

## Storage Time and Eggshell Colour of Pheasant Eggs vs. the Number of Blastodermal Cells and Hatchability Results

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The aim of this study was to investigate the number of the embryo blastodermal cells and hatchability of pheasant from eggs of different eggshell colours depending on the length of the storage period before hatching. On the day of collection, dark-brown and olive eggs were characterised by a similar and significantly higher (by about 48%) number of embryo BCs in comparison with light-brown and blue eggs. Dark-brown eggs stored longer than one day had the highest, while blue-shelled eggs the lowest, number of BCs. The number of BCs found in eggs with blue and light-brown coloured eggshells stored for 10 days was similar and significantly lower (by 27.7%) in comparison with dark-brown eggs. With the lengthening of the storage period, the number of blastodermal cells in eggs of all eggshell colours declined as a result of necrobiosis. In comparison with the dark-brown and olive-shelled eggs, eggs with blue eggshells had higher (by about 7.0%) weight loss during the 21 days until hatching. The dark-brown and olive eggs were found to have a 10.3% higher proportion of eggs considered as fertilised in comparison with the blue-shelled eggs. Eggs with dark-brown shells stored for 2-4 days prior to hatching, in comparison with blue-shelled eggs, had a higher proportion of fertilised eggs. The dark-brown and olive eggs stored for 7 and more days before hatching possessed a higher value of this trait in comparison with the eggs of light-brown and blue eggshells ( $\bar{x} = 80.9$  against 66.4%). The highest drop in the share of fertilised eggs, which amounted on average to 3.25% for each day of storage, was observed in the blue-shelled eggs. The dark-brown eggs stored for 7 days before being placed in an incubator had higher hatchability from fertilised eggs (by 17.8%) in comparison with the eggs with blue eggshells. In the case of eggs stored for 8 to 10 days, values for this trait were higher for the dark-brown and olive-coloured eggs than for the blue-shelled eggs. The highest mean decrease of chick hatchability from fertilised eggs was observed in the case of the blue-shelled eggs (7.93% for each day). The dark-brown eggs had significantly higher (by about 22.0%) chick hatchability from fertilised eggs than the blue-shelled eggs. Moreover, the dark-brown and olive eggs, in comparison with the blue-shelled eggs, were characterised by a significantly higher hatchability after each period of storage before incubation. The highest negative trend-cycle was observed for eggs with blue shells, while the smallest – for olive-shelled eggs. Directly after laying, pheasant eggs differed with regard to the developmental advancement of the blastodermal embryo depending on eggshell colour. Longer storage time caused the numbers of blastodermal cells in eggs to decrease. The group with blue shells had a lower proportion of fertilised eggs and lower hatching results than the dark-brown eggshell group. It was also demonstrated that the value of hatchability indices decreased significantly irrespective of eggshell colour after seven days of storage prior to hatching.

Key words: Pheasant, blastodermal cells, hatchability, incubation.

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Profitability of farm pheasant rearing depends, first and foremost, on the reproductive possibilities of the pheasants, i.e. the obtained number of eggs and, consequently, the number of healthy chicks. The results of artificial hatchings have not been satis-

factory so far (DEEMING & WADLAND 2001; KIRIKCI *et al.* 2003). This is caused by a number of different factors, among others, differences in eggshell colour (HULET *et al.* 1978; KRYSZTIANIAK *et al.* 2000) as well as the length of the storage period prior to hatching.

The relationship between the colour of pheasant eggs and hatchability has not been adequately explained. Nevertheless, in some European countries eggs with blue shells are not intended for hatching because they are considered to be of worse quality (RICHARDS & DEEMING 2001). HULET *et al.* (1985) as well as KOZUSZEK and KONTECKA (2005) demonstrated that hatching results were better for pheasant eggs with dark-brown and olive shells in comparison with those recorded in the case of eggs with light-brown and blue shells. Similar results were obtained in the case of laying hens in which eggs of light shells were characterised by lower fertilisation rate and worse hatchability results than eggs of darker shell colour (ZGŁOBICA & WEŻYK 1995). On the other hand, a correlation was found between the eggshell colour and some physical traits of eggs (SCOTT & SILVERSIDES 2000; KRYSZTIANIAK & KONTECKA 2002; SILVERSIDES & BUDGELL 2004) which can indirectly influence chick hatching.

