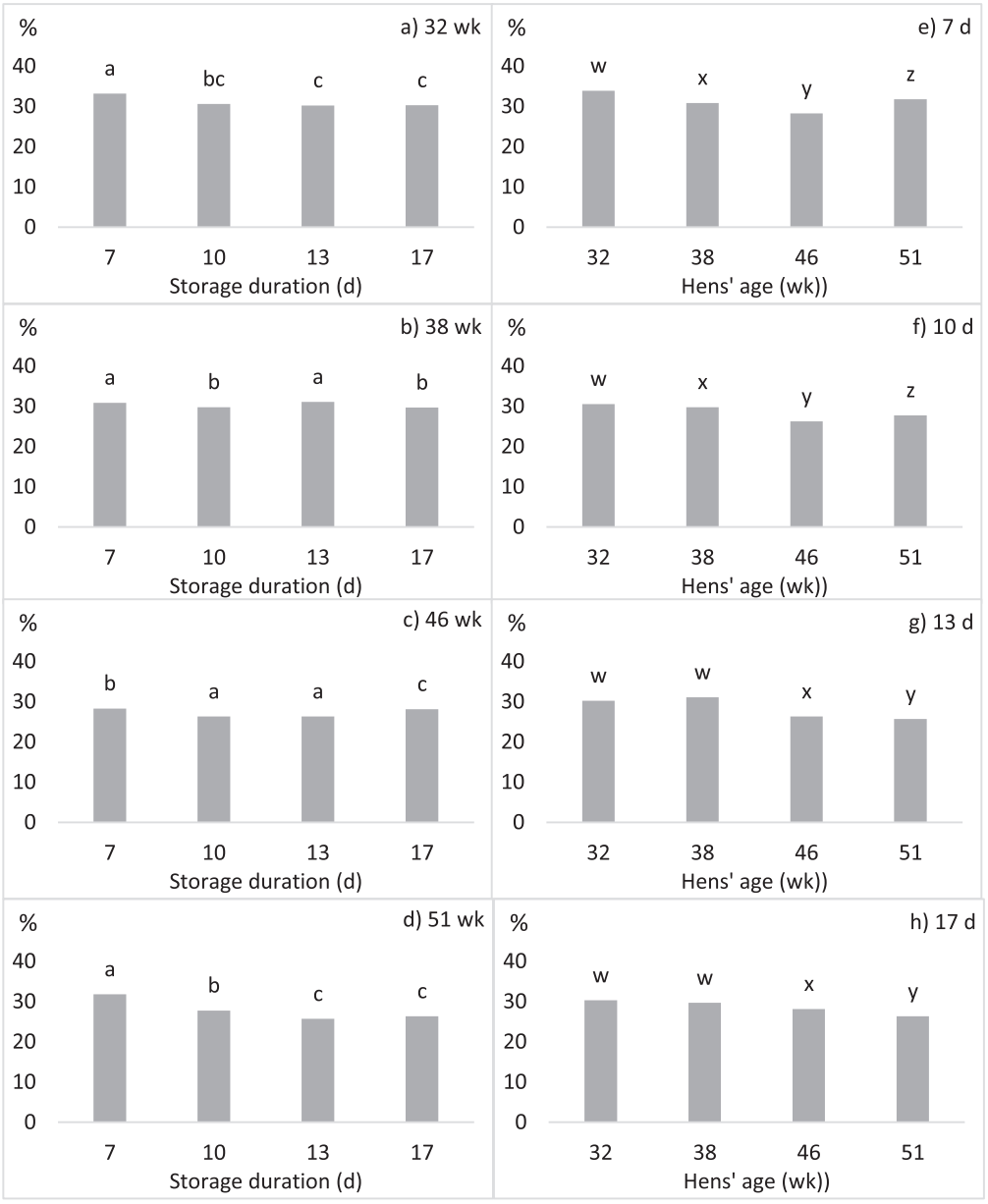

Figure 2. Relative body weight of turkey embryos (%) in eggs laid by hens aged 32−51 wk, stored for 7−17 d, on 21th day of incubation—interaction (means for subgroups). a-c- means for subgroups values followed by different letters differ significantly; ($P < 0.05$). w-z - means for subgroups values followed by different letters differ significantly ($P < 0.05$).

