Animal Science Papers and Reports vol. 26 (2008) no. 2, 129-140
Institute of Genetics and Animal Breeding, Jastrzębiec, Poland

Shell microstructure and hatchability of turkey eggs

Emilia Mróz[1], Katarzyna Michalak[1], Andrzej Faruga[1], Jarosław O. Horbańczuk[2], Aneta Orłowska[1]

[1] Department of Poultry Science, Faculty of Animal Bioengineering, University of Warmia and Mazury in Olsztyn, M. Oczapowskiego 5, 10-719 Olsztyn, Poland

[2] Polish Academy of Sciences Institute of Genetics and Animal Breeding, Jastrzębiec, 05-552 Wólka Kosowska, Poland

(Received November 11, 2007; accepted February 26, 2008)

The study aimed at determining the hatchability of turkey eggs as related to the shell surface characteristics (regular shells, rough shells and pigment-spotted shells – group 1, 2 and 3, respectively). A total of 17 590 eggs of Broad-breasted White Turkeys, marked individually, were considered during the peak of the laying season. Shell microstructure was examined of 60 eggs from each group, using a scanning electron microscope while hatchability was estimated based on fertilization rate, percentage of dead embryos and hatching rate (healthy poults only).

The main differences between the shells from group 1 and 2 included different size of cuticular plates, thicker crystal layer, thinner palisade and mammillary layers, curved walls of the mammillae, and thicker fibres of the inner shell membrane. Eggshells from group 3 showed a significantly thicker crystal layer and curving fibres of the inner shell membrane compared to group 1 shells. The hatching rate of set eggs was highest (77.15%) in group 1. Embryo mortality rates were by 4.9 and 5.4 per cent points higher in eggs from groups 2 and 3, respectively, than in eggs from group 1. The hatchabilities of rough-shelled eggs (group 2) and eggs with pigment spots (group 3) appeared lower than of eggs with regular shells (group 1).

KEY WORDS: turkey / eggshell / hatchability / microstructure

Intensively farmed laying hens often lay eggs with structural faults due to the fact that breeding work is directed primarily towards increased egg production. Eggshell abnormalities may also be a consequence of diseases or improper housing and management conditions [Solomon and Watt 1990, Fraser *et al*. 1995, Roberts *et al*.1995, Szczerbińska 1995, Mróz 1996, 1998, Roberts and Nolan 1997, Malec *et al*.

2002, Świątkiewicz 2005, Michalak and Mróz 2006, Mróz *et al*. 2007a]. The moment of the occurrence of morphological and chemical changes of an eggshell may be determined based on its multilayer structure. The functional properties of an eggshell (gas and vapour permeability) may be assessed during storage and incubation. Normal and abnormal eggshells differ with respect to morphology, chemical composition and functional properties [Arias and Fernandez 1995, Malec 1999, Michalak and Mróz 2006, Mróz *et al*. 2007a].

Certain traits of the eggshell may indicate its structural faults [Solomon 1991, 1996, Arias and Fernandez 1995, Malec 1999, Malec *et al*. 1999, Szczerbińska 2002, Krystianiak *et al*. 2005, Mróz *et al*. 2007a]. These are:

- bodies A and B in the mammillary layer having no connection with the inner shell membrane;
- thickened or curved walls of the mammillae, decreasing or increasing the spaces between them;
- shapes of the mammillae untypical of a given species;
- cracking of eggshell layers and no visible boundaries between them;
- large cuticular plates or no cuticular plates present;
- lack of pigmentation or large pigment spots;
- deposits of eggshell mass of various size and structure [Solomon 1991, 1996, Arias and Fernandez 1995, Malec 1999, Malec *et al*.1999, Szczerbińska 2002, Krystianiak *et al*. 2005, Mróz *et al*. 2007a].
- thick and curved fibres of the inner shell membrane [Michalak and Mróz 2006];
- elevated calcium, magnesium, phosphorus and trace elements content of the eggshell surface with damaged cuticle [Malec 1999].