It is well known that with the lengthening of the egg storage period before hatching, unfavourable physico-chemical changes take place in the egg content (SCOTT & SILVERSIDES 2000; SAMLI *et al.* 2005; JONES & MUSGROVE 2005) and increased penetration of microorganisms into eggs is observed (MESSENS *et al.* 2006) and these, in turn, may lead to the deterioration of hatchability results (DORN *et al.* 1982; FASENKO *et al.* 2001a; TONA *et al.* 2004). Investigations carried out on chickens showed that the hatchability of eggs stored for 3 days before setting was by about 8.2 % higher than from eggs which were stored for 6 days. In the case of Japanese quail, no significant differences were observed in hatchability from eggs stored for 1, 3, 5 and 7 days, nevertheless the hatchability was about 1 % higher in the case of eggs stored for 1 day than for 7 days (PETEK *et al.* 2005). On the other hand, in African ostriches, hatching results deteriorated when eggs were stored for longer than 7 days before setting (WILSON *et al.* 1997). So far, no unequivocal information is available on how long pheasant eggs intended for hatching can be stored. In practice, they are frequently set into the incubator even after 10 days of storage.

Investigations on blastodermal cells (BCs) have been conducted for many years (ETCHES *et al.* 1997; BLOOM *et al.* 1998; LI *et al.* 2002; BEDNARCZYK *et al.* 2003). In experiments carried out on chickens, it was found that numbers of blastodermal cells decreased together with the lengthening of the egg storage period (KOZUSZEK *et al.* 2004). Moreover, it was demonstrated that in comparison

with eggs with cream and cream-white shells, eggs with blue and white egg shells were characterised by smaller numbers of BCs.

The aim of this study was to investigate numbers of embryo blastodermal cells and pheasant hatchability from eggs of different eggshell colour in relation to the length of egg storage before hatching. In addition, an attempt was made to determine the optimal storage time of pheasant eggs before their setting.

## Material and Methods

The experimental material comprised pheasant eggs derived from the farm of the Export Hunting Corporation in Maniszewo. One-year old birds were kept in aviaries (harem-type mating; 1 male and 9 females) and fed complete diets containing 11.7 MJ/kg metabolisable energy, 18.0 % crude protein and 3.05 % total calcium.

### Assessment of the number of embryo blastodermal cells (BCs)

A total of 1760 eggs, i.e. four groups of 440 eggs, of the following colours: dark-brown, light-brown, olive and blue were selected from all collected eggs. From each of the four colour groups, 40 eggs were selected and subjected to evaluation on the day of laying (0 days of storage). The remaining number of eggs (1600 eggs) was divided (within each colour group) into 10 subgroups and stored for 1 to 10 days in a chamber with constant temperature and relative humidity of 15°C and 70%, respectively. The number of eggs in the subgroup amounted to 40. Embryo blastodermal cells were isolated using the method of CHEŁMOŃSKA *et al.* (1997) and CZIRÓK *et al.* (2002). After breaking the egg and separating the yolk, a disc of blotting-paper was placed on the blastodisk and cut out with the blastodisk attached to it and washed using 2 ml PBS physiological saline into a test tube. Next, the obtained suspension was centrifuged for 5 minutes (2000 rpm). After centrifugation, the blastodermal cells were deposited as the top layer. The cells were counted in a Bürker chamber according to the method described by BOMSKI (1983) using for this purpose a microscope and the MultiscanBase v. 8.08 computer image analysis program (CIA). The number of BCs in 1 mm<sup>3</sup> suspension was calculated according to the following formula:

$$\text{Number of BCs in 1 mm}^3 = \frac{\text{number of BCs in 0.256 mm}^3 \text{ suspension} \times 1 \text{ mm}^3}{0.256 \text{ mm}^3}$$

### Evaluation of hatching results and egg weight losses during hatching

The experimental hatchings were carried out during the reproductive season of pheasants in the hatchery of the Maniszewo Farm. From over 20 000 eggs, 8448 eggs of similar weight (2112 eggs in each of the following colour categories: dark-brown, light-brown, olive and blue) were selected on the day of laying. Moreover, each eggshell colour group was further divided into 11 subgroups of 192 eggs each. Eggs from the first subgroup were set for hatching on the day of laying (0 days of storage), while the remaining subgroups were stored for 1 to 10 days in the warehouse of the hatchery at a temperature of about 15°C and relative humidity of 65%. Egg disinfection was carried out using an air ionizer which remained on throughout the storage period. Experimental eggs were weighed before setting and then disinfected using formaldehyde vapours. Hatching was conducted in BIOS OB-45 type incubators employing the hatching technique and parameters adopted by the hatchery of the Maniszewo Farm. Until day 21 of hatching, eggs were kept in the setting compartment in which the temperature was adjusted to 37.7°C, relative humidity maintained at 49-52% and, during this period, eggs were turned every hour by an angle of 90°. On the 7<sup>th</sup> and 21<sup>st</sup> day of hatching, the eggs were candled in order to eliminate infertile eggs as well as eggs with dead embryos. In addition, on day 21 of hatching, 30 eggs from each colour group were randomly selected and weighed and then transferred into the hatching compartment in which the temperature was 37.6°C and relative humidity was 75-82%. Chicks hatched between day 24 and 26 of incubation.