Clear increasing or decreasing trends in the RBW of embryos with hen's age were not found (Figures 1 and 2). The values of RBW were least stable in eggs laid by 46-wk-old hens, which suggests less efficient nutrient utilization by embryos in this group. The present results corroborate the findings of Mróz et al. (2019) who reported the highest percentage of embryos with various defects in eggs laid by hens aged 46 wk. On d 24 of incubation (Figure 3), the RBW of embryos in eggs laid by hens aged 32 and 46 wk was lowest in the group of eggs stored for 17 d ($P < 0.05$). No clear trends were noted in the RBW of embryos in eggs laid by hens aged 38 and 51 wk, as in the case of eggs stored for 17 d−regardless of the age of the laying hens. One of the methods to reduce the negative effects of long-term storage has been to incubate eggs for short periods before storage. In both turkey and chicken eggs, this technique has been successful in improving the hatchability of long-term stored eggs (Christensen et al., 2003).

Yolk sac weight was affected by hen's age on all analyzed days of incubation ($P < 0.001$, Table 2). The effect of egg storage duration on yolk sac weight was noted only on d 9 of incubation ($P < 0.001$). An interaction between hen's age and egg storage duration (Table 2) was observed on d 9 of incubation. After 7, 10, and 13 d of storage, yolk sac weight was lowest ($P < 0.05$) in eggs laid by hens aged 46 wk (Figure 4). After 17 d of storage, yolk sac weight was significantly higher ($P < 0.05$) in eggs laid by hens aged 32 wk than in the remaining groups. In the group of 32-wk-old hens, yolk sac weight was comparable in eggs stored for 7, 10, and 13 d ($P > 0.05$), and it was lower than after 17 of storage ($P < 0.05$). The values of yolk sac weight were lowest in eggs laid by hens aged 38 wk and stored for 10 d ($P < 0.05$), in eggs laid by hens aged 46 d and stored