During incubation water loss (evaporation) is higher in eggs with abnormal than with proper shells (13.47-12.21% *vs*. 11.71%) – Mróz *et al*. [2007a]. Increased water loss is followed by increased embryo mortality [Mróz 1998, Malec 1999, Malec *et al*. 2002, Mróz *et al*. 2002ab]. Turkey embryo mortality rates in the groups of eggs without shell pigmentation and eggs with rough shells are higher by 8.56% and 2.9%, respectively, than in the group of eggs with proper quality of shells [Mróz *et al*. 2007b].

Disturbances in the shell formation process in turkeys are quite common, as confirmed by a high number of eggs which differ from regular eggs in terms of pigment colour, pigmentation pattern or shell surface [Mróz 1996, 1998]. The number of eggs with shell surface faults depends on the origin of turkeys, reaching up to 10% in heavy-type birds [Mróz *et al*. 2002a, 2007a] and up to 39% in medium- and light-type birds [Mróz *et al*. 1997, Mróz 1998]. Structural faults of eggshells can significantly decrease the reproductive performance of turkeys.

Professional literature provides information on the shell surface characteristics of an ideal turkey egg [Michalak and Mróz 2006, Mróz *et al*. 2007a], as well as on certain types and morphology of structural defects of an eggshell [Mróz 1998, Michalak and Mróz 2006]. However, there are no data available on the microstructure of rough shells and shells with pigment spots, which account for 16.3-19.3% and 1.21-2.28% of all turkey eggs [Mróz 1998]. Although many publications stress the importance of shell structure quality, turkey eggs with rough shells or shells with unevenly distributed pigment are still widely used for incubation, due to a still too low number of eggs yielded or unawareness of their low biological quality [Mróz 1996, 1998, Mróz *et al*. 2002b, 2007b].

The objective of the present study was to determine the hatchability of turkey eggs of different shell surface characteristics (eggs with regular shells, rough-shelled eggs and eggs with pigment spots).

Material and methods

The material comprised 17 590 hatching eggs of Broad-breasted White turkeys, yielded during two weeks over the peak of the laying season. Eggs differing in shell surface characteristics were allocated organoleptically to three groups: 1 – with regular (proper) shells, 2 – with rough shells, 3 – with pigment spots on the shell surface. All eggs were marked individually on 143 trays. Good-quality (regular) shells were light-brown or cream-coloured, with brown spots uniformly distributed over the surface (Fig. 1A). Rough shells had a few pigment spots only (Fig. 1B). Shells with pigment spots had large brown spots of various shape in the central part of an egg (Fig. 1C). A detailed description of the surfaces of eggs representing particular groups was given by [Mróz 1998].

Examined were 60 randomly selected eggs from each group. The eggs were opened, their shells emptied and rinsed thoroughly with water to remove all the albumen. Samples of shells with membranes were taken from the central part of an egg. The membrane was separated from the shell. Next the samples were attached to the stabilizers, gold-coated with a JEOL Fine Coater, JCF-1200, and viewed under a scanning electron microscope (JSM –531OLV, JEOL, 25kv) to obtain the image of the surface area and cross-sectional area. The structure characteristics common to all 60 sampled eggs from each group were determined based on microscope images. Images of the surface area and cross-sectional area, representative of each group, were photographed at 350 x magnification, whereas those of the inner shell membrane were photographed at 1500 x magnification. In the current report pictures are presented of the analysed types of eggshells and inner membranes.

Two incubation series were carried out at one-week intervals in PETERSIME incubators in accordance with the relevant technological standards for turkeys. The percentages of: unfertilized eggs, eggs containing dead embryos, eggs containing living but unhatched embryos, poults with physical defects and healthy poults hatched of set eggs were determined at the completion of incubation. The results were processed statistically using a one-factor analysis of variance, calculating means and coefficients of variation.