On the basis of the obtained data and taking into account the division of eggs into groups (eggshell colour) and subgroups (length of egg storage period), the following parameters were calculated:

1. Egg fertilisation (%); eggs were considered fertilised after the elimination of those which did not exhibit signs of embryo development (infertile and apparently infertile eggs) during the first candling,
2. Hatching (%) from the set and fertilised eggs,
3. Egg weight loss during hatching (g and %) from the difference of egg weight before setting and on day 21 of hatching.

### Statistical calculations

Mean values ( $\bar{x}$ ) and standard errors (SEM) were calculated for the obtained results concerning the number of blastodermal cells, weight of eggs be-

fore setting and egg weight losses during hatching. Differences between the egg groups of different eggshell colour and the length of the storage period with regard to the number of BCs were determined by a two-way ANOVA. Differences between egg groups of different eggshell colour with reference to weight and its loss during hatching were calculated using a one-way ANOVA. A Fisher's test was employed to verify statistically the significance of differences between values of the examined traits.

Differences between egg groups of different eggshell colour and the length of the storage period regarding their fertilisation and hatchability were estimated using the fraction test (GREŃ 1976). Differences between egg groups of different eggshell colour with regard to their fertilisation and hatching results were calculated using a one-way ANOVA, whereas significance of differences of these traits was verified by Fisher's tests. The performed calculations were conducted using the SAS<sup>®</sup> v. 9.1 statistical package.

Changes in the number of blastodermal cells as well as indices of fertilisation and chick hatchability from eggs of different eggshell colour in relation to the length of the storage period were presented in the form of regression equations and were used to plot a trait linear trends-cycle according to the formula given by ZAJĄC (1988):

$$y_t = a + b_t,$$

in which:

- a – trait value at zero period,
- b – (directional) regression coefficient  
expressing the daily change of the trait value,
- t – time expressed.

### Results

Data from the day of egg collection (0 days of storage) reveal that eggs with a dark-brown and olive eggshell colour were characterised by a similar and statistically greater (by about 48%) number of BCs in comparison with eggs from the remaining two eggshell colour groups (Table 1). Dark-brown eggs had the highest mean number of BCs in eggs stored for more than 1 day. On the other hand, the smallest values for this trait in consecutive days were recorded for eggs with blue coloured shells. In comparison with the dark-brown eggs, blue- and light-brown-shelled eggs stored for 10 days were found to have similar and a significantly smaller (by about 27.7%) number of BCs. Longer egg storage time led to a decline in the number of blastodermal cells as a result of dying but this decline was significant only in eggs with dark-brown and olive shells in which, on average, 11.3 blasto-

Table 1

Number of blastodermal cells (bcs per 1 mm<sup>3</sup>) in pheasant eggs of different eggshell colour depending on storage time

Storage time (days)	Eggshell colour							
	Dark-brown (n=440)		Light-brown (n=440)		Olive (n=440)		Blue (n=440)	
	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM
0	286.4 a	16.2	159.4 b	11.4	252.0 a	14.8	99.8 c	8.2
1	251.5 a	15.4	149.7 b	10.3	233.1 a	15.3	81.1 c	6.8
2	242.4 a	20.1	133.4 b	9.4	227.5 a	14.5	65.6 c	5.3
3	231.1 a	14.0	127.8 b	7.6	183.1 c	12.9	61.6 d	6.9
4	219.1 a	16.1	107.0 b	7.3	167.4 c	12.1	57.4 d	5.6
5	286.8 a	11.5	82.3 b	7.7	138.9 c	11.6	53.5 d	6.1
6	258.4 a	9.1	77.7 b	6.1	132.3 c	10.6	46.8 d	6.1
7	244.8 a	10.8	73.8 b	5.0	87.5 b	10.3	35.1 c	4.9
8	213.6 a	10.4	65.4 b	6.8	72.7 b	8.0	26.1 c	3.7
9	90.2 a	9.5	28.1 b	4.4	52.9 c	6.0	18.6 b	3.1
10	66.8 a	7.5	24.0 bc	3.4	33.0 b	4.8	13.0 c	2.1

Mean values designated in rows with different letters differ significantly at the level  $P \leq 0.05$ .