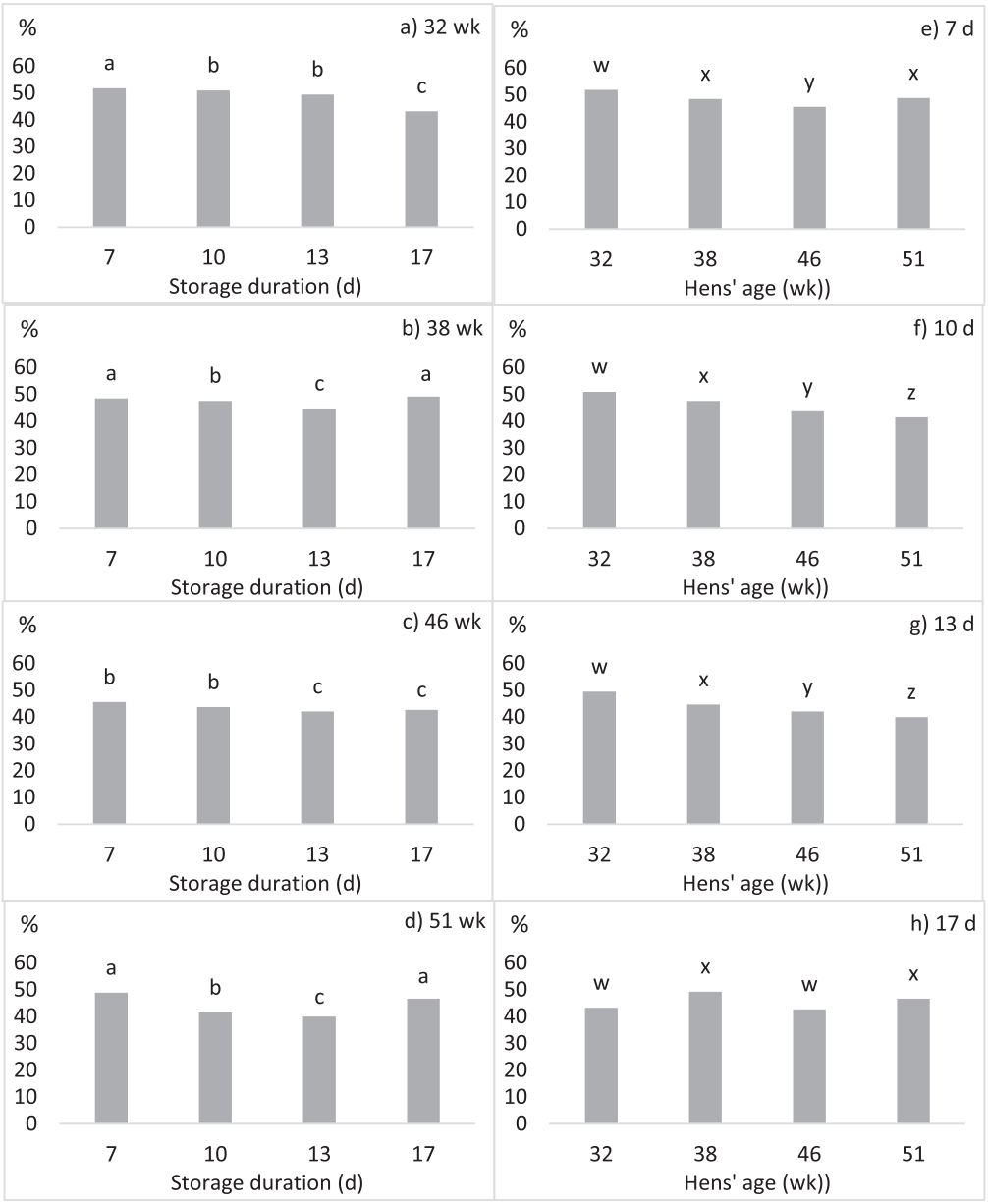

Figure 3. Relative body weight of turkey embryos (%) in eggs laid by hens aged 32−51 wk, stored for 7−17 d, on 24th day of incubation − interaction (means for subgroups). a-c- means for subgroups values followed by different letters differ significantly; ($P < 0.05$). w-z - means for subgroups values followed by different letters differ significantly ($P < 0.05$).

for 10 and 13 d, and in eggs laid by hens aged 51 wk and stored for 7 and 17 d. Ding and Lilburn (1996) found that the size of the yolk sac in turkey embryos decreased from d 13 of incubation. On incubation d 15, 21, and 24, yolk sac weight was higher in eggs laid by older hens (aged 46 and 51 wk) than in eggs laid by younger hens (aged 32 and 38 wk; Table 2).

The effects of hen's age ($P < 0.001$) and egg storage duration ($P < 0.001$) on RWY were noted on all analyzed days of incubation (Table 2). The interaction between the factors was noted on d 9 ($P = 0.016$) and 24 of incubation ($P = 0.010$). On d 9 of incubation (Figure 5), RWY was lowest in eggs laid by hens aged 46 wk and stored for 7, 10, and 13 d. After 17 d of storage, RWY was lower in eggs laid by older hens, aged 46 and 51 wk ($P < 0.05$), and similar values were noted in both groups ($P > 0.05$). On d 15 of incubation, RWY was highest in eggs laid by hens aged 51 wk ($P < 0.05$), and similar values were found in eggs laid by hens aged 46 wk. No significant differences in RWY were observed in eggs stored for 7 and 10 d, but the noted values were lower ($P < 0.05$) than those in eggs stored for 13 and 17 d. On d 21 of incubation, RWY was lower in eggs laid by younger hens (aged 32 and 38 wk) than in eggs laid by older hens (aged 46 and 51 wk, $P < 0.05$). The values of RWY in eggs stored for 7 were lower ($P < 0.05$) than in eggs stored for 10, 13 d and 17 d. As reported by De Oliveira (2008), the last stages of embryonic development are crucial for turkeys because their metabolism shifts to accommodate post-hatch survival and growth. An interaction between the experimental factors was noted on d 24 of incubation ($P < 0.001$). After 7 d of storage, RWY

Table 2. Weight of the yolk sac (g), relative weight of the yolk sac (%), relative weight of unused albumen (%) in eggs laid by hens aged 32−51 wk, stored for 7 to 17 d (mean and SEM).