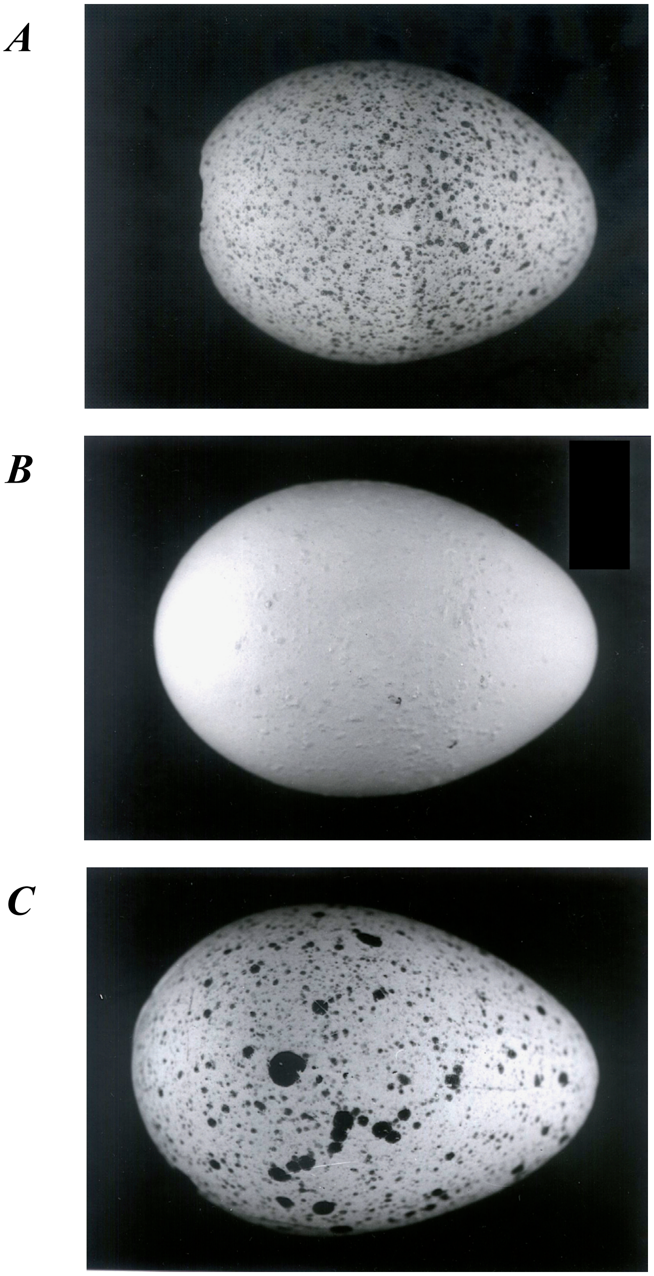


Fig. 1. Hatching eggs. A – regular shell, B – roughness, C – pigment spots.

Results and discussion

A cuticle with numerous tiny cracks, which looks like a torn-up net, covers the entire surface of good-quality eggshells. Pores are observed rarely on the shell surface (Fig. 2 A). On rough shells the cuticle is distributed over some places like on good-quality shells, whereas at other places it exhibits slab-like structure with cracks, which are much wider than those on normal-quality shells. Numerous tiny infiltrations of shell mass form thickened areas which resemble vesicles (Fig. 2 B-b). The surface of eggshells with pigment spots does not differ from the surface of regular eggshells, except for in pigment-covered areas (Fig. 2 C-d). The surface structure of rough shells and shell with pigment spots suggest that the glands responsible for the production of shell mass and pigment continued to function although the next shell layer (cuticle) had already been formed. The reasons for this phenomenon have not been clarified, but it seems that the egg moving backwards in the duct before being laid could stimulate further secretion from those glands.

The crystal layer was rarely observed in regular (good-quality) shells (group 1). In group 2 the layer was well visible under the cuticle (Fig. 3-e), while in shells with pigment spots (group 3) it was thick at some places, like in regular quality shells. The palisade layer had spongy structure in eggs from group 1 and 3. In rough-shelled eggs the palisade layer was composed of vesicles and other forms (Fig. 3-f). The mammillae were filled with spongy mass. Vesicles of this layer were small in regular shells, and much bigger in shells with pigment spots (Fig 3-g). In rough shells the mammillary layer was thinner, the walls of mammillae were curved, and the inner part of mammillae was hardly distinguishable. The tested shells differed in thickness. Group 2 shells were found to be the thinnest, due to the malfunction of the pseudo-uterine glands or accelerated egg laying (Fig. 3 B).

