Animal Science Papers and Reports vol. 23 (2005) no. 1, 5-14
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Quality and ultrastructure of eggshell and hatchability of eggs in relation to eggshell colour in pheasants

Stanisława Krystianiak, Radosław Kożuszek, Helena Kontecka, Sebastian Nowaczewski

Department of Poultry Breeding,
The August Cieszkowski Agricultural University of Poznań
Witosa 45, 61-693 Poznań, Poland

(Received October 27, 2004; accepted January 5, 2005)

A total of 990 ring-necked pheasant hatching eggs were examined in relation to their shell colour –dark-brown, light-brown, olive, blue. Eggshell thickness, water vapour conductance and number of pores per 0,25 cm^2 of eggshell were analysed in 285 eggs. Moreover, shell ultrastructure was examined of six eggs of each colour and three incubation sets were carried out with 681 eggs. Shells of blue eggs were found thinner, had higher water vapour conductance and showed higher density of pores than those of remaining three colours. In addition, structural abnormalities of the blue eggshells were found. Blue eggs were characterized by lower fertilization rate, greater weight loss to day 21 of incubation and poorer hatchability. It is concluded that blue-shelled pheasant eggs should be eliminated when selecting eggs for incubation.

KEY WORDS: eggshell / hatchability / pheasant / ultrastructure

Profitability of pheasant rearing depends, among other things, on their reproductive capacity, *i.e*. the number of eggs laid, and, as a result, on the number of chicks hatched. Avian hatchability results depend largely on the quality of eggs, and especially their shells [Dohnal *et al.* 1989, Szczerbińska 1997]. Some physical traits of eggs were shown to be related to eggshell colour [Krystianiak and Kontecka 2002] and hatchability [Dohnal *et al*. 1989, Mróz and Pudyszak 2000]. The eggshell is composed of the palisade and mamillary layers which both affect its structure and thickness [Fraser *et al.* 1999, Malec *et al.* 1999] and thus the conditions for embryo development. Thinner eggshells are associated with poorer hatchability [McDaniel *et al.* 1979]. Roberts *et al.*

[1995] report that thinner eggshells are characterized by the early joining of mamillary cones, which reduces the mamillary layer of the shell. In addition, Carnarius *et al.* [1996] showed that chicken eggshell resistance is related to the thickness of the mamillary layer. Bunk and Ballown [1978] found that the mamillary layer of eggs with poorer shell quality shows some structural abnormalities. Attached to the shell are two membranes – inner and outer [Solomon 1994]. Analysis of eggshells from different strains of chicken revealed significant differences in the thickness of outer shell membrane and its fibres [Gielecki *et al.* 1995].

Pheasants lay eggs of different shell colour: dark-brown, light-brown, olive and blue [Krystianiak *et al.* 2000] and the unsatisfactory reproductive results in pheasants may probably be ascribed to differences in eggshell structure and quality related to and expressed by eggshell colour. The objective of this study was to analyse the eggshell quality and hatchability from pheasant eggs as related to eggshell colour.

Material and methods

The study was performed in year 2000 during the reproductive period of ring-necked pheasants at a farm belonging to the Agricultural University of Poznań. Two-year-old birds (10 males and 50 females) were kept in ten aviaries (1 male and 5 females) and fed a complete diet containing 11.7 MJ/kg metabolizable energy, 19.1% crude protein (N×6.25) and 2.6% total calcium.

Eggs were collected on 3th, 8th, and 13th week of productive season. Shell quality was tested in a total of 285 eggs of different colour (82 dark-brown, 78 light-brown, 79 olive, 46 blue). Prior to analysis, eggs were weighed with an accuracy of 0.01 g. Shell quality analysis accounted for water vapour conductance [Ar *et al.* 1974], the number of pores per 0.25 cm^2 [Tyler 1953] and shell area [Paganelli *et al.* 1974]. For the determination of water vapour conductance eggs stored for 6 days in an incubator (25°C) were weighed daily in the presence of KOH (used as water vapour absorber). Ultrastructure of both the *s*hell and the outer shell membrane of the eggs collected at week 8 of productive season (6 eggs from each colour group) was analysed with a scanning electronic microscope as described by Richards and Deeming [2001]. One fragment of shell, 0.25 cm^2 in area, was obtained from the air-sac end of each egg. After removing the inner membrane, shell fragments were covered with a layer of carbon and gold and pasted to a stabilizer. Analysis covered 20 measurements taken on each preparation to determine the shell cross-section (palisade to mamillary layer ratio). Shell thickness, diameter of outer shell membrane fibres, and outer shell membrane density were also determined. Density of the outer shell membrane was determined after binarization of the microscopic image, as a per cent of fibres in relation to the area of the image analysed. Measurements were made using the Computer Image Analysis System MultiScan v.4.01.