Specification n = 20		Incubation day 9	15	21	24
Weight of the yolk sac (g)					
Hen's age (weeks)	32	49.12	26.55[a]	18.63[a]	15.90[a]
	38	47.70	27.60[a]	21.31[b]	18.31[a]
	46	43.57	30.61[b]	25.02[c]	21.68[b]
	51	52.31	30.94[b]	24.76[c]	22.56[b]
Storage time duration (days)	7	48.16	28.62	22.49	19.09
	10	46.74	28.60	22.53	19.69
	13	47.72	29.18	22.48	20.07
	17	50.08	28.25	22.20	19.62
SEM		1.231	0.433	0.397	0.405
P-value					
Hen's age (A)		<0.001	<0.001	<0.001	<0.001
Storage time duration (T)		<0.001	0.948	0.173	0.310
Interaction (A x T)		<0.001	0.151	0.118	0.716
Relative weight of the yolk sac (%)					
Hen's age (weeks)	32	56.22	30.37[a]	21.32[a]	18.27
	38	50.64	29.32[b]	22.61[b]	19.44
	46	44.73	31.41[c]	25.67[c]	22.25
	51	53.37	31.51[c]	25.25[c]	23.02
Storage time duration (days)	7	50.35	29.81[w]	23.38[w]	19.83
	10	49.56	30.21[w]	23.73[x]	20.72
	13	51.08	31.15[x]	23.94[x]	21.36
	17	53.98	31.43[x]	23.80[x]	20.99
SEM		0.091	0.097	0.085	0.051
P-value					
Hen's age (A)		<0.001	<0.001	<0.001	<0.001
Storage time duration (T)		<0.001	0.023	0.016	0.004
Interaction (A x T)		0.016	0.173	0.147	<0.010
Relative weight of unused albumen on day 24 of incubation (%)					
Hen's age (weeks)	32				0.02[a]
	38				0.01[a]
	46				0.28[b]
	51				0.95[c]
Storage time duration (days)	7				0.04[w]
	10				0.15[x]
	13				0.24[x]
	17				0.93[y]
SEM					0.032
P-value					
Hen's age (A)					0.017
Storage time duration (T)					0.007
Interaction (A x T)					0.081

[a-c]−The mean values of hen's age (A) or
[w-y]Egg storage duration (T) followed by different letters differ significantly ($P < 0.05$).

was lowest in eggs laid by hens aged 32 wk (Figure 6, $P < 0.05$). A similar trend was observed after 10, 13, and 17 d of storage. Since the embryo absorbs nutrients from the yolk sac, RWY decreases during incubation (Ding and Lilburn, 1996).

Previous research has demonstrated that albumen utilization by turkey embryos ends on d 24 of incubation, before the initiation of yolk sac absorption (Tullett and Deeming 1987; Moran, 2007; De Oliveira et al., 2008). According to Anandh et al. (2012), early- and mid-lay eggs differ significantly in terms of physical and chemical characteristics. The cited authors found that an increase in yolk percentage and changes in albumen can be important physical factors that directly affect the survival and performance of embryos. Ghane et al. (2015) reported a positive and strong relationship between hen's age and egg size and weight, but they also suggested that the changes in egg weight are not linear and occur predominantly in early- and mid-cycle eggs. In the authors' opinion, the age and body weight of hens at the onset of egg production have a direct and positive effect on egg size and the production of small eggs throughout the production cycle. Moreover, the most significant differences are related to the albumen and yolk ratios, which in turn significantly impact the chemical composition of eggs, especially early- and mid-lay eggs (Iqbal et al., 2016; Marzec et al., 2019). The albumen height decreases with hen's age, even if egg weight and the total albumen content of the egg increase in fresh eggs (Silversides and Scott, 2001). However, when eggs of the same