In eggs with regular shells (group 1) the inner shell membrane consisted of fibres of different thickness, forming a dense web. In group 2 shells the fibres of the inner membrane were thicker than in regular shells. The inner shell membrane of group 3 shells was characterized by very thick fibres. Curved fibres with uneven walls are indicative of certain functional disorders of the oviduct isthmus (Fig. 4 C-i).

The structure of rough eggshells, in contrast to the structure of the other types of eggshells, had large cuticular plates and broad cuticular cracks as well as a thicker crystal layer. Identical structural changes in eggshells of poor quality were observed in laying hens [Solomon 1991, Malec 1999], pheasants [Krystianiak *et al*. 2005] and turkeys [Michalak and Mróz 2006]. In turkeys as well as in other bird species the crystal layer can be found only in certain parts of the eggshell [Solomon 1991, Szczerbińska 2002]. Similarly as in granular shells and shells without surface pigmentation, a thicker crystal layer was observed in rough shells and shells with pigment spots [Michalak and Mróz 2006]. Thinner palisade and mammillary layers as well as the lack of clear-cut boundaries between those layers are indicative of certain structural defects of an eggshell. The thinnest fibres were recorded in turkey eggs with good quality shells, which corroborates the reports concerning chicken and pheasants [Krystianiak *et al*.

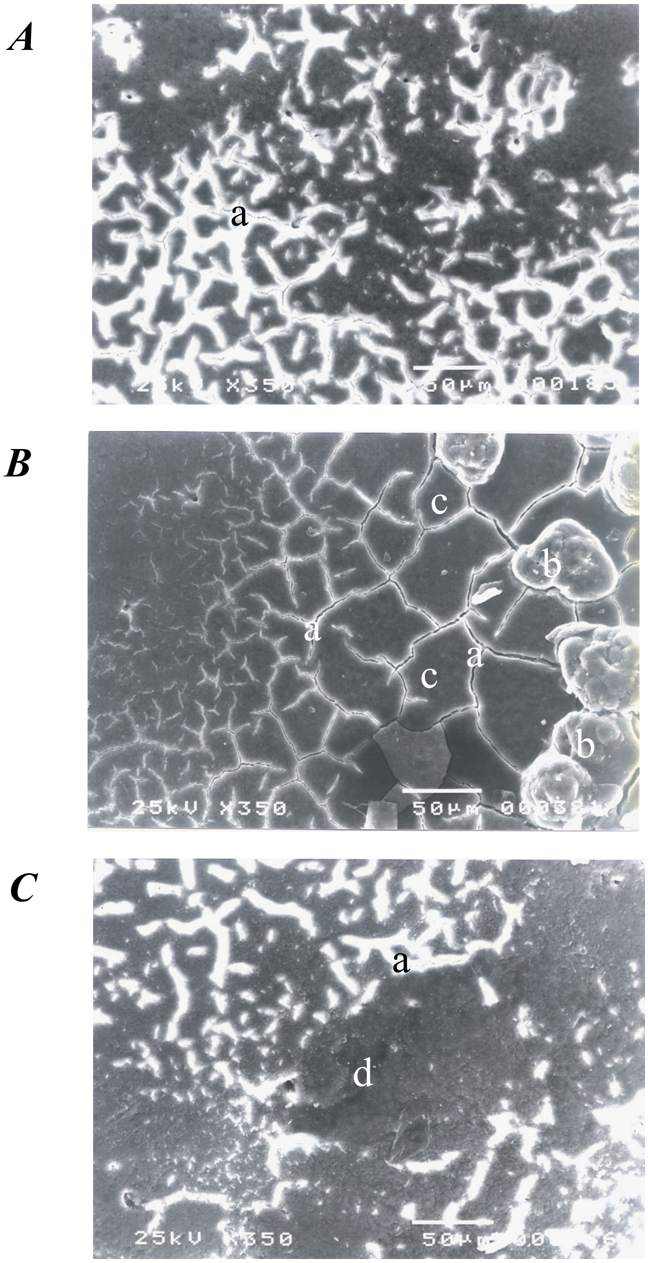
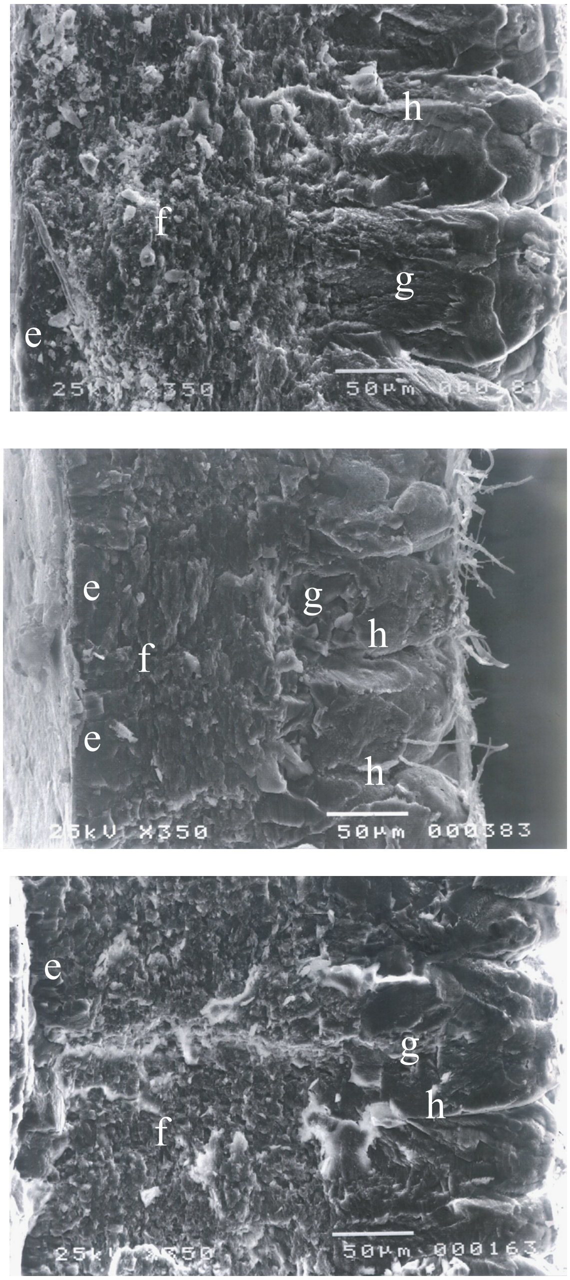