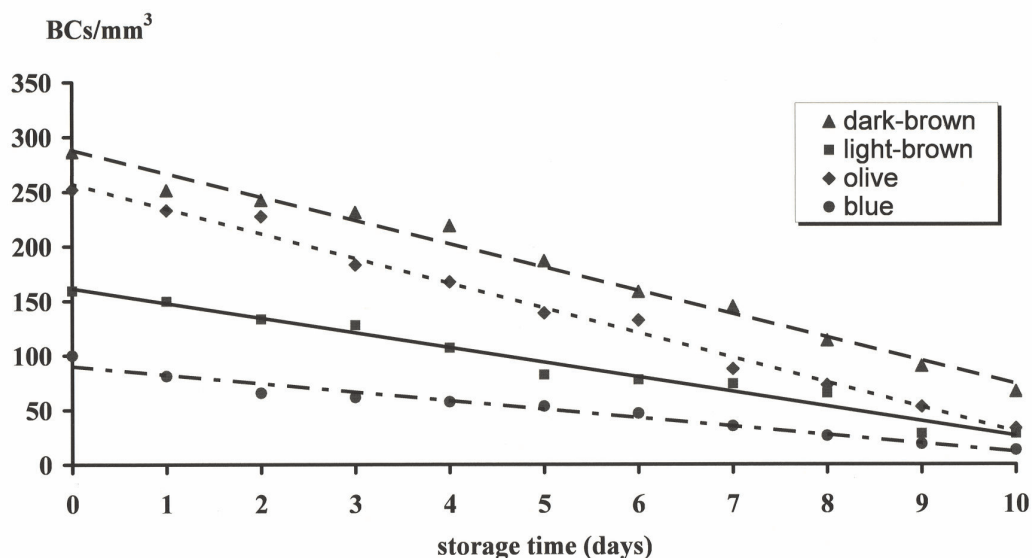


Fig. 1. Trend-cycle of blastodermal cell number in pheasant eggs of different shell colour depending on storage time. Dark-brown eggs:  $y = 299.10 - 10.74x^*$ ; light-brown eggs:  $y = 168.70 - 6.84x$ ; olive eggs:  $y = 272.2 - 11.80x^*$ ; blue eggs:  $y = 94.10 - 3.94x$ .  
\*Trend-cycle statistically significant

dermal cells died every day (Fig. 1). Blue-shelled eggs were characterised by the slowest rate of BC necrobiosis, namely 3.94 cells per each day of storage.

The eggs with dark-brown shells were heaviest, whereas olive-shelled eggs were lightest (Table 2). This difference was statistically significant and amounted to 2.02 g. On the other hand, in compari-

son with dark-brown and olive coloured eggs, the blue-shelled eggs were characterised by approximately 7.0% higher weight loss during 21 days of hatching. The smallest mean weight loss (about 12.2%) was recorded in the group of olive-shelled eggs.

The percentage of eggs considered as fertilised stored for 1 to 10 days prior to setting is presented

Table 2

Egg weight and egg weight loss during incubation depending on eggshell colour

Traits	Eggshell colour							
	Dark-brown (n=330)		Light-brown (n=330)		Olive (n=330)		Blue (n=330)	
	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM	$\bar{x}$	SEM
Egg weight (g)	33.99 a	0.45	32.88 ab	0.59	31.97 b	0.50	32.08 b	0.48
Egg weight on 21st day of incubation (g)	29.48 a	0.32	27.60b	0.46	27.98 b	0.39	25.68 c	0.62
Egg weight lost during 21st days of incubation (g)	4.52 a	0.50	5.28 ab	0.70	3.99 a	0.52	6.40 b	0.76
Egg weight lost during 21st days of incubation (%)	12.99 a	1.35	15.56 ab	1.88	12.18 a	1.46	19.56 b	2.19

Mean values designated in rows with different letters differ significantly at the level  $P \leq 0.05$ .

Table 3

Mean values of fertility and hatchability results for pheasant eggs of different eggshell colour depending on storage time before hatching

Storage time	Egg fertilisation (%)				Hatching from fertilised eggs (%)				Hatching from set eggs (%)			
	Eggshell colour											
	Dark-brown	Light-brown	Olive	Blue	Dark-brown	Light-brown	Olive	Blue	Dark-brown	Light-brown	Olive	Blue
0	95.8 ADa	92.7 Bb	93.7 Cc	89.6 Da	96.7 Abb	87.6 Aa	94.4 Ca	83.1 BCc	92.7 ABb	81.2 Aa	88.5 Ca	74.5 BCc
1	93.2 a	89.1 b	94.8 b	90.6 c	97.8 ABC	87.7 Aa	92.3 BDa	82.2 CD	91.1 AB	78.1 AC	87.5 CD	74.5 BD
2	93.7 ab	87.0 a	89.1 c	86.5 b	91.1 Ab	85.6 Ba	92.4 Ca	72.3 ABCc	85.4 ABb	74.5 Aa	82.3 Cc	62.5 BCa
3	92.2 a	87.5 b	86.5 c	84.4 a	87.6 A	81.5 B	84.9 C	72.2 A	80.7 Aa	71.3 Bab	73.4 Ac	60.9 ABbc
4	90.6 a	85.4 b	87.0 c	82.3 a	81.6 ABCb	66.5 Aa	72.4 BDc	57.0 CDa	74.0 ABCa	56.8 Ab	63.4 Bc	46.9 Cc
5	89.1 Aa	82.3 Bb	86.5 Cc	76.6 Ac	67.9 Ac	63.3 Ba	62.6 Cb	50.3 Aab	60.4 A	52.1 B	54.2 C	38.5 ABC
6	87.0 Aa	78.6 Bb	84.9 Cc	75.0 Ac	62.3 A	57.6 B	59.5 C	43.0 ABC	54.2 A	45.3 B	50.5 C	32.3 ABC
7	85.9 ABa	75.0 Ab	83.8 Cb	70.3 BCc	54.5 A	50.0 B	52.2 C	37.0 ABC	46.9 A	37.5 A	43.8 B	26.0 AB
8	81.2 AB	68.7 AC	80.7 CD	64.1 BD	48.1 Ac	38.6 Ca	45.8 Bb	27.6 ABa	39.1 ABb	26.1 ACa	37.0 CDc	17.7 BDa
9	79.2 AB	66.1 AC	79.2 CD	59.4 BD	45.4 ABb	29.1 ACa	42.8 Da	10.5 BCDC	35.9 AB	19.3 ACD	33.9 CE	6.2 BDE
10	77.6 AB	64.1 AC	79.7 CD	63.5 BD	34.2 AB	14.6 AC	28.1 CD	9.8 BD	26.6 AB	9.4 AC	22.4 CD	6.2 BD
Mean	87.7 ac	79.7 c	86.0 b	76.6 ab	69.7 a	60.2 b	66.1 c	49.5 d	62.5 a	50.1 b	57.9 c	40.6 a