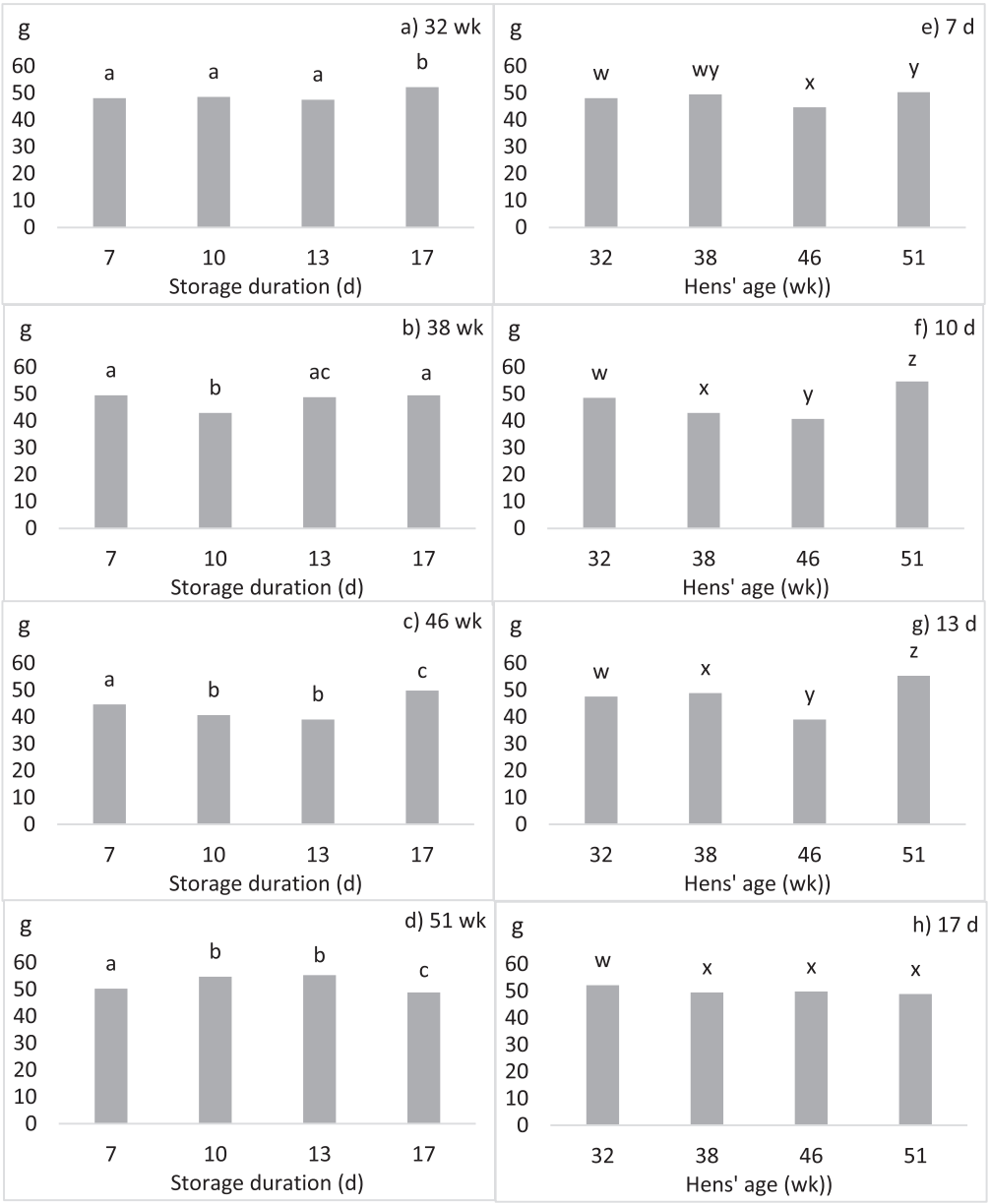

Figure 4. Weight of the yolk sac (g), in eggs laid by hens aged 32−51 wk, stored for 7−17 d on 9th day of incubation—interaction (means for subgroups). a-c- means for subgroups values followed by different letters differ significantly ($P < 0.05$). w-z - means for subgroups values followed by different letters differ significantly ($P < 0.05$).

breed and breeder age are heavier, this is mainly due to an increase in the amount of albumen (Everaert et al., 2008), resulting in a proportional decrease in yolk weight and an increase in albumen weight with egg weight (Ho et al., 2011). Other researchers (Stępińska et al., 2017) reported that the nutritional value of eggs decreased with hen's age, along with a deterioration in reproductive performance. The above findings indicate that the total protein content of the yolk and albumen decreases during the laying season.

In the present study, the amount of unused albumen was small on d 24 of incubation in eggs laid by younger hens (aged 32 and 38 wk) and in eggs stored for short periods. Hen's age ($P = 0.017$) and egg storage duration ($P = 0.007$) exerted a significant effect on RWA on d 24 of incubation (Table 2). The largest amount of unused albumen was found in eggs laid by hens aged 51 wk ($P < 0.05$) and stored for 17 d ($P < 0.05$). Ghane et al. (2015) also demonstrated that early lay eggs had more dense and thicker albumen, but eggs laid in the late stages of the production cycle had higher moisture content, and the albumen was more fluid. The slower rate of albumen absorption in eggs laid by hens aged 51 wk and stored for 17 d could result from the high water content of eggs, pointing to the adverse effects of hen's age and egg storage time on the reproductive performance of turkeys (Stępińska et al., 2012). During embryo development, albumen proteins flow into the amniotic cavity and the yolk sac, and they reach the embryo's digestive tract, and serve as the main protein source for tissue synthesis. Therefore, the residual albumen that had not been utilized prior to yolk sac absorption contributes to embryo