Fig. 2. The cuticle on the surface. A – regular shell, B − roughness, C − pigmented spots.

A

B

C



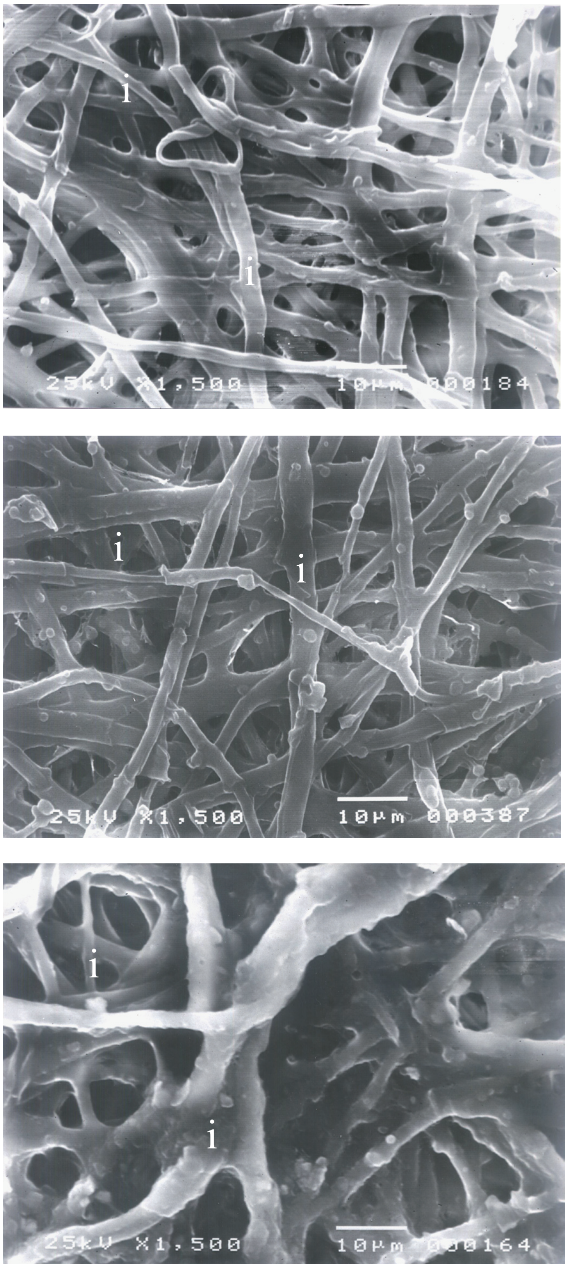
e – crystal layer
f – palisade layer
g – mammillary layer centre
h – mammillary wall