Three incubation sets were carried out between week 4 and 8 of egg laying season and a total of 681 eggs were set (229 dark-brown, 276 light-brown, 147 olive and 29 blue). Eggs were collected over seven consecutive days and stored at 13°C and 70% relative humidity. Eggs were weighed before setting into the BIOS BA-134 incubator and on day 21 of incubation. Temperature in the setter was 37.5°C and relative humidity 75%. In the hatcher, the respective values were 37.3°C and 90%. Until day 21 of incubation, eggs were turned every hour at an angle of 90°. On day 10, the eggs were candled to eliminate those unfertilized or with dead embryos. Per cent loss in egg weight on day 21 of incubation in relation to its weight before setting was calculated. Based on these results, egg fertilization and hatchability parametres were found.

One-way analysis of variance was used for statistical calculations. Significant differences between the eggs ($P \leq 0.05$) with different shell colour were evaluated with the Fisher test.

Results and discussion

Blue eggs weighed significantly less than eggs with other shell colours and were characterized by significantly thinner shells (by 45.9 mm on average) than olive and dark-brown eggs (Tab. 1). These results are similar to those reported by Hulet *et al.* [1985] and Richards and Deeming [2001].

The highest water vapour conductance of the shell was found in blue eggs (Tab. 1). Ar and Rahn [1985] reported a higher value (6.92 mg H_2O/day × torr) in ring-necked pheasant eggshells regardless of their colour. However, in the paper cited the eggs analysed were heavier than in this study (by 0.8 g on average) and thus had greater shell surface, which may explain the difference between this study and the paper cited.

Mean total number of pores in pheasant eggshells was 4661 (Tab. 1) being lower by over 2700 from that reported by Ar and Rahn [1985]. Such a large difference could be due to both the difference in egg size and the environmental effects, for Tullett [1984] reported that the reduced number of pores in the shell may be a way in which layers adapt to unfavourable climatic conditions. Total number of pores in blue eggshells was significantly higher (by 443) compared to the other colour groups of eggs.

Analysis of the shell cross-section showed that the palisade and mamillary layers were clearly marked except for the blue shells (Fig. 1). The mamillary layer of blue eggshells was thinner than of shells of remaining colours, corroborating the results of Richards and Deeming [2001]. In the present study we found, similarly to Richards and Deeming [2001], that weakly distinct mammillae tended to join early in the mamillary layer of blue shells, which resulted in the mamillary layer being shallower. They were also characterized by the largest contact surface with the outer shell membrane compared to the papillae of eggshells of remaining colours. Blue shells had significantly thinner fibres of the outer shell membrane (by 0.15 μm on average) compared to eggs of other colours (Tab. 1, Fig. 2). Richards and Deeming [2001] found greater mean thickness of fibres of pheasant eggshells than in the present study, regardless of shell colour. No significant differences in the density of outer shell membrane were shown among pheasant eggs of different colours (Tab. 1).

Table 1. Means and their standard deviations (SD) for weight, shell traits and outer shell membrane traits of pheasant eggs in relation to shell colour

Eggshell colour		Egg weight (g)	Eggshell thickness (µm)	Water vapour conductance (mg H_2O/day ×torr)	Eggshell area (cm²)	No. of pores per 0.25 cm² shell area	Total no. of pores per shell	Diameter of eggshell outer membrane fibres (µm)	Eggshell outer membrane thickness (per cent of fibres in relation to the area of the image analysed)
Dark-brown	mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Light-brown	mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Olive	mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Blue	mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Overall	mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

[a,b] Within columns means bearing different superscripts differ significantly at P≤0.05.