Mean values designated in rows with the same letters differ significantly at the level:

small letter  $P \leq 0.05$

capital  $P \leq 0.01$

in Table 3 and, in the form of trend-cycles, in Figs 2-4. Mean values of this trait for eggs intended for hatching on the day of collection ranged from 89.6% to 95.8%, depending on the eggshell colour, while in the case of eggs set after 10-day storage – from 63.5% to 79.7%. Generally speaking, a

higher proportion of dark-brown and olive-shelled eggs were fertilised than blue-shelled eggs. This difference was statistically significant and reached 10.3%. Eggs with dark-brown shells stored for 2-4 days prior to hatching, in comparison with blue-shelled eggs, were characterised by a higher pro-



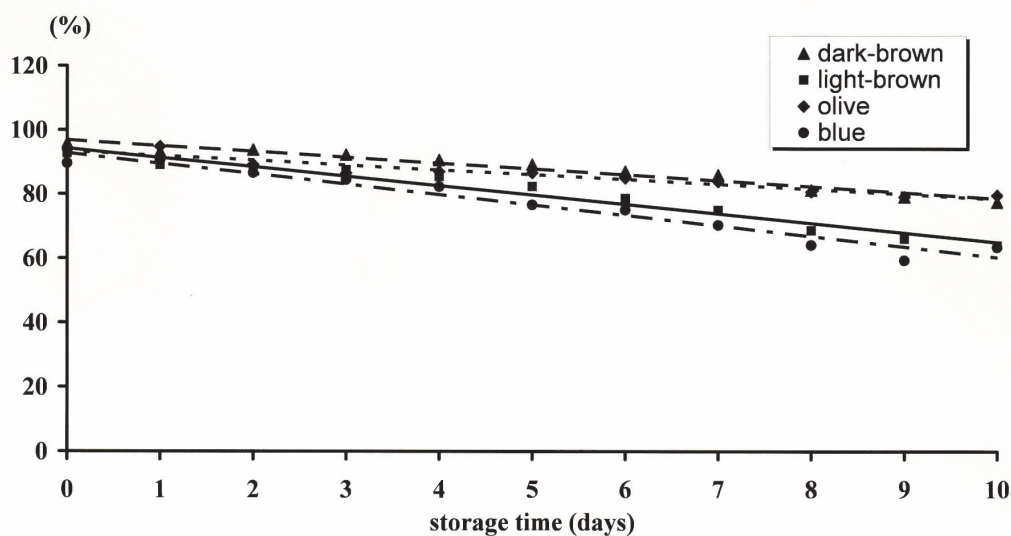


Fig. 2. Trend-cycle of fertilisation of pheasant eggs of different shell colour depending on storage time. Dark-brown eggs:  $y = 96.90 - 1.82x$ ; light-brown eggs:  $y = 94.31 - 2.92x^*$ ; olive eggs:  $y = 92.54 - 1.78x$ ; blue eggs:  $y = 92.84 - 3.25x^*$ . \*Trend-cycle statistically significant.