Fig. 3. The cross section of: A – regular shell, B − roughness, C − pigmented spots.

A

B

C



i - fibres

Fig. 4. The fibres of undershell membrane. A – regular shell, B − roughness, C − pigmented spots.

2005, Malec 1999, Michalak and Mróz 2006, Mróz *et al*. 2007a]. Thickened areas on the surface of an eggshell, large cuticular plates and pigment spots are a consequence of the malfunction of the pseudo-uterus at the final stage of shell formation.

Eggs with regular shells surface accounted for only 69.18% of all tested eggs. Apart from rough-shelled eggs and eggs with pigment spots, 10.64% of eggshells showed other surface defects. The eggs from shell microstructure groups differed, but not significantly, with respect to hatchability indicators (Tab. 1). The lack of significant differences resulted from wide variation within the analysed traits. The lower final hatching rates were affected by considerable differences in the per cent of dead embryos in particular groups. The hatchability of eggs with pigmented spots on shells as well as of rough-shelled eggs was lower by 7.47 and 5.23 per cent points, respectively, than of eggs with regular (normal) quality shells. Hatching rates intercepting the shell surface characteristics indicate a low biological value of eggs with rough shells and pigment spots, which is in accordance with earlier reports [Mróz *et al*. 1997, Michalak and Mróz 2006, Mróz *et a*l. 2007a]. Comparable hatching rates were reported for low-quality turkey eggs and turkey eggs stored for 7 days or longer [Mróz *et a*l. 2002b, 2004]. Other authors stress that negative effect of uneven shell pigmentation on the final hatching rate is stronger than are irregularities and bumps on the surface of an eggshell [Krystianiak *et al*. 2005, Malec 1999], which was also confirmed by the present study.

Table 1. Incubation indicators

Item		Eggshell quality group 1 (regular)	2 (shell roughness)	3 (pigmented spots)
Hatching eggs				
number (pcs)		12170	3297	253
percent as related to eggs set		69.18	18.74	1.44
Unfertilized eggs (%)	mean	6.50	6.82	8.55
	V	16.16	20.45	25.81
Embryos dead before day 10 (%)	mean	6.25	8.40	8.17
	V	11.40	19.26	22.29
Embryos dead after day 10 plus unhatched poults plus poults hatched with physical defects (%)	mean	10.10	12.86	13.60
	V	13.74	23.61	31.63
Poults hatched (%)	mean	77.15	71.92	69.68
	V	28.57	33.50	41.00

V – variation coefficient.

The surface characteristics of an eggshell reflect its inner structure. The main differences between regular (*i.e*. of good quality) eggshells and rough eggshells include:

– different size of cuticular plates;
– thicker crystal layer;
– thinner palisade and mammillary layers;
– shell mass of various size and shape in the palisade layer;
– lack of visible central parts in the mammillae;
– curved walls of the mammillae;
– thicker fibres of the inner shell membrane.

Eggshells with pigment spots, in contrast to regular quality eggshells, are characterized by:

– significantly thicker crystal layer;
– vesicles in the central part of the mammillae;
– thicker and curving fibres of the inner shell membrane.