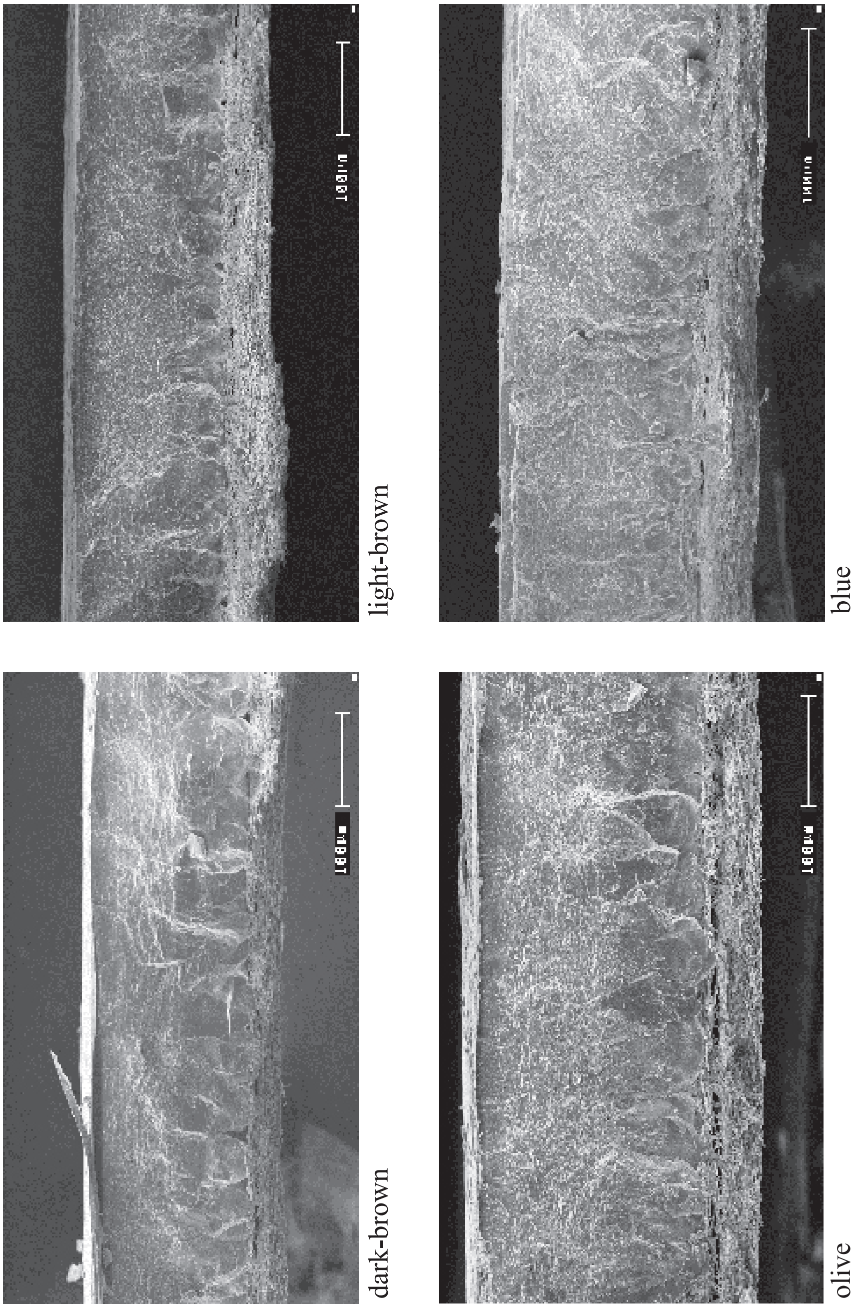

Fig. 1. Cross-section of pheasant eggshells of different colours (× 400).