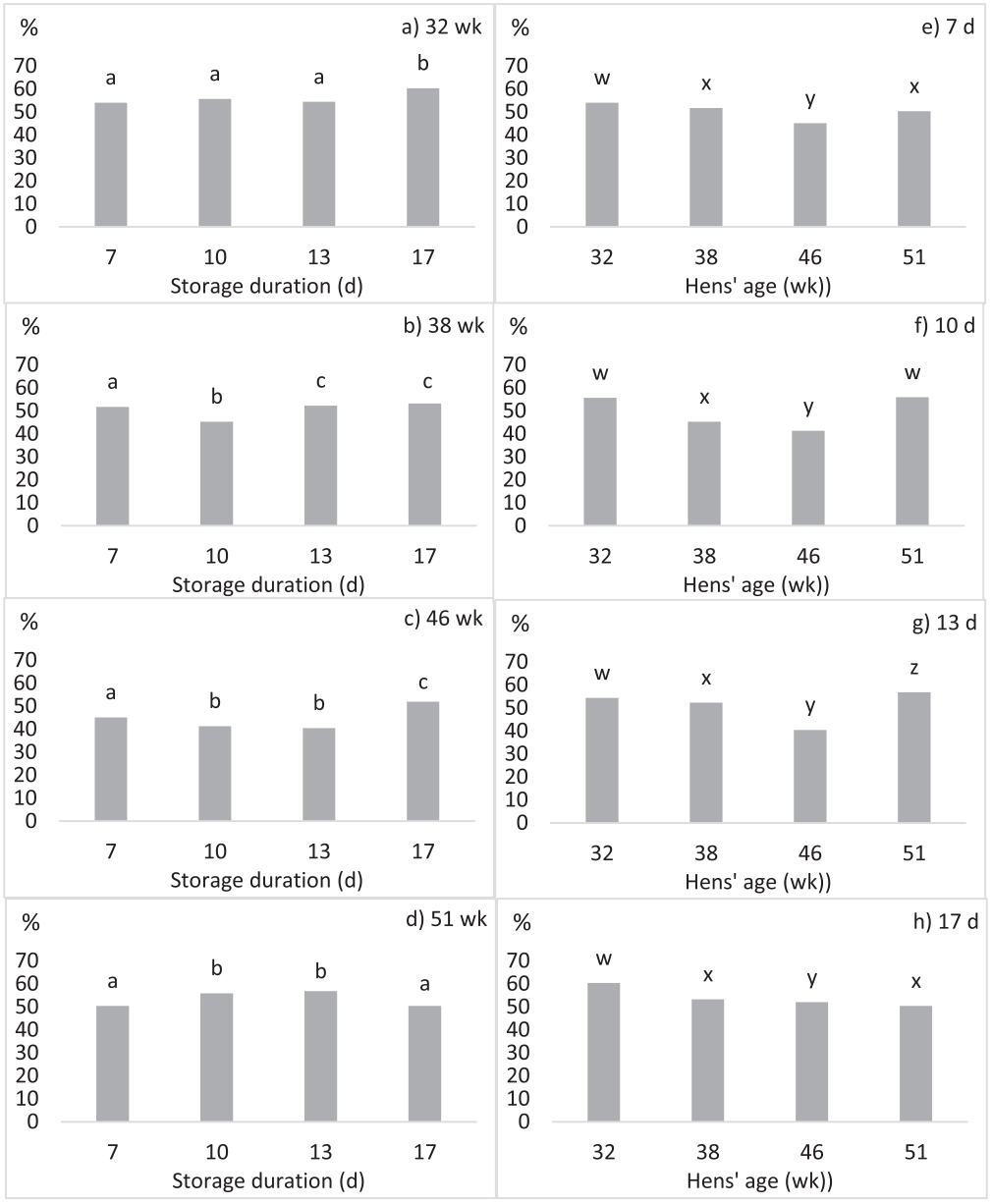

Figure 5. Relative weight of the yolk sac (%) in eggs laid by hens aged 32−51 wk, stored for 7−17 d, on 9th day of incubation—interaction (means for subgroups). a-c- means for subgroups values followed by different letters differ significantly ($P < 0.05$). w-z - means for subgroups values followed by different letters differ significantly ($P < 0.05$).

malnutrition Willems et al. (2014). In the work of Nasri et al. (2020), prolonged storage duration resulted in lower chicken quality in both young and old breeders. However, the interaction between storage duration and breeder age on multiple chicken quality parameters is not clear. According to the cited authors, a short egg storage period may improve the hatchability of eggs laid by younger hens, but not by older hens. Moreover, eggs from older breeders, compared with those from younger breeders, are more susceptible to the negative impact of prolonged storage.

According to Rocha et al. (2013), if eggs have to be stored for prolonged periods, the adoption of management practices such as storing the egg with the thin tip down, egg turning during storage, and prestorage incubation may contribute to reducing the negative effect of prolonged storage on the incubation yield.

In conclusion, numerous interactions in selected growth parameters of turkey embryos indicate that the quality of hatching eggs changes with hen's age, affecting their suitability for long-term storage under standard conditions. Most of the noted changes were not linear, which makes it difficult to interpret the results of this study. However, they suggest that eggs laid by younger breeders should not be stored for longer periods due to undesirable changes in RWY and RWA. Further research is needed to determine the underlying mechanisms of changes in egg quality in order to modify storage conditions and optimize the use of hatching eggs, taking into account hen's age and egg storage duration.