The hatchability of rough-shelled eggs and eggs with pigment spots was lower, in comparison to eggs with normal quality shells.

REFERENCES

1. ARIAS J.L., FERNANDEZ M.S., 1995 – Role of extracellular matrix on eggshell formation. Proceedings of the VI European Symposium on the Quality of Eggs and Egg Products. September 25-29, Spain, 89-96.
2. FRASER A., BAIN M.M., SOLOMON S.E., 1995 – The structural organisation and functional properties of eggshells from alternative housing systems. Proceedings of the VI European Symposium on the Quality of Eggs and Egg Products. September 25-29, Spain, 141-146.
3. KRYSTIANIAK S. KOŻUSZEK R., KONTECKA H., NOWACZEWSKI S., 2005 – Quality and ultrastructure of eggshell and hatchability of eggs in relation to eggshell colour in pheasant. ***Animal Science Papers and Reports*** 23(1), 5-14 .
4. MALEC H., 1999 – Biologiczna wartość kurzych jaj wylęgowych o różnej zawartości porfiryny. In Polish, summary in English. ***Zeszyty Naukowe AR w Krakowie.*** Rozprawy 251.
5. MALEC H., BORZEMSKA W., NIEDZIÓŁKA J., 1999 – Ultrastruktura powierzchni skorupy jaj u niosek chorych na kolibakteriozę. In Polish, summary in English. ***Medycyna Weterynaryjna*** 3, 172-175.
6. MALEC H., BORZEMSKA W., NIEDZIÓŁKA J., PIJARSKA I., 2002 – Wartość wylęgowa jaj o tzw. skorupach z naddatkami . In Polish, summary in English. ***Medycyna Weterynaryjna*** 1, 49-51.
7. MICHALAK K., MRÓZ E., 2006 – Ultrastructure of the turkey hatching egg shell. ***Polish Journal of Natural Sciences*** *21* (2) 671-689.
8. MRÓZ E., 1996 – Niektóre cechy morfologiczne powierzchni skorupy jaj indyczych i ich związek z wylęgowością. In Polish, summary in English. ***Zeszyty Naukowe Przeglądu Hodowlanego*** 24, 77-84.
9. MRÓZ E., 1998 – Studia nad zmiennością cech powierzchni skorupy jaj indyczych i ich związkiem z wylęgowością. In Polish, summary in English. ***Dissertations and Monographs*** 4. University of Agriculture and Technology in Olsztyn.
10. MRÓZ E., MICHALAK K., ORŁOWSKA A., 2007a – Hatchability of turkey eggs as dependent on shell ultrastructure. ***Polish Journal of Natural Sciences*** 22(1), 31-42.
11. MRÓZ E., MICHALAK K., ORŁOWSKA A., 2007b – Embryo mortality and poult quality depend on the shell structure of turkey hatching eggs. ***Animal Science Papers and Reports*** 25, 3:1-12.