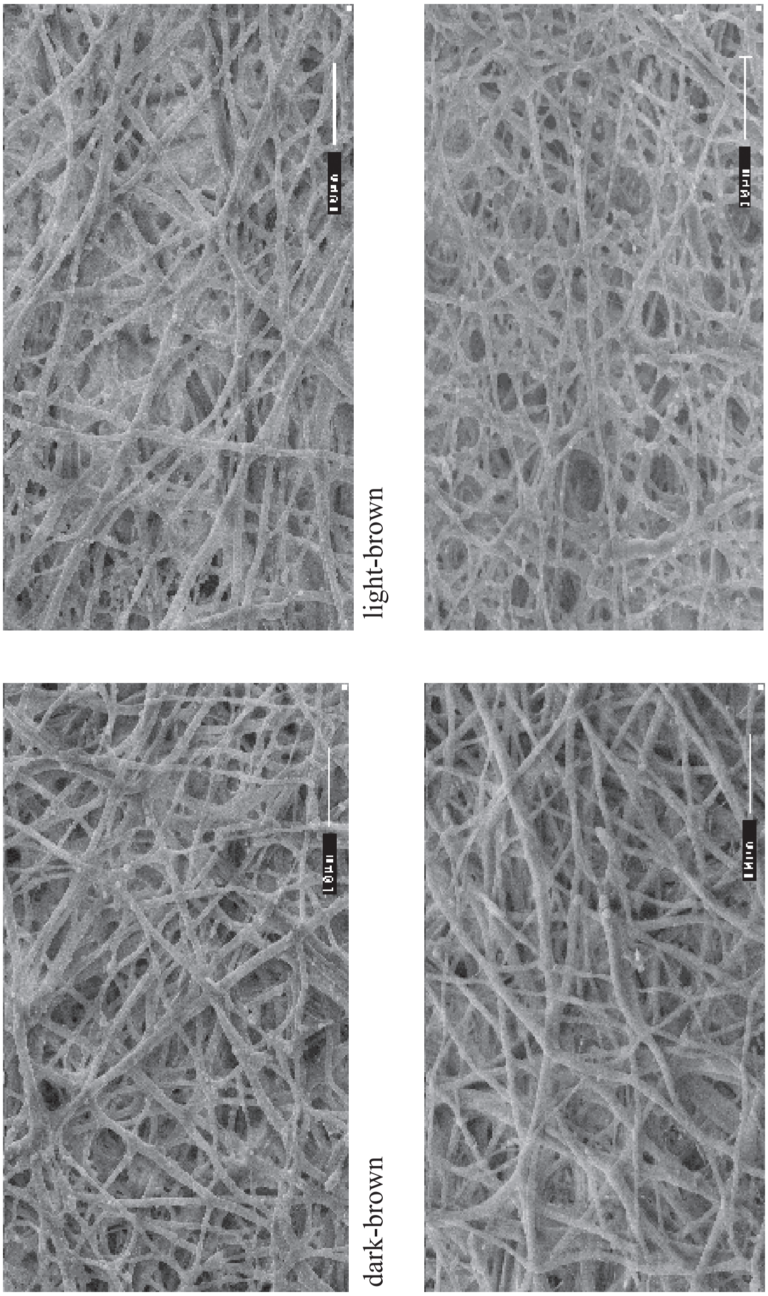

Fig. 2. Shell outer membrane of pheasant eggs of different colours, inside view (× 300).

Table 1. Means and their standard deviations (SD) for weight, weight loss, fertilisation and hatchability of pheasant eggs in relation to shell colour

Eggshell colour		Egg weight (g)	Egg weight loss to day 21 of incubation (%)	Egg fertilisation (%)	Hatchability from fertilised eggs (%)	Hatchability from set eggs (%)
Dark-brown	mean	[illegible]^a	[illegible]^a	[illegible]^a	[illegible]^a	[illegible]^a
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Light-brown	mean	[illegible]^a	[illegible]^b	[illegible]^a	[illegible]^a	[illegible]^a
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Olive	mean	[illegible]^a	[illegible]^a	[illegible]^a	[illegible]^a	[illegible]^a
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Blue	mean	[illegible]^b	[illegible]^b	[illegible]^b	[illegible]^b	[illegible]^b
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Overall	mean	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
(n=[illegible])	SD	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

a,b Within columns means bearing different superscripts differ significantly at P≤[illegible]