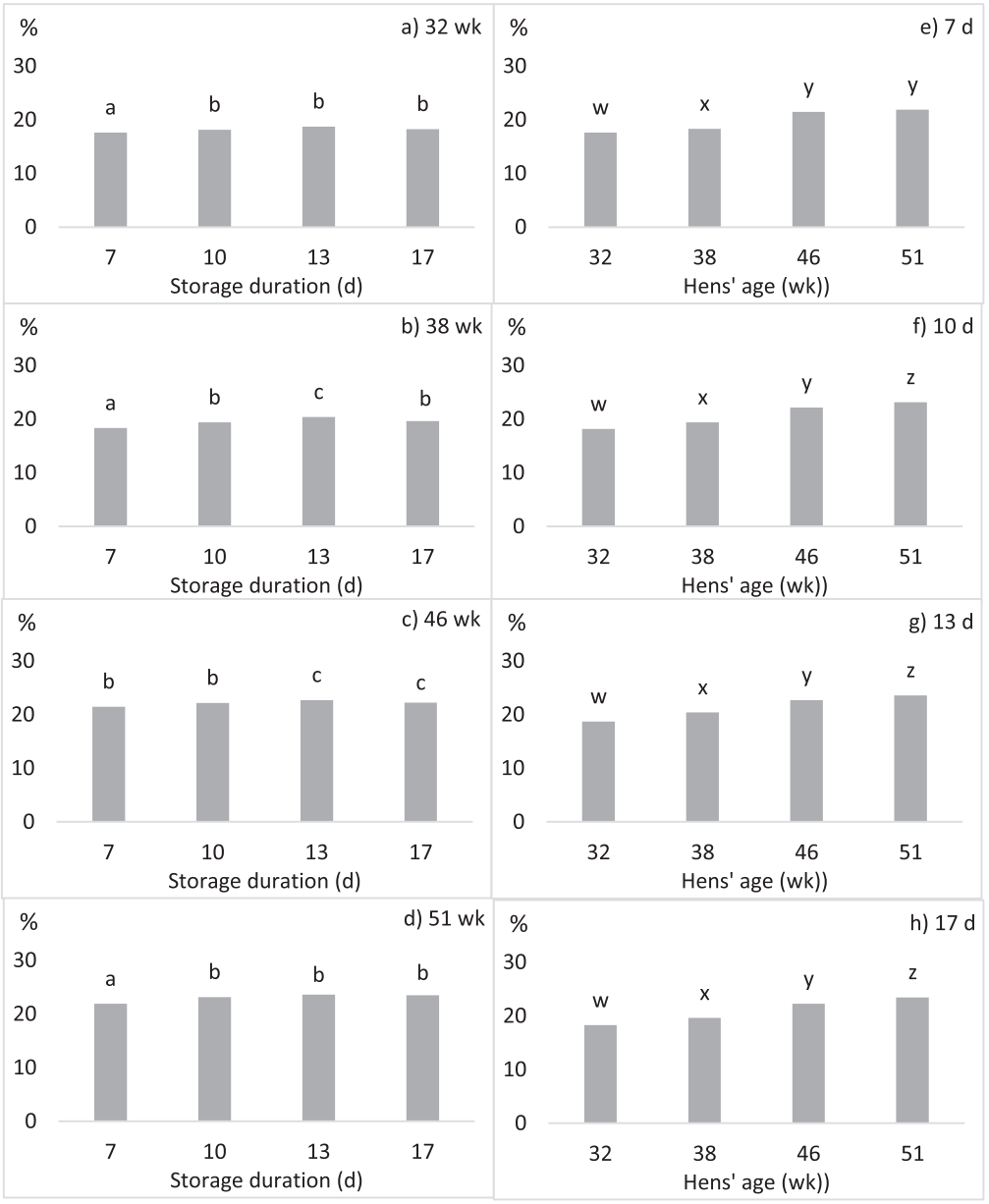

Figure 6. Relative weight of the yolk sac (%) in eggs laid by hens aged 32−51 wk, stored for 7−17 d, on 24th day of incubation—interaction (means for subgroups). a-c- means for subgroups values followed by different letters differ significantly ($P < 0.05$). w-z - means for subgroups values followed by different letters differ significantly ($P < 0.05$).

DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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