12. MRÓZ E., ORŁOWSKA A., REITER K., 2004 – Czas przechowywania jaj indyczych a wyniki wylęgu i jakość indycząt. In: Towaroznawstwo żywności i przedmiotów użytku. (K.A. Skibniewska, Ed.). In Polish. University of Warmia and Mazury in Olsztyn : 143-153.
13. MRÓZ E., PUCHAJDA H., PUDYSZAK K., 2002a – Structure and pigmentation of eggshell and biological value of turkey hatching eggs. ***Polish Journal of Natural Sciences*** 10(1) 141-152.
14. MRÓZ E., PUCHAJDA H., MICHALAK K., PUDYSZAK K., 2002b – Analiza biologiczna wylęgowości jaj indyczych . In Polish, summary in English. ***Roczniki Naukowe Zootechniki*****,** Suplement 16, 61-66.
15. MRÓZ E., PUDYSZAK K., PUCHAJDA H., 1997 **–** Wpływ różnych czynników na jakość skorupy i wylęgowość jaj indyków (The influence of different factors upon eggshell quality and hatchability of turkey eggs). In Polish, summary in English **. *Zeszyty Naukowe Przeglądu Hodowlanego*** 31, 59-66.
16. 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 VI European Symposium on the Quality of Eggs and Egg Products. September 25-29, Spain,: 107- 115.
17. ROBERTS J.R., NOLAN J.V., 1997 – Egg and eggshell quality in five strains of laying hens and the effect of calcium source and hen age. Proceedings of the VII European Symposium on the Quality of Eggs and Egg Products. September 21-26, Poland, 38-44.
18. SOLOMON S.E, WATT J.M., 1990 – Influence of stress on eggshell structure. ***Poultry International***, September, 12-14.
19. SOLOMON S. E., 1991 **–** Egg and eggshell quality . Wolfe Publishing Limited. London.
20. SOLOMON S.E., 1996 – The eggshell a barier? ***Turkeys*** 44(4), 8-10.
21. SZCZERBIŃSKA D., 1995 – Skorupa jaja a wylęgowość i ich związek z wiekiem i pochodzeniem kur (An eggshell and hatchability and their relation to age and origin of hens). PhD Thesis. In Polish, summary in English. Agricultural University of Szczecin. Rozprawa doktorska, AR w Szczecinie.
22. SZCZERBIŃSKA D., 2002 – Charakterystyka użytkowości reprodukcyjnej emu (Dromaius Novaehollandiae) ze szczególnym uwzględnieniem ultrastruktury skorupy i jej związku ze wskaźnikami wylęgowości jaj (Reproduction in emu (Dromiaius Novaehollandiae) with special reference to eggshell structure and its relation to hatchability). In Polish, summary in English. Rozprawy 210, AR w Szczecinie**.**
23. ŚWIĄTKIEWICZ S., 2005 – Wpływ poziomu oraz formy cynku i manganu w paszy dla niosek na jakość skorup jaj i kości oraz wydalanie Zn i Mn (The effect of level and form of Zn and Mn in feed for laying hens on eggshell and bone quality and Zn and Mn excretion). Thesis. Rozprawa habilitacyjna. In Polish, summary in English. ***Roczniki Naukowe Zootechniki*** 22.

Emilia Mróz, Katarzyna Michalak, Andrzej Faruga,
Jarosław O. Horbańczuk., Aneta Orłowska

Budowa skorupy a zdolność wylęgowa jaj indyczych

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

Celem pracy była ocena zależności między cechami mikrostruktury powierzchni skorup jaj indyczych, charakteryzujących się powierzchnią wzorcową (grupa 1), chropowatą (grupa 2) i plamiście pigmentowaną (grupa 3) a ich zdolnością wylęgową. Do badań użyto 17 590 jaj indyków białych szerokopierśnych w szczycie nieśności, wśród których wyróżniono i oznakowano indywidualnie wymienione typy powierzchni skorupy. Budowę skorup oceniono za pomocą mikroskopu skaningowego na 60 jajach z każdej grupy, a zdolność wylęgową jaj na podstawie procentów zapłodnienia, zamarłych zarodków i wylęgu prawidłowo zbudowanych indycząt.

Skorupy chropowate różniły się od wzorcowych różną wielkością płytek kutykuli, grubszymi warstwami krystalicznymi, cieńszymi warstwami palisadowymi i brodawkowymi, brodawkami o powyginanych ścianach i grubszymi włóknami błony podskorupowej. Skorupy z plamami pigmentu miały znacznie grubsze warstwy krystaliczne i bardziej poskręcane włókna błony podskorupowej w porównaniu ze skorupami wzorcowymi. Wylęgowość z jaj nałożonych była największa w grupie o wzorcowej powierzchni skorupy – 77,15% – jednak nie różniła się istotnie od stwierdzonej w pozostałych grupach. Śmiertelność zarodków w jajach o powierzchni chropowatej i z plamami pigmentu okazała się nieistotnie wyższa (odpowiednio o 4,9 i 5,4 punktu procentowego) od procentu zarodków zamarłych w grupie jaj o powierzchni prawidłowej. Wnioskuje się, że zdolność wylęgowa jaj indyczych o skorupie chropowatej, a także plamiście pigmentowanej jest niższa niż jaj o skorupie prawidłowej (wzorcowej).