Fertilization rate was by 62.3 per cent points (pp) lower in blue than in eggs of remaining colours (Tab. 2). Hulet *et al.* [1985] reported no association between pheasant shell colour and egg fertilization rate, although they found the latter to be the lowest in blue eggs, differing by 6.3 pp on average from the other eggs. Mean hatchability of chicks from fertilized and set eggs was 74.7% and 54.3% respectively. These values were similar to those (75.2% and 53.4%) reported by Krystianiak *et al.* [1999] and by 17.3 and 8.8 pp better than the values given by Hulet *et al*. [1978]. In the present study, blue-shelled eggs were characterized by lower hatchability from fertilized eggs (by about 58 pp) compared to the other egg colour groups. These indicators are similar to those of Hulet *et al.* [1978], who showed lower (by 24 pp) hatchability from blue eggs compared to the other egg colour groups. In the present study, blue eggs were characterized by the greatest weight loss (over 7%) to day 21 of incubation compared to the eggs of remaining shell colours. Poorer hatchability of chicks from blue eggs could result from intensive evaporation of water, which led to the excessive loss of egg weight during incubation. Ar and Rahn [1980] report that the total egg weight loss must not exceed 15% to ensure the maximum embryo survival possible.

The thinner shell of greater porosity and of greater water vapour conductance found in blue eggs, indicates its inferior quality compared to the shell of eggs of remaining three colours. In addition, the analysis of blue eggshell ultrastructure showed a shallower mamillary layer, while the excessive contact surface between the papillae of this layer and the shell membrane points to a lack of coherence between these layers, and thus an abnormal structure of the shell. All these factors contributed to poorer hatchability results from blue eggs, which in turn indicates that they should be eliminated when being selected for incubation.

REFERENCES

1. AR A., PAGANELLI C.V., REEVES R.B., GREENE D.G., RAHN H., 1974 – The avian egg: water vapor conductance, shell thickness, and functional pore area. **C*ondor*** 7, 153-158.
2. AR A., RAHN H.**,** 1980 – Water in the avian egg: Overall budget of incubation. ***American Zoologist*** 20, 373-384.
3. AR A., RAHN H.**,** 1985 – Pores in avian eggshells: Gas conductance, gas exchange and embryonic growth rate. ***Respiration Physiology*** 61, 1-20.
4. BUNK M.J., BALLOWN S.L., 1978 – Ultrastructure of the mammillary region of low structure strength avian eggshells. ***Poultry Science*** 57**,** 639-647.
5. CARNARIUS K.M., CONRAD K.M., MAST M.G., MACNEIL J.H., 1996 – Relationship of eggshell ultrastructure and shell strength to the soundness of shell eggs. ***Poultry Science*** 75, 656-663.
6. DOHNAL J.M., KIEŁCZEWSKI K., POŚPIECH M., ŁAKOTA P.**,** 1989 – Genetyczna zależność między jakością skorupy a wylęgowością jaj kur nieśnych (Genetic relationship between egg shell quality and hatchability of eggs from laying hens). In Polish, summary in English. ***Zeszyty Naukowe Drobiarstwa*** 6, 5-11.
7. FRASER A.C., CUSACK M., SOLOMON S.E., BAIN M.M.**,** 1999 – Genetic influences on the ultrastructural integrity and protein chemistry of broiler breeder eggshells. Proceedings of the 8th European Symposium on the Quality of Eggs and Egg Products, 19-23 September, Bologna, Italy, 31-36.