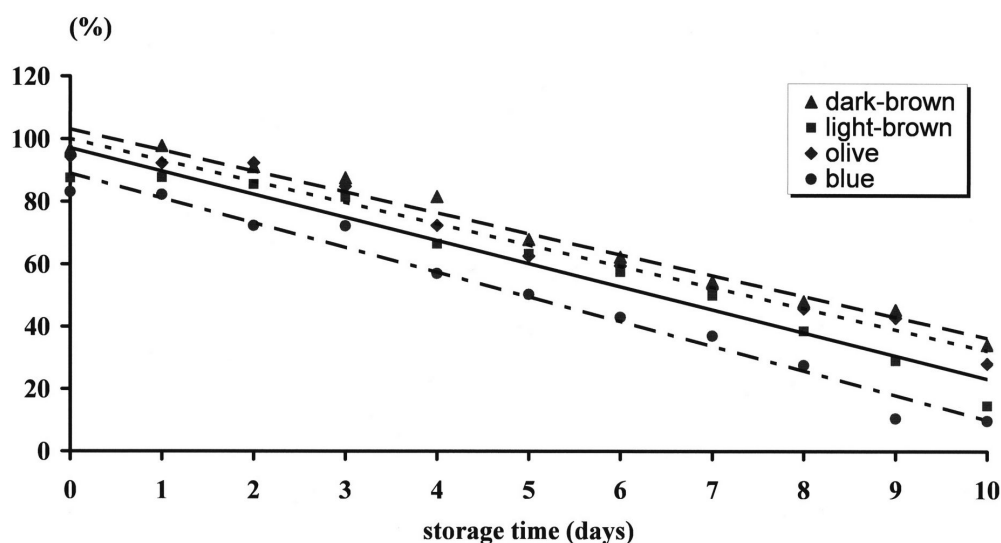


Fig. 3. Trend-cycle of hatching from fertilised pheasant eggs of different shell colour depending on storage time. Dark-brown eggs:  $y = 103.67 - 6.70x^*$ ; light-brown eggs:  $y = 97.11 - 7.38x^*$ ; olive eggs:  $y = 100.11 - 6.80x^*$ ; blue eggs:  $y = 89.17 - 7.93x^*$ . \*Trend-cycle statistically significant.

portion of fertilised eggs. The proportion of eggs considered as fertilised stored for 5 to 6 days prior to setting was significantly higher in eggs with dark-brown and olive shells (by about 11.1%) in comparison with the blue-shelled eggs. On the other hand, eggs from these groups stored for 7 and more days before setting had higher values of this trait in comparison with light-brown and blue eggs (= 80.9 against 66.4%). It is evident from the data presented in Figure 2 that with the lengthening of

egg storage time the number of eggs considered as fertilised decreased, with blue-shelled eggs having the greatest decline of this trait amounting to, on average, 3.25% for each day of storage.

Mean hatching results from fertilised eggs set into the incubator on the day they were laid (0 days of storage) ranged from 83.1% to 96.7%, depending on the eggshell colour, with the highest hatch observed in dark-brown eggs, while the lowest in blue-shelled eggs. On the other hand, mean hatch-

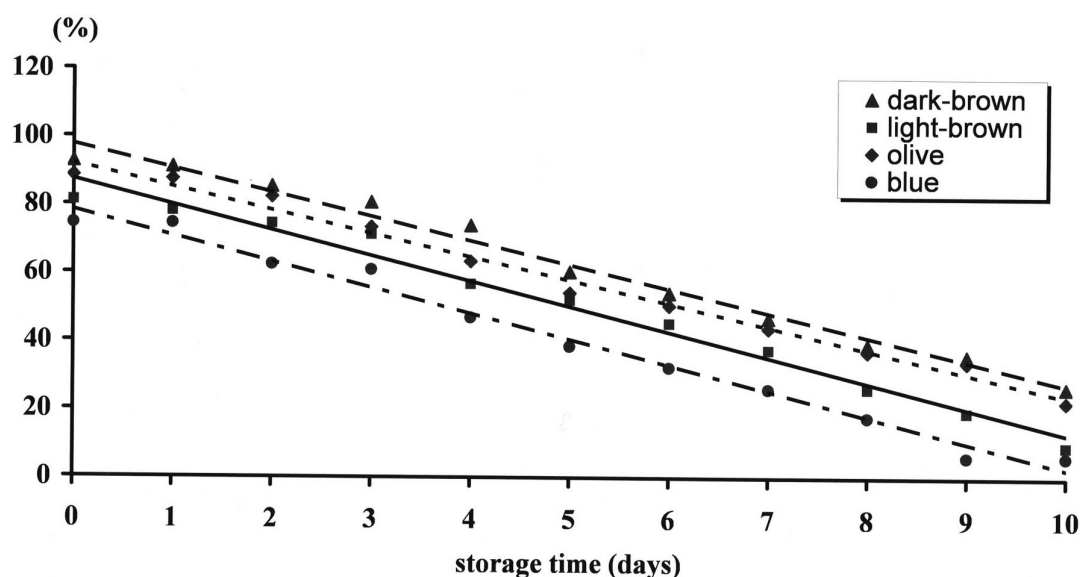


Fig. 4. Trend-cycle of hatching from set pheasant eggs of different shell colour depending on storage time. Dark-brown eggs:  $y = 97.80 - 7.07x^*$ ; light-brown eggs:  $y = 87.37 - 7.44x^*$ ; olive eggs:  $y = 93.47 - 6.08x^*$ ; blue eggs:  $y = 78.44 - 7.58x^*$ . \*Trend-cycle statistically significant

ing results from fertilised eggs set for hatching after 10-day storage fluctuated from 9.8% to 34.2%. Dark-brown eggs stored for 7 days before incubation were characterised by a better hatch from fertilised eggs in comparison with the eggs with blue eggshells. This difference was statistically significant and, on average, amounted to 17.8%. In the case of eggs stored for 8 to 10 days, values of this trait were found higher for dark-brown and olive-coloured eggs than for blue-shelled eggs. A significantly higher mean decrease of hatchability from fertilised eggs was observed in the case of blue-shelled eggs (7.93% for each day). This value was 1.23% higher in comparison with the lowest value determined for eggs with dark-brown eggshells (Fig. 3).

Dark-brown set eggs had significantly higher hatchability (by about 22.0%, Table 3) than the blue-shelled eggs. Moreover, dark-brown and olive eggs, in comparison with the blue-shelled eggs, were characterised by a significantly higher hatchability after each period of storage before hatching. On average, this difference amounted to 19.6%. The highest negative trend-cycle was observed for eggs with blue shells while the smallest was determined for olive-shelled eggs with the difference amounting to only 1.5 percentage points (Fig. 4).

## Discussion

Eggs with dark-coloured eggshells stored for a period of 0 to 10 days, in comparison with their

light-coloured counterparts, were characterised by higher numbers of BCs already on the day of laying. According to BORZEMSKA (2005), necrobiosis of blastodermal cells in the oviduct, and hence a smaller number of BCs after laying, can be caused by, among others, the occurrence of disease, penetration of external toxins into the egg, improper environmental conditions, poorly balanced nutrition as well as by lethal genes. The smallest numbers of BCs in consecutive days of the experiment were found for the blue-shelled eggs, while the highest value was determined in the dark-brown ones. It was also shown that numbers of BCs declined with the lengthening of the storage period. In studies on the numbers of embryo BCs in pheasant eggs stored for 1 to 7 days, KOZUSZEK and KONTECKA (2004) found that BC numbers decreased with the passage of storage time. In addition, the same researchers demonstrated that the highest mean number of BCs were observed in eggs examined 1 day after storage. Moreover, in turkey embryos, BAKST and AKUFFO (2002) found that the lengthening of the egg storage time (over 10 days) caused the blastoderm to become asymmetric and weak, i.e. the number of cells decreased.

In investigations carried out on chickens, it was demonstrated that eggs with dark-shells derived from the green-legged partridge were characterised by greater mean numbers of BCs than blue-coloured eggs obtained from the Araucana (KOZUSZEK *et al.* 2004). It should also be mentioned that in the case of the green-legged partridge, more BCs were determined in dark-coloured eggs than

in eggs with light shells. HUTT (1968) claims that brown pigment occurs primarily in the external part of the eggshell, whereas the blue colour penetrates through the entire shell thickness. Moreover, Warren and Conrad (after HULET *et al.* 1985) found that majority of the brown pigment appears during the last few hours of the egg's stay in the shell gland and the quantity of the pigment deposited in the shell is proportional to the length of time the egg stays in this section of the oviduct. Therefore, the shorter the egg stays in the oviduct, the less brown pigment is deposited on it. Hence, it can be said that the observed smaller number of blastodermal cells in pheasant eggs with blue eggshells can be attributed to their shorter stay in the oviduct and, consequently, their less advanced embryonic development.

The highest proportion of fertilised eggs was observed in eggs with dark-brown eggshells, while blue-shelled eggs exhibited the lowest level of fertilisation. Similar results were reported by HULET *et al.* (1985), MRÓZ and PUDYSZAK (2000), KRYSZTIANIAK *et al.* (2005), KU NIACKA (2005) and KU NIACKA *et al.* (2005) confirming that eggs with dark eggshells were characterised by higher fertilisation in comparison with light-coloured eggs. In our experiments, blue-shelled eggs had lower hatchability than the remaining groups. Similar results for pheasants were obtained by HULET *et al.* (1978; 1985) who also demonstrated that the best hatching from the set and fertilised eggs was achieved in the case of dark-brown and olive-coloured eggs and worse results were recorded for light-brown and blue-coloured eggs. KRYSZTIANIAK *et al.* (2005) reported inferior hatching from fertilised and set eggs for eggs with blue eggshells. On the other hand, MRÓZ and PUDYSZAK (2000), KU NIACKA (2005) as well as KU NIACKA *et al.* (2005) showed better hatching from eggs with darker eggshells.