8. GIELECKI J., GACEK W., ROSIŃSKI A., ELMINOWSKA-WENDA G., CYWA-BENKO K., 1995 – Cyfrowa analiza ultrastruktury zewnętrznej błony skorupowej. Materiały II Sympozjum Polskiej Grupy Badań Struktury Kości. Warszawa 10 czerwca, 15-16. Digital image analysis of the outer egg-shell membrane. In Polish, summary in English.
9. HULET R.M., MARQUEZ B.J., MOLLE E., FLEGAL C.J., 1978 – The relationship of pheasant egg color and hatchability. ***Poultry Science*** 57, 1146.
10. HULET R.M., FLEGAL C.J., CARPENTER G.H., CHAMPION L.R., 1985 – Effect of eggshell color and thickness on hatchability in Chinese ring-necked pheasants. ***Poultry Science*** 64, 235-237.
11. KRYSTIANIAK S., NOWACZEWSKI S., KONTECKA H., TORGOWSKI J., 1999 – Cechy reprodukcyjne bażantów łownych utrzymywanych w wolierach (Reproductive traits of pheasants kept in aviaries). In Polish. Prace Komisji Nauk Rolniczych i Komisji Nauk Leśnych Polskiego Towarzystwa Przyjaciół Nauk, 87, 171-177.
12. KRYSTIANIAK S., KONTECKA H., WĘŻYK S., 2000 – Jakość jaj bażantów łownych w zależności od barwy skorupy (Pheasant´s eggs quality depending on its eggshell colour). In Polish, summary in English. ***Zeszyty Naukowe Przeglądu Hodowlanego*** 49, 501.
13. KRYSTIANIAK S., KONTECKA H., 2002 – Jakość skorup jaj w zależności od jej barwy i okresu nieśności bażantów (Eggshell quality depending on its colour and laying period of pheasants). In Polish, summary in English. ***Roczniki Naukowe Zootechniki*** Supplement l, 16, 131-135.
14. MALEC H., BORZEMSKA W., ROMANISZYN K., 1999 – Zmiany w ultrastrukturze przekroju skorupy jaja u niosek chorych na kolibakteriozę (Changes in ultrastructure of eggshell layers infected by colibacillosis). In Polish, summary in English. ***Medycyna Weterynaryjna*** 55 (4), 268-271.
15. McDANIEL G.R., ROLAND D.A., COLEMAN A.M., 1979 – The effect of eggshell quality on hatchability and embryonic mortality. ***Poultry Science*** 58 (1), 10-13.
16. MRÓZ E., PUDYSZAK K., 2000 – Barwa skorupy jaja bażanta łownego i jej wpływ na wylęgowość (Pheasant eggshell colour and its effect on hatchability). In Polish, summary in English. ***Zeszyty Naukowe Przeglądu Hodowlanego*** 49, 459-466.
17. PAGANELLI C.V., OLSZOWKA A., AR A., 1974 – The avian egg: surface area, volume, and density. ***Condor*** 76 (3), 319-325.
18. ROBERTS J.R., BRACKPOOL C.E., SOLOMON S.E., 1995 – The ultrastructure of good and poor quality eggshells from Australian layer strains. Proceedings of the 6th European Symposium on the Quality of Eggs and Egg Products. September 25-29, Zaragozza, Spain, 107-115.
19. RICHARDS P.D.G., DEEMING D.C., 2001 – Correlation between shell colour and ultrastructure in pheasant eggs. ***British Poultry Science*** 42, 338-343.
20. SOLOMON S.E., 1994 – Egg shells: Patterns of mineralisation. ***Poultry International*** 33 (7), 50-53.
21. SZCZERBIŃSKA D., 1997 – Skorupa jaja a wylęgowość i ich związek z wiekiem kur (Relationship between age, eggshell and hatchability in layers). In Polish, summary in English. ***Zeszyty Naukowe Akademii Rolniczej w Szczecinie***, 176 (Zootechnika) 34, 119-125.
22. TULLETT S.G. 1984 – The porosity of avian eggshells. ***Comparative Biochemistry and Physiology*** 78 A, 1, 5-13.
23. TYLER C., 1953 – Studies on egg shells. II – A Method for marking and counting pores. ***Journal of the Science of Food and Agriculture*** 4, 266 -272.

Stanisława Krystianiak, Radosław Kożuszek,
Helena Kontecka, Sebastian Nowaczewski

Barwa, jakość i ultrastruktura skorupy jaj bażancich oraz związek jej barwy z wylęgowością

S t r e s z c z e n i e

Materiał stanowiło 990 jaj wylęgowych bażanta łownego podzielonych na cztery grupy, zależnie od barwy skorupy (ciemnobrązowa, jasnobrązowa, oliwkowa, niebieska). W 285 jajach określono grubość skorupy, przepuszczalność przez nią pary wodnej oraz liczbę porów na 0,25 cm^2 powierzchni, a nadto ultrastrukturę skorupy (w sześciu jajach każdej barwy). Przeprowadzono także trzy lęgi, do których wybrano łącznie 681 jaj. Wykazano, że skorupa jaj niebieskich była cieńsza i cechowała ją większa przepuszczalność pary wodnej, a także większa liczba porów na jednostce powierzchni w porównaniu ze skorupami jaj pozostałych trzech kolorów. W budowie skorupy jaj niebieskich stwierdzono odchylenia strukturalne Jaja te charakteryzowała także gorsza wylęgowość oraz większa strata masy do 21 dnia lęgu. Uzyskane wyniki wskazują na celowość eliminacji jaj o niebieskich skorupach przy wyborze bażancich jaj do lęgu.