In our experiments, it was found that with the lengthening of the egg storage period before setting, irrespective of the eggshell colour, the hatching results of chicks decreased. Also KOZUSZEK and KONTECKA (2005) found a smaller percent of eggs considered as fertilised and worse results of hatching (on average by 10.6 and 18.4 %, respectively) from dark-brown and light-brown as well as blue-shelled eggs stored for 4 days in comparison with eggs set for hatching on the day of laying. WOODARD and MORZENI (1975) demonstrated that pheasant eggs set for hatching after storage for 1 to 7 days were characterised by a significantly better hatchability than those which were stored longer. On the other hand, KU NIACKA (2005) demonstrated that pheasant eggs stored for 3 to 9 days before hatching had higher fertilisation values than those stored for 10 to 11 days. What is interesting is that eggs set for hatching after 1 to 2-day storage were characterised by even up to

13.2% lower fertilisation than eggs stored longer. This author also demonstrated that a higher hatchability from set and fertilised eggs was found in eggs set for hatching following 3-6 days of storage. On the other hand, eggs of broiler breeders stored for 4 days before setting were characterised by 17.5 and 17.2% higher hatching from set and fertilised eggs than those stored for 14 days (FASENKO *et al.* 2001b).

BORZEMSKA (2005) maintains that chicken eggs are not ready for hatching directly after laying and should be set into incubators 3-4 days after laying. Quoting results of other investigations, the above researcher claims that during the first two days after laying, various egg structures become stabilised, such as layers of the albumen and chalaza, loose fibres of the inner shell membrane become compact and shell calcification is finalised. In addition, chicken egg incubation without earlier storage leads to weaker gas exchange for blastodermal cells situated at the point of contact under an excessively thick layer of albumen (BENTON *et al.* after BORZEMSKA 2005). The deterioration of hatchability associated with increased embryo necrobiosis in eggs can be caused by the significant worsening of internal egg traits together with prolonged egg storage before hatching, leading to decreased biological value (TONA *et al.* 2004; SAMLI *et al.* 2005). In experiments carried out on chickens it was demonstrated that hatchability decreased together with the lengthening of storage period before egg setting (MEIJERHOF *et al.* 1994; KUURMAN *et al.* 2002). On the other hand, SKRZYDLEWSKI and PAWLAK (1993) showed that eggs of Pekin ducks stored for 4 and 7 days before setting had lower hatching results than those stored for 1 day. Similar relationships were shown in experiments carried out on other poultry species (FASENKO *et al.* 2001a; PETEK *et al.* 2005).

In our studies, better hatchability results from fertilised eggs, in comparison with blue-shelled eggs, were recorded for the dark brown eggs set both on the day of laying as well as those stored for 1 to 10 days. Similar results were recorded in the case of hatching from set eggs. The lowest hatching rates, both from the fertilised and set eggs, were demonstrated for light-brown and blue-shelled eggs stored for 10 days. In addition, it was found that values of these traits declined significantly together with the lengthening of the storage period of pheasant eggs, in particular for blue-shelled eggs. In experiments conducted by KOZUSZEK and KONTECKA (2005), the lowest hatchability recorded for fertilised eggs ( $\bar{x} = 52.5\%$ ) was also obtained for blue-shelled eggs following a 4-day storage.

Lower hatchability achieved in the case of blue-shelled eggs in comparison with eggs of other shell



colours could have been caused by, among others, excessive water loss during hatching. This, in turn, could be attributed to the fact that blue-shelled eggs have a thinner shell and greater number of pores. In the presented experiments, the loss of weight in blue-shelled eggs was the highest and reached 19.6%. KRYSZTIANIAK *et al.* (2005) reported similar results; by day 21 of hatching, blue-shelled eggs pheasant eggs lost the highest, i.e. 20.3%, amount of their initial weight. In addition, the above mentioned researchers showed that blue-shelled eggs had the highest eggshell water vapour conductance, associated with smaller eggshell thickness and greater number of pores. Furthermore, blue-shelled eggs were found to possess structural deviations in their shell ultrastructure.

In summary, it can be said that directly after laying, pheasant eggs differed with regard to the advancement of the blastodermal embryo development depending on eggshell colour. Blue-shelled eggs were characterised by the smallest number of BCs. With the lengthening of storage time, numbers of blastodermal cells in eggs decreased but the necrobiosis rate of BCs was faster in eggs with dark-brown and olive eggshells. Blue-shelled pheasant eggs had lower fertilisation and worse hatchability results than eggs with dark-brown eggshells. It was also demonstrated that the value of hatchability indices decreased significantly irrespective of the eggshell colour after 7 days of storage prior to hatching. Bearing in mind the observed deteriorating hatchability results with the lengthening of the storage period of pheasant eggs, it is recommended that eggs should be placed in the incubator directly after collection, i.e. not later than 7 days after collection. It is also worth emphasizing that blue-shelled pheasant eggs, due to their significantly poorer hatching results in comparison with others, should not be set for hatching if they were stored for more than 3 days.

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